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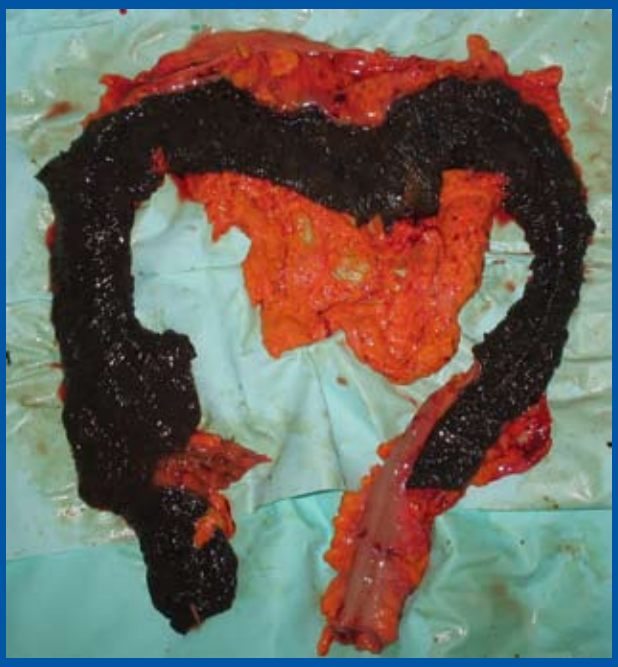

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The Ulster Medical Journal

The Journal of the Ulster Medical Society. First published in 1932.
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Transactions of the Belfast Clinical and Pathological Society (1854-1860)

The Ulster Medical Society was founded in 1862
by the amalgamation of the Belfast Medical Society (founded 1806)
and the Belfast Clinical and Pathological Society (founded 1853)

Editorial

The Hirsch Index and measuring the quality of scientific papers

Quantifying the quality of a scientists output is difficult. Publishing papers in journals gets increasingly more difficult as standards increase and publishers compete to survive in a cut throat world. The universities recently completed the latest research assessment exercise (RAE). Submissions included the 'best four papers' – usually based on impact factor. Journal impact factor is measured by taking two years of journal issues and dividing the number of papers published into the number of citations – the higher the impact, the 'better quality' the paper. This method has several disadvantages – often the best paper that impacts on scientific practice and changes things for the better, may not be published in a high citation journal favouring cutting edge science that may contribute to treatments some years later.

JE Hirsch recently proposed a scientific index – now known as the Hirsch index *h* – which is defined as the number of papers with a citation number higher or equal to *h*, as a useful index to characterise the scientific output of a researcher[1] – (Box 1). For example, Stephen W Hawking has published several physics papers and 62 of these have been cited at least 62 times giving him an *h* index of 62. The index has the advantage of allowing easy comparison between researchers of different ages in different fields, and helps measure the importance of their work. Prominent researchers in medicine with a high *h* index include Robert Gallo ($h = 154$) for HIV research and Bert Vogelstein ($h = 151$) for colon cancer research. Hirsch has suggested that based on a typical *h* value, professorial status could be conferred at $h \sim 18$, with entry to senior lecturers or tenured posts at h ~10-12. Those bright academics gaining coveted membership of the US National Academy of Science could be $h \sim 45$, and so on for other elite bodies. The next RAE may be using some *h* indices rather than impact factors in an attempt to be fairer.

Box 1 – Hirsch index *h*

> A Scientist has index *h* if *h* of his/her N_p papers have at least *h* citations each, and the other ($N_p - h$) papers have no more than *h* citations each

Accessing published research is also improving. The *Ulster Medical Journal* is now on PubMedCentral (PMC) – the US National Institutes of Health (NIH) digital archive of biomedical and life sciences journal literature. This will allow increased access to articles in the journal and issues will be posted on the PMC server (www.pubmedcentral.nih.gov/) some months after the publication of the paper and online copy of the journal to subscribers and society members. The entire 2006 and 2007 volumes are now available, and with a Wellcome Trust grant, the entire back file since 1932 has been shipped to PMC in the USA for digitization in 2008.

Two articles in this issue of the journal deal with two eminent Professors of a bygone era who would have scored well in a RAE if there had been one[2,3]. The first – James Lorrain Smith - the fairly eccentric foundation holder of the Musgrave professorship of pathology – did some interesting and cutting edge experiments with JS Haldane (father of the biologist JBS Haldane), and established Belfast as a leader in respiratory research in the early part of last century. Some experiments on himself and Haldane were downright dangerous with hindsight and clearly with the safety regulations of today such self experimentation would not be possible. With the help of PMC, you can read the original articles he published with free access in the Journal of Physiology from 1894 onwards.

The second, John Edgar Morison – was the longest serving editor of the Ulster Medical Journal[4] from 1951 – 1984. He established the journal on Medline and when former editor David Hadden and I visited him and his wife Ellen at home two years ago, John Edgar was delighted with progress on the journal and the fact that I had managed to get a Wellcome grant to put the journal onto PMC. He always enjoyed the editing and encouraging young authors and correcting their grammar and fledgling works for publication. He told us 'the only time editing the journal was a nuisance was when I was revising one of my textbooks, otherwise it was a complete joy to edit'. The journal only exceptionally publishes obituaries – minimum criteria being either a former editor or an honorary fellow of the Ulster Medical Society – John Edgar Morison was both.

We pass our condolences to his wife Ellen, and also our thanks to Ellen for tolerating his editing and substantial contribution to both the journal and the Ulster Medical Society over so many years.

Patrick J Morrison

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Footnote - PJM has an $h = 17$ and feels he has justified holding his honorary Professorships according to a Hirsch threshold of $h = 18$ for salaried Professors. Hirsch says nothing about *h* indices for honorary posts so holding down a full time busy NHS job whilst editing a journal and other such things should perhaps be factored in for honorary post holders in case Universities start getting ideas.

LIST OF REFEREES FOR 2007

We pass on our sincere thanks to all of our referees for 2007.

Dr Derek Allen
Dr Pooler Archbold
Dr Robin Ashe
Mr Paul Blair
Dr Declan Bradley
Professor J Bridges
Dr Norman Campbell
Dr Jonathan Cash
Professor Chee Soong
Mr Kishor Choudhari
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Obituary

Professor John Edgar Morison OBE, MD, DSc, FRCOG, FRCPath (1912-2007)

J Denis Biggart, Dorothy M Hayes, Ingrid V Allen

Dr JE Morison, later to become Honorary Professor of Histopathology, died on 5th September, 2007 aged 95, after a short illness. To all his colleagues he was familiarly known as 'John Edgar'. He gave outstanding service to the Ulster Medical Society, acting as editor of the Ulster Medical Journal for 32 years (1951-1984). Professor DAD Montgomery was his co-editor from 1975-1984. In his review of "The Editors of the UMJ"[1], Professor David Hadden has commented on how the journal prospered under his guidance, becoming internationally recognised "with citations in Current Contents as well as the Index Medicus, the predecessor of Medline". His broad knowledge of pathology helped greatly in the assessment of the worthiness of submitted articles. He was a stickler for the use of good English and grammar, often by himself undertaking major rewriting if he considered the basic content worthwhile. In 1974-1975 he became President of the Ulster Medical Society[2] and in 1979 was made an Honorary Fellow. He also valued his Honorary Fellowships of the Ulster Surgical Club and the Ulster Obstetric Society. He was a founder member of the Paediatric Pathology Club in 1955 which evolved into the Paediatric Pathology Society in 1962.

He was born in Banbridge in 1912 and educated at Banbridge Academy. In 1929 he entered the Medical Faculty of Queen's University, where he pursued a distinguished undergraduate career graduating in 1935, MB BCh BAO with Honours and First Place Scholarship. In 1937 he joined the University Department of Pathology as a research assistant and was made lecturer in 1942. His theses earned an MD with gold medal in 1940 and a DSc in 1951. Despite heavy teaching and diagnostic work, he published many papers in pathology and bacteriology. It was at this time that he became interested in the pathology of the neonatal period and was awarded a Rockerfeller Travelling Fellowship to Harvard Medical School at Boston Children's Hospital (1946-1947). Returning to Belfast he was appointed Reader and admitted to the pre-NHS visiting staff of the Royal Victoria Hospital. Undoubtedly his most outstanding academic contribution was his authorship of the pioneering book "Foetal and Neonatal Pathology" first published in 1951 with new editions in 1963 and 1971. These were translated into Italian, Spanish and Japanese. Thereafter, wherever in the world Queen's graduates travelled, they were amazed by how John Edgar's book was regarded as a unique masterpiece in the field of neonatal pathology, which had placed Belfast firmly on the international map. In 1960 his international reputation was borne out by an invitation from the British Council to undertake a ten week visit to Uruguay, Argentina and Brazil, where there was an awakening interest in perinatal problems. His contacts in North America were maintained by visits and he acted as Guest Professor at Illinois University in Chicago.

In 1954 he resigned his Readership in the University to become an NHS consultant histopathologist, based in the Central Laboratory on the City Hospital site. For more than ten years he provided a superb single-handed postal biopsy service to the rural district hospitals in Northern Ireland and even found time to travel to these hospitals to perform autopsies on the more puzzling cases. Based on his encyclopaedic knowledge, his reports were noteworthy for their detailed advice on the treatment and prognosis of the rarer diseases. The clinicians recognised that in John Edgar they had an intellectual friend to whom they could always turn for help. This was of particular importance in the pre-1960 era before the establishment of medical libraries in the provincial hospitals. It was not until 1965 that he acquired the able support of another pathologist, Dr Dorothy Hayes, who became his co-consultant in 1971. Together, with the help of highly skilled technical staff, they coped with an enormous workload which grew to 24,000 surgical biopsies per year, prior to laboratory decentralisation in the early 1970s and the setting up of separate laboratories in the major provincial hospitals. A shortage of pathology trainees delayed his retirement until 1984 when he was seventy-two. He was awarded an OBE for his services to medicine.

His interests included travel, gardening, photography, collecting antique Irish glass and restoring old furniture. He leaves a wife, Ellen, three children and six grandchildren.

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Ulster Med J 2008; **77 (1)** 4-5

Commentary

The White Paper and regulatory reforms: Beginning the end of professional self-regulation for doctors.

Kishor A Choudhari

Accepted 28 November 2007

The White Paper *Trust, Assurance and Safety - The Regulation of Health Professionals in the 21st Century,* published by the Government[1] in 2007 proposes several changes in the composition and functioning of the councils that regulate health professionals, including the General Medical Council (GMC). Some of these changes include -

I. ASSURANCE OF INDEPENDENCE IN THE GOVERNANCE AND ACCOUNTABILITY OF THE PROFESSIONAL REGULATORS.

To achieve this, the Paper proposes:

- Parity of membership between lay and professional members for the regulators to be and seen to be independent and impartial, with enhanced accountability to the Parliament.
- Independent appointment of the council members than election to dispel the perception that councils are overly sympathetic to the profession they regulate
- Reducing the size of the councils and making them more-board like to enable them to focus more effectively on strategy and the oversight of their executives.
- Deferring mergers of the professional regulatory bodies, at least until 2011

II. INTRODUCTION OF AN EFFECTIVE SYSTEM OF REVALIDATION

The White Paper also outlines robust revalidatory mechanisms for all statutorily regulated health professionals who will periodically be required to demonstrate their fitness to practise. There are two core components to the proposed revalidation – r*elicensure* and *recertification*.

a) For r*elicensure*, all doctors will have a licence to practise to remain on the medical register, to be renewed every five years. This will be based on annual appraisal system which will be modified to have a summative (judgemental) element in addition to the current formative (developmental) structure. A 360° feedback system will also be piloted in England.

b) *Specialist re-certification* will apply to specialist doctors, including general practitioners requiring them to meet the standards set and assessed by the medical Royal colleges and respective specialist societies.

III. TO ADDRESS CONCERNS AT LOCAL AND NATIONAL LEVELS

It is recognised that the current system for tackling poor performance has a "regulatory gap" whereby a doctor may not inspire confidence of his colleagues or employers, but his or her performance is not so poor that referral to the GMC is indicated. To bridge this gap two changes are proposed at local level –

- Introduction of "GMC affiliates" (mostly senior clinicians) at a regional level in England, and at a national level in Scotland, Wales and Northern Ireland.
- A system of "recorded concerns" against a doctor's GMC registration

At the national level, as suggested by Dame Janet Smith in the fifth Shipman report[2], two changes are proposed-

- Use of "civil standard of proof" (on the balance of probabilities), with a sliding scale, instead of currently used "criminal standard of proof" (beyond reasonable doubt) in GMC's fitness to practise cases for doctors.
- Disassociation of the GMC's roles of investigation and prosecution from adjudication to ensure complete public and professional confidence.

Both the modifications are accepted by the GMC, are due to be implemented soon and have generated considerable debate and anxiety.

There is little disagreement that the professional self-regulation in place over last 150 years since the inception of the GMC is not adequate to protect our patients. It is also widely recognised that recent enquiring over the last decade including Bristol, Shipman, Ayling, Neale and other similar investigations incriminating the medical profession have significantly eroded public confidence in the medical profession. This has prompted the Government to launch strong regulatory measures to identify and tackle poorly performing doctors at an early stage. While these measures are also meant to have a supporting function in addition to a disciplinary role- with options for rehabilitation and re-training, the Government's focus is centred primarily on

Consultant Neurosurgeon, Regional Neurosciences Unit, Royal Victoria Hospital, Belfast BT12 6BA, United Kingdom.

kishor.choudhari@belfasttrust.hscni.net

the safety of our patients. The White Paper[1] also recognises benefits of a "three-board model" covering undergraduate, postgraduate education and continuing professional development. It is hoped that all the above measures, once implemented and fully operational, will help us restore confidence of the public, profession and politicians in the medical profession. One can therefore foresee overwhelming support among the public, patient-organisations and media for these sweeping reforms in the regulation of health-care professionals, especially doctors. Many might even consider them to be perhaps long overdue.

As majority of doctors provide excellent quality of care, these measures aimed at a relative minority are bound to be perceived to be harsh and heavy-handed on the medical profession in general. For example, the changes in the standard of required proof required in fitness to practise cases from criminal to civil category may result in more erasures from the medical registers, although the GMC denies such a possibility[3]. The GMC envisages restrictions placed on practice of more number of doctors than increase in suspension rates due to these changes[3]. There is also a concern that the over-regulated medical environment may generate a culture of fear among doctors. This may, in turn, force them to focus on being politically correct than on concentrating on patient's well-being, and also to practise defensive medicine- a change already noticeable over last few years. There is no disagreement with Sir Liam Donaldson's assertion that "in 2006 every patient is entitled to a good doctor" (*Good Doctors, Safer Patients*)[4], but there is no universally agreed and widely understood definition of what exactly a good doctor is[5]. In the longer term, these changes may reflect in early retirements, disillusioned doctors opting for alternative careers, lack of motivation and depletion of innovation in the medical practice. There would also be little incentive to work hard in clinical practice, as the harder one works and the more patients one treats, more mistakes one is likely to make. In surgical specialties, surgeons may shy away from undertaking complicated and inherently risky cases – surely not a step in forward direction. Further increasing bureaucracy and paper-work in the appraisal-revalidation process is unlikely to make us better doctors. The proposed modifications in the professional regulation are not convincing enough to ensure that genuine poor-performers are indeed filtered before it is too late. In fact, the crux question that remains unanswered is- whether the proposed radical reforms in the existing system of professional regulation will necessarily identify more poorly performing doctors as envisaged by the Government, or will it merely portray more number of doctors to be poorly-performing?

Like it or loath it, it is clear that these reforms are here to stay. They can be considered as marking the end of self-regulation for medical professionals. If embraced by the profession in the right spirit, and implemented effectively, they will hopefully enable us to strike the right balance between professional independence and regulation, and eventually make it a win-win situation for all NHS stakeholders.

Conflict of interest- None to declare

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Ulster Med J 2008; **77 (1)** 6-17

Review

Carpal tunnel syndrome

Somaiah Aroori, Roy AJ Spence *

Accepted 1 November 2007

SUMMARY

Carpal tunnel syndrome is one of the most common peripheral neuropathies. It affects mainly middle aged women. In the majority of patients the exact cause and pathogenesis of CTS is unclear. Although several occupations have been linked to increased incidence and prevalence of CTS the evidence is not clear. Occupational CTS is uncommon and it is essential to exclude all other causes particularly the intrinsic factors such as obesity before attributing it to occupation. The risk of CTS is high in occupations involving exposure to high pressure, high force, repetitive work, and vibrating tools. The classic symptoms of CTS include nocturnal pain associated with tingling and numbness in the distribution of median nerve in the hand. There are several physical examination tests that will help in the diagnosis of CTS but none of these tests are diagnostic on their own. The gold standard test is nerve conduction studies. However, they are also associated with false positive and false negative results. The diagnosis of CTS should be based on history, physical examination and results of electrophysiological studies. The patient with mild symptoms of CTS can be managed with conservative treatment, particularly local injection of steroids. However, in moderate to severe cases, surgery is the only treatment that provides cure. The basic principle of surgery is to increase the volume of the carpal tunnel by dividing transverse carpal ligament to release the pressure on the median nerve. Apart from early recovery and return to work there is no significant difference in terms of early and late complications and long-term pain relief between endoscopic and open carpal tunnel surgery.

INTRODUCTION

Carpal tunnel syndrome (CTS) is one of the most common upper limb compression neuropathies [1-5]. CTS account for approximately 90% of all entrapment neuropathies. It is due to an entrapment of the median nerve in the carpal tunnel at the wrist (Figure 1). An estimated one million adults from the United States (annually) have CTS requiring medical treatment[6] and the cost to the Health Care system is high. In 1995, Palmer *et al* estimated that between 400,000 and 500,000 cases of CTS require operative treatment annually in the States, with an economic cost in excess of $2 billion per year[7]. The surgical decompression rates for UK are 43 to 74 per 100,000 per year [8].

The incidence and prevalence varies, 0.125% - 1% and 5 -16%, depending upon the criteria used for the diagnosis[6, 9-14]. It is a condition of middle-aged individuals and affects females more often than males. Since its first description by Phalen in the 1950s[15], several studies have reported marked female preponderance and a peak incidence around 55 to 60 years[6,11,16]. In the first population based study, Stevens *et al* noted that the mean age at diagnosis was 50 years for men and 51 years for women[11]. In a recent surveillance study from Canterbury and Huddersfield, UK, Bland *et al* reported an annual incidence of 139.4 cases per 100,000 in females and 67.2 cases per 100,000 in males, with a female to male ratio of 2.07[17].

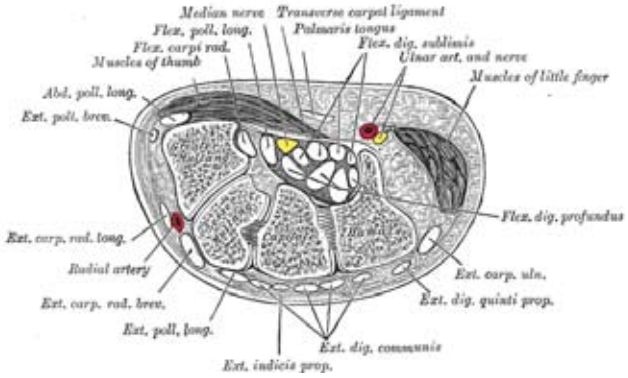

Fig 1. Cross section across wrist (Reproduced, with permission from Bartleby.com: Gray, Henry. Anatomy of the Human Body. Philadelphia: Lea & Febiger, 1918.)

It is one of the most widely recognised occupational health conditions; particularly in industries where work involves high force/pressure and the repetitive use of vibrating tools. Einhorn and Leddy estimated an incidence of 1% in the general population and 5% of workers in certain industries which require repetitive use of the hands and wrists[12]. In 1999, the US Bureau of Labour Statistics, reported that the median number of days away from work was highest for CTS (27days) when compared to any other major disabling illnesses and injuries[18]. In addition, estimates by the National Institute for Occupational Safety and Health (NIOSH) suggest that 15 to 20% of Americans are at risk of developing Cumulative Trauma Disorders (CTDs) [19].

AETIOLOGY

There are two distinct varieties of CTS - acute and chronic. The acute form is relatively uncommon and is due to a rapid

Department of Surgery, Level-2, Belfast HSC Trust, Lisburn Road, Belfast, BT9 7AB, United Kingdom

Correspondence to Professor Spence

roy.spence@bch.n-i.nhs.uk

TABLE I.

Various non-occupational causes of Carpal Tunnel Syndrome

A. Local causes
• Inflammatory: e.g. tenosynovitis, histoplasma fungal infection, hypertrophic synovium • Trauma: e.g. Colles' fracture, dislocation of one of the carpal bones • Tumours: e.g. Haemangioma, cyst, ganglion, lipoma, neuroma etc. • Anatomical anomalies: e.g. thickened transverse carpal ligament, bony abnormalities, abnormal muscle bellies, persistent median artery etc.
B. Regional causes
• Osteoarthritis • Rheumatoid arthritis • Amyloidosis • Gout
C. Systemic causes
• Diabetes • Obesity • Hypothyroidism • Pregnancy • Menopause • Systemic lupus erythematosus • Scleroderma • Dermatomyositis • Renal failure • Long-term haemodialysis • Acromegaly • Multiple myeloma • Sarcoidosis • Leukemia • Alcoholism • Haemophilia

and sustained rise of pressure in the carpal tunnel. This is most commonly associated with a fracture of the radius as Sir James Paget described in 1854[19]. It is also associated with burns, coagulopathy, local infection and injections. The chronic form is much more common and symptoms can persist for months to years. However, in only 50% of cases is the cause identified, and can be divided into local, regional and systemic causes as summarised in Table I. Carpal tunnel syndrome is common in pregnant women[20-23] It is commonly diagnosed during third trimester of pregnancy and it is often bilateral. In the majority of patients symptoms will resolve either spontaneously or will respond to conservative treatment after delivery[20,24,25].

ROLE OF OCCUPATION

CTS is the most common form of Repetitive Trauma Disorder (RTD). In 1995, there were approximately 308,000 trauma-related musculoskeletal disorders, representing nearly 62% of all occupational illness cases reported to the US Bureau of Labour Statistics[26]. Brain *et al* were the first to implicate occupation as a causal factor in CTS[27]. At risk occupations include, grinders, cashiers, and meat packers, workers sewing car seats, aircraft engineers, grocery store workers, and small part assembly liners.

The physical factors implicated and extensively studied in relation to occupational CTS include repetition, force, posture, external pressure, and vibration. Repetition is the most widely recognized risk factor for occupational CTS. In epidemiological studies high repetition is defined either by the frequency of the task or the percentage of time spent on repetitive work. A high repetitive job is defined as one which involves the repetitive use of awkward wrist movements lasting less than 30s or when more than 50% of work time is spent performing tasks that involve repetitive awkward wrist movements[28].

Experimental studies have shown a higher incidence of CTS in workers who are involved in high force and repetitive work compared to workers who are not[28-31]. Silverstein *et al* examined the association between high force / repetitive movements and CTS among 652 workers from 39 jobs from seven different industrial areas[28]. The authors noted a prevalence of 5.6% among workers in high force and high repetitive jobs compared to 0.6% among workers in low force and low repetitive jobs. The authors showed occupation to be a risk factor only when high force and high repetition are present, but the accuracy of their estimated ratio of 15.5 (95% confidence interval, 1.7-141.5) suffered from a small sample size. High repetitiveness seems to be a greater risk factor than high force but neither was statistically significant alone[28]. In a case–control study, Armstrong & Chaffin compared patients with CTS to asymptomatic people amongst 18 sewing machine operators and noted that cases that used pinch grip (opposition of the thumb and the distal joints of four fingers) exerted more force than controls[32]. The authors also noted that cases tended to use non-neutral postures more often and exerted more force in these postures[32].

Several epidemiological studies have shown that force is an independent risk factor for CTS, but the dose–response relationship is not clear. In a cross-sectional study by Latko *et al*, a dose–response relationship was observed between the prevalence of CTS and level of repetition[33]. In the study by Silverstein *et al* [28] force was a weaker risk factor than repetition, but in the report by Chiang *et al*[29] force was a stronger risk factor than repetition. Force and repetition increased the risk of CTS in a cumulative way in the Silverstein study. The odds ratio for high force and high repetition group was 15.5 in the Silverstein study. However, Chiang *et al* showed odds ratio for repetition was 1.1 and did not find a significant association between repetition and force[29].

Several studies examining carpal tunnel pressure (CTP) in healthy subjects indicate that the greatest increase in CTP occurs following wrist flexion and extension[34,35]. In an experimental study on 17 healthy volunteers, Rempel *et al* measured intra-compartmental pressures using a saline filled catheter introduced into the carpal tunnel[36]. The authors noted highest mean intra- compartmental pressure (55mm Hg)

during full supination and 90 degree metacarpophalangeal (MCP) joint flexion and lowest pressure at 45 degrees of pronation and 45 degrees of MCP joint flexion. The authors speculated that the increased carpal tunnel pressure at full supination and at 90 degree MCP flexion changes the orientation of the tendons, thereby, increasing the volume of the carpal tunnel predisposing to CTS. The authors suggested that the lowest pressures achieved by position should be considered in job and tool design[36]. In a review of 15 cross-sectional studies involving 32 occupational or exposure groups and six-case studies, Hagberg *et al* noted a high prevalence of CTS in occupations requiring high force and high repetitive manual movements[37]. In a review, NIOSH found a strong association between physical factors such as force, repetition, and vibration but did not find a stronger association between non-neutral postures and CTS[38].

Occupational risk factors alone do not explain the occurrence of CTS and it is proposed that a combination of several factors is involved. The majority of CTS is attributable to patient related factors (intrinsic risk factors). Several studies have noted that the occurrence of CTS is correlated with unhealthy habits and lifestyle[39,40]. This was supported by an analysis that showed that 81.5% of the explainable variation in electro-physiologically defined CTS was attributable to body mass index, age, and wrist depth to width ratio, whereas only 8.29% was due to job related factors[41]. In a study comparing workers with and without CTS, Nathan and colleagues noted that there was a 19% greater lifetime use of tobacco, 75% greater history of alcohol abuse, and 5% greater use of caffeine in workers with CTS[36]. Furthermore, the authors reported that, current tobacco, caffeine, and alcohol consumption independently predicted 5% of the risk for CTS in female workers[42]. Several studies have noted high incidence of CTS in patients with high body mass index[39,43-45]. Garland *et al* found that gender was a more predictive risk factor for CTS than exposure to high risk occupations[46]. In a series of 654 hands with CTS, Phalen did not observe any relation between CTS and occupation. Furthermore, he argued that occupational trauma is seldom the precipitating factor in the production of CTS[15]. It is important to establish the nature of risk factor and the interaction between intrinsic and extrinsic factors. In a longitudinal study of predictors of CTS in industrial workers over a period of 17yrs, Nathan *et al* did not find an obvious relationship between the incidence of carpal tunnel syndrome and repetitive work. However, the authors noted high incidence of carpal tunnel syndrome in overweight people and in females[47].

One of the major drawbacks of studies that show a positive association between occupation and CTS is the wide variety of criteria used to diagnose CTS. Studies conducted in the 1980s depended on patients' self-reported symptoms and physical signs to establish the diagnosis of CTS. Physical signs in CTS have poor reproducibility and poor correlation with symptoms. Ideally, the diagnosis of CTS should be based on combination of symptoms, physical signs and nerve conduction studies. Furthermore, studies relied upon patients to report the degree of occupational exposure. Spielholz *et al* showed that direct observation and direct measurement of working practices are much more reliable methods of assessment of occupational exposure leading to CTS[48]. The most often sited publication linking the occupation exposure to high repetitive and force and the increased incidence of CTS relied on patient reported symptoms and physical examination for the diagnosis of CTS. Furthermore, the authors also did not define what constitutes high force and repetition[28].

Despite the use of much more rigorous methods to establish the diagnosis of CTS, conflicting results were published in the 1990s linking occupation and CTS. Stetson *et al* examined workers from several industries and noted significantly lower sensory amplitudes and longer motor and sensory latencies on nerve conduction studies in occupations involving high repetition and force[49]. Osorio *et al* studied 56 grocery store workers and found strong associations between forceful and repetitive wrist movements and the prevalence of CTS[50]. However, several other studies did not find any substantial evidence linking specific occupations and the prevalence or severity of impaired sensory conduction of the median nerve at the carpal tunnel[42,51]. Moore and Garg *et al* videotaped work practices of workers from a pot-processing factory. The authors subsequently reviewed workers' medical records and identified all patients with various upper limb neuropathies including CTS. The authors found no significant association between ergonomic factors and the CTS (Relative risk = 2.8, P = 0.44, 23)[52]. However, the most recent systematic literature review on the role of occupation in carpal tunnel syndrome by Palmer *et al,* found that the regular use of hand-held vibrating tools increased the risk of CTS by more than 2-fold[53]. The authors also found substantial evidence for high risk of CTS in occupations requiring high repetitive flexion and extension at wrist and also forceful grip[53]. However, the authors did not find evidence between the work on keyboard and computers and CTS.

PATHOPHYSIOLOGY

The exact pathogenesis of CTS is not clear. Several theories have been put forward to explain the symptoms and impaired nerve conduction studies. The most popular ones are mechanical compression, micro-vascular insufficiency, and vibration theories. According to mechanical compression theory, symptoms of CTS are due to compression of the median nerve in the carpal tunnel. The major drawback of this theory is that it explains the consequences of compression of the nerve but does not explain the underlying aetiology of mechanical compression. Brain and colleagues attributed the symptoms of CTS to spontaneous median nerve compression in the carpal tunnel[27]. The term 'spontaneous' was used due to lack of clear association between wrist joint deformities and symptoms. The compression was believed to be mediated by several factors such as exertion strain, overuse, hyperfunction, repeated or prolonged wrist extension, prolonged grasping of tools, and unaccustomed manual work[27].

The micro-vascular insufficiency theory proposes that the lack of blood supply leads to depletion of nutrients and oxygen to the nerve causing it to slowly lose its ability to transmit nerve impulses. Scar and fibrous tissue eventually develop within the nerve. Depending on the severity of injury, changes in the nerve and muscles may be permanent. The characteristic symptoms of CTS, particularly tingling, numbness and acute pain, along with acute and reversible loss nerve conduction are thought to be secondary to ischemia of the affected nerve segment. Seiler *et al* showed (by laser Doppler flowmetry) how normal pulsatile blood flow within the median nerve

was restored within 1 min of transverse carpal ligament release. The authors concluded that ischemia likely plays a significant role in the aetiology of CTS[54]. A number of experimental studies support the theory of ischemia due to externally applied compression and due to increased pressure in the carpal tunnel[30]. The development of ischemia and, therefore, symptoms, will vary according to the integrity of the blood supply of the nerve and the systolic blood pressure. Kiernan *et al* found that the conduction slowing in the median nerve can be explained by ischemic compression alone and may not always be attributable to disturbed myelination[55]. Tucci *et al* noted five times higher levels of interleukin-6, maloionaldehyde bis- (diethyl acetal) and prostaglandin E2 at the time of surgery in patients with CTS compared to asymptomatic volunteers[56]. The authors concluded that such alteration may be the result of oxidative changes following repetitive ischemia and reperfusion injury.

According to the vibration theory the symptoms of CTS could be due to the effects of long-term use of vibrating tools on the median nerve in the carpal tunnel[30]. Lundborg *et al* noted epineural oedema in the median nerve within days following exposure to vibrating hand-held tools. Furthermore, the authors also noted similar change following mechanical, ischemic, and chemical trauma. Interestingly, the authors also report animal studies that show a temporary accumulation of smooth axoplasmic structures and deranged axoplasmic structures following a short exposure to a vibrating force[57]. These changes were first noted in unmyelinated fibres that serve sympathetic activity; a loss of which could reduce micro-vascular flow to the median nerve leading to disruption of its myelin sheath and decreased motor conduction velocity[57].

CLINICAL FEATURES

The symptoms vary depending upon the severity of the disease. In early stages, patients usually complain of symptoms due to the involvement of the sensory component of the median nerve and only later report symptoms from involvement of motor fibres. The most common symptom is burning pain associated with tingling and numbness in the distribution of median nerve distal to wrist. The portion of the hand involved is classically the thumb, index and middle fingers, and radial half of the ring finger. Patients are often awoken by pain in the middle of the night and report hanging their hand out of bed or shaking it vigorously in order to relieve their pain. Patients may report pain, tingling and numbness of the whole hand, but careful questioning will identify that the little finger is rarely involved as it is innervated by the ulnar nerve. Occasionally, however, all five fingers can be involved if the ulnar nerve is affected at same time. Symptoms of nocturnal paraesthesia are reported to be 51-96% sensitive and 27-68% specific[58-61]. Less common symptoms include a feeling of clumsiness and weakness in the affected hand that is often made worse by activity or work. Patients may also complain of pain radiating to the forearm, elbow or even the shoulder. In some patients shoulder pain may be the presenting symptom but they will never have any objective evidence of sensory changes above the wrist.

In Kendall's series of 327 patients, 313 (95.7%) reported paresthesia; 118 (38%) reported nocturnal symptoms only, 178 (58%) reported symptoms during the day and night, but worse at night, and 17 (5%) reported symptoms during the day only[62]. In the Yamaguchi *et al* series, 99% of the 433 surgical patients reported paresthesia[63]. In Phalen's experience, the typical history was that of a gradual onset of numbness and paresthesia[15].

SIGNS

Several tests have been described which help in the diagnosis of CTS. None of these tests are diagnostic on their own. Most of the tests are complementary to each other rather than diagnostic of CTS. A combination of symptoms, signs and diagnostic tests should be taken into account when the diagnosis of CTS is made. The presence or absence of characteristic physical findings has limited diagnostic value. The various tests are Tinel's sign, Phalen's sign, square wrist sign, closed fist sign, flick sign, Katz hand diagram, flexion and extension of wrist test, pressure provocation test, and tourniquet test. There are limited studies that have evaluated the diagnostic use of square wrist sign, flick sign, closed fist sign, and tourniquet test and hence these tests are not discussed in detail in this article. However, it is sufficient to say that prior to routine use of these tests; further evidence is required to support their effectiveness in the diagnosis of CTS. Diminished pinprick sensation (hypalgesia) in the distribution of median nerve compared to the pinprick sensation over the ipsilateral little finger is a very useful diagnostic test in patients with CTS than abnormalities of other sensory modalities.

Tinel's sign

In this test, the examiner taps lightly over the site of the median nerve at the distal wrist crease. Development of tingling or discomfort in the fingers supplied by the median nerve constitutes a positive sign. Tinel described this sign in 1915[64]. He noted that a tingling sensation occurred when an injured nerve was percussed over its proximal stump and speculated that this was a sign of axonal degeneration and intended his sign to be used in patients after blunt traumatic injury to follow the course of the regenerating nerve[65-67]. Tinel's sign is not a precise test and several factors can influence the outcome of the test. Firstly, its efficacy is reduced, as patients with CTS will have continually regenerating nerves at the distal wrist crease. The other limiting factor is the amount of pressure used to elicit the sign. Testing technique is important when the physician is eliciting Tinel's sign, and subtle differences in test performance probably account for some of the discrepancies in reported prevalence. It is difficult to quantify precisely how much pressure should be used to elicit the sign. The use of too much force or a sharp blow over a normal median nerve will produce finger tingling. This must not be interpreted as the presence of Tinel's sign. The Tinel's sign is associated with sensitivities of 23% to 67%, and specificities of 55% to 100%[59,60,65,67-71]. In a review, Kuschner *et al* summarised the frequency of Tinel's sign and reported that it is positive from 8% to 100% of CTS patients[67]. Tinel's sign is the least accurate test according to Mondelli *et al,* who did not find a combination of signs more useful than a single sign alone[71].

Phalen's test

Phalen and Kendrick described this test in 1957[15]. Flexion of the wrist causes compression of the nerve between the

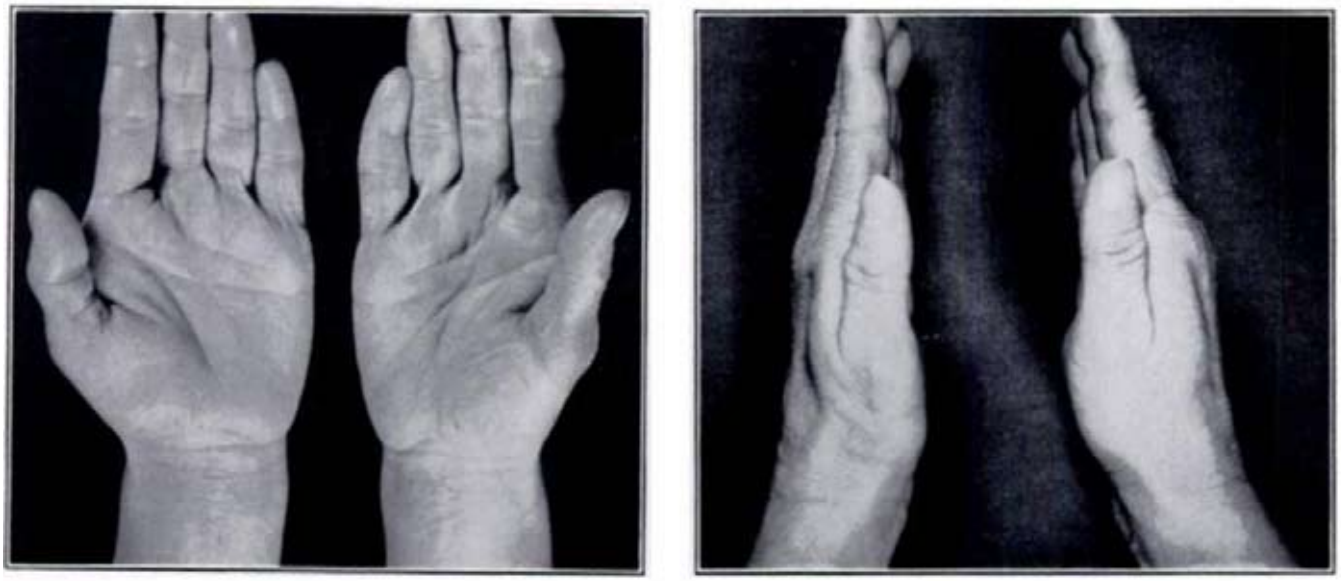

Fig 2. Picture showing moderate thenar atrophy of the left hand in a woman with bilateral carpal tunnel syndrome
(Reproduced, with permission from George S. Phalen. The Carpal-Tunnel Syndrome: Seventeen Years' Experience In Diagnosis And Treatment Of Six Hundred Fifty-Four Hands. J Bone Joint Surg Am 1966;48:211)

transverse carpal ligament (TCL) and flexor tendons in the carpal tunnel, causing paresthesia in the median nerve distribution[70,72] reproducing the patient's symptoms. Phalen performed the test by having the patient hold the forearm vertically with the elbows resting on the table and then allowing both hands to drop with complete wrist flexion for approximately one minute. The test is considered positive when paresthesia develops in less than one minute. Patients with advanced CTS often note paresthesia in less than 20 seconds. The reported sensitivity ranges between 10% and 91% and specificity between 33% and 100%[59,60,68, 69,73-75].

Katz hand diagram

This is a self–administered diagram, which depicts both the dorsal and palmar aspect of the patient's hands and arms. Patients use this diagram to mark the specific location of their symptoms, characterising them as pain, numbness or tingling, or other. The diagnosis is graded as classic, probable, possible or unlikely to be CTS based on criteria that appear in the hand diagram[60,76]. In diagrams classified as classic or probable the sensitivity of the test is 80% and the specificity is 90% for the diagnosis of CTS[76,77]. Katz himself reported a sensitivity of 64% and a specificity of 73%[76].

Square wrist sign

Kuhlman *et al* reported that a square-shaped wrist, where the anterior-posterior dimension of the wrist (at the distal wrist crease) divided by the medio-lateral dimension is greater than 0.70[65,78] and weakness of the abductor pollicis brevis were the two most sensitive signs (69 and 66% respectively). This test is associated with a sensitivity of 47% to 69% and specificity of 73% to 83%[65,78].

The tethered Median Nerve stress test

LaBan described this test in 1986. It is performed by hyper-extending the supinated wrist and the distal interphalangeal joint of the index finger for a minute. Patients with chronic carpal tunnel syndrome experience pain on the volar aspect of proximal forearm[79]. LaBan noted that hyper extension of index finger causes distal excursion of the median nerve more than hyperextension of the adjacent fingers[80]. Raudino evaluated this test in 140 patients with electro- physiologically confirmed CTS and noted that the test was positive in 60 hands (42.8%) compared to the 56.4% positive rate with Phalen's sign and 42% positive rate with the Tinel's sign[81].

Pressure provocation test

A positive result in this test is the presence of pain, tingling and numbness in the distribution of the median nerve when the examiner presses with his/her thumb on the palmar aspect of the patient's wrist at the level of the carpal tunnel for 60 seconds. The test is seldom positive. The reported sensitivity is between 28% and 63% and specificity is between 33% and 74%[59,65,72,73].

Tourniquet test

A positive result is the development of paresthesia in the distribution of the median nerve when a blood pressure cuff around the patient's arm is inflated to above systolic pressure for a minute or two. The irritated and compressed median nerve is thought to be more susceptible to ischemia than the normal median nerve. However, even normal individuals can also develop the same symptoms and it is difficult to evaluate, especially in mild cases of CTS. The tourniquet's test sensitivity lies between 21% and 52% with a specificity between 36% and 87%[59,69].

MOTOR EXAMINATION

Thenar atrophy is a late sign and signifies significant functional loss. Finger weakness associated with an inability to pinch or frequent dropping of grasped objects follows involvement of the motor component. Long-term involvement leads to thenar muscle atrophy (Figs 2 and 3) with associated loss of thumb abduction and opposition strength. Diminished sensation to pinprick in the median nerve distribution always precedes thenar atrophy. Thenar atrophy is seldom noticed by

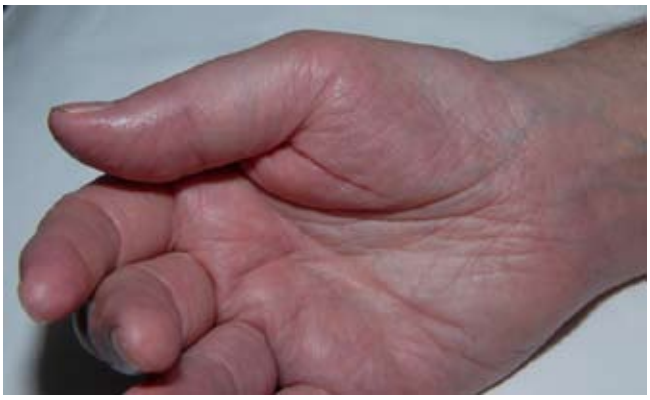

Fig 3. Picture showing moderate thenar atrophy of the right hand in a man with bilateral carpal tunnel syndrome

patients and may not be obvious even to the examiner when examined by looking down onto the palm. However, it will be readily appreciated by comparing both palms together (Fig 2)[15]**.** In Phalen's series the atrophy of abductor pollicis brevis, opponens pollicis and flexor pollicis brevis was noted in 41% of hands[15]. Abductor pollicis brevis is the most commonly affected muscle and testing its function is useful in making the diagnosis of CTS.

DIAGNOSIS

Symptoms are often difficult to interpret in patients with CTS. Patients often have difficulty in describing their symptoms, and physicians may have difficulty in interpreting the symptoms. Self-administered tests, such as the Katz diagrams may help reduce these potential sources of errors and bias. The combination of clinical symptoms and signs with electro-diagnostic findings is the most valid way of diagnosing CTS[15,82,83].

The role of Nerve Conduction Studies

The nerve conduction studies (NCS) measure the sensory and motor nerve conduction velocity in the median nerve at the level of the wrist. The sensory component of the median nerve is affected much earlier than the motor component and in early stages of CTS there is usually a delay in the sensory nerve conduction velocity. Sensory nerve conduction delay is measured by placing an electrode near the base of the ring finger following which the median nerve is stimulated approximately 13cm proximal to the recording electrode. The antidromic sensory potentials are recorded and measured. The motor nerve conduction velocity from elbow to wrist is measured using surface electrodes.

Median nerve conduction studies are the gold standard diagnostic tests with sensitivities between 49% and 84% and specificities of 95% and 99%[84-86]. In entrapment neuropathies there will be a delay in the conduction velocity at the point of compression due to the demyelination of the nerve. In patients with clinical symptoms suggestive of CTS with normal sensory conduction velocity, measurement of both motor and sensory conduction velocity increases the diagnostic yield by 10%[87]. Chang *et al* found that, in patients with normal sensory and motor conduction velocities, measuring the latency between the median and ulnar nerve for the ring finger and comparing it to the median and radial nerve latency for the thumb increases the diagnostic yield by another 10%[87].

NCS not only allow a diagnosis of CTS but also help in the diagnosis of other conditions presenting with similar symptoms e.g. cervical radiculopathy, polyneuropathy, other median nerve entrapment syndromes[74,88-90].

Although nerve conduction studies are the gold standard test for the confirmation of diagnosis of CTS, they have certain limitations. A small percentage of asymptomatic individuals can have positive NCS. Similarly, a small percentage of patients can have negative NCS despite symptoms suggestive of CTS. Atroshi *et al* randomly surveyed 2466 individuals in Sweden to find out the incidence of CTS in general population[9]. 14.4% complained of pain, tingling and numbness in the distribution of the median nerve. However, only 4.9% of individuals with neuropathic symptoms had positive NCS. Furthermore, 18% of asymptomatic subjects had abnormal NCS[9]. Bingham *et al* examined 1021 applicants for industrial jobs and noted that 17.5% of the job applicants had abnormal NCS[91]. However, only 10% of these applicants actually had symptoms suggestive of CTS. In severe CTS cases, NCS results may not correlate with the clinical findings due to the varying nature of the impairment in different nerve fibres. In addition, nerve conduction studies will not accurately predict the recovery following release of the carpal tunnel, though neither do any of the other investigations predict this with any certainty[91]. Therefore these studies suggest that NCS alone should not be used to diagnose, rather it should be based on presence of clinical symptoms, physical findings and positive nerve conduction studies taken together.

The role of ultrasound in the diagnosis of carpal tunnel syndrome

The diagnosis of CTS is based mainly on clinical symptoms and signs and nerve conduction studies. However, as 13-27% of patients will have a normal NCS[92], alternative diagnostic tests such as ultrasound (US) and magnetic resonance imaging (MRI) are useful.

In a prospective study, Keles examined the role of US in 35 patients with a NCS confirmed diagnosis of CTS and compared it to 40 normal wrists. US measured the cross-sectional area (CSA) of the median nerve, bowing of the flexor retinaculum (FR) and flattening of the flexor retinaculum[93]. The CSA of the median nerve and bowing of the FR were significantly increased in patients with NCS positive CTS when compared to controls. The flattening of the FR had no correlation with diagnosis of CTS. Koyuncuoglu studied the role of US in 59 patients with clinical diagnosis of CTS with negative NCS findings by comparing their results with US findings in 30 normal wrists. They found a CSA of larger than 10.5mm in 18 patients compared to one wrist in the control group[94]. El Miedany *et al* compared the results of US with NCS in a group of patients with CTS against a control group and observed a high degree of correlation between the US findings and NCS in diagnosing and in assessing the severity of CTS. US also helped to identify the underlying cause of CTS and thus facilitated planning of treatment[95].

TREATMENT

There are several treatment options and they can be broadly categorised into surgical and non-surgical. Non-surgical methods are effective in patients with mild to moderate CTS.

They are indicated in patients with no muscle weakness or atrophy, absent denervation (on electromyography needle examination), and with only a mild abnormality on nerve conduction studies[96]. Pregnant women with CTS rarely require surgical treatment. In the majority of patients symptoms will resolve either spontaneously or will respond to conservative treatment after delivery[23-25].

The various non-surgical methods include: use of hand brace, splinting of the wrist, ultrasonic therapy, laser therapy, oral steroids, non-steroid anti-inflammatory drugs (NSAIDs), oral vitamin B6, local injection of corticosteroids with, or without, insulin, work place modifications and yoga etc. Description of the role of each type of non-surgical treatment option is beyond the scope of this review. In the recent Cochrane review, O'Connor *et al* looked at the available evidence about the role of various non-surgical treatment options in mild to moderate CTS[97]. They concluded that a significant short-term benefit could be gained with oral steroids, wrist splinting, local ultrasound therapy, yoga and carpal bone mobilization. However, the authors did not find any evidence to support the role of other treatment methods such as the use of a hand brace, exercises, usage of ergonomic key boards, oral diuretics and oral NSAIDs[97].

Steroid injection into the wrist is often successful. It may cause symptoms to worsen temporarily but can produce complete or significant pain relief in 60 to 70% of patients for weeks to years[96,98-100]. In a randomized, single blind controlled study Hui *et al* evaluated the role of steroid injection in patients with idiopathic CTS confirmed by NCS[101]. The primary outcome of the study was symptomatic relief measured by a global symptom score, which rated symptoms on a scale of 0 (no symptoms) to 50 (most severe). The authors randomized 50 patients, 25 into steroid and 25 into the open surgical group. The authors noted greater symptomatic relief in surgical group at 20 weeks follow-up. Furthermore, surgical decompression resulted in greater improvement in median nerve distal motor latencies and sensory nerve conduction velocity [101]. One of the major complications of steroid injection is iatrogenic injury to the median nerve. The safest location of injection is not clear. Though Racasan *et al* report that the safest location for the steroid injection is through the flexor retinaculum tendon[102]. Agarwal *et al* evaluated the role of methyl prednisolone acetate injection in 40 patients with mild idiopathic CTS. Patients were evaluated at 3 and 12 months[103]. The authors noted marked improvement of symptoms in 93.7% patients at 3 months follow up. Furthermore, they also found a significant improvement in the mean values of the distal motor and sensory latency at the wrist at 3 months. At 16 months median follow up 79% continued to have improvement in their symptoms and only 16.6% patients suffered a relapse of their symptoms following an initial response[103]. In a randomized controlled trial, Ly-Pen *et al* compared the role of local steroid injection with open surgery. The authors noted that local steroid injection was better than surgical decompression for the symptomatic relief from nocturnal paresthesia at 3 and 6 months. At 12 months follow up local steroids injection was as effective as surgical decompression[104].

Surgery

Surgery consists of division of transverse carpal ligament. This reduces the pressure on the median nerve by increasing the space in the carpal tunnel. Surgery is indicated in almost all patients with moderate to severe CTS. An absolute indication for CT release (CTR) is muscular atrophy[105]. Two different types of surgical approaches are in use for the treatment of CTS; open and endoscopic release. Open CTR (OCTR) is the traditional option and still the recommended method of surgical treatment for idiopathic CTS. It was first performed by Herbert Galloway in 1924, though since then several modifications have been made to refine it[106]. The classic OCTR uses a curved longitudinal inter-thenar incision, approximately 4 to 5 cm in length[107]. It involves opening of subcutaneous tissue, superficial fascia and transverse carpal ligament and 2 to 3 cm of distal forearm fascia under direct vision. The canal also inspected for mass lesions and anatomical abnormalities.

Open carpal tunnel release is easy to perform and in majority of patients it leads to good symptomatic relief with a low complication rate**.** In a series of 32 patients who underwent OCTR over a period of four years, 88% of patients reported good functional and symptomatic improvement[108]. The well recognised early complications are incomplete release of TCL, neuropraxia or injury to the median or ulnar nerve, inadvertent entry to Guyon's canal (the tunnel between the pisiform and hamate bone and the ligament connecting both bones), injury to the palmar cutaneous or recurrent motor branch of the median nerve and injury to the superficial palmar arch or ulnar artery[109]. These complications are rare as surgery is performed under direct vision. The late complications are scar tenderness, loss of grip strength, pillar pain, and rarely reflex sympathetic dystrophy and bow stringing of flexor tendons. Pillar pain is a frequent complication of both open and endoscopic release procedures. The pillar pain is characterised by pain or tenderness in the thenar or hypothenar eminence or radial and ulnar tenderness. The incidence varies between 6 and 36%[110,111]. It delays resumption of daily activities, return to work, and causes emotional distress all leading to an increased cost to health care system[112]. The exact aetiology of pillar pain is not clear. However, the pain could be secondary to alteration of the carpal arch structures[113], oedema of the tissues superficial to TCL, injury to the cutaneous branches of the palm[114], or could be due to relaxation of the muscles of opposition and pinch following sectioning of TCL[115].

To minimise post-operative complications and reduce length of hospital stay, several modifications to the length, location and shape of the incision in OCTR have been described. One of the modifications of classical OCTR is to make a limited transverse incision of ≤ 2cm in the same location as classical OCTR. Another modification is a limited open release performed by Atik *et al* in 2001[116]. The overall success rate of OCTR is more than 95% with a complication rate of less than 3%[117]. Studies have found no difference between patients who undergo bilateral simultaneous OCTR when compared to patients who undergo consecutive operations in terms of the post-operative complication rate, hospital stay, time to return work and the overall cost[118].

As in other fields of surgery, less invasive techniques have been introduced into carpal tunnel surgery to facilitate earlier return to work and reduce post-operative pain and the first endoscopic carpal tunnel release was performed by Okutsu

and his colleagues in Japan in 1987[119]. Since its introduction several modifications of the technique have been described in the literature. There are several endoscopic approaches but the underlying principle is the same; to release transverse carpal ligament. ECTR techniques can be broadly divided into single portal and dual portal techniques depending on the number of ports used to access the carpal tunnel. The two most commonly used techniques are single-portal technique described by Agee[121] and two-portal technique described by Chow[120]. It is beyond the scope of this review to go into details of each technique. The reported success rates for surgical treatment range from 70 to 90%. In an extensive review of all articles on ECTR covering six different types of techniques, Jimenez *et al* found that the endoscopic release techniques offer similar success and complication rate as open surgery[117]. The overall success rate for ECTR was 96.52% with a complication rate of 2.67% and a failure rate of 2.61%[117]. The most common complications noted by the authors were paresthesia of the ulnar and median nerves, injury to superficial palmar arch, reflex sympathetic dystrophy, flexor tendons lacerations and incomplete division of TCL[117]. The Cochrane database group reviewed all available evidence from randomized controlled trials comparing various surgical techniques in terms of efficacy in relieving symptoms, promoting early return to work and post-operative complications and found no strong evidence to favour alternative surgical techniques against the standard open technique. Specifically, they found conflicting evidence in support of endoscopic release leading to an earlier return to work and/or activities of daily living when compared to open CTR[122]. These findings have been replicated by another meta-analysis study of randomized controlled trials comparing endoscopic and open carpal tunnel decompression which also found no conclusive evidence favouring ECTR with regard to symptom relief and return to work[123]. However, they found that ECTR was associated with reduced scar tenderness and increase in pinch grip and pinch strength at 12 weeks follow up[123].

Evaluation of response following surgery

One of the major problems in assessing the effectiveness of various treatment methods is a lack of agreed outcome assessment criteria. Several outcome assessment measures have been used to measure the outcome or effectiveness of treatment and can be broadly divided into those that assess patient bodily activities and function and those that assess activity and participation. The most commonly used are self-reported symptoms questionnaires, hand diagrams, records of daily activity at work and at leisure time, return to work, complications, NCS, and quality of life questionnaires. Some patient outcome questionnaires assess the whole upper limb function rather than the wrist alone e.g. the patient evaluation measure (PEM)[124] and the Disabilities of the Arm, Shoulder and hand (DASH)[125]. Short-Form-36 (SF-36) measures generic quality of life[126]. Whilst the only disease specific questionnaire that assesses both functional and activity and participation outcome measures is Boston Carpal Tunnel Questionnaire (BCTQ)[127].

The role of NCS in predicting the outcome of surgery is not clear. In a randomized controlled trial, Schrijver *et al* compared the outcome measures for the severity of complaints with results of NCS and found that nerve conduction studies improved significantly at 12 months[128]. However, there was only a modest correlation between the improvement in NCS and relief of symptoms following surgery. The authors concluded that patients do not require routine nerve conduction studies following CTR. However, they have recommended the routine use NCS when studying the effects of treatment for CTS [128]. Several other studies have shown that NCS improve following surgery [129]. Rider *et al* used a patients rated survey, gap detection test and a rapid pinch and release task to evaluate the response following the carpal tunnel release. The authors found significant improvements in the performance of these tests within the short-term following surgery. He concluded that these tests are good alternatives to NCS in assessing outcome following surgery. The gap detection sensory thresholds test estimates minimum width needed for detection of gap on smooth work surface by probing with one finger[130]. The gap detection test measures the functional aspects of carpal tunnel syndrome e.g. detection of scratches and surface defects. The rapid pinch and release test measures psychosomatic performance in terms of speed and force control by using an Aluminum strain gauge dynamometer[131,132].

In a review of 28 randomized controlled trials of surgical treatment for CTS, Joersch- Herold *et a*l noted that the self-reported symptom resolution, grip or pinch strength and return to work were the more frequently assessed outcomes measures. In addition, the authors noted that majority of the studies used quality of life measures such as SF-36 that measured the psychosocial impact of CTS. Authors concluded that the majority of the studies used outcome measures that mainly assess body structure and functional outcomes but not outcome measures of activity and participation[133,134]. In a systematic review of 92 studies published over a period of 11 years, Gummesson *et al* noted that only 4.1% of studies reported outcomes on activity and participation. Furthermore, the studies which included health-related quality of life limited the outcome measure to number of days taken to return to work[134].

The BCTQ questionnaire was first introduced in 1993 by Levine *et al*[135]. BCTQ is a carpal tunnel syndrome specific outcome assessment questionnaire. It assesses not only the severity of symptoms but also the functional status in patients who have undergone carpal tunnel release. The BCTQ questionnaire has two components. The first part is a Symptom Severity Scale with 11 questions. The second is Functional Status Scale with 8 items that are rated for degree of difficulty on a five-point scale. Each scale generates a final score that ranges from 1 to 5, with a higher score indicating a greater disability. BCTQ has been used extensively as an outcome measure following CTS treatment. It is highly reliable, reproducible and a valid outcome assessment tool. In a systematic review of 10 studies which examined the psychometric properties of BCTQ, Leite *et al* concluded that BCTQ is highly reliable, responsive, and should replace any other non-standardized methods of assessment[127].

CONCLUSIONS

Occupational CTS is uncommon and it is essential to exclude all other causes particularly the intrinsic factors such as obesity before attributing it to occupation. The risk of CTS is high in occupations involving exposure to high pressure,

high force, repetitive work, and vibrating tools. The diagnosis of CTS should be based on symptoms and signs and nerve conduction studies. Surgery is the only treatment that provides cure in moderate to severe cases. Apart from early recovery and return to work there is no difference in the early and late complications and the outcome between open and endoscopic surgical decompression.

The authors have no conflict of interest.

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Paper

Timeliness of diagnosis in Motor Neurone Disease: a population-based study.

Colette Donaghy[1], Alison Dick[1], Orla Hardiman[2], Victor Patterson[1]

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SUMMARY

Following the observation from our experience with the Northern Ireland Motor Neurone Disease (MND) register that excessive delays appeared to exist in the diagnosis of patients with MND, we performed a population-based study of the length and factors involved in the diagnostic process. In 73 patients we found that the median time to diagnosis from symptom onset was 15.6 months, being shorter in bulbar onset patients and longer in females or those presenting with non-specific gait disturbance. We divided this interval into three time periods – symptom onset to first medical contact, first medical contact to neurology referral and neurology referral to diagnosis. The time period from first medical contact to neurology referral was the longest of the three periods studied indicating that appropriate timely referral of patients to neurologists was responsible for the greatest delay in making a diagnosis of MND. We propose that improving the accessibility of neurological services could potentially reduce the time to diagnosis by at least three months.

INTRODUCTION

From our experience in setting up the Northern Ireland Motor Neurone Disease (MND) register, in August 2004, we felt that excessive delay existed in the diagnosis of patients with MND. Timeliness is an essential component of high quality health care[1] particularly in such a devastating diagnosis as MND. Earlier diagnosis in patients could mean earlier commencement on riluzole, the only licensed treatment for MND, as well as a greater opportunity to become enrolled in clinical drug trials. It is also likely that significant psychological stress accompanies the wait for a diagnosis. A study by Johnston et al[2] reported that the majority of MND patients described positive aspects of being given their diagnosis, particularly because they now had a 'label' for their condition. Furthermore, earlier diagnosis allows patients more time to make personal and financial adjustments and make plans for the future, including the modification of their home to cope with impending disability.

The latency from symptom onset to diagnosis in MND documented in the literature has shown little improvement or change over the last 40 years and figures range from 10.6-17.5 months[3-13]. Two recent studies[4,10] looked at the factors leading to such delay. The first study[10] ascertained patients solely via the Motor Neurone Disease Association (MNDA) and collected data directly from patients. Using this method one cannot assess the validity of the diagnoses and in addition it is very unlikely that patients will be able to recall accurately the details of their diagnostic process. Although the second study[4] used data from structured case reports completed by consultant neurologists we feel the most robust and accurate method to examine the diagnostic process in MND would be to review GP (General Practitioner) records. In the vast majority of patients, the GP would be the first point of contact for patients and GP records would contain all correspondence between various hospital specialists. In addition, our study has the added advantage of being population-based due to a well maintained register of MND patients in Northern Ireland.

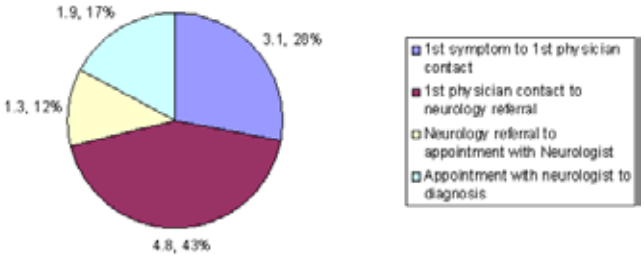

Fig 1. Median time periods (months)

Using the MNDA's 'Standards of Care' document (figure 1), we performed a population-based case note review to study the length of the diagnostic process of MND and the contributing factors using both primary care and hospital records.

METHODS

We used the MNDA's 'Standards of Care' for our study. Ethical committee approval was obtained for the setting up of an MND register. We identified all patients from the Northern Ireland MND register prevalent on 1st January 2006. All were diagnosed by a consultant neurologist and fulfilled the original El Escorial criteria, and gave written informed consent for their medical notes to be examined. All GP and hospital notes were examined (by CD and AD). Data was collected using a

1 Department of Neurology, Royal Victoria Hospital, Belfast, BT12 6BA UK.

2 Department of Neurology, Beaumont Hospital, Dublin, Ireland

Correspondence to: Dr Donaghy

Donaghy1a@hotmail.com

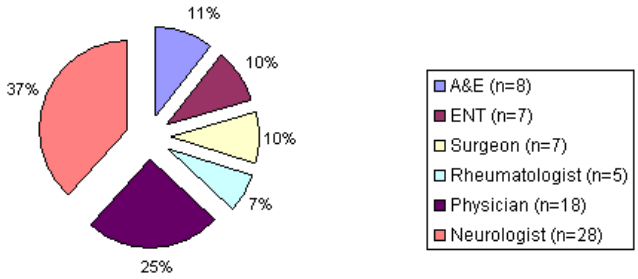

Fig 2. 1st Specialist seen on referral from 1st medical contact

structured form that focused on the time periods from initial medical contact to diagnosis as well as the nature or specialty of the physicians involved

We used median instead of mean values in calculating time periods because the prevalent population (figure 2) included some long living MND patients who had presented insidiously.

RESULTS

There were 83 patients with MND prevalent on the register on the 1st January 2006 and we were able to include 73 patients providing 88% ascertainment. We were unable to obtain consent from nine patients while another had no primary care records available due to their diagnosis being made outside Northern Ireland. These cases were excluded. Ascertainment of cases was 99.5% based on unpublished results from a capture-recapture analysis performed on a prevalence study on 30th June 2005. Capture-recapture analysis is a method of counting the total number of cases within a population from two or more overlapping incomplete but distinct sources which allows estimation of the number of unobserved cases and can determine the completeness of ascertainment[14]. We used multiple sources to ascertain cases and minimise bias.

The median time to diagnosis from symptom onset (TTD) was 15.6 months and the time period from first physician contact to neurology referral was proportionately the longest (figure 1). There was no correlation between TTD and age. TTD was longer in females, 20.9 months, compared to males, 13.9 months, and this was due largely to the time period from neurology referral to diagnosis (2.7 months in males and 5.2 in females).

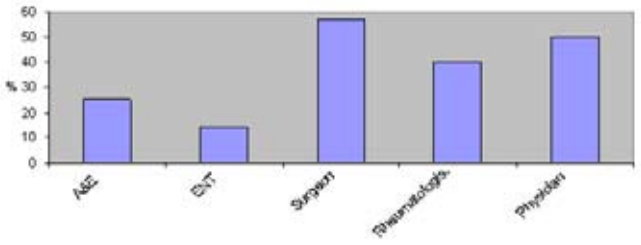

Fig 3. Appropriate onward referral from non-neurologists

The impact of the physicians involved

The GP was the first physician contacted in 67/73 or 92% of cases. Three patients attended the Accident and Emergency department when presenting first and for two it was unknown. One patient who had frontotemporal dementia associated MND presented to a psychiatrist with concerns from her family that she had depression. The first specialist seen was the neurologist in 28/73 (37%) cases (figure 2). Appropriate onward referral to neurologists from the first specialist seen ranged from 14 to 57%. Referral was lowest in those seen by ENT surgeons (7 patients, figure 3). The highest level of appropriate onward referral was by surgeons (orthopaedic and neurosurgeons) at 57%. One might have expected that physicians would have referred more than 50% of the patients they saw onto a neurologist.

TABLE I

Standards of care used for the study

Standards of care to achieve quality of life for people affected by Motor Neurone Disease
Before diagnosis:
Speed in acquiring the correct diagnosis through
(a) Early recognition of symptoms which might suggest the diagnosis
(b) Earliest possible assessment by neurologist

The impact of site of onset of disease and presenting symptoms

TTD was shorter in bulbar onset (13.5 months) as compared to limb onset disease (17 months) particularly the time period from neurology referral to diagnosis which was shorter in bulbar onset (2.2 months) as compared to limb onset disease (4.6 months). The TTD in bulbar onset disease was 24.7 months for those initially referred to an ENT surgeon (7/19), and only 4.9 months for those referred directly to a neurologist (3/19) and 12.2 months for the remaining 9/19 patients referred to other specialists.

Although responsible for only 8% of patients, non-specific gait disturbance was associated with the longest TTD due to a delay within all time periods (Table III). This group presented with gait disturbance without evidence of weakness or foot drop, and tended to have upper motor neurone predominant MND. Whilst MND is believed to be a painless condition many patients do complain of pain not associated with identifiable trauma as was the case in four of our patient group.

TABLE II.

Demographics & Clinical details

Male : Female ratio	1.4 : 1
Site of disease onset	Bulbar (n=19) Limb (n=52) Cognitive (n=2)
Mean age at symptom onset	60 years (SD 13.7)
Mean age at diagnosis	61.8 years (SD13.6)

TABLE III.

Median time periods dependent on nature of predominant 1st symptom

Time periods	Median time (months)					
	Weakness (n=36, 50%)	Bulbar (n=19, 26%)	Cognitive (n=2, 3%)	Non-specific gait disturbance (n=6, 8%)	Fasciculation / Cramps (n=6, 8%)	Pain (n=4, 5%)
Symptom onset to 1st physician contact	2.6	2.0	5.8	11.0	9.6	0.1
1st physician contact to neurology referral	4.2	4.8	5.4	9.6	2.4	10.7
Neurology referral to diagnosis	4.8	2.2	2.2	13.3	2.7	0.8
Symptom onset to diagnosis (TDD)	15.5	13.5	13.1	33.6	15.6	13.0

Misdiagnosis

The final diagnosis of MND was given to the patient by a neurologist in 69/73 (95%) of cases and a general physician in 4/73 (5%) of cases although all patients had their diagnosis confirmed by a neurologist as is appropriate. Initial incorrect diagnoses were made in 20/73 cases (27%). These falsely negative diagnoses were made by neurologists in 7 of the 69 patients, by GPs in 3 before onward referral and by non-neurology specialists in 10 of the 45 cases seen before being referred onto neurologists. Table IV lists the range of diagnoses. TTD was longer in the group who were misdiagnosed (26.3 months) when compared to those who were not (13.8 months). No correlation was found between misdiagnosis and age.

DISCUSSION

The TTD in our study was 15.6 months and the longest median time period responsible for this latency was that time spent with a physician before a neurology referral was made (4.8 months). Only 37% of patients were referred to a neurologist as the first specialist and of those referred to non-neurologists only 40% were appropriately referred on to a neurologist. If 80% of patients were to be referred to a neurologist as the first specialist then the median TTD could be reduced by just over 3 months.

When one considers that the current survival of MND in Ireland is 16.4 months from diagnosis until death[15], a wait of 15.6 months from symptom onset to diagnosis appears excessive. The MNDA's standards of care before diagnosis (table I), detail that speed in achieving a diagnosis should be attained through 'early recognition of symptoms' and 'earliest possible assessment by a neurologist'. These two key areas appear to be responsible for the greatest delay in this patient group. Our results indicate that GPs and non-neurology specialists are not referring patients to neurologists quickly enough, in particular ENT surgeons. This may be due to a combination of poor recognition of neurological signs and symptoms however it is more likely that GPs are disillusioned with the waiting times for neurology outpatient appointments (6 – 12 months at the time of this study) and simply try to find other specialists that might be able to help. Nonetheless presentations such as non-painful gait disturbance, progressive bulbar dysfunction and fasciculations, when they are associated with wasting or weakness, always necessitate neurological assessment. ENT surgeons need also to be aware that progressive bulbar dysfunction not due to a structural cause requires urgent referral to a neurologist.

What can one learn from this study and what can be done to help? The time period from presentation to neurology referral is the best place to target improvements - GPs need to refer appropriate patients directly to neurologists. We know that new neurological outpatients are more efficiently managed by neurologists than general physicians[16]. Furthermore

TABLE IV.

Falsely negative diagnoses

Diagnoses
Cervical Spondylosis
Nothing
Neuropathy
Lumbar disc prolapse
Capsulitis of shoulder joint
Myelopathy
Multiple sclerosis
Parkinson's Disease
Stroke
Carpal Tunnel Syndrome
Vascular pseudobulbar palsy
Osteoarthritis
Rhinitis
Depression

the results from this study indicate that neurologists are successfully prioritising referrals on patients with MND, with a median waiting time of 1.3 months from neurology referral to appointment with the neurologist, and GPs should be encouraged by this. Ultimately making neurologists more accessible would encourage GPs to make appropriate referrals for patients with neurological symptoms or signs. One approach that might help would be the introduction of an email triage system as examined by Patterson et al[17]. This allows a neurologist to deal with appropriate GP referrals using email and shorten the time for a clinic appointment. The recent establishment of the Northern Ireland MND Care Centre may also help as the care centre coordinator can accept referrals from non-neurologists concerning patients with suspected MND and 'fast track' them through the system.

The strengths of our study are that this is a population-based study with a high level of ascertainment and both GP and hospital records were used to allow as accurate information to be collected as possible. This robust methodology is lacking from previous studies. The weakness of this study is that it is from a single region and does not necessarily reflect practice in the rest of the UK and beyond. It is likely that similar trends exist within the rest of the UK.

Our TTD was consistent with that documented in the literature of 10.6-17.5 months[3-13] although our misdiagnosis rate of 27% was at the lower end of reported figures 27-61%[4,10,18,19]. In a survey involving Germany, Spain, Italy, USA, Brazil and Argentina[4], 63% of patients were referred directly to a Neurologist from the GP. In a study performed in England and Wales the reported rate was 47%[10]. Although these rates seem much better than our 37%, these studies were not population based. If the reported rates in these studies were indeed representative of the population from which they were drawn it may be in part due to the increased accessibility of neurologists in some European countries compared to Northern Ireland. Both studies noted that the main delay appeared to be in the time period from presentation to neurology referral.

With improvements in the accessibility of neurological services we feel that the median time period from presentation to neurology referral of 4.8 months could be reduced by three months or more. As this represents approximately 10% of the average survival of MND patients from symptom onset, it would be an effort worth making.

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Conflict of interest – none declared.

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Paper

Open Carpal Tunnel Release – still a safe and effective operation

Stephen A Badger, Mark E O'Donnell, Jagannath M Sherigar, Peter Connolly, Roy AJ Spence

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ABSTRACT

Background: Carpal tunnel syndrome is a common cause of neurological symptomatology. Surgical decompression remains the treatment of choice in patients not responding to conservative therapies. The aim of this study was to assess the effectiveness of standard open decompression by analysis of symptomatic and functional improvement and to assess whether a general surgeon can still perform this operation safely.

Patients and methods: Patients undergoing standard open carpal tunnel release by a single general surgeon were recruited. A self-administered Boston questionnaire was used to assess symptom severity and functional status pre- and post-surgical intervention.

Results: Forty-seven patients (51 hands) underwent carpal tunnel release and 32 patients completed the questionnaire. 88% had a significant reduction in the symptom severity score, while improvement in function status score was achieved in 79% of patients. Mean symptom severity score improved from 3.41 points preoperatively to 1.85 ($p<0.0001$) points at the last follow up examination, while the mean function status score improved from 2.73 to 1.99 points ($p<0.0001$). Outcome was poor in six patients with slight worsening of either symptom or function status score. Three patients were treated conservatively for minor wound infection without long-term sequelae.

Discussion: Standard open carpal tunnel release still provides efficacious symptomatic relief with a low risk of associated complications when performed by a general surgeon.

Key words: Carpal tunnel release, symptomatic, functional, improvement

INTRODUCTION

Carpal tunnel syndrome is a common focal peripheral neuropathy. A raised intracarpal canal pressure results in median nerve compression and impaired nerve perfusion that leads to discomfort and parathesia in the one or both hands[1]. Traditional treatment modalities include physiotherapy, steroid injections and various surgical options[2-4]. Surgery is generally preferred in severe cases of carpal tunnel syndrome and should be considered when carpal tunnel syndrome does not respond to conservative measures. The standard surgical approach uses a palmar curvilinear incision to facilitate division of the transverse carpal ligament and its overlying structures. Recent modifications to this approach have been developed in an attempt to attenuate scar formation and to facilitate an earlier return to normal daily activities and the workplace[4-6]. There is, however, no consensus on the most effective method of treatment. We reviewed symptom resolution and functional improvement in patients following standard carpal tunnel release.

PATIENTS AND METHODS

All patients who had carpal tunnel release performed by a single surgeon from January 2001 to December 2004 were included in the study. Age was expressed as median and range. Previous studies have reported a plateau in symptomatic and functional improvement approximately six months post-carpal tunnel release when assessed by a validated questionnaire.[7] For this retrospective study, a nine month follow-up period was adopted to ensure full capture of any aspect of patient improvement.

A review was made of the case records of all the patients for predisposing conditions, pre-operative investigations, operative procedure and the post-operative complications. The Boston carpal tunnel questionnaire was used to evaluate patient outcome[8]. This is a patient oriented and self-administered questionnaire with strong internal consistency, reproducibility and validity[8]. As the study was retrospective in nature, the patients completed the pre-operative and post-operative questionnaires by recall.

The symptom severity scale includes 11 items from six clinical areas critical to evaluation of carpal tunnel syndrome, such as pain, numbness and nocturnal symptoms. Each item score ranges from 1 (mild) to 5 (severe). The functional state scale evaluates hand function with respect to eight daily routine activities, such as driving, sports and working with

Department of Surgery, Level 2, Belfast City Hospital Lisburn Road, Belfast, BT9 7AB

Correspondence to Mr Badger 21 Marlo Heights, Bangor, BT19 6NQ

stephenbadger@btinternet.com

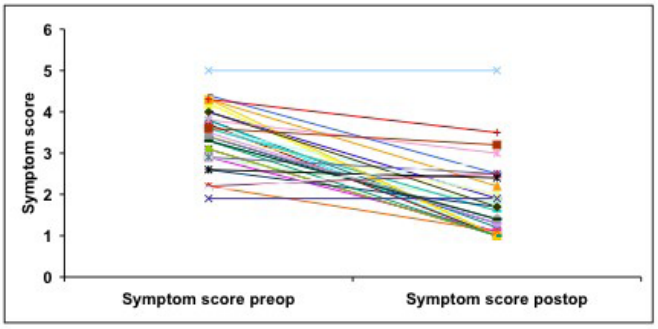

Fig 1. Symptom Score of 34 patients

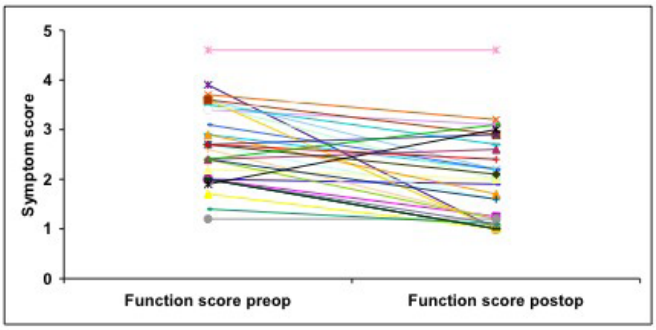

Fig 2. Functional Score of 34 patients

tools, Each item score ranges from 1 (no difficulty) to 5 (can not perform the activity). Each summative score is calculated as the mean with standard deviation of the scores of individual items[8]. Severe impairment is indicated by a high score. A post-operative change in the Boston questionnaire score was then determined and subsequently compared to the pre-surgical initial score. At the end of the questionnaire patients were asked to rate their contentment with the surgery as being very satisfied, satisfied, neither satisfied nor dissatisfied, or dissatisfied.

Response to clinical change was assessed by comparison of the pre-operative and post-operative scores. The scores are expressed as mean and standard deviation. The ability of the questionnaire to detect change was expressed as the effect size. This effect size, as used elsewhere with the questionnaire, was calculated by determining the mean difference between the pre-operative and post-operative scores and then dividing this difference by standard deviation of the difference. Therefore a large effect size indicated a more significant improvement in the clinical condition of the patient, compared to a small effect size.

RESULTS

All patients in the study had a clinical diagnosis of Carpal Tunnel Syndrome (CTS), which was defined as the presence of two subjective symptoms (numbness, tingling in the median nerve distribution). Physical examination included Phalen's and Tinel's Test. A pre-operative nerve conduction study was diagnostic of CTS in all patients. A total of 17 patients had one or more associated predisposing factors or conditions (renal failure = 2; rheumatoid arthritis = 7; diabetes = 8; thyroid dysfunction = 4).

Forty-seven patients (10 male) underwent the procedure during the study period, with 4 having bilateral surgery on separate occasions. The median age was 59 years (range 34 - 96 yrs), with a mean follow-up period of 17 months (range 9 to 36 months). Decompression of the carpal tunnel was performed using a standard open technique, which involved a 3-4cm curvilinear incision. The palmar fascia and flexor retinaculum was then divided under direct vision, taking care to protect the recurrent motor branch. Three patients developed minor wound infection postoperatively and were treated conservatively with good healing and no long-term sequelae.

Thirty-two of 47 patients (68%) successfully completed both pre-and post-operative Boston questionnaires and were included in the final study analysis. Two patients had bilateral surgeries and each procedure was considered independently during data analysis. The pre-operative symptom severity score was 3.41 points (± 0.72). The score improved post-operatively to 1.85 points (± 0.89) indicating substantial response to clinical change ($p<0.0001$). Therefore the difference resulting from surgery, as expressed by the effect size was 1.55. The symptom score improved in 88%, while it remained unchanged in 6% and worsened in 6% (Fig. 1). The preoperative functional status score was 2.73 points (± 0.80), compared with post-operative score of 1.99 points (± 0.94) and the effect size was 0.83 ($p<0.0001$). The functional score improved in 79% of patients, was unchanged in 6% and deteriorated in 14% (Fig. 2).

Two patient's hand symptoms remained the same even after the surgery. Functional status slightly worsened (difference in functional scale < 0.3) after the operation in six patients, although the symptoms improved in three of these, giving a mixed picture. Four individuals had a history of either rheumatoid arthritis involving the hand, renal failure, diabetes mellitus or gout. All patients stated they were either satisfied or very satisfied with surgery except three for whom functional status failed to improve after the operation.

DISCUSSION

Carpal tunnel decompression with division of the transverse carpal ligament is a successful procedure for the treatment of carpal tunnel syndrome[9,10]. Controversy still exists regarding the choice of surgery for this condition. Three different surgical techniques have been described in the literature – the classic or standard approach, endoscopic approach either single or multiport, and limited incision approach[4,12]. The traditional approach with long palmar incision has been criticised for greater scar tenderness and longer time to return to work[3,4]. In an effort to overcome this, endoscopic and limited incision approaches have emerged.

Although the endoscopic carpal tunnel release has been demonstrated to reduce recovery time, a previous study raised concerns about an increased rate of complications[11]. However with the improvement in the endoscopic techniques, fewer complications, such as persistent weakness and scar tenderness have now been demonstrated[5,6,11,13,15]. Klein *et al*[13] conducted a prospective study to evaluate the safety and functional outcome of limited incision technique and concluded that this procedure is an effective method for releasing standard carpal tunnel syndrome and is associated with significant symptom relief, minimal scar tenderness and an improvement in overall hand function. However it has

the limitation of not able to perform additional procedures, if required with inadequate exposure after conversion to a longer incision.

Surgical success for carpal tunnel release is achieved in most cases, but subjective evaluation appears to provide the best outcome measures for carpal tunnel syndrome[16]. We used the Boston Questionnaire scales, which have been demonstrated as a valid and reliable assessment tool for hand dysfunction, having previously been used to study the effectiveness of the open carpal tunnel release[8].

The Boston questionnaire symptoms showed an overall improvement in 88% of hands. In general the effect sizes for symptomatic improvement are considered moderate when greater than 0.5 and those of more than 0.8 indicates a large effect as indicated previously[8]. The symptom scale had a very large effect size revealing a significant post-operative improvement and the function scale showed medium responsiveness. Although this may be surprising the presence of significant co-morbidities in some of these patients may explain the lack of apparent success in the function change.

Three patients developed minor wound infection after the operation and were treated conservatively by their own doctor. Seventeen patients in our study were diagnosed to have one or more than one associated predisposing conditions. Four of those with poor outcome had at least one associated predisposing conditions. There are mixed reports with diabetic patients exhibiting a trend towards less pain relief post operatively[18]. Mondelli *et al*[19] compared the results of carpal tunnel release in patients with diabetes mellitus and idiopathic carpal tunnel syndrome and concluded that the patients with diabetes have the same probability of positive surgical outcome as patients with idiopathic carpal tunnel syndrome.

A Cochrane review concluded that there is no strong evidence supporting the need for replacement of standard open carpal tunnel repair by existing alternative surgical procedures[17]. None of the existing alternatives to standard open carpal tunnel release offer better relief for carpal tunnel syndrome in the short term or the long term and there is conflicting evidence about whether endoscopic carpal tunnel release results in earlier return to work and / or activities of daily living.

The limitations of this study are the small patient numbers and that the data on preoperative status were obtained retrospectively, and thus recollections will be less accurate than prospectively collected data. This weakens the nature of the evidence produced as it leaves potential for recall bias, thereby limiting the strength of the conclusions. However, it appears that greater satisfaction with the result was associated with greater improvement in the score, for both the severity of symptoms and functional status. Patients dissatisfied with the operation had worsening of the scores after the operation.

CONCLUSION

Standard open carpal tunnel release remains a safe and effective method of treatment. It is not very technically demanding, results in satisfied patients and can be performed safely by general surgeons with experience.

The authors have no conflict of interest

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Paper

Germline MSH6 mutations are more prevalent in endometrial cancer patient cohorts than Hereditary Non Polyposis Colorectal Cancer cohorts.

Lisa A Devlin[1], Colin A Graham[1], John H Price[2], Patrick J Morrison[1].

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ABSTRACT

Objective: To determine and compare the prevalence of MSH6 (a mismatch repair gene) mutations in a cohort of families with Hereditary Non-Polyposis Colorectal Cancer (HNPCC), and in an unselected cohort of endometrial cancer patients (EC).

Design: Two patient cohorts participated in the study. A cohort of HNPCC families who were known to the Regional Medical Genetics department, and an unselected cohort of patients with a history of EC. All participants received genetic counselling on the implications of molecular testing, and blood was taken for DNA extraction with consent. All samples underwent sequencing and Multiple Ligation probe analysis (MLPA) for mutations in MSH6.

Populations: DNA from one hundred and forty-three probands from HNPCC families and 125 patients with EC were included in the study.

Methods: Molecular analysis of DNA in all participants from both cohorts for mutations in MSH6.

Outcome measures: Prevalence of pathogenic mutations in MSH6.

Results: A truncating mutation in MSH6 was identified in 3.8% (95% CI 1.0-9.5%) of patients in the endometrial cancer cohort, and 2.6% (95% CI 0.5-7.4%) of patients in the HNPCC cohort. A missense mutation was identified in 2.9% and 4.4% of the same cohorts respectively. No genomic rearrangements in MSH6 were identified.

Conclusion: MSH6 mutations are more common in EC patients than HNPCC families. Genomic rearrangements do not contribute to a significant proportion of mutations in MSH6, but missense variants are relatively common and their pathogenicity can be uncertain. HNPCC families may be ascertained through an individual presenting with EC, and recognition of these families is important so that appropriate cancer surveillance can be put in place.

Key Words: Endometrial, Cancer, MSH6, HNPCC.

INTRODUCTION

HNPCC is an autosomal dominant highly penetrant cancer-susceptibility syndrome caused by germline mutations in one of the DNA mismatch repair (MMR) genes, namely MLH1, MSH2 and MSH6[1]. Affected individuals have a predisposition to developing early onset colorectal cancer (CRC) and other HNPCC associated cancers, particularly endometrial cancer (EC)[2].

Diagnosis of HNPCC is dependent on familial clustering of CRC's, and other HNPCC related cancers, early onset cancers, and synchronous and metachronous cancers. Associated with a life time cancer risk of up to 80% [3,4], early diagnosis enables at risk family members to be enrolled in a cancer surveillance programme, thus reducing mortality and morbidity [5-7].

The Amsterdam criteria, developed in 1991 by the International Collaborative Group on Hereditary Non-polyposis Colorectal Cancer (ICG-HNPCC)[8], and subsequently revised in 1999[9], are not diagnostic, but can be used to standardise HNPCC families for comparative multi-centre studies (see Boxes 1 and 2).

MLH1 and MSH2 mutations account for the majority of known mutations in HNPCC families, and can represent between 25%[10] and 49% of Amsterdam criteria positive families[11]. Higher mutation detection rates of 86% have been published, but this may be as a result of founder mutations[12]. MSH6 mutations were first reported in HNPCC kindreds in 1997[13,14], and are less prevalent in HNPCC cohorts with MSH6 mutations estimated to represent approximately 10%

Departments of [1]Medical Genetics and [2]Gynaecological Oncology, Belfast City Hospital, Belfast HSC Trust, Belfast, BT9 7AB, United Kingdom

Correspondence to Dr Devlin, Department of Immunology, Royal Victoria Hospital, Belfast HSC Trust, Grosvenor Road, Belfast BT12 6BA, UK.

lisa.devlin1@belfasttrust.hscni.net

Box 1:

Amsterdam criteria I

There should be at least three relatives with histologically verified CRC; all of the following criteria should be present:
• One should be a first degree relative of the other two; • At least two successive generations should be affected; • At least one CRC should be diagnosed before age 50; • FAP should be excluded in the CRC case; • Tumours should be verified by pathological examination

Box 2:

Amsterdam criteria II

At least three relatives with an HNPCC associated cancer *
• One affected person is a first degree relative of the other two • At least two successive generations are affected • At least one person was diagnosed before the age of 50 years • Familial adenomatous polyposis has been excluded • Tumours have been verified by pathological examination

*large bowel, endometrium, small bowel, ureter, or renal pelvis, though not including stomach ovary, brain, bladder or skin

of all MMR mutations in HNPCC families[15,16]. Between 2-5% of HNPCC families including Amsterdam I, Amsterdam II, or 'HNPCC like' will have a germline mutation in MSH6[15,17,18]. Mutations have been described in PMS2 and PMS1 in HNPCC kindreds but have not been found to contribute to a significant proportion of families[19,20].

In comparison to MLH1 and MSH2, the phenotype of MSH6 is characterised by a later age of onset of CRC, incomplete penetrance, and a higher risk and later age of onset of EC in female MSH6 carriers[15,21]. MSH6 mutation carriers may be missed amongst analysis of HNPCC families if the Amsterdam criteria are used as selection criteria[22] It is likely that MSH6 mutations may occur at a higher prevalence in a cohort of EC patients in comparison to HNPCC cohorts that have been selected by the Amsterdam criteria which are characteristic of HNPCC families with a mutation in MLH1 or MSH2. A few studies have looked at the prevalence of MSH6 mutations in EC patients with estimates between 1.7% of patients with EC less than 50 years[23], and 4.7% identified in EC patients un-selected for age or family history[24].

In this study we sought to determine the prevalence of MSH6 mutations in our Northern Ireland HNPCC cohort with less restrictive inclusion criteria than the Amsterdam II criteria, in an attempt to include as many MSH6 phenotype families as possible given the probable later onset of colon cancers; and also determine the prevalence of mutations in an unselected cohort of EC patients.

METHODS

Subject Recruitment

The study was granted ethical approval by the Office for Research Ethics Committees Northern Ireland (ORECNI). Two patient cohorts were recruited; HNPCC and endometrial cancer patient cohorts. Sample sizes were calculated from previous studies with estimated prevalence figures of MSH6 of 9%[16] and 8%[25] respectively in each cohort, giving a target size of 197 cases for an estimate of prevalence with 95% confidence intervals no wider than +/- 4% for the HNPCC cohort and a target size of 177 cases for an estimate of prevalence with 95% confidence intervals no wider than +/- 4% for the endometrial cohort.

The HNPCC cohort was known to the regional genetics department, and had received genetic counselling in the past, with blood taken for diagnostic testing of MLH1 and MSH2 or DNA storage. All families who met the Amsterdam I and II criteria were included. In addition, inclusion criteria was extended to families with a clustering of CRC, or other HNPCC related cancer, with at least three affected family members (age not restricted), or at least two family members if the age of onset was below 50 years with pedigrees suggestive of autosomal dominant inheritance, or an individual with CRC diagnosed less than 35 years – similar to the Bethesda criteria. Probands were contacted with information regarding the study, and a consent form with a stamped addressed envelope (SAE) to return if they wished to participate. One hundred and forty-three participants in total were recruited to this cohort 35 meeting the Amsterdam I, 6 Amsterdam II, and 102 classified as 'HNPCC like'.

Patients with a history of EC (back to 01/01/99) were identified by means of a pathology coding database covering all patients from the Eastern Health Board in Northern Ireland and recruited consecutively.

All potential participants were contacted with a participant information sheet, with a detachable reply slip, to be returned in the enclosed SAE, for those keen to participate. For patients with returned reply slips, a clinic appointment was offered to discuss the study with genetic counselling, and obtain medical details, family history, informed consent and a blood sample for DNA extraction. One hundred and eighty-eight potential participants were contacted, and one hundred and twenty-five participants were recruited to this cohort. Age ranged from twenty-six to eighty-four, with a mean age of 58.9 years. Mean body mass index (BMI) in this cohort was 30.65, ranging from 18.64-53.15.

MSH6 Sequencing

All DNA was or had been extracted by the Nucleic Acid Extraction Centre (NAEC), Belfast City Hospital, and stored at -80˚C. Working dilutions in X1 TE were made at 5ng/µl.

All ten exons and at least 20 base pair (bp) of flanking intronic sequence was subject to direct sequencing analysis. PCR products for nine of ten exons of MSH6 were obtained using Applied Biosystems VariantSEQr™ Requencing System (product number: RSS000012234_02). All reactions were carried out using standard reaction mix and conditions as determined by ABI. Exon 1 primers were as follows:

TABLE I:

Number of mutations identified in each cohort

	HNPCC (115)	95% CI	Endometrial (105)	95% CI	Total 220
Truncating	3 (2.6%)	0.5% - 7.4%	4 (3.8%)	1.0% - 9.5%	7 (3.2%)
Missense	5 (4.4%)		3 (2.9%)		8 (3.6%)
Total	8 (7.0%)	3.1% - 13.2%	7 (6.7%)	2.7% - 13.3%	15 (6.8%)

1F PCR; TCCGTCCGACAGAACGGTTG, 1R PCR; ATGCTCCAGACTCGACCCG, using a standard 25µl reaction mix with 3.4µl of 25mm $MgCl_2$ and 0.4µl of 5U/µl Expand DNA polymerase (ABI) at an annealing temperature of 60°C.

All PCR products were subject to clean up using ExoSAP-IT®† to remove excess primer dimer, unincorporated dNTPs, and non-specific DNA products. Sequencing reactions were carried out using BigDye® terminator Ready Reaction Mix v1.1 from ABI®, according to manufacturer's instructions.

Additional primers were required to sequence exons 7 and 8 because of a poly T at the 5′ end of exon 7, which resulted in slippage during the sequencing reaction, and polymorphisms situated at the 3′, (c.3646 +35_38delATCT) of exon 7, and 5′, (c.3647 -51_-35 del 17), and 3′ (c.3802-42insT) end of exon 8, which made sequence of the exons unreadable when the polymorphisms were present in the heterozygous state. Additional sequencing primers for exon 7 (7F Seq; TTGTGATTTTTTTTTTTTAAG, 7R Seq; TAGTCTTCAAATGAGAAG) and 8 (8F Seq; GAGTTACTTCCTTATGCA , 8R Seq; GAAGTGCCCTCTCAAAAAACC) were designed. Electrophoresis was carried out by the Queen's University Belfast genomic core facility on an ABI 3730 DNA analyser.

MLPA Analysis

All samples were subject to MLPA analysis using SALSA MLPA KIT POO8 MSH6/PMS2 from MRC-Holland. Reaction mix and conditions are as determined by MRC-Holland. Electrophoresis was carried on an ABI 3100 Avant DNA analyzer using a GeneScan™ - **500 ROX**™ size standard. From the raw data generated, peak heights of each amplification product were exported to Excel worksheets designed by Dr Andrew Wallace, National Genetics Reference Laboratory, Manchester, so that the result of each sample could be 'normalised'.

Statistical Methods

The cohort sizes necessary to obtain estimates with adequate precision were initially calculated using a Normal approximation to the binomial sampling distribution provided by the StatCalc program in the EpiInfo package (http://www.cdc.gov/EpiInfo/). The Stata package (http://www.stata.com) was used to give the exact binomial confidence limits for a proportion (Table I).

RESULTS

MSH6

Good quality sequence was obtained for all 10 exons of MSH6 in 220 participants, 115 from HNPCC cohort, and 105 from endometrial cohort. Results with exact binomial confidence limits for a proportion are shown in table I. Given that the pathogenicity of the missense mutations identified has yet to be determined, arguably the proportion of the truncating mutations is more relevant.

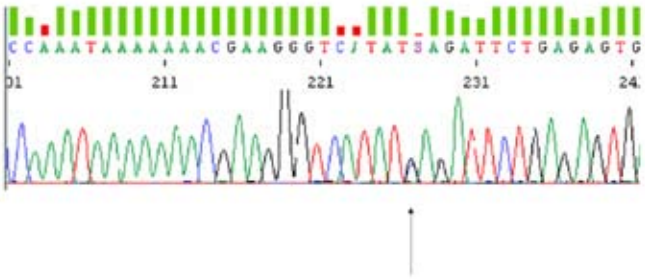

Fig 1. Sequencing analysis showing a truncating mutation c.755 C>G (p.Ser252X) in exon 4 of MSH6

All sequences were viewed with reference to a control sample (with a known mutation in MLH1 or MSH2), and reference sequences (www.ensemble.org, www.ncbi.nlm.nih.gov). All variants are described with reference to den Dunnen *et al*[26].

TABLE II:

Truncating mutations identified

Nucleotide Change	Protein Change	Mutation	Exon	Cohort	Classification
c.642 C>A	(p.Tyr214X)	Truncating	4	HNPCC	HNPCC like
c.755 C>G (figure 1)	(p.Ser252X)	Truncating	4	EC	HNPCC like
c.755 C>G	(p.Ser252X)	Truncating	4	EC	Not HNPCC
c.3103 C>T	(p.Arg1035X)	Truncating	4	EC	HNPCC like
c.3261 delC	(p.Pro1087Pro fs X3)	Truncating	5	HNPCC	HNPCC like
c.3840_3846delGGAGACT	(p.Gln1280_Thr1282>GlnfsX45)	Truncating	9	HNPCC	HNPCC like
c.3938_3941dupTTCA	(p.Gln1314HisfsX14)	Truncating	9	EC	Amsterdam II

Table III:

Missense variants identified

Nucleotide Change	Protein Change	Polyphen prediction	Exon	Cohort	Classification
c.663 A>C	(p.Glu221Asp)	Benign	4	EC	HNPCC like
[c.866 G>A]+[c.867C>A]	[p.Gly289Asp]+[p.Gly289Gly].	Benign	4	HNPCC	HNPCC like
c.1508 C>G	(p.Ser503Cys)	Possibly damaging	4	HNPCC	Amsterdam I
c.1508 C>G	(p.Ser503Cys)	Possibly damaging	4	EC	Not HNPCC
c.1739 C>T	(p.Ser580Leu)	probably damaging	4	HNPCC	HNPCC like
c.3217 C>T	(p.Pro1073Ser)	Benign	5	HNPCC	Amsterdam II
c.3929 G>C	(p.Glu1310Asp)	Benign	9	HNPCC	HNPCC like
c.3963 A>T	(p.Arg1321Ser)	possibly damaging	9	EC	HNPCC like

All truncating and missense variants identified are shown in tables II and III (example figure 1) with their corresponding cohort and family classification. Participants from the endometrial cohort with a variant identified were also classified according to Amsterdam I, II, 'HNPCC like', or, a family history that was not in keeping with HNPCC. All missense variants were subject to analysis by PolyPhen (www.coot.embl.de/PolyPhen/), a tool that predicts the potential impact of an amino acid substitution on the structure and function of a human protein (see table III for predictions). Further population, family and functional studies were not carried out to evaluate the missense variants but further work is planned.

MLPA

All samples underwent MLPA analysis. Out of 268 successful MLPA results, no aberration in copy number was identified.

DISCUSSION

Fifteen variants in all were identified from the two cohorts, seven of which resulted in premature STOP codon (truncating mutations), and were therefore considered pathogenic. A further eight missense mutations were identified, of which the functional significance is not known at this time. A summary of results is shown in table I. At the time of study design, only estimates of total prevalence were available[16,25] and the cohort sizes actually attained in the study were smaller than planned. This is reflected in lower precision (wider confidence limits) in the estimates than had originally been specified.

HNPCC Cohort

A definite pathogenic mutation was identified in 2.6% of the HNPCC cohort. These results are comparable with other studies carried out on Amsterdam, Amsterdam II and 'HNPCC like' families – estimates range between 2% and 5% - who have a germline mutation in MSH6[15,17,18]. Further work on the missense variants is required to determine their pathogenicity, as the yield of MSH6 mutations in the HNPCC cohort could increase up to 7% if these are found to be significant.

Endometrial cohort

A truncating germline mutation was identified in 3.8% of the endometrial cohort. This is higher than that obtained for the HNPCC cohort of whom the majority were 'HNPCC like', thereby broadening the criteria to fit with the described characteristics of a MSH6 phenotype[15,21]. This prevalence figure is also likely to increase following further work (including immunohistochemistry), being carried out on the missense variants as some of these may be pathogenic.

There are few studies looking at MSH6 in EC. Comparison can be difficult to make between studies because of pre-selection of some study groups of EC patients by age restriction or tumour microsatellite instability. Goodfellow[26] estimated the minimum prevalence of inherited MSH6 mutations in EC to be 1.6%, from a sub-population of an EC cohort, selected for molecular analysis, the majority of which showed tumour microsatellite instability (MSI). A comparable figure is seen by Berends[23] one MSH6 mutation identified in a cohort of 58 EC patients diagnosed less than 50 years whose families fulfilled the Amsterdam II criteria. Higher figures of 4.7%[24], and 8.3%[25], have been observed in EC cohorts not restricted by age or the limitations of the Amsterdam II criteria, but where the majority of tumours exhibit MSI, although the significance of the latter study will be limited by its relatively small cohort.

As well as the heterogeneous populations studied, the variability in frequency of MSH6 mutations in both HNPCC and endometrial cohorts can also be accounted for by the sensitivity of techniques used to identify variants, the use of MSI and IHC to target molecular screening of MMR genes, and the interpretation of missense mutations which occur relatively frequently in MSH6[23]. Founder mutations in certain populations can also contribute to higher than average prevalence rates of MMR genes[12]. Further work on functional, population and family studies is required to determine the pathogenicity of the eight missense mutations identified.

Genomic Rearrangements

Genomic rearrangements account for between 17%[28] to 54.8% of pathogenic mutations in MLH1 and MSH2 in HNPCC families[29]. The prevalence of genomic rearrangements in MSH6 is less well studied, but it had been estimated that rearrangements may account for 10-20% of mutations in MSH6[30]. No genomic rearrangements were identified in our HNPCC cohort consistent with findings by Charbonnier[31]and Wagner[18]. Likewise genomic rearrangements were not detected in any of our EC patients in keeping with findings by Ollikaninen[32]. Studies that have identified MSH6 genomic rearrangements have been particularly large HNPCC

TABLE IV:

Classification of families with a MSH6 variant identified

	Truncating	Missense
Amsterdam I	0	1
Amsterdam II	1	1
'HNPCC like'	5	5
Not HNPCC	1	1

cohorts[33], or EC cohorts pre-selected by MSI[24], yielding a genomic rearrangement in MSH6 in less than 1% of the chosen population.

Promoter Region

Sequencing is highly sensitive for detection of mutations in the coding regions; however the promoter region of MSH6 was not sequenced in this study.

Previous studies have mainly concentrated on coding regions, and exonic / intronic boundaries. Two deletions of the MSH6 promoter region have been described in HNPCC families[22,33], but other studies looking at the promoter region of MSH6 in EC cohorts[27] or in HNPCC patients negative for a mutation in MLH1 or MSH2[17], did not identify any pathogenic mutations. Studies looking specifically at the promoter region of the more prevalent MMR genes, MLH1 and MSH2, identified three possible pathogenic mutations in the promoter area in 141 HNPCC patients and patients with early onset CRC (<45 years)[34]. Given that MSH6 mutations occur at a relatively low rate in both HNPCC and EC patients, we can assume that mutations of the promoter region in either cohort are unlikely to significantly alter the prevalence figures calculated from this study.

Redundancy of MSH6 mutations

Although germline mutations in MSH6 are distributed throughout the length of MSH6 displaying little redundancy, the majority of pathogenic mutations identified are in exon 4[15], with fifty-seven percent of truncating mutations identified in this study (4/7) occurring in exon 4, the largest of MSH6 exons, indicating that analysis of MSH6 in HNPCC families without a known mutation should commence at exon 4.

MSH6 phenotype

None of the truncating mutations identified in this study met the original Amsterdam criteria, the majority having a 'HNPCC like' phenotype, with a later age of cancer onset, and non-penetrance in family members (Table IV). This further supports current evidence that application of the Amsterdam criteria to HNPCC families to select for molecular testing will result in a significant proportion of MSH6 mutations being missed[15,22].

Previously unidentified HNPCC families

Eighteen out of 125 participants (14.4%) from the endometrial cohort had a significant previously unidentified HNPCC phenotype. Five of these participants had a variant identified, three truncating mutations and two missense mutations. These findings are in keeping with findings from other studies where previously unidentified HNPCC families have been ascertained through an individual with EC[23]. Increased awareness of HNPCC and other hereditary cancer syndromes amongst physicians/surgeons directly involved with the care of cancer patients such as gynaecologists, surgeons, oncologists, and general practitioners is essential for their identification.

Endometrial Cancer

HNPCC, traditionally identified as a condition with a genetic predisposition to CRC, has now been recognised as conferring a significant risk of EC to females, particularly those with a mutation in MSH6. In addition to other Mendelian inherited syndromes with a predisposition to EC such as Muir Torré, Cowden and Turcot syndrome, there are families who show a clustering of EC alone that do not have an identifiable molecular basis. Un-identified genes or predisposing low penetrant polymorphisms may contribute. The importance of environmental factors conferring a risk to the development of EC cannot be underestimated. Obesity is associated with increased levels of endogenous oestrogens, and is a significant risk factor for the development of EC. Mean BMI in the endometrial cohort was 30.65 and ranged from 18.64-53.15, with only 28 (22.4%) of participants having a BMI within the normal range (<25). In comparison it is estimated that 44% of UK females over 16 years have a BMI within the normal range. (Figures published by the Department of Health and estimated by the Health Survey for England 2003; www.dh.gov.uk).

The mean BMI for the four participants from the endometrial cohort with a truncating mutation in MSH6 was 26.2, ranging from 22.36-31.05. One of these participants had a BMI in the overweight range, and one had a BMI in the obese range. It is likely that obesity has an additive effect to the underlying risk form a MMR mutation, but larger studies would be required to determine this.

CONCLUSION

From this study we have identified the minimum prevalence of pathogenic mutations in MSH6 to be higher in an unselected cohort of EC patients, than a cohort of HNPCC patients who have been selected by criteria 'widened' from the traditional Amsterdam II criteria, in keeping with the described phenotype of MSH6. Unlike the other more common MMR genes, genomic rearrangements do not contribute to a large proportion of mutations in MSH6.

HNPCC families may not be identified if patients present with HNPCC associated cancers, such as gynaecological cancer, rather than the more commonly recognised phenotype of CRC. Clinicians should be vigilant to this possibility when presented with a history of endometrial cancer in young women. Further work on immunohistochemistry of possible missense variants may increase the true frequency of mutations in MSH6.

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Paper

Sex-differences in lung cancer cell-types? An epidemiologic study in Ireland

Zubair Kabir[1,2], Gregory N Connolly[1], Luke Clancy[2]

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ABSTRACT

Objective: This study assesses the epidemiological pattern of lung cancer cell-types in Ireland, with identification of any underlying gender variations.

Methods: Lung cancer incidence data, including the major cell-types: squamous-cell-carcinoma (SCC), adenocarcinoma (AC), small-cell-lung-carcinoma (SCLC) and large-cell-carcinoma (LCC) were obtained from the national cancer registry (1994-2000), together with individual characteristics, such as age, gender, smoking status, and the year of diagnosis. Age-standardised incidence rates (ASIR), male-to-female (M: F) rate ratios (RR) of ASIR for SCC and AC, as well as RR of AC: SCC according to smoking status for both sexes, were estimated. Estimated-annual-percent-changes for each of the cell-types were calculated.

Results: AC incidence in females is rising annually (8.5%, p=0.008) from 1994 to 2000, while SCC is declining (-5.4%, p=0.01) in males. M: F ratios of ASIR are consistently greater than 'one', but converging recently. RR of AC: SCC is also approaching 'unity' across both sexes, irrespective of the smoking status

Conclusions: An apparent increase in lung AC incidence in females was observed in Ireland that might indicate some local environmental risk factors, in addition to changing smoking habits. The study findings do not support the hypothesis that females in general are at higher risk for lung cancer development, but tobacco and histologic-specific susceptibility cannot be ruled out.

Key words: Histology; Incidence; Lung cancer; Ireland; Smoking

INTRODUCTION

Lung cancer occurs in multiple histological cell-types. The four major cell-types include squamous cell carcinoma (SCC), adenocarcinoma (AC), large cell carcinoma (LCC), and small cell carcinoma (SCLC). Together, these four major cell-types account for >90% of lung cancer cases in the United States (US)[1]. Despite extensive research, the mechanisms leading to these different types of lung cancer remain uncertain. Over recent decades there have been both geographical and temporal changes in the distribution of lung cancer cell-types[2-4]. Knowledge of these modifications may help to recognize any underlying new aetiological and pathological mechanisms of lung cancer.

Lung adenocarcinoma has become the leading cell-type in North America[2], Europe[3] and Asia[4].This increase may partly be artefactual and involve several biases, or may be a real change[5]. Geographical and temporal trends also differ in males and females. A recent birth-cohort study in the US concluded that males and females may be 'equally' susceptible to developing lung cancer from a given amount of cigarette smoking, rather than supporting the hypothesis that females are more susceptible to developing lung cancer[6]. This was reinforced in a recent prospective study[7]. Nonetheless, the gender susceptibility to developing lung cancer is debatable, and is still speculated to be associated with gender differences in their background risk profiles[8].

To date, no such temporal variations in lung cancer incidence by major cell-types have been identified in the Republic of Ireland. Therefore, the overall aim of this study is to assess the epidemiological pattern of lung cancer cell-types in Ireland, with identification of any underlying gender and/or temporal variations.

METHODS

Source of lung cancer incidence data

The Irish National Cancer Registry Board based in Cork has been registering lung cancer incident cases from January 1st, 1994[9]. More than 90% of cancer cases are histopathologically verified, and the Registry has a centralised system of uniform data collection and quality assessment[9]. However, for lung cancer cases only 75% could be verified histo-pathologically[9]. At the time of this study, all lung cancer incident cases (on an individual basis) registered from 1994 to 2000 were

[1] Harvard School of Public Health, Division of Public Health Practice, 401 Park Drive, Landmark Center, 3rd Floor (East), Boston, MA 02215, USA

[2] Research Institute for a Tobacco Free Society, The Digital Depot, The Digital Hub

Thomas Street, Dublin 8. Ireland.

Correspondence to: Dr Zubair Kabir

zkabir@hsph.harvard.edu

obtained from the Registry. Specific individual covariates, such as age, gender, year of diagnosis, and smoking status (smokers, non-smoker or former smokers) were also collected for further analyses. Based on the morphology codes of the WHO International Histological Classification of Tumors[10], invasive carcinomas of lung [ICD Codes: 9 (162) and 10 (C34)] were categorized into four major cell-types. They are: SCC (ICD-O: 8051-52, 8070-76), SCLC (ICD-O: 8041-45), LCC (ICD-O: 8011-12, 8020-21, 8030-33), and AC (ICD-O: 8050, 8140-246, 8260-571).

Estimation of age-standardised incidence rates (ASIR)

Incidence rates for total lung cancer cases in both sexes, together with the major cell-types, were age-standardised to the European Standard Population for better comparison. The estimated-annual-percent-changes (EAPC) in rates for each cell-type were calculated, using generalised log-linear regression model. The annual rates are adjusted for the gender and age-specific annual smoking prevalence of the Irish population for the year 1994, as the baseline year. The annual gender and age-specific smoking prevalence for the year 1994 was obtained from the publication of Lee and colleagues[11].

Estimation of age-standardised incidence rate ratios (RR)

Annual male-to-female RR (with 95% confidence intervals: CI) from 1994 to 2000 was estimated for SCC, AC, and for total lung cancer cases. The male-to-female ratios (with 95% CI) were calculated using a spreadsheet (quick-calc) developed by Rothman[12]. Ratios more than one would generally indicate that males have higher lung cancer rates, thereby the less likelihood of supporting the hypothesis that females are more susceptible to developing lung cancer. Likewise, annual rate ratios of AC: SCC for both sexes was calculated according to their smoking status.

RESULTS

In total, 10,514 lung cancer incident cases (6,823 in males, 3,691 in females) were registered in the Republic of Ireland from 1994 to 2000. Of these, SCC was the most frequent cell-type in both males (34%) and females (22%), while AC was relatively high among female populations across all the periods studied (18% vs. 14% in males). The frequency of SCLC was also high in females (17% vs. 12% in males); LCC was the least frequent cell-type across both sexes (3%). In all our analyses where appropriate, we have combined both former and current smokers as ever-smokers for better estimates.

The overall ASIR across all the periods studied was higher in males (on an average 500 cases / 10,000 smokers) than in females (on an average 300 cases / 10,000 smokers). Total lung cancer incidence is significantly increasing annually (2%, p=0.001) in females, while males show an annual decline (-2.4%, p=0.058). In females, there is a significant annual rise (8.5%, p=0.008) in AC incidence, which translates into an absolute increase from 30 cases/10,000 smokers in the year 1994 to 45 cases/10,000 smokers in the year 2000 (figure 1).

Table I shows the annual male-to-female (M: F) age-adjusted population-standardised incidence rate ratios (RR) for AC, SCC and total lung cancer cases. Statistically significant higher RR was observed among ever-smokers. Also, there is gradual convergence in RR in the most recent periods, suggesting an increasing trend among the females. The ratios were relatively low among never-smokers, with very wide confidence intervals and unstable estimates, probably due to small numbers.

In table II, almost all the age-standardised incidence rate ratios (RR) of AC: SCC are less than 'one', especially in smokers of

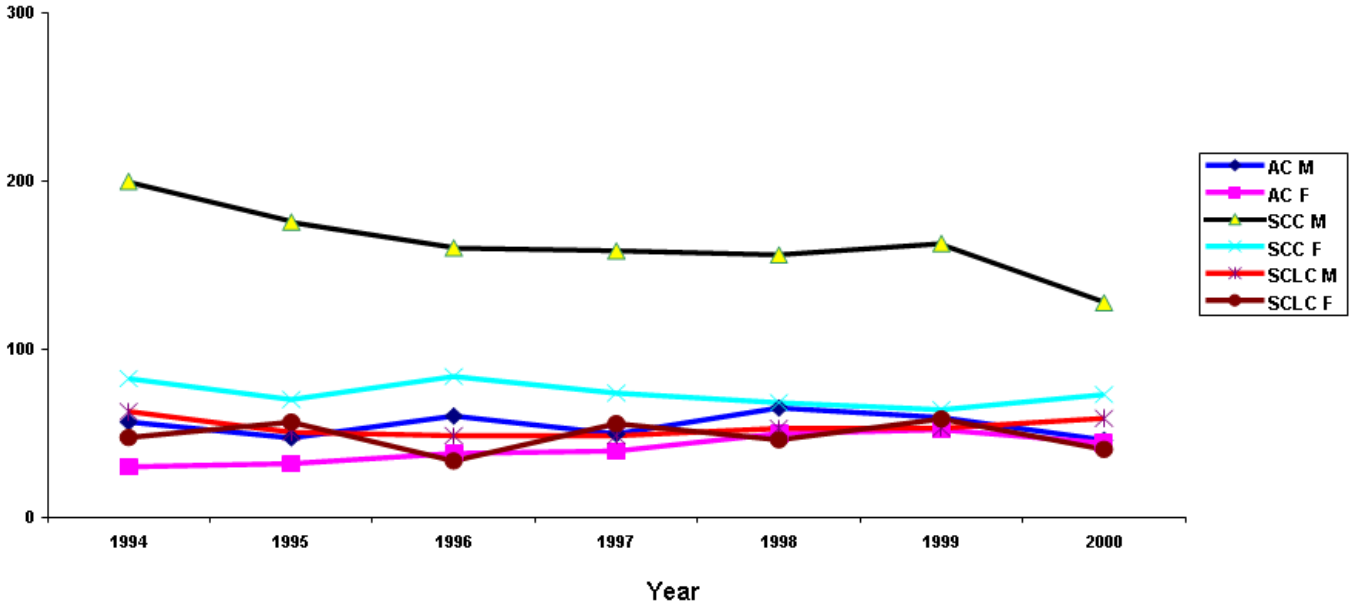

Fig 1. Age-standardised annual incidence rates of lung cancer cell-types in the Republic of Ireland from 1994 to 2000 per 10,000 smokers

AC M = Lung adenocarcinoma in males; AC F = Lung adenocarcinoma in females; SCC M = Squamous cell carcinoma in males; SCC F = Squamous cell carcinoma in females; SCLC M = Small cell lung carcinoma in males; SCLC F = Small cell lung carcinoma in females

Table I.

M: F Standardized Incidence Rate Ratios (age-adjusted) in ever (former and current combined) and never-smokers for total lung cancer cases, Lung Adenocarcinoma (AC) and Squamous cell carcinoma (SCC) cases.

		All cases	AC	SCC
	Year	*RR (95% CI)*	*RR (95% CI)*	*RR (95% CI)*
Ever-Smokers				
	1994	1.94 (1.78, 2.12)	1.87 (1.42, 2.43)	2.41 (2.09, 2.78)
	1995	1.70 (1.55, 1.86)	1.47 (1.07, 1.93)	2.50 (2.15, 2.90)
	1996	1.66 (1.52, 1.82)	1.57 (1.19, 2.01)	1.91 (1.62, 2.23)
	1997	1.55 (1.40, 1.70)	1.26 (0.93, 1.66)	2.14 (1.81, 2.49)
	1998	1.62 (1.47, 1.77)	1.30 (1.00, 1.65)	2.28 (1.94, 2.67)
	1999	1.54 (1.40, 1.68)	1.14 (0.87, 1.47)	2.54 (2.16, 2.96)
	2000	1.40 (1.27, 1.54)	1.04 (0.76, 1.38)	1.74 (1.46, 2.08)
Never-Smokers				
	1994	1.02 (0.51, 1.72)	1.43 (0.44, 3.15)	1.63 (0.51, 3.65)
	1995	1.42 (0.74, 2.39)	1.47 (0.33, 4.61)	2.20 (0.41, 5.84)
	1996	1.69 (0.86, 2.91)	0.71 (0.12, 3.44)	3.18 (0.99, 9.31)
	1997	0.95 (0.42, 1.73)	0.39 (0.01, 3.10)	1.37 (0.33, 4.61)
	1998	1.53 (0.83, 2.55)	0.74 (0.08, 2.33)	6.00 (1.56, NC*)
	1999	1.14 (0.60, 2.04)	1.39 (0.13, 4.01)	4.00 (1.09, NC*)
	2000	1.22 (0.68, 2.10)	1.03 (0.19, 2.74)	2.06 (0.39, 5.48)

***** NC: Could not be calculated because of extreme values

Table II.

Age-Standardised Incidence Rate Ratios (RR) of Lung Adenocarcinoma: Squamous cell carcinoma (AC: SCC) in ever (former and current combined) and never-smokers by gender distribution.

	Ever-Smokers		**Never-Smokers**	
	Male	**Female**	**Male**	**Female**
Year	***RR (95% CI)***	***RR (95% CI)***	***RR (95% CI)***	***RR (95%CI)***
1994	0.3 (0.2, 0.4)	0.4 (0.3, 0.5)	1.0 (0.4, 2.0)	1.2 (0.3, 3.2)
1995	0.3 (0.2, 0.3)	0.5 (0.3, 0.6)	0.8 (0.2, 2.3)	1.3 (0.2, 4.8)
1996	0.4 (0.3, 0.5)	0.5 (0.3, 0.6)	0.4 (0.1, 1.8)	1.9 (0.2, 6.6)
1997	0.3 (0.2, 0.4)	0.5 (0.4, 0.7)	0.3 (0.02, 1.8)	0.9 (0.1, 3.8)
1998	0.4 (0.3, 0.5)	0.7 (0.6, 0.9)	0.5 (0.08, 1.5)	4.4 (0.9, NC*)
1999	0.4 (0.3, 0.5)	0.8 (0.6, 1.0)	0.6 (0.90, 1.6)	1.8 (0.2, 7.2)
2000	0.4 (0.3, 0.5)	0.6 (0.5, 0.7)	1.0 (0.2, 2.3)	2.0 (0.4, 5.5)

* NC: Could not be calculated because of extreme values

both sexes, suggesting that SCC incidence is still high among the Irish ever-smokers. However, the more recent rate ratios of AC: SCC is approaching 'unity' in female ever-smokers, indicating a recent annual rise in AC incidence in females (table I). Such ratios are also relatively high in female never-smokers, and are not statistically significant.

DISCUSSION

Our study has two important findings. Firstly, our findings may indicate a real increase in lung AC incidence in females from 1994 to 2000 in Ireland, consistent with other industrialised nations.[2-4] The gradual convergence in ASIR (table I), with approaching 'unity' ratios between AC and SCC (table II), also suggests that the observed increase in AC incidence is less likely due to the proportionate declining SCC incidence. Secondly, all estimates (rates and ratios) indicate that females in general are unlikely to have a greater susceptibility to developing overall lung cancer, although the ratios are changing recently. This is consistent with a few of the recent observations[6, 7].

The main strength of our study is the analysis of lung cancer incidence data rather than lung cancer mortality data, although the trend analysis was apparently short. Our study did show that the total lung cancer incidence was significantly increasing in females, but the fact that only 75% of lung cancer cases were histologically verified using the Irish Cancer Registry Data could have had an impact on the study findings. Another weakness is the lack of comprehensive smoking data for the individual patients analysed. However, the population smoking data used in our study for the estimation of the proportions of smokers and never-smokers in Ireland for the baseline year, 1994 for analysing time-trends was from Lee and colleagues' publication[11], and the quality of such smoking data has recently been reviewed[13].

The increase in lung AC incidence is also less likely due to changing diagnostic techniques or better diagnostic facilities, because the period studied was relatively short. Secondly, evidence suggests that a rise in AC incidence could be antecedent to diagnostic interventions[14]. Thirdly, the histopathological criteria for diagnosis and classification have not changed during the study period. Fourthly, the WHO's re-classification of LCC in 1999

is unlikely to influence the AC trend[10], because our data suggest an opposite trend in AC incidence between the sexes from 1994 to 2000 (figure 1).

In Ireland, the overall survival rate in lung cancer has not improved significantly (from 8.2% in 1994 to 9.0% in 2001)[9]. However, evidence suggests that females with non-small cell lung carcinoma can have better survival, following both surgery and chemotherapy[15,16]. This emphasises that females may respond differently to tobacco-specific carcinogens for certain cell-types[17,18]. Several molecular studies have also suggested that sex-differences in lung cancer biology do exist. Examples include, females having higher DNA adduct levels[19], an increased *CYP1A1* expression[19], a decreased DNA repair capacity[20] and an increased incidence of K-*ras* gene mutations[21]. A novel oestrogen receptor β was also detected in lung tumours[22], although both exogenous and endogenous oestrogens might be involved in lung AC development[23]. All these indicate that oestrogen signalling could have a biological role in lung carcinogenesis.

Unlike the earlier notions of lung AC being more common among never-smokers, recent evidence suggests a stronger association with smoking, especially in former smokers[24]. Because only 50% of the cigarettes in the late 1960s were 'filter-tipped' in Ireland[11], any underlying change in female smoking habits is less likely to contribute to the recent lung AC incidence increase, similar to a recent study[25]. Despite small effects, potential environmental risk factor such as air quality can have some role[26]. High residential radon levels have also been reported in Ireland[27]. Lung AC is strongly associated with asbestos exposure levels[28], which also coincides with the increased mesothelioma incidence in Ireland[29]. In summary, rapid urbanization coupled with recent lifestyle changes can potentially explain the changing lung AC incidence patterns[30, 31].

In March 2004, the Republic of Ireland introduced a comprehensive workplace smoking ban[32], with Northern Ireland being the latest to follow suit[33]. If lung AC is indeed strongly associated with smoking exposure levels, then a dramatic fall in lung AC incidence over the next few years post-ban will certainly confirm the apparent increase seen in Ireland. In addition to tobacco-specific carcinogen susceptibility and gender variations in nicotine addiction levels[33], local environmental factors potentially contributing to such an apparent increase need to be identified, integrating traditional epidemiological approaches with modern molecular techniques[32]. To conclude, our study findings do not support the hypothesis that females are at a greater risk of developing lung cancer, but histologic-specific lung cancer susceptibility cannot be ruled out.

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The authors have no conflict of interest.

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Technical Note

Complete mechanical circulatory support using ventricular assist devices for post-cardiotomy biventricular failure.

Nnamdi Nwaejike[1], Pramod Bonde[2], Gianfranco Campalani[2]

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ABSTRACT

Cardiopulmonary bypass (CPB) allows operations on the heart in a motionless and bloodless field while reducing cardiac workload and sustaining systemic and coronary perfusion. Failure to wean from CPB remains a significant problem. Results from recent large registry data have shown dramatic improvement in the survival following ventricular assistance for post cardiotomy failure if instituted early. We show how post-cardiotomy heart failure can be successfully treated by proactive use of biventricular assist devices (BIVAD).

INTRODUCTION

Cardiopulmonary bypass (CPB) allows operations on the heart in a motionless and bloodless field while reducing cardiac workload and sustaining systemic and coronary perfusion. It has gained widespread acceptance but failure to wean from CPB remains a significant problem. Factors determining post-cardiotomy failure include: perioperative ischaemia, complications of PCI, patients presenting with acute coronary syndrome, post-infarct VSD, inadequate myocardial protection, diffuse non-graftable disease, or complications associated with a chronically pressure and volume overloaded ventricle. This can result in a significant amount of hibernating and stunned myocardium.

Post-cardiotomy heart failure can be reversible but carries a high risk of mortality. The initial four decades after the advent of cardiac surgery witnessed a gradual decrease in the incidence of post-cardiotomy failure as a result of improved peri-operative care and better myocardial preservation strategies, however, in the last decade the incidence has gradually started to rise as the case mix for cardiac surgery has become increasingly complex[1]. We have seen a dramatic change in the referral pattern of patients being offered cardiac surgery; many have high risk profiles that would have been denied intervention a decade or two ago.

We present a case of post-cardiotomy failure in a patient with unstable angina with rest pain being treated by biventricular assist devices (BIVAD). This is the first adult case to survive with BIVAD use following cardiac surgery in the province.

CASE

A 67-year-old man was admitted to the Department of Cardiac Surgery for urgent CABG. Three weeks earlier, he was admitted to the Coronary Care Unit (CCU) with unstable angina and a Non-ST segment myocardial infarction. Angiography revealed triple vessel disease and impaired left ventricular function (Ejection fraction 18%). His operation was deferred on initial admission due to MRSA grown from the right groin and he continued to experience chest pain on minimal exertion daily, with activity limited to bedside movements only.

Fig 1. Photograph depicting the close up of a Biomedicus (Medtronic Inc) pump with the inlet and outlet connectors. Two pumps were used, one for the right ventricular bypass and one for the left ventricular bypass.

In theatre soon after intubation, he experienced an asystolic arrest and a rapid median sternotomy was performed with CPB. Antegrade cold blood cardioplegia was instituted to arrest and protect the heart and systemic hypothermia to 28°C was used. The left long saphenous vein was used to bypass the Left anterior descending artery (LAD), obtuse marginal artery (OM) and the posterior descending artery (PDA). The patient was re-warmed and an attempt was made to wean off CPB. This was unsuccessful as the heart was barely able to maintain a systolic blood pressure of 60mmHg, with CVP of 20mmHg and wedge pressure 20mmHg despite adequate preload, high inotropic and intra aortic balloon pump (IABP) support. A second attempt was made after giving the heart 20mins on

[1]Department of Vascular and Endovascular Surgery, Barts and The London NHS Trust, The Royal London Hospital, London E1 1BB, UK, and [2]Department of Cardiac Surgery, Royal Victoria Hospital, Belfast, Northern Ireland, UK

Correspondence to Mr Bonde,

prambond@hotmail.com

bypass to recover but the left ventricle struggled and the right ventricle showed signs of dilatation and dysfunction. The clinical scenario suggested bi-ventricular failure.

A decision was made to support both ventricles by establishing temporary left and right heart bypass using two Biomedicus (Medtronic Inc; Minneapolis) pumps as Biventricular assist devices (BIVAD). Right heart bypass was achieved by the right atrial two-stage cannula for venous drainage and a 24 French aortic cannula (pulmonary artery cannula) placed in the right ventricular outflow tract and advanced in the main pulmonary artery for return. Left heart bypass was achieved by inserting a 38 French angled anvil cannula (left atrial cannula) through a purse string suture in the right superior pulmonary vein and the inflow cannula was the aortic 24 French cannula initially used for CPB (Fig. 1). With all cannulae in place and secured, heart assist was established and acceptable flow rates in the region of 2.2 lt/min/m^2 achieved (Figs. 2 and 3). All catecholamines except a continuous infusion of nor-epinephrine, milrinone and inhaled nitric oxide were discontinued, the patients' acidosis and lactate levels normalised with 8 hours of BIVAD support.

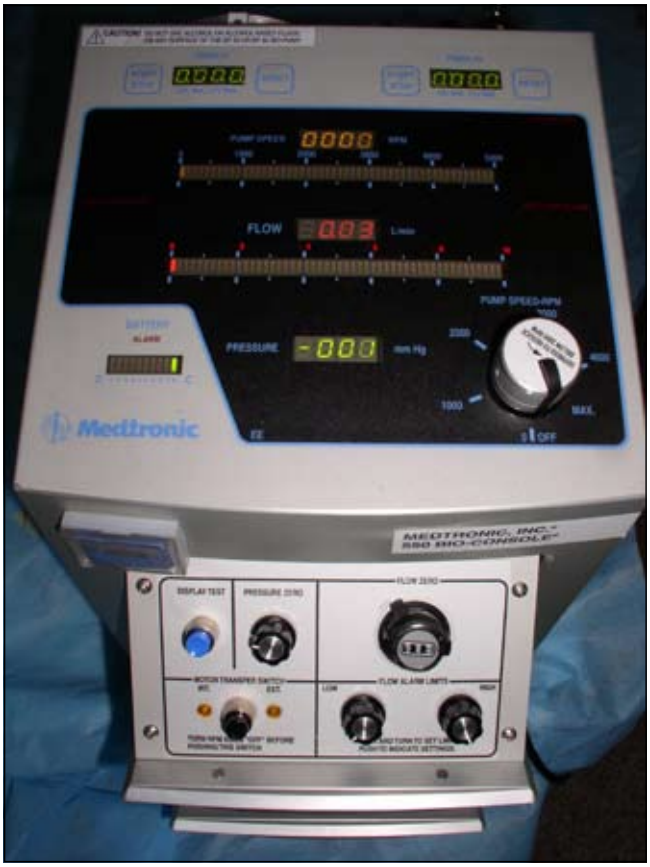

Fig 2. Photograph of the console of the Biomedicus pump (Medtronic Inc), two independent consoles controlled the Biomedicus pumps for flow and revolutions per minute.

The patient developed a coagulopathy due in part to rapid heparinisation and prolonged CPB and required substantial infusions of coagulation products. The chest was left open and packed with sterile gauze and he had repeated re-explorations over the next 24 hours to identify and correct surgical bleeding points as they became apparent.

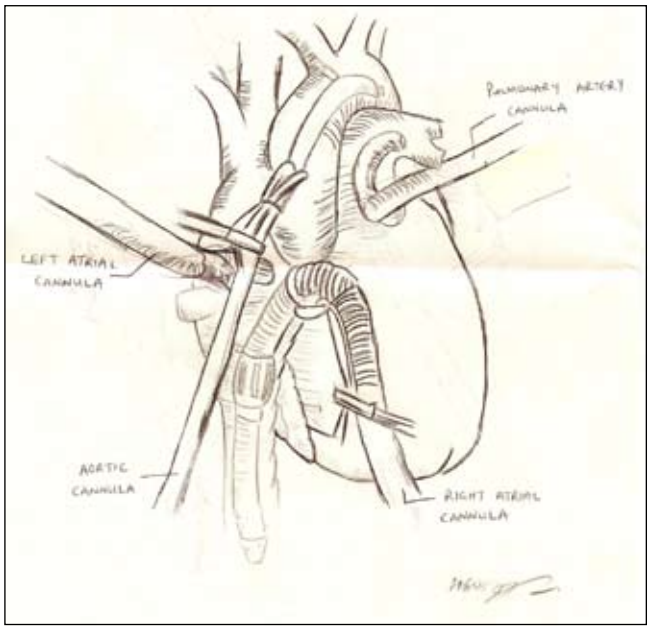

Fig 3. Illustration depicting the insertion of the cannulas for BIVAD.

After 48 hours an improvement in ventricular function was observed on trial of weaning, so BIVAD support was weaned off and removed. The sternum was left open with the skin closed to allow rapid re-exploration if required. By day 4 the sternotomy could be closed without haemodynamic compromise and on day 5, IABP was removed. He made steady improvement and was extubated on day 11 with a minitracheostomy tube placed to aid aspiration of bronchial secretions. He was discharged home on day 43. On review at six weeks, cardiac and respiratory parameters, were satisfactory but an abdominal mass was palpated. He underwent resection for a localised ileal stricture and anastomoses four months from his initial cardiac surgical procedure. At last follow-up he was pain-free and able to walk 2 miles daily.

DISCUSSION

This case demonstrates a not too infrequent scenario of a significantly stunned myocardium in spite of adequate myocardial revascularisation in a patient with ongoing ischaemia peri-operatively. Currently about 6% of patients develop post-cardiotomy ventricular failure. Phosphodiesterase inhibitors, catecholamines and intra-aortic balloon pump (IABP) are frequently employed for treating post-cardiotomy failure and are successful in the majority of patients. However, 1% of patients undergoing cardiac surgery develop intractable ventricular failure, which is non-responsive to catecholamine and IABP therapy[1]. Traditionally, ventricular assist devices were reserved as a last resort for these types of patients, due to earlier experience of mortality in excess of 75% with the use of assist devices[2,3].

The Biomedicus pump (Medtronic Inc) used consists of valveless rotator cones that impart a circular motion to incoming blood by viscous drag and constrained vortex principles generating pressure and flow. This assembly is housed in a polycarbonate cone shaped container with inlet and outlet. These pumps are afterload dependent unlike the roller type pump. They can be used to bypass both the

right and left ventricle, are widely available and are easy to operate. They are driven by a magnetic impeller that has no direct contact with the blood within. A separate console allows the operator to control the revolutions and set alarms. Conventional bypass cannulas are used together with bypass tubing to connect the ventricle to the pump outside the body.

A recent comprehensive review of contemporary practice reveals a dramatic improvement in survival following use of ventricular assist devices in patients needing post cardiotomy support in several North American centres[4]. This is mainly attributed to earlier recognition and use of assist devices prior to irreversible ventricular failure, improving the overall survival to discharge by ~50%. Early implantation of an assist device capable of supporting adequate flow and allowing the heart to rest may improve results and allow recovery of the stunned myocardium[1-4]. There is an argument for pro-active use of ventricular assist devices early, to gain maximum advantage by allowing the ventricle to recover.

The use of ventricular assist devices is not without technical problems and complications. The *R*andomized *E*valuation of *M*echanical *A*ssistance for the *T*reatment of *C*ongestive *H*eart Failure (REMATCH) trial was designed to compare the results of mechanical assistance versus the medical management for patients suffering from heart failure[5]. This trial results show device infection was 28%; bleeding events were 42%, and device malfunction 35%. This must be balanced with the overwhelming survival benefit of mechanical support. In the LVAD group, the corresponding survival rates were 48% at 12 months and 23% at 24 months, representing a relative risk reduction of death of 48% over the two-year period[5].

Similar randomised data is lacking in post-cardiotomy failure, due to the ethical issues involved in randomising such patients, but; there is ample data from bridge to recovery and bridge to transplant ventricular assist therapy in the context of post-cardiotomy failure[1,4].

The centrifugal pumps used in this case are easily available in most units without dedicated heart failure facilities and are easy to use and maintain. As the patient profiles accepted for coronary revascularisation are undergoing rapid changes towards performing revascularisation procedures in a high-risk population; the use of such devices pro-actively to help the myocardium recover is prudent. Patients can recover to lead a normal life after postcardiotomy heart failure if VAD therapy is instituted early.

ACKNOWLEDGEMENTS:

We would like to thank our ICU nursing staff, technicians, anaesthetists and especially our perfusionists, Julie Rea, Nicola McConkey, Carolyn Reid and Ann Panayotou. No conflict of interest declared.

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Medical History

Pulmonary Oxygen Toxicity: Investigation and Mentoring

John Hedley-Whyte

Accepted 31 July 2007

SUMMARY

At sea level oxygen is toxic to man when breathed for more than twenty-four hours at a percentage greater than about forty percent. Pulmonary pathology is the first manifestation in subjects with previously normal lungs. In patients with pre-existing lung disease the results are often additive. There is, however, great variation in response from subject to subject and between patients. Queen's Belfast and Harvard University Medical School have been the sites of seminal investigations. Mentoring at both universities is due to training at the University of Copenhagen.

Key words: Pulmonary Oxygen Toxicity

INTRODUCTION

Prescribing the correct dose of oxygen remains a serious concern with still unresolved quandaries in patient management. The descriptions and investigative work of James Lorrain Smith on oxygen toxicity, while at Queen's College, Belfast at the turn of the nineteenth and twentieth centuries has stood the test of many further investigations[1-4](Fig. 1).

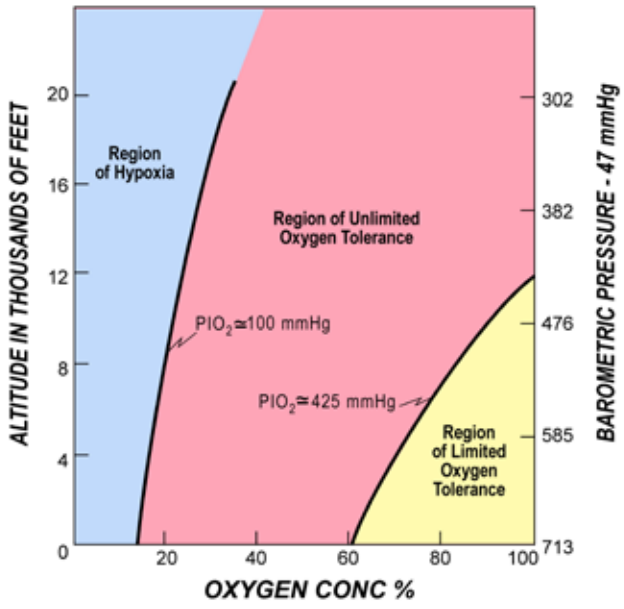

Figure 1. The inspired oxygen tension rather than the inspired oxygen concentration determines pulmonary oxygen toxicity (Modified from Becker-Freyseng H, Clamman HG. Zur Frage der Sauerstoffvegiftung. *Klin Wehnschr* 1939;**18**:1382-5[42] and Hedley-Whyte J, Winter PM. Oxygen therapy. *Clin Pharmacol Ther* 1967;**8(5):**696-737[4]).

LORRAIN SMITH AND JS HALDANE

James Lorrain Smith was born in 1862 to a talented family where his father, Walter, was a Free Church of Scotland minister in Half Morton just north of Gretna Green[1,5]. An elder sister, Annie, became so distinguished that in 1888 she was employed, sub rosa, at the British Museum, paid from special funds[5]. James went to Edinburgh University graduating in medicine in 1889, and immediately went to work with Sir John Scott Burdon Sanderson's Regius Professorial Unit at Oxford. There he joined John Scott Haldane who had graduated in medicine at Edinburgh University in 1884. Haldane was the Regius's maternal nephew[6].

Lorrain Smith became Walker Student in Pathology at Cambridge and later Demonstrator under Professor Charles Roy, who sent him to von Recklinghausen's laboratory at Strasburg to study histology and to Christian Bohr's laboratory at Copenhagen[1]. In 1892 and 1893 Lorrain Smith and Haldane carried out "some research in the laboratory under Bohr's direction". "Far more important was getting into personal touch with Bohr himself", wrote Haldane subsequently[7]. In 1894 Lorrain Smith was appointed Lecturer in Pathology at Queen's College, Belfast, where he remained until 1904; in 1901 he was promoted to Professor[1]. During this decade working in Belfast, Lorrain Smith and Haldane laid the foundations of the eminence of British respiratory physiology[1,8-14].

Lorrain Smith was exactly correct in writing in 1897, "We may in the study of oxygen tension in various pathophysiological conditions not only find the explanation of various phenomena of respiratory disease but also obtain data for estimating the clinical significance of disturbance to the respiratory functions in these conditions[10]". By 1899 Lorrain Smith had demonstrated that oxygen at up to 41 percent of an atmosphere is well tolerated. At seventy to eighty percent inspired oxygen, fifty percent of mice are dead at the end of the week[12]. "Mice have a remarkable power of recovering from the effects of high inspired oxygen. The same is probably true of man," wrote Lorrain Smith.

FALSE TRACK

In their investigations of the transport of oxygen from the airways into blood, Bohr, Haldane and Lorrain Smith erroneously espoused active oxygen secretion into the blood,

David S Sheridan Professor of Anaesthesia and Respiratory Therapy
Harvard University, 1400 VFW Parkway, Boston, MA 02132-4927. USA
Correspondence to Prof. Hedley-Whyte
john_hedley-whyte@hms.harvard.edu

generally finding arterial oxygen tension to be higher than alveolar. The causes were the lack of reliable methods of measuring oxygen tension in blood[9]. Maybe the purity of the oxygen was a problem. Cylinders were supplied by the Scottish and Irish Oxygen Company. "The gas was manufactured by the peroxide of barium method and contained no impurity except nitrogen[9,10]." Almost pure oxygen produced by rectification for welding was not available.

THE KROGHS' RESPONSE

It was not until 1910 that Marie Krogh, the wife of Christian Bohr's successor August Krogh, proved that oxygen diffuses across the alveolar capillary membrane[15,16]. The Kroghs wrote seven different dissertations in proving diffusion to be the only method of alveolar-capillary transit. In the last of the seven theses, August Krogh wrote "I shall be obliged in the following pages to combat the views of my teacher Professor Bohr…Real progress, made during the last twenty years in the knowledge of the processes in the lungs, is mainly due to his labours and to the refinement of methods which he has introduced"[17].

HALDANE AND OXFORD

Why did JS Haldane in his uncle's department as Lecturer in Physiology, University of Oxford, Grocers' Company Research Scholar, do his research work in Professor Edmund Albert Letts' Chemical Laboratory of Queen's College, Belfast? Were the facilities better in Belfast as in Copenhagen? Letts was Professor at Queen's from 1879 to 1917[18]. Probably it was at least in part due to Lorrain Smith's return in 1894 to Belfast from Oxford and Cambridge and Continental Europe. But there is another possible cause, dissatisfaction with the Oxford scientific milieu[19].

In 1903 Haldane's uncle's intention to resign became known. Sanderson and his colleagues, chiefly Francis Gotch, Haldane and Arthur Thomson[20] were desirous that one of their own group should continue Sanderson's tradition. The London graduates expressed themselves in favour of a clinician, preferably an Oxonian: they met in London on January 5, 1904 and stated in *The Times:* "The Regius Professor of Medicine should be held by a physician who is representative of Medicine in its widest sense"—a statement inimical to the prospects of candidates: Reader, James Ritchie, a pathologist in Sanderson's group or Haldane. Pamphleteering began, London versus Oxford[20-21].

HARVARD

At this time, Mr. Charles W Eliot, President of Harvard University, tried to get William Osler to come to Harvard[19]. Osler gave the Ingersoll Lecture on Science and Mortality. Mr Eliot after the lecture "Expressed himself as greatly disappointed[19]." Osler's wife and mother-in-law, a Bostonian, also were disapproving, "Willie should not 'rub the calf of his leg with his other foot to stir up ideas'," said his wife at Eliot's informal reception after the lecture[19]. The Oslers' future was settled by Arthur J Balfour who as Prime Minister nominated Osler to King Edward VII, as Regius Professor[19]. Oxford, presumably with Osler's connivance, made Haldane a Reader in 1907, a decade after his FRS. Haldane removed his research work to a structure in his North Oxford garden after Lorrain Smith left Belfast.

Osler in July 1906 visited Professor Lorrain Smith now translated to Victoria University, Manchester[1,22]. Lorrain Smith had founded the 'new' Pathological Society of Great Britain and Ireland and was chief host at the Society's inaugural meeting[22].

In 1913, Francis Gotch, having been made a Waynflete Professor of Physiology in 1905, died. Osler was the Chairman of the Board of Electors for the succession[23]. They chose Charles S Sherrington. Haldane was most disappointed, but remained a Fellow of New College.

PERSONAL INTERACTIONS WITH THE CONSEQUENCES OF THE WORK OF BOHR, HALDANE AND LORRAIN SMITH

In 1931 as a senior Harvard Medical student, Henry K Beecher won the Warren Triennial Prize of the Massachusetts General Hospital for two papers on the effect of surgery on gas exchange in man[24,25]. He subsequently received a Moseley Travelling Fellowship to work in the laboratory of Augustus Krogh who had won his Nobel Prize in 1920[26]. After joining Beecher's Anaesthesia Laboratory of the Harvard Medical School at the Massachusetts General Hospital in 1960, we continued work initiated a lifetime before by Lorrain Smith[2-4,27-29](fig.1). This continuation was enormously helped by recent fabrication of polarographic oxygen[30,31] and carbon dioxide electrodes employing semi-permeable membranes[32-34]. Incidentally, US patent requests were denied to the developers because of prior use of the methodology by John R Pappenheimer, Higginson Professor of Physiology, Harvard University[33].

We investigated the effect of intrapulmonary shunting as described by Sackur in 1897*[1,35] and Christian Bohr's 1905 values for oxygen solubility in solutions: values which had been superseded[36]. We found Bohr's measurements and principles superior to those quoted in the Handbook of Chemistry and Physics[37]. We used a Haldane apparatus, a successor technique to those Haldane and Lorrain Smith had developed in Belfast in 1895 and 1896[8,9]. Our values, validating the principles of Bohr are in more recent handbooks[38,39]. Aage Bohr wrote to us in 1964 to welcome validation of his grandfather's work[40]. In 1967 we were asked to review and update Lorrain Smith and Haldane's work on the effects of oxygen[29,41].

Further work on the effects of inspired oxygen over 42 percent[42] needs to be done on patients with very large right to left intrapulmonary shunts (fig. 2)[3], on patients with altered cholesterol metabolism[43] and in patients with intracranial pathology[44]. The genetic basis of the variability of pulmonary pathologic response to oxygen needs to be explored.

END OF AN ERA

John S Haldane died in March 1936, a Companion of Honour, as was his sister Elizabeth. His brother Richard, twice Lord Chancellor, was a Viscount with an Order of Merit[6]. JS Haldane wrote James Lorrain Smith's 1931 obituary[1]. James's sister Annie was, in 1904, among the first women elected to

* P. Sackur was a member of the Pharmacology Institute of the University of Breslau (now Wroclaw, Poland). The university library was totally burned by the Red Army on May 10, 1945.

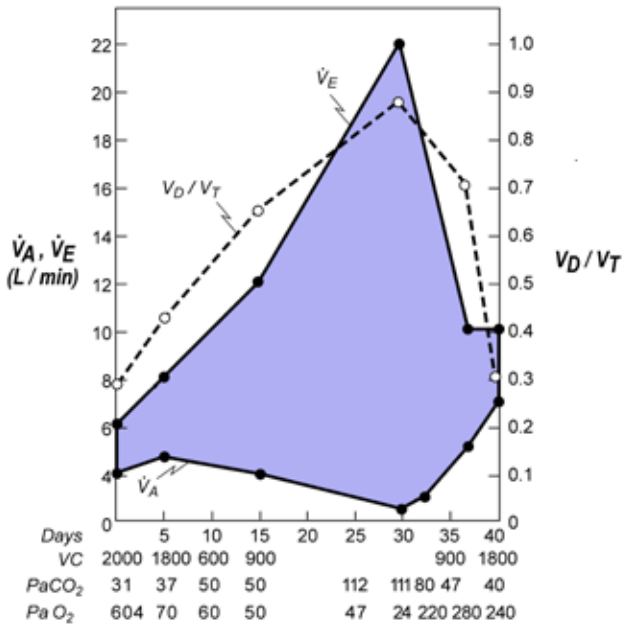

Figure 2. Increase in ventilation and inspired oxygen tension requirements in respiratory failure due to pneumonia and ARDS.

During respiratory failure in a patient with ARDS and pneumonia despite minute ventilation (V_E) of more than 20 liters per minute provided on the thirtieth hospital day by a constant volume ventilator, effective ventilation (alveolar ventilation V_A) was only 2.5 liters per minute. Ineffective ventilation (dead space ventilation) is shown in blue. The ratio of dead space to tidal volume (V_D/V_T) is a measure of lung inefficiency (and consolidation in this patient). For two weeks this man had to be ventilated with 100 percent oxygen and heavily sedated to decrease oxygen consumption, and even so his arterial oxygen tension (P_aO_2) was consistently under 50 mm of mercury. The 60 percent intrapulmonary shunt, venous to arterial diminished as the patient recovered. Six months later the patient was in excellent health with no exercise limitation (Reproduced by permission from the *New England Journal of Medicine*. Adapted from Hedley-Whyte J. Control of the uptake of oxygen. *New Engl J Med* 1968;**279(21)**:1152-8[3]).

the Linnaean Society. She became an OBE in 1934[5]. Christian Bohr died in 1911. Subsequently his son Niels (1922) and grandson Aage (1976) won Nobel Prizes for physics.

Marie Krogh died under German occupation, in 1943. In 1946 August Krogh visited Harvard University. He lectured both at the College and Medical School. His daughter, Bodil Schmidt-Nielsen, has written that he was very pleased with this visit to Cambridge and Boston. He was entertained, in turn, by the five Harvard University Professors and Department Heads whom he and Marie had trained: James Howard Means, Jackson Professor of Clinical Medicine from 1923; Cecil K Drinker, Professor of Physiology from 1923 and Dean of the School of Public Health Science from 1935; Edward D. Churchill, John Homans Professor of Surgery from 1931; Henry K Beecher, Henry Isaiah Dorr Professor of Research in Anaesthesia from 1941; and Eugene Landis, George Higginson Professor of Physiology, who had succeeded Walter B Cannon in 1943[45]. August Krogh died in 1949[46].

Henry K Beecher subsequently recruited University of Copenhagen graduates Henrik H Bendixen (graduated 1951) and Henning Pontoppidan (graduated 1952), to the Harvard Anaesthesia Laboratories of the Massachusetts General Hospital[27,47]. This Copenhagen-trained trio illuminated, in the laboratories and at parties, with wit and anecdote, stories of Lorrain Smith and JS Haldane, the Bohrs and the Kroghs.

We have twice previously reported Queen's support of the endeavours of Harvard Medical School: first during World War II[48*], secondly during the 1950's and 60's[51]. In this third example, the influence was inherited from John S Haldane and James Lorrain Smith's work at Queen's Belfast between 1894 and 1904.

The author has no conflict of interest

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* My father[48] told me that Lorrain Smith had been Musgrave Professor. The Musgrave Chair in Pathology at Queen's College Belfast was founded by James Musgrave (1826-1904) a native of Lisburn, County Antrim who had established a firm of patent stove-makers and ironmongers in Belfast[49]. In 1901 Lorrain Smith was appointed the first Musgrave Professor of Pathology[50].

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Annual Oration

More Than The Sum Of Its Parts

Royal Victoria Hospital, Thursday 4th October 2007

Stanley A Hawkins

Ladies and gentlemen it is a particular honour to present the Annual Oration. My title is "More than the sum of its parts". The sub-title is "the Brain". The Annual Oration has been running regularly since the nineteenth century. By tradition it is held on the first Thursday of October each year. The first recorded oration was in 1827 by Dr James McDonnell, the next recorded oration was in 1852 by Dr Andrew Malcolm[1]. Following his untimely death, to honour his name, his medical colleagues endowed the funds for a competitive prize for junior clinical students. I had the honour of winning this prize in the spring of 1969, so the first oration that I attended was during that year. Following the prize giving Dr Robert Marshall, a retired cardiologist came up to me and said he had won the same prize when he was a young man. He wished me well and said I had a bright future. As I approach my sixtieth birthday, I still like to feel that I have a bright future.

Two neurologists have previously presented the annual oration. The first was Dr Sydney Allison in 1941 while serving in the Royal Naval Volunteer Reserve[2]. His title was "Medicine and the Navy". The second neurologist was Dr Harold Millar in 1975; his subject was "The Medical Library"[3]. As neither of them talked about the brain, the field is clear for me.

Fig 1. Portrait of René Descartes by Frans Hals from the Louvre, Paris

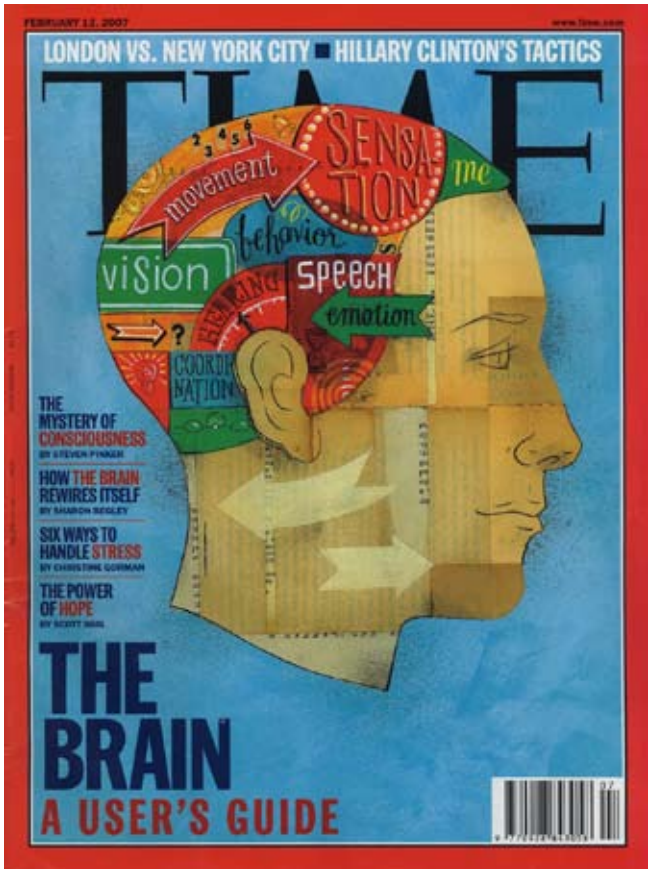

Fig 2. Front cover of Time Magazine Feb12th 2007

Looking at this very distinguished audience, I am filled with a sense of unreality. I am not sure if I am dreaming: if I am going to wake in a few moments with my lovely wife Fiona mopping my brow saying "don't worry dear, it was only a nightmare". This caused me to think, how do we know that what we are experiencing is reality rather than a dream? This is not an original thought. Three hundred years ago it occurred to René Decartes, the father of modern philosophy (fig 1). As we will see later in this talk, neuroscientists and philosophers are still struggling with the concepts of consciousness.

It is my impression that most people take their brains for granted. Having taught every Belfast medical graduate for the last 26 years, I am familiar with a sense of mental blankness in our students after the long summer break. Occasionally we

Consultant / Reader in Neurology, Royal Hospitals Trust, Grosvenor Road, Belfast BT12 6BA, United Kingdom.

s.hawkins@qub.ac.uk

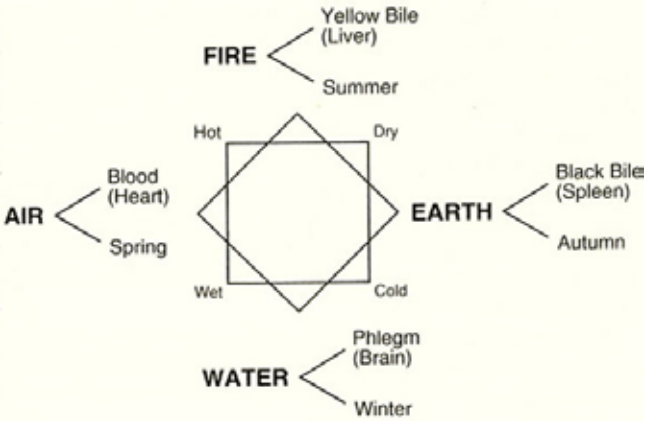

Fig 3. Schema of ancient Greeks' concepts of the balance of humours

see the brain referred to the in the news media, for example "Time Magazine" from February 2007 (fig 2), also from the same month the front page of the "Guardian" featured a story about modern brain imaging.

I would like you to ponder this quotation: "*Men ought to know that from the brain and from the brain only arise our pleasures and joys, laughter and jests, as well as our sorrows, pains, griefs and tears, through it in particular we see, hear and distinguish the ugly from the beautiful, the bad from the good, the pleasant from the unpleasant, it is the same thing that makes us mad or delirious and inspires us with dread or fear*".

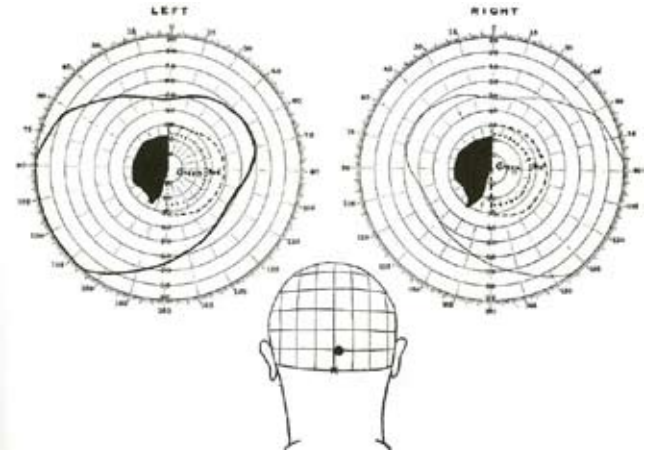

Fig 4. Illustration from a paper by Dr Gordon Holmes. In *Br J Ophthalmology* 1915

This was not written last year, but around 400 BC by Hippocrates[4]. His thoughts on the brain sound surprisingly modern but should be put in the context of how the ancient Greeks thought of human physiology. They thought there were four fluids in the body, and they felt that madness came from excess moisture in the brain. The four fluids were: blood, yellow bile, phlegm and black bile (fig 3). Blood, phlegm and yellow bile are familiar to us. Phlegm was thought to emanate from the brain via the pituitary gland and thence into the nasal passages. Black bile is the only fluid that does not exist but its translation into Latin "melancholia" still exists in modern language.

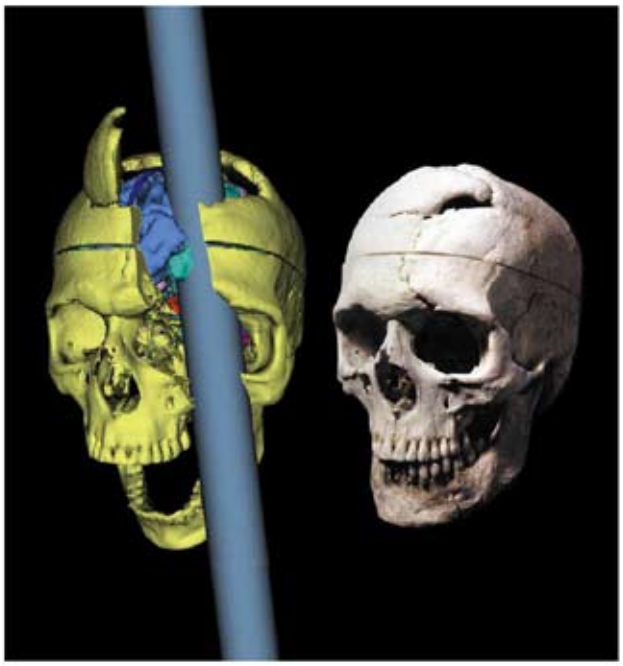

Fig 5. Reconstruction of the track of the tamping iron through the head of Phineas Gage. From Ratiu P, Talos IF *New England J Med* 2004;**351:**e21.

The ancient Greeks had taboos about human dissection. Around 160 AD the Greek, Galen dissected many animal species, but not humans. Treating surviving gladiators in Greece and Rome after their contests was the basis of his knowledge of human anatomy. René Descartes lived from 1596 to 1650; he was the most distinguished philosopher of his day and the father of modern scientific method[5]. Following the example of Andreas Vesalius (1514-1564), Professor of Anatomy at Padua, Descartes dissected the brain. He was puzzled that most of the brain existed in two mirrored halves, apart from certain mid-line structures like the pineal gland. From his musings he concluded that the pineal gland was the seat of the soul and the centre of consciousness. Descartes felt that as conscious individuals, we must feel pain. The saying "Cogito ergo sum" was his most memorable quotation. Thomas Willis (1621-1675) in Oxford took the study of the anatomy of the human brain to a higher level. His friend Christopher Wren drafted some of the illustrations of dissections of the brain.

When I started my preclinical studies of anatomy in 1966, 41 years ago, I was inspired by Professor Thomas (Thos) Harrison and I read the anatomy of the nervous system by Ranson and Clarke[6] in great detail. This book drew on the researches of Ramón y Cajal on the cellular anatomy of the brain. Brodmann discovered that the cytoarchitecture of the cortex varies from place to place, and as a result he inferred that the function of the cortex must vary from place to place. As a student of physiology, in 1968-9, I studied the papers of Hodgkin and Huxley based on the giant axon of squid caught off Plymouth. They worked out the basis of the action potential, and as a result won the Nobel Prize for medicine and physiology in 1963. When I entered clinical studies, I was enthused by the neurologist Dr Louis Hurwitz. He was an inspirational teacher, who died in 1971, during my final year. I have already paid tribute to him and my predecessors

in Belfast neurology in the presidential address to the Ulster Medical Society[7]. Having been trained in London, Paris and New York, Louis Hurwitz followed the best traditions of British and French neurologists.

The pre-eminent British neurologist of the last century was Sir Gordon Holmes, a man born Castlebellingham, County Louth into a family of Yorkshire ancestry. Following Trinity College Dublin he went on to study in Frankfurt and London. During the First World War he served in the RAMC in France. He made his mark by studying the effects of brain damage in the victims of head injuries (fig 4) sustained during trench warfare[8]. The study of individuals who had survived brain injuries, strokes and tumours was how clinical neurology was first developed.

Fig 6. Department of Neuroradiology, RVH, circa 1974, showing Dr Harry Shepherd and Mr Tom Fannin looking at cerebral angiograms

The story of Phineas Gage (1823-1860) is a wonderful example of an early study of a damaged brain[9]. In 1848, when he was working on the construction of the railways in Vermont, North America a premature explosion caused a metal tamping iron 109cm long and 3cm wide to be projected through his head, landing more than thirty metres away (fig 5). Quite fortuitously he survived without losing consciousness. His lucidity was short lived and he entered coma for several weeks. Eventually he recovered from cerebral abcesses and meningitis, but his personality had changed. Prior to the accident he was conscientious and diligent. Following the injury he became foul-mouthed and erratic. Despite living for another 11 years, he was never his former self. We now know that the change in his pattern of behaviour is typical of frontal lobe damage - the first clear example to be described.

In 1974 I entered my clinical training as a registrar in neurology, my teachers were Jo Lyttle, Michael Swallow and Harold Millar, all gifted men who brought their very remarkable talents to the practice and teaching of clinical neurology. I worked in Quin House and Claremont Street Hospital. Professor Frank Pantridge when he heard that I was interested in neurology, encouraged me to think of cardiology instead. He said: "*Hawkins, when your heart stops you are dead. I know people walking around Belfast who have shown no sign of cerebral activity for years*". I was aware of the distinguished work of my predecessors Harold Millar and Sydney Allison in the field of multiple sclerosis. Dr Orla Gray, in our department has recently found that the risk of developing MS in women in N. Ireland is 1 in 130.

Since the techniques for imaging the brain and spinal cord were very rudimentary in 1974-5, the emphasis was on the clinical history and examination. We had isotope brain scans that to our eyes appear primitive. We had a good neuro-radiology department (fig 6). Cerebral angiograms were performed by direct stab into the common carotid artery. This allowed us to demonstrate the swelling caused by malignant brain tumours and arterial venous malformations. Occasionally we would perform lumbar air encephalograms. This procedure involved removing 30mls of CSF and injecting 30mls of air, somersaulting the patient on a special chair and taking X-rays of the brain (fig 7). Another technique was myelography where we injected oil-soluble dye, into the spinal fluid and took pictures of the spinal cord by negative contrast. Occasionally the dye was run up into the head and we took X-rays of the cerebral ventricles.

In 1975 the first CT scanner was installed in Belfast. We were astonished at the quality of the results that now to our modern eyes look very fuzzy. Modern CT scanners produce images that can show abnormalities the size of a grain of rice within the brain. I had a patient who we felt had a spinal tumour. She had to travel to England for MRI imaging prior to planned surgery. She worked as a primary school teacher in Carrickmannon, Co Down. Her headmistress, Mrs Montgomery was instrumental in setting up a charity to assist in the provision of the first MRI scanner in Northern Ireland. In 1993 the scanner was installed. The diagnosis of multiple sclerosis was made more accurate and reliable (fig 8). Some cynics said that clinical neurology would be dead following the introduction of these imaging techniques. MRI scans did not provide us with the diagnosis in every case. In 1995 a patient who had an entirely normal MRI scan of the brain reported at the time, was dead within six months with an irreversible neurological illness which was subsequently found to be variant CJD - the first case in Ireland.

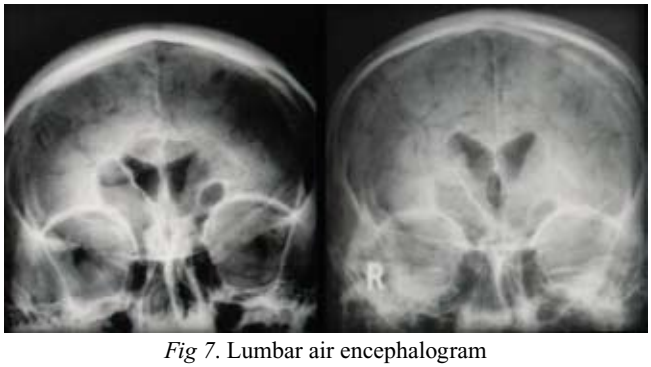

Fig 7. Lumbar air encephalogram

During the last 30 years there have been very substantial advances in clinical neurosciences. Can there ever have been a more interesting era to practice clinical neurology? Our understanding of how the brain interprets visual images, smell, hearing, touch and taste has been revolutionised e.g. the Nobel Prize for Medicine and Physiology in 2004 was awarded to Axel and Buck for their studies on odorant receptors. Our

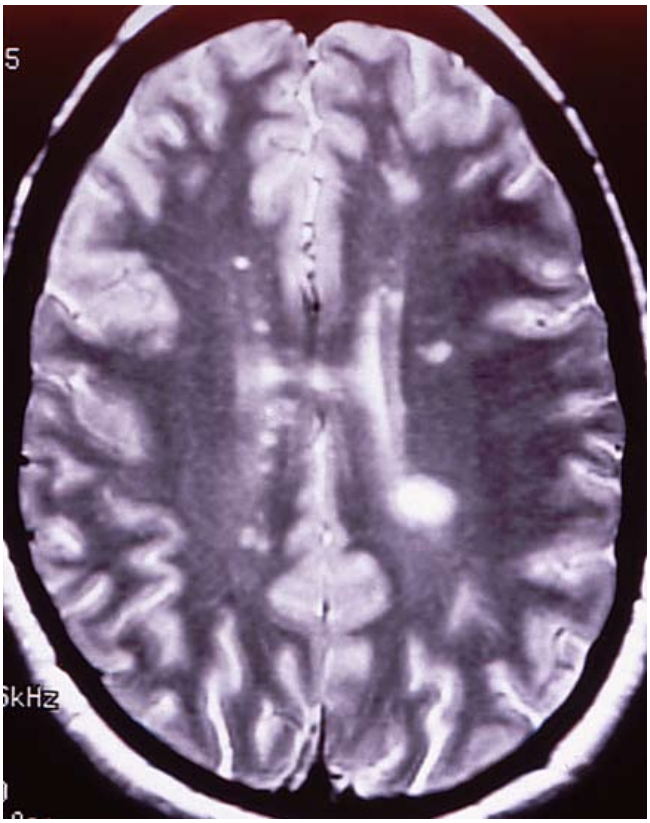

Fig 8. MRI in a case of multiple sclerosis

understanding of the organisation of posture and movement has been clarified. Our understanding of language has been revolutionised. We are starting to understand how memories are processed, stored and retrieved within the brain. For decades the prevailing dogma in neurosciences was that adult human brain had matured and was fixed in form and function. By the time children mature into adulthood it was felt that the brain is rigid and incapable of change. This view held that genes and development dictate that one cluster of neurones will process signals from the eye, another, the fingers of the left hand and they will do nothing else until the individual dies. We now know that the adult brain is capable of learning and making new connections and this is called neuro-plasticity. Also there have been amazing advances in cell biology. It is possible to grow neurones in cell culture and study their physiology in great detail. It is also possible now to transplant neuronal stem cells into the brains of animals and humans in order to ameliorate clinical conditions. Also, studies of neuropathology have been revolutionised by a range of techniques.

The amazing advances in clinical neurosciences have been based on our understanding of imaging of the brain, CT, MRI, and PET scans. Also, we have entered the age of genetic engineering. The structure of DNA was discovered by Watson and Crick, and published in Nature in April 1953[10]. In 1961, Brenner and Crick discovered the genetic code was made up of nucleotide triplets[11]. A rapid method of gene sequencing by Fred Sanger in Cambridge and Walter Gilbert in the United States was discovered in the late 1970's. The Human Genome Project produced results that were published in Nature and in Science in February 2001 (fig 9). We now know that the human genome consists of about 30,000 genes, fewer than was originally thought. 6000 of these are expressed exclusively in the brain.

The brain weighs about 1.4-1.5 kg. Seventy percent of its weight is water. The cerebral cortex is the main computing and storage facility within the brain. If the cerebral cortex were spread out it would be about the same surface area as a large table napkin although somewhat thicker. It contains about 100 billion nerve cells or neurons. When you include the additional supporting tissue the total cell count of the brain is 1 trillion cells. It has been estimated that there are one quadrillion (10^{16}) synaptic transmissions per second[12].

Fig 9. Front cover of Nature Feb 15th 2001

The lobes of the cerebral hemispheres are merely named after the bones that overly them. There is some functional significance to this, but it is rather limited. Modern imaging techniques have enabled us to study functional correlations better, showing that important connections cross the borders of the lobes. Damage around the Sylvian fissure is likely to result in language deficits. The first modern studies of language were performed in France and Germany. Paul Broca and Karl Wernicke found that faulty language was a consequence of damage to the left cerebral hemisphere. Broca lived in France between 1824 and 1880. He discovered that damage in the frontal lobe of the left cerebral hemisphere made it impossible for a man called Leborgne to say anything other than "Tan". Wernicke, from Germany later discovered that damage to the posterior part of the left cerebral hemisphere made it impossible for a person to understand speech, either speech that was spoken to him or his own spoken words.

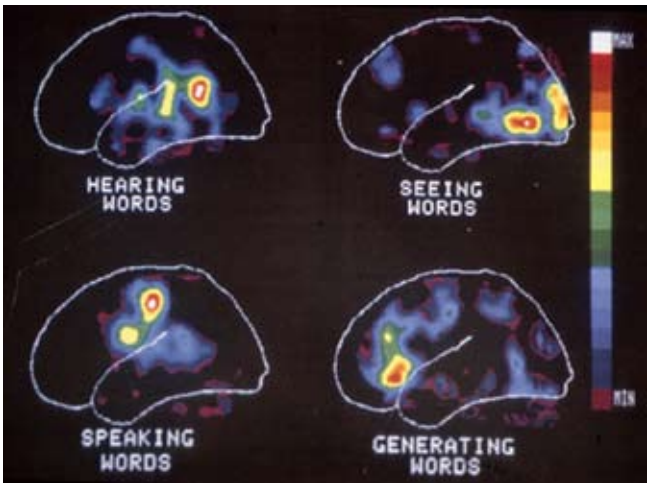

Fig 10. PET scans of cortical anatomy of processing of single words. From Petersen SE *et al. Nature* 1988;**331:**585

There are two steps in language production. First of all hearing is interpreted in the upper part of the temporal lobe, the visual cortex, at the back of the brain is in the occipital lobe. Interpretation of the written word starts there, then words are generated in the frontal lobe. Most of us communicate all the time by speech but we don't really think about how we do it.

In the Boston School in 1960's and 1970's Frank Benson and Norman Geshwind worked out that language understanding and production is modular, different parts of the brain having different functions. They worked this out through the study of damaged brains[13]. In order to study the brains of their patients at autopsy, their strategy was that the researcher had to live longer than his or her patients. This was difficult. A better approach was necessary.

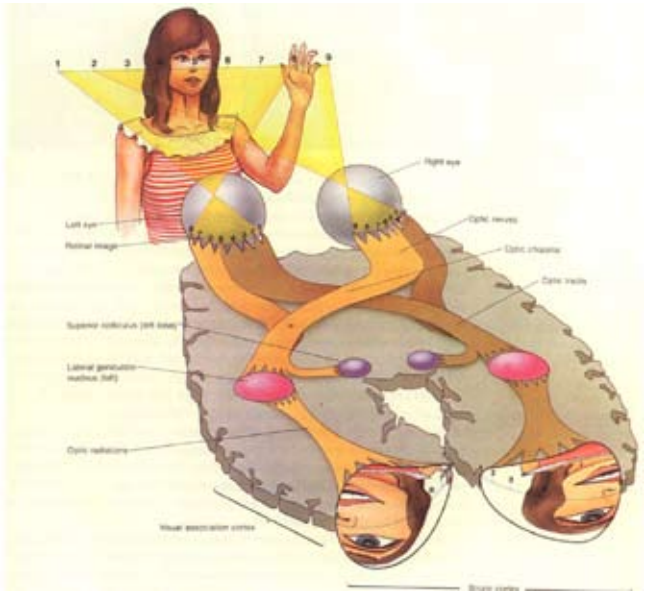

Fig 11. Visual fields. After Frisby 1979

A major advance has been the PET scan. Positrons are positive electrons. They are produced by very unstable nuclei, with short half-lives. Oxygen[15] and Fluorine[18] are very unstable isotopes that degrade, causing positrons to be emitted. When a positron encounters an electron both particles are annihilated producing two gamma rays at 180 degrees to each other. A co-incidence processing unit registers simultaneous detection of these gamma rays and the images are produced in a computer. Differences between the original resting scans and during stimulation show the occipital cortex lighting up when the subject looks at an object.

John Mazziotta in UCLA in the 1980's produced images

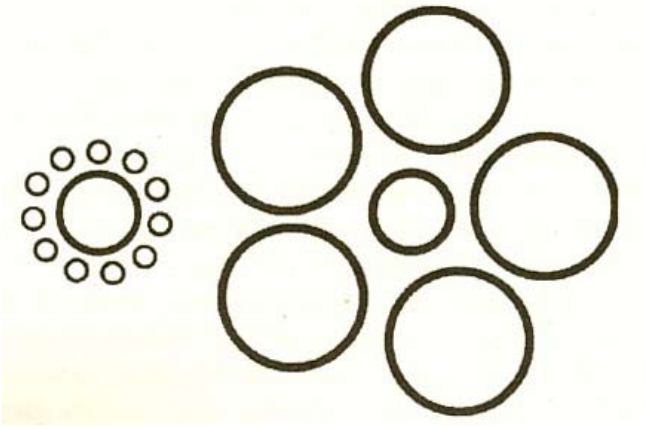

Fig 12. Circles

showing focal changes in metabolism in the occipital lobes where a patient has eyes closed, has eyes open and then is looking at a complex scene. He also produced images of a volunteer listening to music. This showed increased metabolic activity in the right perisylvian region. For some decades before these studies it had been felt that the interpretation of melody was situated in the right "non-dominant" hemisphere in right-handed people. This was the first time that it was demonstrated to be true in living intact individuals. He also produced a scan of someone with a low-grade astrocytoma, a primary brain tumour at the back of the brain. During a focal epileptic seizure there is focal increase in metabolism in the tumour.

Experiments in living volunteers in St Loius during the 1990's showed different parts of the brain lighting up. Hearing words, seeing words, speaking words and generating words (fig 10) showed modular function in discrete locations[14]. This demonstrated how the modular nature of language function, proposed by the Boston school through the study of people with damaged brains had been validated in living normal volunteers.

The linguist Noam Chomsky is interested in our innate ability to learn and produce language[15]. In Chomsky's view all languages share a universal structure or grammar and this universal grammar seems to be pre-programmed into our brains rather than something that is learned through teaching and experience. There are approximately 5000 known human languages. It appears that they all obey certain common rules that are innate. The rapid development of language in children is a wonder to all parents. Typically at about 10 – 12 months babies produce their first recognisable words. New words come slowly at first but then typically at about 15 – 20 months the rate begins to accelerate. Between the ages of 2 and 17 years a typical person learns about 60,000 words, an average of 10 new words per day[16].

Following on from this came functional imaging using MRI. The principle is that where there is increased metabolism

in different parts of the brain there is increased blood flow. Modern MRI scanners can measure this. Oxyhaemoglobin is present in increased amounts and this has different MRI decay characteristics. It is possible to show how local changes in brain metabolic activity varies in response to differing stimuli.

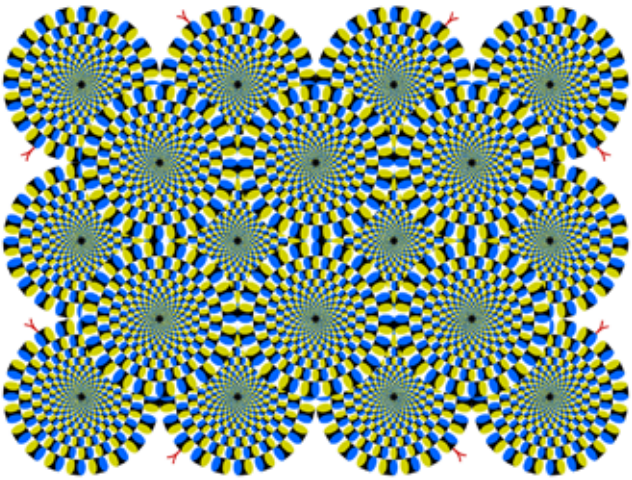

Fig 13. Akiyoshi illusion

Our understanding of the visual system has been transformed over the last century, but especially in the last thirty years. The retinae at the backs of our eyes receive images of our environment in two dimensions, up-side down (fig 11). The brain enables us to perceive our surroundings the right way up and with a perspective of depth and colour. We are conditioned to believe what we think we see. Our perceptions are conditioned by what we expect to see. The mechanisms of visual perception are complex. There are cells in the cortex that respond to stimuli in different orientations and there are other cells the respond to motion only in particular directions. In addition there are cells that respond to faces. In the 1960's researchers tracked how we recognise faces. The eyes dart momentarily from the eyes to the mouth, which are the major points of recognition in peoples' faces.

The distinguished neuroscientist Semir Zeki has spent a lifetime investigating the perception and processing of visual imagery. He reckons that 1/3 of the cerebral cortex is taken up with interpreting and processing visual imagery. He discovered that the primary visual cortex which is at the back of the brain first identified before the days of Gordon Holmes, is a primary staging point for cortical processing. Colour is processed in a separate area called V4 and motion is processed in a site on the lateral surface of the occipital lobe called V5[17]. It is of particular interest that colour perception is perceived before motion perception by a matter of 80 milliseconds. There are perhaps 30 different modules of visual perception. The question of what binds them all together into a consciously recognised whole is imperfectly understood. Colour, faces, language and interpretation of the human body are processed in different parts of the brain[18].

An illusion of two circles surrounded by rings of circles of different sizes confuses the brain to imagine that the inner circles are different sizes, when in fact they are the same size (fig 12). Hermann's grid - shows bouncing grey dots at the intersections of lines. The bouncing dots are generated in our brains. Our perception of colour is changed and altered by the context in which colour is viewed. Also, you can get illusions of movement. This is because when the eyes look at complex images they dart from side to side and this can stimulate the motion centre giving an illusion of movement (fig 13).

The next illusion shows a domino effect. The images are two-dimensional, but because of the way our brains are wired up the dominos appear to have concave and convex additions to the surface. When this is turned upside down the reverse appears (fig 14). Our brains are conditioned to perceive that light comes from on high and it appears as if the additions on the dominos are convex and concave. The famous Thatcher illusion is worthy of mention (fig 15). Margaret Thatcher looks more normal upside down than she does the correct way up. This was first described by Thompson[19]. Illusions arise because the brain interprets the world in relation to stores of memories of perceptions.

In the Guardian newspaper that I quoted earlier, reference is made to a publication by Haynes in Nature Neurosciences showing different parts of the brain lighting up when an individual looks at a place compared to a face[20]. The Guardian was concerned with the effects on civil liberties if such techniques are used as new lie detectors. Using modern MRI imaging it is possible to investigate emotional changes within the brain investigating romantic versus maternal love[21]. The changes fit in with the distribution of neurotransmitters. Variations in brain metabolism generated by our perception of beauty can be detected.

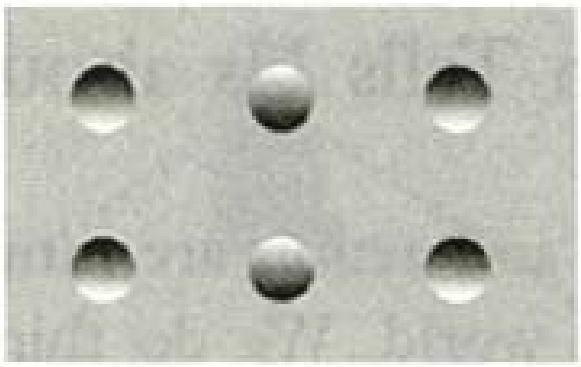

Fig 14. Dominos – from Frith C. Making up the mind. Blackwell, Oxford, 2007. Invert the page and the concave become convex and vice versa.

Earlier I made reference to neuroplasticity. PET scans of the hippocampus of London taxi drivers have shown that the longer people had worked driving taxis in London the greater the metabolism in the hippocampus, indicating that the metabolic activity of the hippocampus and indeed its size was increased by the learning experience of navigating London streets[22]. Implanted dopaminergic neurons in the basal ganglia of patients with Parkinson's disease survive multiply and make new connections[23]. Professor VS Ramachandran has studied the perception of phantom limbs in people who had amputations. Sensation interpreted by the brain as coming from the phantom limb arise when the face or the shoulder

are touched with a cotton bud. The hand area of the sensory cortex makes new connections[24].

Women who have had treatment for leukaemia with bone marrow transfusions from their brothers have been found to have male cells incorporating the male Y chromosome in adult neurons, indicating that stem cells coming from males transplanted into female patients are capable of becoming functional mature neurons and glial cells. 1% neurones and 1-2% of glial cells came from the brother's bone marrow stem cells[25]. It is not recorded whether after the transplants, the ladies expressed a greater interest in football.

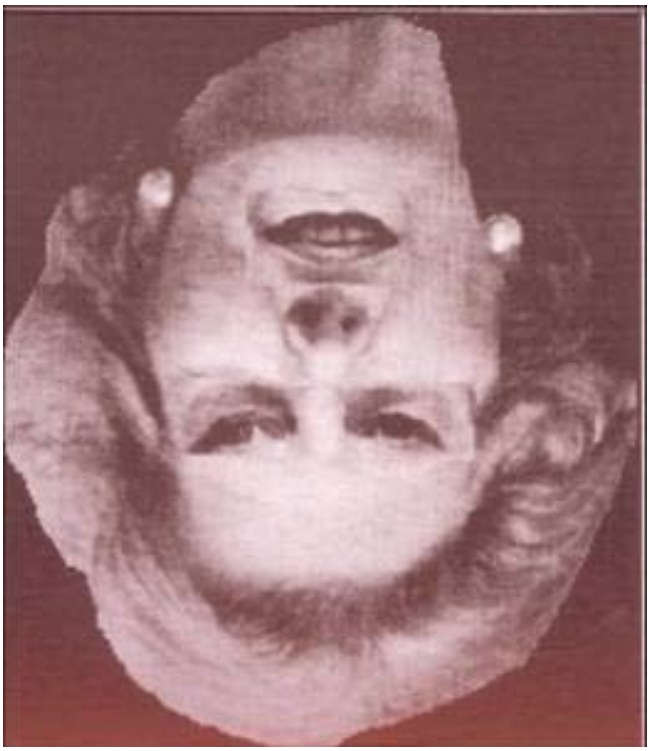

Fig 15. Margaret Thatcher – head inverted, but mouth and eyes right way up. Invert the page, and she looks very odd!

In my preparation for this talk I read the life history of Eric Kandel who won the Nobel Prize for medicine and physiology in 2000. He became interested in how the brain, behaviour and memory are controlled. Kandel feels that in the last century much was done to work out how the brain works and during the 21st century much work will done on the mechanisms of the mind[26].

Going back to our impressions of the mind, René Descartes said, "Cogito ergo sum", "I think therefore I am". There are more sceptics now and some might say "I think, therefore I am, I think" and our students who use text messages say "I text, th4 I iz".

The debate on consciousness is essentially a division between dualism and materialism. Dualists feel there is a split between mind and brain or body and soul. This thread has been present in philosophy for hundreds of years. Famous dualists were Popper and Eccles, who were friends and collaborators. Karl Popper was a philosopher, and John Eccles was a neurophysiologist who shared in the 1963 Nobel Prize. Materialism and materialists feel that neural activity accounts for everything and when neural activity stops – what?

Much has been written about consciousness. Wittgenstein felt that there was an unbridgeable gulf between the brain and the mind. Bernard Baars, an American cognitive neuroscientist said that "performing research on consciousness and the mind was the last taboo" and neuroscientist Antonio Damasio, an American neurologist said that consciousness is "a story that we tell ourselves". Daniel Dennett, said that "the brain is part of a clever robot" and others such as the columnist, Tom Wolfe, who has promoted an entirely materialistic point of view stated that "the soul has died"[27].

David Chalmers, the Australian philosopher says that there are two steps in trying to understand consciousness - the "easy problem" and the "hard problem". The easy problem is to distinguish conscious to unconscious brain processing, and the hard problem is – how subjective experiences arise from neuronal activity. Does it feel the same for me as for you? Am I dreaming? – and - What is reality?[28]

Fig 16. Portrait of Sir Hans Sloane, Ulster Medical Society Rooms, with permission from the Ulster Medical Society.

This has been my discourse over how the brain and mind works and I want to try and draw my talk to some conclusion for medical students. Thomas Sydenham taught Sir Hans Sloane (Fig 16) who was born in Killyleagh, Co Down and Sydenham said to Sloane when he wanted to study botany – "*You must concentrate on the bed side, it is there alone that you will learn disease*"[29]. At the end of the day clinical medicine involves an interaction between an individual doctor and an individual patient. Listening to a patient's history is absolutely fundamental. That is the story of their life and

the account of their symptoms. I tell students that we do not treat diseases; we treat people with diseases. You cannot be a physician unless you enjoy meeting people. While diagnostic techniques have become more humane than injecting air around the brain, and treatments are improving all the time, you must still put your patients first. It is also important to feel that there is a purpose to life.

Finally "More than a Sum of its Parts" the hospital. There are around 350 consultants, 550 junior doctors, 2000 nurses and 2000 administrative and clerical staff. It is possible for sick patients to obtain expert advice from colleagues at any hour of the day or night, whether they are officially on call or not. The reputation of the hospital depends on the excellence of medical and nursing staff. We also depend heavily on the commitment of our unsung heroes, the secretarial and administrative staff. Without them, the hospital could not function. The Royal Victoria Hospital has been in existence since 1797, some 210 years. It has survived many changes with its spirit undiminished. I am confident it will outlast the current fashion in management conglomeration.

Many orations start with a quote from William Osler - I finish with one. In a busy professional life, it is essential to have outside interests. On hobbies he said *"no man is really happy or safe without one, and it makes little difference what the outside interest may be – botany, beetles or butterflies, roses, tulips or irises, fishing, mountaineering or antiquities – anything will do"*[30]. I hope I have convinced you that the brain is greater than the sum of its parts.

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Case Report

A Case of Cutaneous Vasculitis with Underlying Hepatitis C and Cryoglobulinaemia

Cheryl Groves, Clare Devereux, Clifford McMillan.

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ABSTRACT

We report a 74 year old lady presenting with cutaneous leukocytoclastic vasculitis. The underlying aetiology was established as chronic hepatitis C infection with associated cryoglobulinaemia. This presented clinically as recurrent cutaneous vasculitic eruptions with absence of any other clinical manifestations. In this case, antiviral treatment to eradicate hepatitis C virus (HCV) was deemed inappropriate given the low necroinflammatory score determined by liver biopsy, absence of other systemic sequelae of cryoglobulinaemia and potential risks of therapy given her age. Currently her cutaneous disease is relatively well controlled with intermittent application of potent topical steroids. This case highlights the need to consider hepatitis C as a potential aetiological factor in all patients with cutaneous vasculitis. We suggest that viral hepatitis screening should be routine in all patients presenting with cutaneous vasculitis.

INTRODUCTION

Cutaneous vasculitis is a common diagnosis encountered in routine dermatology practice. Underlying autoimmune diseases, malignancy, drugs and systemic vasculitis are often found to be aetiological factors. Infections also play a major role including streptococcus, staphylococcus, mycobacterium and hepatitis B or C[1,2]. We present a case of occult chronic hepatitis C with associated cryoglobulinaemia manifesting as a cutaneous leukocytoclastic vasculitis in an elderly lady.

CASE REPORT

A 74 year old lady presented to dermatology out-patients with an eight year history of a recurrent tender, non-blanching, palpable, purpuric rash involving her lower limbs (figs 1 and 2). A diagnostic punch biopsy revealed a leukocytoclastic vasculitis. She had no significant medical history and denied systemic upset, recent infections or new medications.

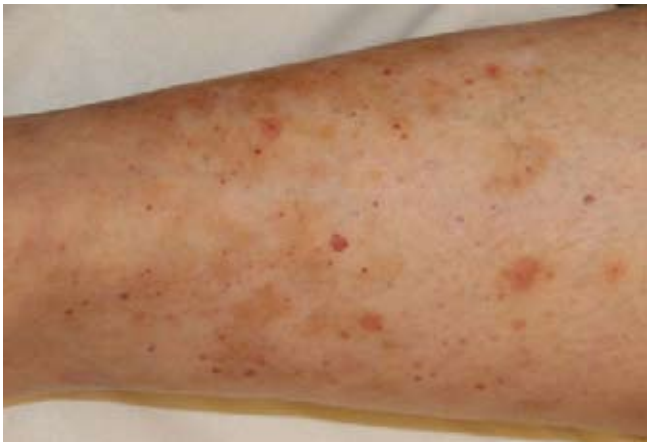

Fig 2. Close up view showing vasculitis

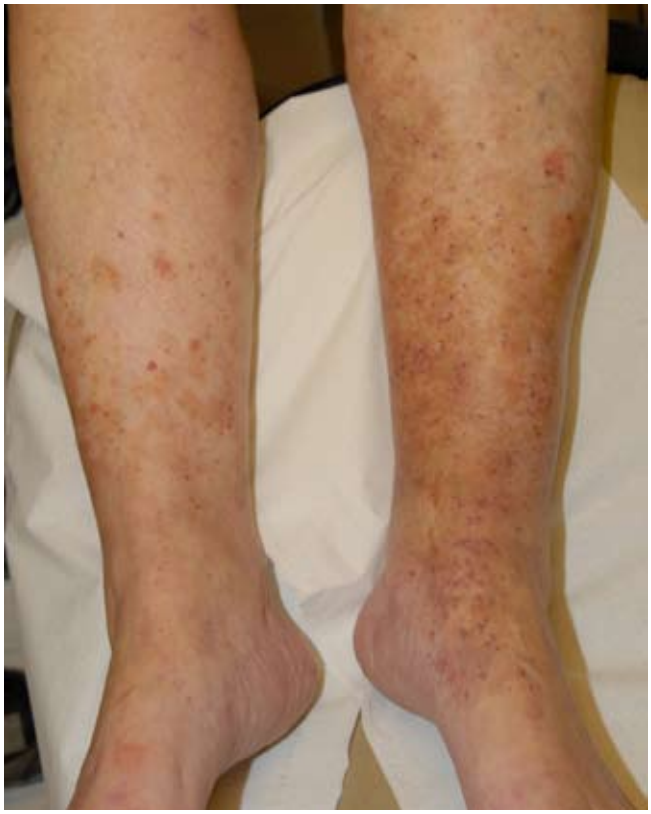

Fig 1. Lower limbs showing vasculitis

Laboratory investigations confirmed normal full blood count, renal, liver and thyroid function with an absence of microscopic haematuria and red cell casts on urinalysis testing. Inflammatory markers, autoimmune screen, double stranded DNA, pANCA and cANCA were all negative and remained so on repeated sampling. Normal anti-streptolysin O

Department of Dermatology, Belfast HSC Trust, Belfast City Hospital Campus, Lisburn Road, Belfast BT9 7AB, United Kingdom

Correspondence to Dr Groves

Cheryl_groves2003@yahoo.com.au

titre, complement and immunoglobulin levels were reported. Chest radiograph was normal. Rheumatoid factor was positive. Chronic hepatitis C virus infection was confirmed by enzyme immunoassay and reverse-transcriptase polymerase chain reaction. The virus was typed by limited sequencing of the 5′ non-coding region of the virus and was confirmed as genotype 2. The HCV specific antibody level was abnormally low, in keeping with immune-complex sequestration.

Given the association between hepatitis C infection and type II [mixed] cryoglobulinaemia, the presence of serum cryoglobulins was sought and detected. A diagnosis of hepatitis C related cryoglobulinaemic cutaneous vasculitis was made. Her only risk factor for contraction of hepatitis C was a blood transfusion received in 1954 in the United States following a spontaneous abortion.

She was referred to the regional hepatology unit for further assessment including consideration of hepatitis C eradication therapy. Subsequent liver biopsy revealed changes consistent with chronic hepatitis C infection with a necroinflammatory score of only 1 out of a possible 8 and a modified staging score of 0. Eradication of hepatitis C virus with interferon 2 alpha plus ribavirin was deemed inappropriate in this patient given the low necroinflammatory score on liver biopsy, the absence of other systemic sequelae of cryoglobulinaemia and potential risks of therapy. Intermittent use of potent topical steroids has to date controlled exacerbations of her cutaneous vasculitis and prevented progression to ulceration.

DISCUSSION

At the time of initial presentation to dermatology out-patients there was no reason to have a clinical suspicion of chronic hepatitis C infection in this otherwise healthy elderly woman. A study of hepatitis C in Northern Ireland by McDougall[3] described 78% of patients as asymptomatic at the time of diagnosis - a figure substantiated by earlier studies[3,4]. An increased awareness of HCV infections' cutaneous manifestations may enhance its chances of detection.

Our patient presented with palpable purpura but this is just one such cutaneous indication of underlying hepatitis C infection. Others include livedo reticularis, urticaria, lichen planus, erythema multiforme, erythema nodosum and porphyria cutanea tarda[5-8].

Subsequent detection of cryoglobulins in our patient confirmed the underlying pathology responsible for the leukocyclastic vasculitis. Cryoglobulins are immunoglobulins that precipitate at temperatures below 37^{0}C and re-dissolve with warming. Cryoglobulinaemia can manifest in two ways. Firstly, by precipitation and obstruction of small blood vessels in the peripheries [feet, hands, nose and ears] resulting in cutaneous ischaemia and possible infarction. Secondly, by deposition as immune complexes thereby initiating a leukocytoclastic vasculitis. Depending on the site of deposition, various clinical entities may arise – palpable purpura, arthritis, glomerulonephritis or peripheral neuropathy. Our patients' vasculitis was limited to cutaneous involvement.

Cryoglobulinaemia is classified into three types. Type I consists of a single monoclonal immunoglobulin [Ig], usually a paraprotein, which is associated with haematological disorders. Type II is characterised by polyclonal IgG rheumatoid factor and monoclonal IgM rheumatoid factor. Polyclonal IgG and IgM rheumatoid factors are found in type III. Types II and III are known as mixed cryoglobulinaemia and can be associated with several conditions. Haematological associations include lymphoma and myeloma. It also exists with autoimmune disorders such as rheumatoid arthritis and systemic lupus. Infections implicated include parasitic, bacterial and viral infections, of which HCV is more common than hepatitis B virus.

Essential mixed cryoglobulinaemia is a term used to describe mixed cryoglobulinaemia occurring without identification of a primary disease. Evidence suggests that HCV infection may well be responsible for a majority of those cases previously defined as 'essential'[9]. Anti-HCV antibodies are found in 70-100% of patients with mixed cryoglobulinaemia[10]. Contrastingly, among patients with chronic HCV infection, one third to one half have serological markers of cryoglobulinaemia but the clinical syndrome of mixed cryoglobulinaemia, as manifested in this case, occurs rarely in only 1-2%[11,12].

The definitive management of hepatitis C related cryoglobulinaemia is by eradication of the virus with subsequent suppression of the cryoglobulinaemic process. Use of pegylated interferon alpha [IFN-alpha] 2a or 2b in combination with ribavirin has been shown to be effective with sustained virological response [defined as undetectable viraemia 24 weeks after the end of therapy] reported as 76% using 24 weeks of IFN-alpha 2a plus ribavirin and 82% using IFN-alpha 2b plus ribavirin for the genotype group applicable to our patient - genotype 2 or 3[13,14].

The decision not to offer IFN-alpha combined with ribavirin therapy to this patient was based on her age, low necroinflammatory score on liver pathology and risk of significant side effects such as myelosuppression, polyarthritis, thyroiditis and peripheral neuropathy. Alternative therapies which have been implemented for cutaneous mixed cryoglobulinaemia are systemic prednisolone, azathioprine, mycophenolate, dapsone or plasmapheresis. More recently, rituximab has been used successfully[15].

Currently, this lady's flares of cutaneous vasculitis are successfully treated to resolution with topical agents only. If, however, the cutaneous features become unresponsive to topical measures or further sequelae of cryoglobulinaemia should develop, further consideration will be given to treatment with pegylated IFN-alpha combined with ribavirin or alternatively a systemic immunosuppressant agent.

In summary, our case report highlights the interesting association between hepatitis C virus, cryoglobulinaemia and leukocytoclastic vasculitis. Heightened awareness of all the cutaneous manifestations of hepatitis C can only serve to increase the diagnostic rate of a notoriously clinically silent infection. Although cutaneous vasculitis is a relatively common clinical presentation, this case emphasises the need to perform full screening investigations to exclude the least prevalent aetiologies and to consider underlying hepatitis C infection, even in those you least suspect.

The authors have no conflict of interest.

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Case Report

Pseudo–obstruction with pitch black colon - A very rare Presentation of Melanosis Coli

Arshad H Malik[1], Syed IH Andrabi[2], Mohammad Niayesh[3]

Accepted 3 July 2007

ABSTRACT

Melanosis coli is associated with an increased risk of colorectal tumors but is not agreed to be a precancerous lesion. The condition has been associated with the ingestion of anthracene laxatives and is believed to be caused by increased epithelial apoptosis. Although melanosis coli is a frequent finding in colonic biopsies and resection specimens, to our knowledge severe jet black melanosis coli with pseudo-obstruction has not been reported in literature. Such gross Melanosis is exceptional and particularly striking.

Key Words: Melanosis coli, pseudo-obstruction, laxative

INTRODUCTION

Laxative use is integrally linked to constipation. Melanosis coli is a brownish pigmentation of colonic mucosa which, since 1933[1], has been related with the persistent ingestion of anthranoid laxatives. Anthranoid laxatives have been found to have mutagenic and carcinogenic effects by in vitro and animal studies[2]. Melanosis coli has also been found in patients who do not use laxatives or suffer from constipation, possibly because of the apoptosis of epithelial cells and their subsequent phagocytosis by macrophages of lamina propria with accumulation of lipofuscin pigment[3]. Few studies have explored a possible connection of anthranoid laxative use and melanosis coli with colorectal carcinoma in humans and the outcome are incongruous[4]. We present a very rare case of severe melanosis coli with pseudo-obstruction which has not been reported in literature to our knowledge.

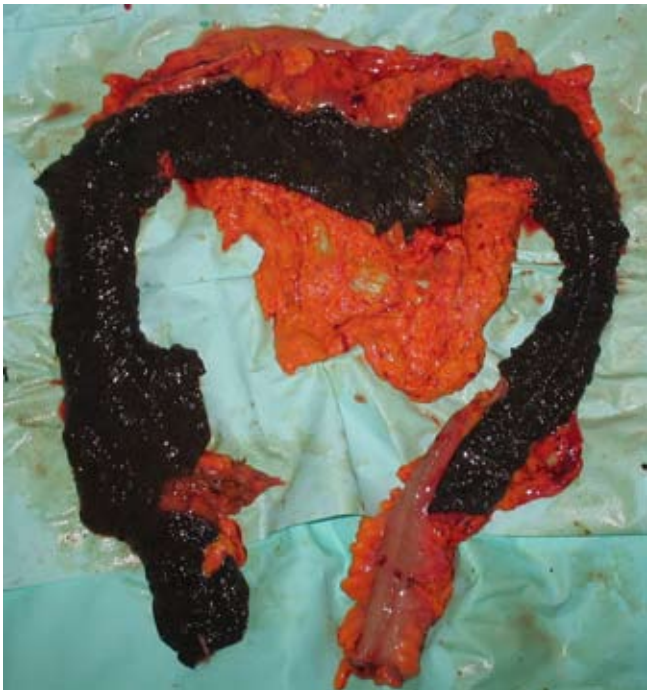

Figure 1: Melanosis coli

CASE REPORT

A 71 year old lady presented with a one week history of gradually worsening abdominal pain. On examination she was pale and the abdominal was distended with sluggish bowel sounds. She was constipated and the clinical signs were suggestive of intestinal obstruction. Blood tests revealed anemia with normal electrolytes. Abdominal radiograph showed distended transverse colon. A CT scan showed distended large bowel with sigmoid diverticulosis. Persistent non-resolution of symptoms lead to exploration which revealed a pitch black colon with black pigment in inferior mesenteric nodes (fig 1). A sub-total colectomy was performed. Histology revealed gross melanosis coli with pigment in mesenteric nodes. Post operatively the patient recovered well.

DISCUSSION

Melanosis coli is a condition usually associated with chronic laxative use in which dark pigment is deposited in the lamina propria of the colon[5]. The pigment deposition results in a distinctive dark brown to black staining of the lining of the large intestine. This condition at times is called pseudomelanosis coli[6] because the pigment deposits consist of a pigment known as lipofuscin and do not contain melanin as implied by the term “melanosis.” Lipofuscin is a cellular pigment that forms when cells are destroyed, frequently called “wear and tear” pigment that can be found all over the body.

The dark color of the intestinal lining may be uniform or patterned, and the pigmentation may be slight - or very marked as in our case. The concentration and pattern of the discoloration may even differ among different sites in the

[1] Department of Surgery, Causeway Hospital, Coleraine, UK,

[2] Craigavon Area Hospital, Portadown, UK, [3]Yeovil Foundation NHS Trust, Somerset, UK

Correspondence to Dr Malik

8 Bellview Court, Coleraine, Northern Ireland, UK.

drarshadmalik@hotmail.com

colon of a patient. The condition may reverse on discontinuing laxative use. The wall of the colon may appear grossly normal, but microscopic examination may show areas of pigment.

Melanosis coli usually results from chronic use of laxatives of the anthranoid group[5], including senna and rhubarb derivatives[7]. Animal studies show that extremely high doses of phenolphthalein led to tumors in animals, but it has never been shown to cause cancers in humans[2]. Chronic laxative use induces melanosis coli and possibly increases colorectal cancer risk[5,8]. Colorectal adenomas are more frequently found in patients with melanosis coli[8].

The anthranoid laxatives pass through the bowel unabsorbed till they reach the large intestine, where they are changed into their active forms[6]. These anthranoid laxatives exert their laxative effect by damaging epithelial cells, which leads directly and indirectly to changes in absorption, secretion and motility[6]. The resulting activation causes injury to the cells in the lining of the intestine and leads to apoptosis (a form of cell death). The apoptotic cells appear as darkly pigmented bodies that are taken up by macrophages. When sufficient cells have been damaged, the distinctive pigmentation of the bowel wall develops[6].

Melanosis coli can be observed during colonoscopy and sigmoidoscopy. Sometimes the diagnosis is made upon histological examination of biopsies taken during endoscopic procedures.

Since there have been preliminary reports suggesting a possible role of anthranoid-containing laxatives in the development of colorectal adenomas and cancer, their use should be discouraged[9] and long term use cannot be recommended[6].

CONCLUSION

It is known that long-term use of anthranoid containing laxatives is the cause of melanosis coli. The presence of melanosis coli might signal an increased risk for the development of colorectal cancer which merits resection of the colon at laparotomy. Chronic misuse of such laxatives should be avoided as other safer laxatives are available.

The authors have no conflict of interest.

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Letters

AN UNUSUAL PRESENTATION OF RECURRENT FOLLICULAR THYROID CARCINOMA

Editor,

Around 1500 new cases of thyroid cancer are diagnosed each year in the United Kingdom, being responsible for around 320 deaths annually[1]. Detecting recurrent thyroid carcinoma is important for the speedy imposition of treatment regimens, but occasionally detecting subsequent disease can be problematic. Bone is the commonest site for distant thyroid carcinoma metastases[2], but other sites such as the parapharyngeal space[3] are not unknown. We describe the case of a patient presenting with recurrent follicular thyroid carcinoma following total thyroidectomy in which bilateral internal jugular vein thrombosis, in association with a right jugulodigastric mass were the main features. We believe that this is the first such report of a recurrent follicular carcinoma of the thyroid presenting in this manner.

Case Report: A 39 year old Caucasian lady presented with a large anterior midline mass and hoarseness due to left vocal cord palsy. Due to airway difficulties a surgical tracheostomy was performed, at which time biopsy was taken. Histology reported a poorly differentiated follicular cell carcinoma of the thyroid, CT scan demonstrating masses in both lobes of the thyroid, but no metastatic spread.

Total thyroidectomy was performed followed by thyroid ablation therapy with radioactive iodine. Fourteen months following thyroidectomy the patient was readmitted to hospital with a diffuse red tender right sided neck swelling centred over the jugulodigastric area. Fibreoptic nasendoscopy was unremarkable. Both her inflammatory markers and white cell count were found to be raised, and intravenous antibiotics were commenced. A CT of the neck was performed, which demonstrated extensive bilateral thrombosis of the internal jugular veins, the left displaying intracranial extension involving the sigmoid sinus. An ultrasound scan revealed changes suggestive of right sided parotiditis, but an MRI added nothing new. With no recurrence of tumour found investigations for other causes of thrombosis were undertaken, but were all negative. Fine needle aspiration cytology of the mass was unhelpful.

Subsequently the patient developed dysphagia, dysarthria, and bilateral impairment of cranial nerves IX, X and XII. Ultrasound scan of the liver, and bone marrow biopsy were undertaken and found to be unremarkable. A PET scan was performed, but highlighted nothing other than the right sided neck lump. An incision biopsy of the right sided neck lump was undertaken, the histology revealing a poorly differentiated necrotic carcinoma similar in appearance to the follicular thyroid carcinoma previously excised. Her condition deteriorated and she died shortly after.

Discussion: Diagnosing recurrent thyroid carcinoma following attempted curative surgery is of paramount importance in order to institute the correct treatment as soon as possible. Recurrence of thyroid carcinomas can be difficult to detect, and while thrombosis of jugular veins has been described as a feature of papillary thyroid carcinomas[4], we believe this is the first description of this occurring with a follicular thyroid carcinoma. Some authors have postulated that thyroid carcinomas may result in a hypercoagulable state[5] and we hope that this report highlights the importance of ruling out recurrence of follicular thyroid carcinoma as a cause of unexplained thrombosis in a patient which has previously undergone curative surgery.

No conflict of interest declared.

Gwyn S Williams,* *Senior House Officer*

Khaliq Rehman, *Senior House Officer*

Kai Leong, *Senior House Officer*

Talvinder Singh, *Senior House Officer*

Department of ENT Surgery, New Cross Hospital, Wolverhampton, West Midlands, United Kingdom

gwynsamuelwilliams@hotmail.com

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SUCCESSFUL ENDOSCOPIC MANAGEMENT OF FRACTURED DORMIA BASKET DURING ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY FOR CHOLEDOCHOLITHIASIS

Editor,

Endoscopic retrograde cholangiopancreatography (ERCP) is a valuable tool in pancreaticobiliary evaluation and treatment. ERCP has become the mainstay in the treatment of choledocholithiasis through sphincterotomy and trawl of the common bile duct with either a balloon or a metal basket being used to retrieve stones. Complications of ERCP and sphincterotomy have been reported to occur in five to ten per cent of cases[1-3], and range from minor bleeding to severe pancreatitis. We report an unusual complication of ERCP with basket fracture and retention followed by recovery of the retained basket at second ERCP.

Case report: A 61-year-old gentleman presented with a 10-day history of nausea, right upper quadrant discomfort, dark urine and pale stools. He had a past medical history of ischaemic heart disease and peptic ulcer disease. There was no history of liver disease or gallstones and no risk factors for jaundice. On examination he was apyrexic, icteric and was mildly tender in the right upper quadrant without rebound or

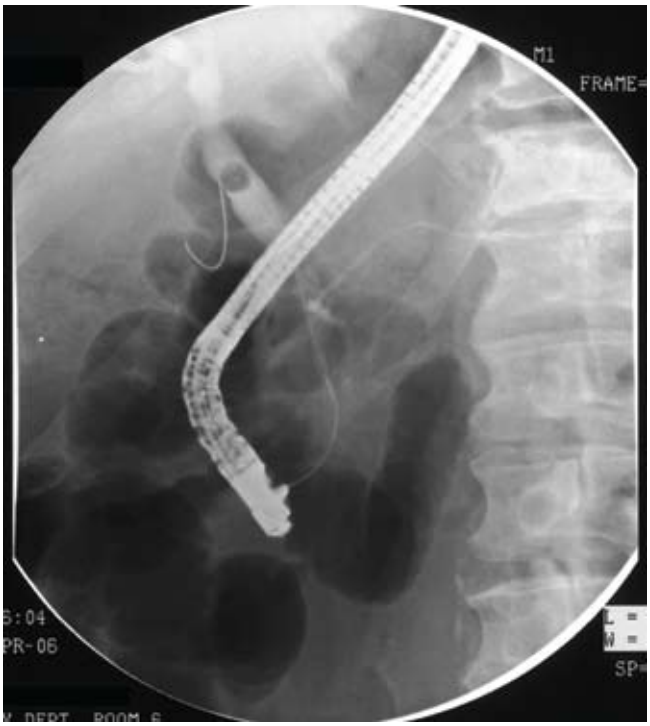

Fig 1. ERCP image of stone engaged in Dormia basket.

guarding. There were no stigmata of chronic liver disease. Initial blood investigations showed Hb 13.4G/dl, WCC 6.34 THOUS/uL, Bilirubin 175umol/l, AST 164 U/L, GGT 603 U/L, ALP 215 U/L. Urea, electrolytes and albumin were within normal limits. Ultrasound scan (USS) of abdomen was performed the day following admission and showed a calculus within the lower common bile duct (CBD). The CBD and intrahepatic ducts were dilated.

As a result of these findings, an ERCP was arranged. ERCP was carried out 4 days following admission. Technique of conscious sedation was employed using midazolam and

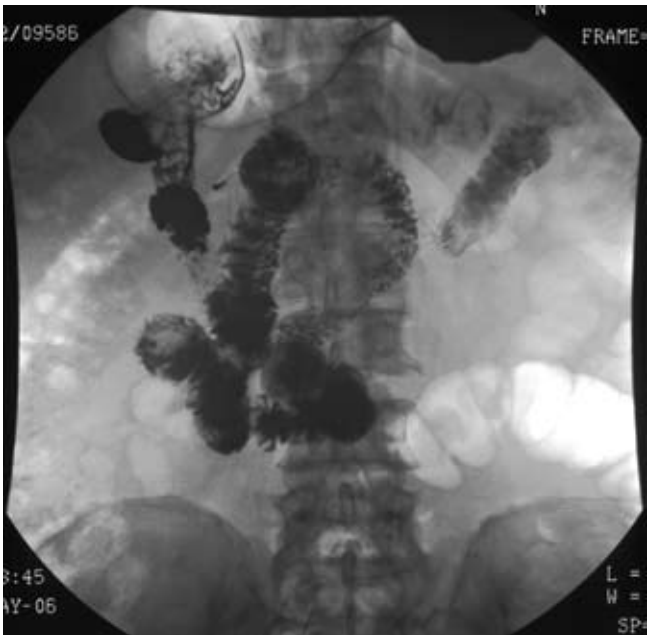

Fig 2. Videofluroscopy image showing contrast in duodenum and fractured basket fragment in CBD.

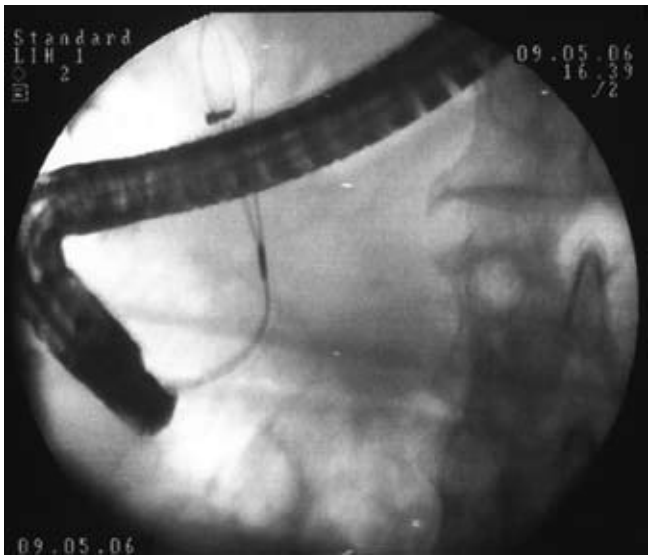

Fig 3. ERCP image of basket fragment engaged in second Dormia basket.

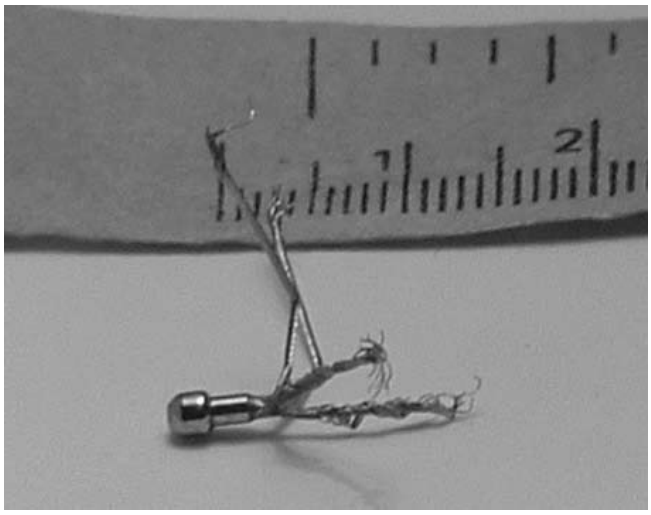

Fig 4. Fragment of fractured basket removed from CBD.

pethidine. Midazolam was titrated to 7mgs and pethidine titrated to 50mgs. Despite this the patient remained agitated throughout the procedure.

Findings were as follows: Ampulla was normal. Pancreatic duct was normal. CBD was dilated to 10-12mm. A single CBD stone approximately 8mm in diameter was present.

An 8mm sphincterotomy was performed. A Dormia basket was placed around the stone. The stone was successfully engaged into the basket (fig.1) but the basket could not be pulled through the ampulla. Subsequently, crushing of the CBD stone with the external lithotripter was attempted. However the patient became extremely agitated and lithotripsy had to be terminated. The end of the impacted basket was cut, the polyethylene sheath was removed and the endoscope withdrawn. It was noted that a portion of the wire had fractured off. The endoscope could not be passed back into the stomach due to the patient's ongoing agitation and the procedure was abandoned.

There was a strong clinical suspicion of retained basket

fragments and the patient was commenced on IV ciprofloxacin. Repeat fluoroscopy with oral contrast confirmed retained basket in the CBD (fig 2).

A second ERCP under general anaesthetic was performed. Cholangiogram demonstrated single calculus which was removed along with the retained fragment of basket (see fig 3). The remaining metal fragment was grasped with a further Dormia basket and removed (fig 4). The patient had no complications post-ERCP and is currently awaiting laparoscopic cholecystectomy.

Discussion: Traction wire or basket fracture, often following stone impaction, is an unusual complication of ERCP and in the past has been managed surgically[4]. Biliary stenting leads to increased risk of cholangitis by disrupting sphincter of Oddi function[5]. Retained metal fragments are likely to similarly disrupt sphincter of Oddi function with subsequent high risk of cholangitis.

Conclusion: We have demonstrated successful medical management of basket fracture with intravenous antibiotics and repeat ERCP facilitating endoscopic removal of the retained fragment. In experienced endoscopic teams this should be considered as an alternative to surgery.

No conflict of interest declared.

Nicholas M Kelly* *ST1 Gastroenterology trainee*

Grant R Caddy, *Consultant Gastroenterologist*

Department of Gastroenterology, Ulster Hospital, Dundonald, Northern Ireland.

* 49 Ravenhill Park, Belfast, BT6 0DG

nickkelly@doctors.net.uk

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APPENDICECTOMY COMPLICATED BY ADDISONS DISEASE

Editor,

Acute appendicitis is the most common surgical emergency. We describe a case in which

a young man underwent appendicectomy but had a complicated postoperative recovery

requiring admission to ICU.

Case report: A 33 year old male presented with a fifteen-hour history of vomiting, diarrhoea, and lower abdominal pain one week after a holiday in Portugal. He had no significant past medical history. On examination he had a tanned appearance, and was tender with guarding and rebound in the right iliac fossa. Rovsing's sign was positive. He proceeded to theatre where the operative findings and subsequent histology confirmed the diagnosis of acute appendicitis.

Over the next 24 hours he had persisting pyrexia and became tachycardic and hypotensive. Examination revealed decreased chest air entry bilaterally and abdominal distension. C reactive protein was increased to 369ug/L, from 5.0ug/L on admission. Electrolyte profile confirmed hyponatraemia. Arterial blood gas sampling showed a metabolic acidosis. He was thought to be septic. The following morning a CT scan of chest and abdomen showed, bilateral pleural effusions with collapse at both lung bases. There was free fluid in the abdomen with dilatation of the small bowel throughout its length. He was thought to have a postoperative ileus, but an atypical pneumonia was also considered.

He was transferred to ICU. Over the next 24 hours the abdominal distention increased and in view of this he returned to theatre. At laparotomy, an inflammatory mass was found in the caecum and terminal ileum, causing small bowel obstruction. A limited right hemicolectomy was done. His general postoperative condition remained poor.

Further discussions with the family revealed that the patient had been of a tanned appearance since he had returned from holiday in a hot climate 10 years previous. The tanned appearance, hyponatraemia, and polyuria raised the possibility of adrenal insufficiency and a Synacthen® test was undertaken. This suggested Addison's disease. Treatment with intravenous hydrocortisone and fludrocortisone lead to an immediate clinical improvement. He was discharged home well five days later.

Discussion: The diagnosis of Addison's disease and then Addisonian crisis in a postoperative patient is one which is fraught with difficulty. Virtually all the signs mimic other more common conditions like post-operative ileus or sepsis. A literature review indicates that these would seem to be the most widely considered initial diagnosis[1]. It has been calculated that some degree of unsuspected adrenal insufficiency is present in up to 1 in 1000 surgical admissions[2], and surgeons should consider this condition when a postoperative patient fails to recover as expected. Abdominal pain as the primary complaint occurs in about 10% although a generalised gastrointestinal upset is much more common. Severe abdominal pain with tenderness mimicking peritonitis is thought to occur in about 7% of cases[2].

Primary adrenocortical failure is usually due to an autoimmune mediated destruction of the adrenal gland which accounts for around 90% of cases. Females are affected two to three times more frequently than males and there is an association with other endocrine deficiencies such as thyroid disease, premature gonadal failure (usually ovarian failure) and type I diabetes[2].

The patient should be treated in the Intensive Care Unit with

standard resuscitation measures of airway control, respiratory support, and cardiovascular monitoring. Normal saline is given intravenously to maintain the circulation, hydrocortisone 100mg is given intravenously 6 hourly and fludrocortisone is administered as a single dose of 100µg orally daily[3]. Patient education is the key to preventing further episodes. Patients need to be fully informed about the condition and counselled with regard to appropriate replacement therapy. It might also be helpful if the patient could wear a Medicalert bracelet and carry a written record of their medications.

The authors have no conflict of interest.

*Michael J. Mullan, SHO General Surgery.

Julie L Scoffield, Consultant Surgeon.

Pawan K. Rajpal, Consultant Surgeon.

Department of Surgery, Antrim Area Hospital, 45 Bush Road, Antrim.

Correspondence to Dr. Michael J. Mullan, 233 Alexandra Park Avenue, Belfast, BT15 3GB.

michaelmullan@doctors.org.uk

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Ulster Med J 2008; **77 (1)** 60-64

Abstracts

Junior Members Forum, Thursday 22 November 2007

North lecture theatre, Medical Biology Centre, Belfast

PROGRAMME

A. PLATFORM PRESENTATIONS

1. Tumour Characteristics of False Negative Imprint Cytology In Patients Undergoing Sentinel Lymph Node Biopsy.

AS McCoubrey, E Murray, I Cameron, S Dace.

Altnagelvin Area Hospital, Londonderry, BT47 6SB

Introduction: Sentinel Lymph Node Biopsy (SLNB) is set to become the standard of care for axillary staging in breast cancer. The aim of this study is to evaluate the tumour characteristics associated with false negative intraoperative imprint cytology.

Methods: Data was recorded prospectively for 105 consecutive patients with clinically node negative breast cancer. All had excision of the breast tumour and SLNB followed by axillary node clearance. Intra-operative imprint cytology was carried out in conjunction with post-operative haematoxylin & eosin (H&E) staining and immunohistochemistry (IHC).

Results: Forty-three patients (41%) had a positive sentinel node diagnosed. Nine cases were negative using imprint cytology. Of those 9 cases, 5 had micrometastases on H&E or IHC. Therefore there were 4 “true” false negative cases (9%). Of those cases that were “true” false negatives, the mean invasive cancer size was 27mm (8-60mm). Fifty percent of the tumours contained lobular elements. Median tumour grade was 2. All of the tumours were oestrogen receptor (ER) positive and 25% were HER-2 positive. Of the 34 remaining cases that were SLNB positive, the mean invasive cancer size was 32mm (12-70mm). Twenty-six percent of the tumours contained lobular elements. Median tumour grade was 2. Eighty-five percent of the tumours were ER positive and 9% were HER-2 positive.

Conclusion: The use of imprint cytology accurately identifies metastatic spread in the majority of patients undergoing SLNB. Although the numbers in this study are small, there are no obvious tumour characteristics associated with false negative imprint cytology in patients having SLNB.

2. Effect of ingestion of food on the inhibition of DPPIV activity by oral metformin in Type 2 diabetes.

Cuthbertson J[1], Patterson S[2], O`Harte FPM[2], Bell PM[1]

[1] Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, Belfast.

[2] School of Biomedical Sciences, University of Ulster, Coleraine

Introduction: The incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) constitute the enteroinsular axis which promotes postprandial insulin secretion. The therapeutic potential of these hormones in diabetes is limited by their rapid inactivation by the enzyme dipeptidylpeptidase-IV (DPP-IV). Here we investigated the acute effects of metformin in the presence and absence of food on DPP-IV activity in Type 2 diabetes.

Methods: Ten subjects with Type 2 diabetes (6 male/4 female, age 65.8±15.8 years (mean ± SEM), body mass index 30.0± 7.5kg/m2, HbA1c 6.3±1.2%) received metformin 1g orally or placebo together with a standard mixed meal (SMM) in a random crossover design. Six subjects reattended fasting and received metformin 1g without a SMM.

Results: Following SMM (n=10), DPP IV activity was not suppressed by metformin compared with placebo (area under curve $AUC_{0\text{-}4h}$ 1574±4 and 1581±8 µmol/min respectively). No differences were observed in plasma glucose, insulin and total GLP-1. After fasting (n=6), DPP IV activity was suppressed ($P<0.02$) when compared to those given metformin with a SMM ($AUC_{0\text{-}4h}$ 1494±9 vs. 1578±4 µmol/min). Metformin plasma levels were significantly higher ($P<0.03$) after fasting than SMM ($AUC^{0\text{-}4h}$ 457±55 vs. 350±66 mcg/ml).

Conclusion: Metformin inhibits DPP IV activity in patients with Type 2 diabetes in the fasting state but not when taken with a standard mixed meal. Metformin plasma concentrations are lower if taken with food. Metformin may have potential for combination therapy with incretin hormones.

3. Prosthetic stent-graft infection following endovascular abdominal aortic aneurysm repair

Muhammad A Sharif[1], Bernard Lee[1], Luk L Lau[1,2] Peter K Ellis[2], Anton J Collins[2], Paul H Blair[2], Chee V Soong[1].

[1.] Department of Vascular and Endovascular Surgery, Belfast City Hospital, Lisburn Road, Belfast, BT9 7AB, Northern Ireland, United Kingdom.

[2.] Department of Vascular Surgery, Royal Victoria Hospital, Grosvenor Road, Belfast, BT7 12 AB, Northern Ireland, United Kingdom.

Objective: The purpose of this report is to discuss the

incidence, diagnosis and management of stent-graft infections following EVAR[1].

Methods: Data were collected from the hospital database and medical case notes for all patients with infected endografts following elective or emergency EVAR for AAA over the last eight years in two university teaching hospitals in Northern Ireland. The data included the patient's age, gender, presentation of sepsis, treatment offered and the ultimate outcome. The diagnosis of graft related sepsis was established by a combination of investigations including inflammatory markers, labelled white cell scan, Computerized Tomography (CT) scan, microbiology cultures and post mortem examination.

Results: Out of a total of 509 patients, including 433 elective repairs and 76 emergency endografts for ruptured AAA, six suffered graft related septic complications. Two patients presented with left psoas abscess and were treated successfully with extra-anatomical bypass and removal of the infected stent-graft. A further two patients presented with infected graft without other evidence of intra-abdominal sepsis: one underwent successful removal of the infected prosthesis with extra-anatomical bypass while the other was treated conservatively and died of progressively worsening sepsis. The fifth patient presented with unexplained fever and died suddenly, with a post-mortem diagnosis of aorto-enteric fistula and ruptured aneurysm. The last patient presented with an aorto-enteric fistula, was treated conservatively in view of concurrent myelodysplasia, and died of possible aneurysm rupture.

Conclusion: This report is to emphasize the need for continued awareness of potential graft-related septic complications in patients undergoing endovascular repair of AAA. Attention to detail with regard to sterility and antibiotic prophylaxis, during stent-grafting and during any secondary interventions, is vital in reducing the risk of infection. In addition, early recognition and prompt treatment are essential for a successful outcome.

1 Sharif MA, Lau LL, Lee B, Ellis PK, Blair PH, Soong CV. Prosthetic stent-graft infection following endovascular abdominal aortic aneurysm repair. *J Vasc Surg* 2007;**46**:442-448.

4. Patients with Primary (Idiopathic) Achalasia Have Circulating Peripheral Blood Mononuclear Immune Cells That Are Hyper- Reactive To the Herpes- Simplex -1 Virus

KW Lau[1], C McCaughey[2], PV Coyle[2], LJ Murray[1], BT Johnston[3]

1 Centre for Clinical and Population Sciences, Queens University of Belfast,

2 Regional Virus Laboratory,

3 Department of Gastroenterology, Royal Victoria Hospital, Belfast, United Kingdom

Introduction: Achalasia is the best characterized oesophageal motor disorder but the aetiology is unknown. The pathology seen in achalasia consists of a decrease in nitric oxide-producing neurones and the presence of an activated T-cell inflammatory infiltrate in the myenteric plexus[1]. Certain Human Leucocyte Antigen (HLA) class II alleles are also more prevalent in patients with primary achalasia[1]. These factors suggest that an autoimmune mechanism may be involved in the pathogenesis of primary achalasia. The stimulus initiating this is unknown but could involve the Herpes simplex -1 virus (HSV -1). A previous study has demonstrated the existence of oesophageal mononuclear immune cells reactive to HSV -1 antigens in an in- vitro setting[2].

Aims & Methods: The aim of this study is to test the hypothesis that circulating peripheral blood mononuclear cells in patients with primary achalasia may be reactive to HSV- 1. Whole blood culture experiments were conducted with heparinised peripheral venous blood obtained from 151 patients with primary achalasia and 118 healthy controls. Whole blood was cultured in the presence of ultraviolet inactivated HSV – 1 (multiplicity of infection of 1 TCID50 / lymphocyte) or conditioned cell culture media. Reactivity of mononuclear cells to viral antigens was quantified by measuring expression of the cytokine gene interferon – gamma using Taqman® Real Time Polymerase Chain Reaction. Data are expressed as cytokine fold change corresponding to ratio of interferon – γ messenger RNA copies produced in antigen stimulated versus unstimulated cells. Interferon- γ fold change was compared between cases and controls using the unpaired student's– t test after log transformation and expressed as median (interquartile range).

Results: The interferon- γ fold change was higher in cases 61.33 (20.54 – 217.00) than controls 49.67 (10.05 – 157.05). Mean fold change difference between cases and controls was 1.66 times (95% confidence interval 1.17 – 2.34, p = 0.02).

Conclusion: The results of this study indicate that mononuclear immune cells hyper- reactive to HSV- 1 are present in the peripheral blood of patients with primary achalasia and may contribute to the pathological changes observed in the myenteric plexus.

1 Park W, Vaezi MF. Etiology and pathogenesis of achalasia: the current understanding. *Am J Gastroenterol* 2005;**100:**1404-14.

2 Castagliuolo I, Brun P, Costantini M, Rizzetto C, Palu G, Costantino M, Baldan N, Zaninotto G. Esophageal achalasia: is the herpes simplex virus really innocent? *J Gastrointest Surg* 2004;**8:**24-30.

5. The value of PSA testing in men older than 65 years

David Connolly[a], Amanda Black[b], Liam J. Murray[b], Anna Gavin[c], Patrick F Keane[a].

a Department of Urology, Belfast City Hospital,

b Cancer Epidemiology & Prevention Research Group, Centre for Clinical and Population Sciences, Queen's University Belfast,

c Northern Ireland Cancer Registry, Queen's University Belfast.

Introduction: Many men ≥65 years old have histological prostate cancer. Only a small proportion may present clinically with the disease and relatively few will die from prostate cancer. We assessed baseline PSA levels and the risk of clinically detected prostate cancer and prostate specific mortality in this population.

Methods: From a regional PSA database, all men aged ≥65 years old who had their first PSA test between 1994 and 1998 were identified. These were followed for prostate cancer diagnosis and mortality until 2003. The absolute risk and hazard ratio for prostate cancer diagnosis and mortality, based

on baseline PSA level, were determined.

Results: 36003 men were included. Mean age was 74.9 years and mean follow-up 5.4 years. 2153 (6.0%) men were diagnosed with prostate cancer. 13074 (36.3%) died, with prostate cancer the cause of death in 673 men (5.1% of deaths). Within age groups, the absolute risk and hazard ratio of cancer increased incrementally with PSA level (Table). Prostate-specific mortality remained low (<5/1000 person years) at all PSA categories <15.0ng/ml. All-cause mortality was similar in PSA categories <10.0ng/ml, and was much greater than prostate-specific mortality across all PSA categories.

Conclusion: The risk of prostate cancer diagnosis and prostate specific mortality is related to baseline PSA level. However, in this age group, death from prostate cancer was infrequent compared to other causes, even when baseline PSA was markedly elevated (up to 20.0ng/ml). A conservative approach to invasive investigation may be appropriate in men older than 65 years.

6. A randomised interventional trial of omega-3-polyunsaturated fatty acids on endothelial function and disease activity in systemic lupus erythematosus.

Stephen A Wright[1,2,3,] Fiona M O'Prey[1], Michelle T McHenry[2,3], William J Leahey[1], Adrian B Devine[1], Emeir M Duffy[2,4], Dennis G Johnston[1], Michael B Finch[2,3], Gary E McVeigh[1], Aubrey L Bell[2,3.]

[1] Department of Therapeutics and Pharmacology, Queens University Belfast, Northern Ireland,

[2] Lupus Research Group, Queens University Belfast, Northern Ireland,

[3] Department of Rheumatology, Musgrave Park Hospital, Belfast, Northern Ireland,

[4] Northern Ireland Centre for Food and Health (NICHE), Department of Biomedical Sciences, University of Ulster, Northern Ireland

Objective: To determine the clinical effect of dietary supplementation with low dose omega-3-polyunsaturated fatty acids on disease activity and endothelial function in patients with systemic lupus erythematosus.

Methods: A 24 week randomised double-blind placebo-controlled parallel trial of the effect of 3g of omega-3-polyunsaturated fatty acids on 60 patients with SLE was performed. Serial measurements of disease activity using the revised Systemic Lupus Activity Measure (SLAM-R) and British Isles Lupus Assessment Group index of disease activity for SLE (BILAG), endothelial function using flow mediated dilation of the brachial artery (FMD), oxidative stress using platelet 8-isoprostanes and analysis of platelet membrane fatty acids were taken at baseline, 12 and 24 weeks.

Results: In the fish oil group there was a significant improvement at 24 weeks in SLAM-R (from 9.4±3.0 to 6.3±2.5, $p<0.001$); in BILAG (from 13.6±6.0 to 6.7±3.8, $p<0.001$); in FMD (from 3.0% (-0.5-8.2) to 8.9% (1.3-16.9), $p<0.001$) and in platelet 8-isoprostanes (from 177pg/mg protein (23 – 387) to 90 pg/mg protein (32 – 182), $p = 0.007$).

Conclusions: Low dose dietary supplementation with omega-3 fish oils in SLE not only has a therapeutic effect on disease activity but also improves endothelial function and reduces oxidative stress and may therefore confer cardiovascular benefits.

7. The systemic effects of cilostazol on exercise-induced lower limb ischaemia-reperfusion in patients with peripheral arterial disease.

ME O'Donnell [1], SA Badger [1], RR Makar [1], J McEneny [2], IS Young [2], LL Lau [1], B Lee [1], RJ Hannon [1], CV Soong [1].

Department of Vascular and Endovascular Surgery, Belfast City Hospital [1] and Department of Medicine, Queen's University Belfast[2], Northern Ireland.

Objectives: The phosphodiesterase-3 inhibitor Cilostazol improves walking distance in peripheral arterial disease

	Absolute rate of cancer / 1000 person years (Hazard Ratio*)					
PSA level	65 - 69	70 - 75	75 - 79	≥80	Prostate specific mortality†	All cause mortality†
0.0-1.99	0.8 (1.0)	1.2 (1.0)	1.5 (1.0)	2.3 (1.0)	0.3 (1.0)	57.7 (1.0)
2.0-3.99	3.2 (3.8)	2.3 (1.9)	2.6 (1.8)	4.4 (1.9)	0.6 (2.3)	61.3 (1.0)
4.0-5.99	7.7 (9.2)	5.7 (4.7)	4.0 (2.7)	5.6 (2.5)	1.1 (3.8)	60.3 (1.0)
6.0-7.99	12.8 (15.3)	10.9 (8.9)	9.8 (6.6)	9.2 (4.0)	1.8 (6.4)	65.0 (1.0)
8.0-9.99	20.1 (23.8)	16.5 (13.4)	10.2 (6.9)	11.2 (5.0)	3.3 (11.8)	64.2 (1.0)
10.0-14.99	22.6 (26.7)	29.1 (23.1)	21.1 (14.0)	15.6 (6.6)	3.9 (13.5)	76.2 (1.1)
15.0-19.99	44.2 (51.3)	37.0 (28.9)	36.3 (23.4)	35.8 (14.6)	8.4 (28.6)	86.6 (1.2)
≥20.0	105.1 (115.2)	107.0 (79.1)	131.6 (77.2)	115.2 (45.8)	34.0 (112.8)	112.9 (1.5)
No. of cancers	476	580	509	588	673	13074
No. of patients	9933	9884	7978	8154	36003	36003

*0.0 - 1.99 used as reference category, †All men, age-adjusted

(PAD) patients through an increase in cyclic AMP levels. The study objective was to assess the effects of cilostazol on the inflammatory response post-exercise in such patients.

Methods: PAD patients were prospectively recruited to a randomised double-blinded, placebo-controlled trial. Baseline clinical data were recorded following medical optimisation. Initial and absolute walking distances were measured on a validated treadmill protocol. Inflammatory response was assessed before and 30-minutes post-exercise by serum lipid hydroperoxide, interleukins 6 and 10, intracellular and vascular cell-adhesion-molecules (I-CAM & V-CAM), highly-selective C-reactive protein (hsCRP) measurement and plasma ascorbate analysis. All tests were at baseline, 6-weeks and 6-months.

Results: 106 PAD patients (72 males) were recruited from August 2004 to August 2006 (overall median age: 66.5, range 37-86). 26 patients were diabetic. Treatment limbs were demographically and medically matched. Patients who received cilostazol, compared to placebo, demonstrated a mean percentage improvement from baseline in absolute claudication distance (77.2% vs. 26.6% at 6 weeks and 161.7% vs. 79.0% at 6 months, $p<0.05$, t-test). There was a reduction from baseline lipid hydroperoxide levels in the cilostazol group compared to an increase in the placebo group before and after exercise (6-weeks: pre-exercise: -11.8% vs. +5.8% and post-exercise: -12.3% vs. +13.9%) (6-months: pre-exercise -15.5% vs. +12.0% and post-exercise: -9.2% vs. +1.9%) ($p<0.01$, MWU-test). Cilostazol significantly reduced I-CAM and V-CAM levels at 24-weeks compared to baseline ($p<0.05$, WSR-test). However, there was no difference between groups for interleukins 6 and 10, ascorbate or hsCRP levels.

Conclusions: Cilostazol is a highly efficacious treatment for improving walking distance in patients with PAD with additional beneficial effects on lower limb ischaemia-reperfusion both before and after walking.

B. POSTER PRESENTATION CASE REPORTS

1. Vasoactive intestinal polypeptide secreting pancreatic tumour (VIPoma): long term survival after orthotopic liver transplantation.

Johnston PC[1], Ardill JE[2], Johnston BM[2], Mc Cance DR[1, 2]

[1] Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, Belfast, BT12 6BA.

[2] Neuroendocrine Tumour Group, Royal Victoria Hospital, Belfast, BT12 6BA.

A 46 year old male presented in 1981 with a two year history of profuse watery diarrhoea, three stone weight loss and fatigue. On examination he appeared gaunt with diffuse muscle weakness. Investigations revealed hypokalaemia (2.5mmol/L; NR 3.5-4.5), achlorhydria and a raised vasoactive intestinal polypeptide (VIP) (1500ng/L; NR 0-100). Abdominal CT showed a 5 cm pancreatic mass but with no focal liver pathology. A distal pancreatectomy was performed. Histology confirmed an islet cell carcinoma (VIPoma).

His symptoms recurred one year post surgery, at which time liver metastases were demonstrated radiologically. He responded initially to three courses of Streptozotocin but ultimately developed resistance. For fifteen years his symptoms were controlled by octreotide injections, initially Sandostatin (subcutaneously) and later Sandostatin LAR. The patient also underwent hepatic chemoembolisation.

By 1997, sixteen years after his initial surgery, treatment failure occurred with a profound deterioration clinically and debilitating diarrhoea. No evidence of extra hepatic disease was found. After extensive discussion he underwent orthotopic liver transplantation which resulted in resolution of his symptoms.

Recurrence was noted two years post transplant in the para-aortic lymph nodes but not in the liver. He remained mildly symptomatic with gradual deterioration of his general health and died 9 years after liver transplantation.

This case is one of the longest reported (25 years) survivors of a VIPoma after initial diagnosis. The case also has several notable features including the absence of liver metastases at diagnosis and the variety of treatment modalities used for symptom control including successful orthotopic liver transplantation.

2. Opsoclonus Myoclonus Syndrome as a paraneoplastic manifestation of benign ovarian teratoma

AS Fitzpatrick, OM Gray, JP McConville, GV McDonnell

Dept of Neurology, Royal Victoria Hospital, Belfast, BT12 6BA

The Opsoclonus Myoclonus Syndrome (OMS) is characterised by nonrhythmic involuntary ocular oscillations, axial and segmentary myoclonia and cerebellar ataxia. It can be a post-infectious, paraneoplastic or idiopathic phenomenon; most commonly associated with neuroblastoma in children and lung or ovarian malignancies in adults. We report the case of a fifteen-year-old girl who presented with subacute onset of opsoclonus and myoclonus, ataxia, nausea and vomiting. Investigation excluded infection, neuroblastoma, chest and breast malignancy but revealed a right-sided benign ovarian teratoma. Paraneoplastic and atypical antibodies including antibodies to neuronal surface antigens and NMDAR antigens were not identified and tumour markers were normal.

The patient was treated with immunomodulatory treatment including intravenous steroids and immunoglobulin but showed the most improvement in response to surgical removal of the teratoma. We discuss OMS as a paraneoplastic manifestation of benign ovarian teratoma. Case reports have suggested a variety of neurological paraneoplastic syndromes associated with this tumour but its association with OMS has not previously been described. We have video evidence pre and post treatment.

3. Macroprolactinomas presenting as nasal polyps: a series of three cases.

PC Johnston, HC Courtney, SJ Hunter, DR McCance

Regional Centre for Diabetes and Endocrinology, Royal Victoria Hospital, Belfast, UK

Intranasal presentations of pituitary tumours are rare. Management can be difficult and delayed due to their location and extension. Macroprolactinomas are uncommon and can

often pursue an aggressive clinical course, including invasion into the nasopharynx.

We describe three cases of prolactinomas that initially presented to the ENT Department as nasal polyps. We describe their clinical features and response to treatment. Recurrence of nasal polyps (patient 1) and radiological evidence of a pituitary mass (patients 2 & 3) prompted testing for a prolactinoma. None of the patients had any signs of hyperprolactinaemia. All have significant residual tumour at follow up, despite prolactin levels approaching the normal range on dopaminergic therapy.

Pituitary tumours that invade the nasal cavity are rare and clinicians should be aware of their existence. Measurement of serum prolactin and immuno-histochemistry for prolactin secreting cells in intranasal tumours should be considered if there is clinical evidence of hyperprolactinaemia or if there is recurrence of the nasal tumour/polyp. This can expedite a diagnosis and prevent delay of treatment with dopamine agonists. Dopaminergic therapy controls excessive prolactin secretion and results in tumour shrinkage in most patients, but treatment may be complicated by dopamine resistance, extensive tumour necrosis and CSF rhinorrhoea.

4. Atypical Addison's Disease. -A case of Familial Glucocorticoid Deficiency.

GM Magee, A Thiraviaraj, CH Courtney, SJ Hunter

Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, Grosvenor Road, Belfast, BT12 6BA

A 2 yr old boy presented initially with hypoglycaemia following a 6h fast prior to an elective surgical procedure. Investigations demonstrated low plasma glucose (1.6mmol/l), undetectable serum insulin, elevated beta hydroxybutyrate (5.2mmol/l) and normal lactate. Plasma amino-acid profile was normal. The dorsum of his hands were pigmented, however a synacthen test demonstrated a normal cortisol response. Ketotic hypoglycaemia was diagnosed, and his parents were given dietary advice. At age 11, he was referred to medical genetics with poor coordination, delayed fine motor skills and a dysmorphic appearance. Chromosomal analysis revealed a normal male karyotype, and no specific diagnosis was made.

He presented at age 18 to our unit with recurrent hypoglycaemia. A 72h fast was terminated after 10 h as the patient was symptomatic with plasma glucose 1.6mmol/l. A synacthen test demonstrated an absent cortisol response and elevated ACTH level. Serum electrolytes were normal, adrenal autoantibodies were absent and very long chain fatty acids were normal. He was commenced on both glucocorticoid and mineralocorticoid replacement and had no further hypoglycaemic episodes.

Later the patient's brother also presented at age 16 with hypoglycaemia when he was unwell with nausea and vomiting. A diagnosis of primary adrenal insufficiency was confirmed - also with normal serum electrolytes and absent adrenal autoantibodies.

Further genetic analysis revealed that both brothers were homozygous for the S74I mutation of the MC2 (melanocortin 2) receptor and a diagnosis of Familial Glucocorticoid Deficiency (FGD) was made. This rare autosomal recessive ACTH insensitivity syndrome responds to glucocorticoid replacement alone.

Ulster Med J 2008; **77 (1)** 65-76

Abstracts

10th Meeting of the Irish Society of Human Genetics, Monday 24th September 2007.

Postgraduate Centre, Belfast City Hospital, Belfast.

PROGRAMME:

10.00 – 11.00	Registration / Tea and coffee
11.00 – 11.01	Welcome
11.01 – 12.00	Spoken presentations Plenary I
12.00 – 13.00	**Keynote address:** "Sweet dreams: using genome wide association methods to find genes for diabetes and obesity" Mark McCarthy, University of Oxford
13.00 – 14.00	Lunch + poster viewing
14.00 – 15.30	Spoken presentations Plenary II
15.30 – 16.00	Tea / coffee & poster viewing
16.00 – 16.15	Business meeting
16.15 – 17.15	**Plenary address:** "Genomic Approaches to Brain Diseases". Guy Rouleau, McGill University
17.15 – 18.00	Wine Reception / Presentations / meeting close

SPOKEN PAPERS:

S1. A prospective study of referrals from the Irish Traveller community to the National Centre for Inherited Metabolic Disorders

AM Murphy, C Halling, SA Lynch, AA Monavari, S Harty, E Crushell, EP Treacy.

The National Centre for Inherited Metabolic Disorders (NCIMD) and National Centre for Medical Genetics (NCMG), Dublin.

Irish Travellers are a nomadic people in whom early marriage, frequent child bearing and consanguinity are cultural norms. They number 22,445, <0.5% of the Irish population, 9.6% of the 1465 patients listed at NCIMD on January 1st 2007 were Travellers. To date 21 different inherited metabolic disorders (IMDs) are reported. Our aim was to prospectively survey all referrals from this community .The study is part of a larger ongoing project to compile a database of "Irish Traveller" genetic disorders to ensure appropriate planning of services and provision of care.

All referrals between January 1st and June 30th 2007 were reviewed, those with a Traveller background identified and the following information sought; source, reason & age of referral, diagnosis & outcome.

Twenty eight (age range 1 day-16 yrs) of the 84 (33%) patients referred were Travellers. 15 new diagnoses of IMDs were established; of which 3 were genetic disorders not previously noted in this population (MSUD, X linked ALD and Hyperinsulinism). Six patients were diagnosed because of a family member with an IMD. Eight are under investigation for suspected metabolic disorder (mitochondrial). The remaining five are thought to have an undiagnosed autosomal recessive disorder because of the presence of multiple affected siblings. Apart from the patient with mucolipidosis, appropriate treatment was commenced once diagnosis was established with a good outcome to date.

This study highlights the huge disease burden imposed by the increased frequency of genetic disorders in consanguinous communities and provides useful epidemiological information for service planning for patients with IMDs with particular reference to the Irish Traveller community

S2. Counselling issues in a family with a presumed non-pathogenic mutation in TSC2.

Crawford H, McKee SA.

The Northern Ireland Tuberous Sclerosis Clinic, Northern Ireland Regional Genetics Centre, Belfast Health and Social Care Trust, A Floor, Belfast City Hospital, Lisburn Road, Belfast BT9 7AB

A diagnosis of Tuberous Sclerosis (TSC) can impact significantly on both the individual and the family. We report a family where the clinical presentation and genetic findings have presented difficulties in approaching management. Case 1 is an 8 year-old boy, who presented at age 8 with mild angiofibromata, but with no history of seizures or learning difficulty. MRI brain scan reported subcortical and periventricular hamartomas, and a small shagreen patch was found on clinical examination. He therefore fulfilled the diagnostic criteria for TSC. Mutation analysis identified a missense change in TSC2 – R261W – which was also found in the child's mother. No other mutations were found, and the mutation is felt to be a rare non-pathogenic variant.

S3. Disorders of cholesterol biosynthesis in Ireland

J Chukwu, C Halling, ATaha, SA Lynch, AA Monavari, EP Treacy, AM Murphy.

The National Centre for Inherited Metabolic Disorders (NCIMD) and The National Centre for Medical Genetics, OLCH, Crumlin Dublin 12. (NCMG)

Seven disorders of cholesterol biosynthesis are recognized of which Smith-Lemli- Opitz syndrome (SLOS) is the most common. These disorders are associated with major developmental malformations, unusual for metabolic diseases.

We reviewed the databases at the NCIMD and NCMG in order to identify all patients with inborn errors of cholesterol synthesis diagnosed in the 10 year period between June 1st 1997 and June 1st 2007. Clinical features (congenital malformations, behavioural

phenotype, growth and developmental profile), biochemical features (plasma sterol profile at diagnosis), genotype, ethnic background and treatment were noted.

Seven patients (age range 3-45yrs) attend the NCIMD making cholesterol pathway defects the 13th most common condition treated here. An additional 6 deceased patients were identified from the database at the NCMGs. All patients had SLOS. None of the other 6 disorders were identified.

The seven patients are being treated with cholesterol supplementation in the form of a powder or egg yolk. The dose of cholesterol supplementation is titrated by monitoring growth, cholesterol and 7 dehydro-cholesterol levels and adjusting levels accordingly. There has been subjective improvement in general well-being and behaviour. Anecdotally parental perception is that supplementation has a huge beneficial effect. Cholesterol biosynthetic disorders are rare disorders in the Irish population but important to recognize as they are partially treatable.

S4. Enzyme Replacement Therapy in Northern Ireland

Fiona J Stewart.

Northern Ireland Regional Genetics Service, Belfast City Hospital, Belfast BT9 7AB Fiona.Stewart@belfasttrust.hscni.net

The first commercially available ERT was imiglucerase for the treatment of Gaucher disease. This was available in Europe from 1997. Since then ERT has become available for Fabry disease, mucopolysaccharidosis type I (Hurler, Hurler-Scheie and Scheie), mucopolysaccharidosis type II (Hunter) and Pompe disease. In Great Britain patients travel to one of the recognised NSCAG centres to receive their ERT. Patients in Northern Ireland have been receiving ERT for nearly 5 years – some initially as part of clinical trials.

Current patients on treatment:

Disease	Total patients	Adult/ children	Male/ female	Infusions
Fabry	8	8 / 0	7 / 1	Fortnightly
MPS I	4	1 / 3	1 / 3	Weekly
MPS II	2	1/1	2/0	Weekly
Pompe	Due to start			

In addition we have treated 4 children with MPS I (Hurler) with a finite course of ERT pre and post bone-marrow transplant. All patients have reported an improvement in their quality of life and clinical improvement has been confirmed by regular assessments and supporting data will be presented. Many of our patients are now on home infusions and this is working very well.

S5. Natural selection and disease susceptibility at the coagulation *F13B* locus

Anthony W Ryan[1,2,3], David Hughes[1], Thomas Ryan[2], Ross McManus[2] and Mark Stoneking[1].

[1] Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, D-04103 Leipzig, Germany.

[2] Department of Clinical Medicine, Trinity College, Dublin and Institute of Molecular Medicine, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8, Ireland.

[3] E: aryan12@tcd.ie

High levels of inter-population differentiation at the coagulation *F13B* locus may be interpreted as evidence of localised natural selection. However, a neutral explanation is also possible. We re-sequenced 4.6kb of the gene, encompassing all exons, splice junctions and 1.4kb of the promoter in African, European and Asian samples. This revealed three major lineages, which correspond to the common protein alleles and differ from each other at a non-synonymous substitution in exon 3 and a novel splice acceptor in intron K. There is evidence that these lineages are not functionally equivalent, a pre-requisite for the action of natural selection. Furthermore, our case-control analyses confirm that variability at this locus modifies susceptibility to myocardial infarct (OR = 1.88 [1.18 – 2.99], P = 0.0047). When our sequence data were combined with additional sequences from the *SeattleSNPs* database, Fu and Li's test for selection suggested a significant departure from neutral expectations (D* = -2.92556, P = 0.02). Patterns of extended haplotype homozygosity from HapMap populations also provide evidence of adaptation ($P < 0.05$). Thus, several independent lines of evidence suggest that the *F13B* locus has been subjected to localised natural selection during recent human evolution. Possible causes of this selection are discussed.

S6. An assessment of Ireland as a population for whole genome association studies

Colm Ó'Dúshláine, Ciara Dolan, Alice Stanton, David Croke, Reetta Kalviainen, Samuel Berkovic, Terry O'Brien, Sanjay Sisodiya, David Goldstein, Derek Morris, Norman Delanty, Gianpiero Cavalleri.

Trinity College Dublin, St James' Hospital, Dublin 8.

The transferability of HapMap SNPs to different populations is a significant factor determining the success of whole-genome association studies. We examined the extent to which the linkage disequilibrium of HapMap SNPs agreed with the same estimates for these SNPs within a number of populations. Comparisons were made for "test" populations of Caucasian individuals from Ireland, UK, Finland and Australia (4424 SNPs genotyped in 1178 individuals, covering 279 genes). Higher overall concordance was observed between HapMap CEPH individuals and Irish and UK populations (Spearman Rho 0.72, $p<0.0005$), the latter also exhibiting the highest level of similarity to each other from pairwise comparisons all our test populations (Spearman Rho 0.76, $p<0.0005$). Similar results were obtained when comparing haplotype diversity (Spearman Rho IRL=0.96, $p<0.0005$; UK=0.97, $p<0.0005$) and tag portability estimates (Spearman Rho IRL=0.55, $p=0.0004$; UK=0.58, $p=0.0002$). These findings have implications for researchers seeking to carry out fine mapping studies of disease susceptibility loci, particularly when these loci are identified from studies where candidate SNPs are derived from a HapMap reference. Specifically, our data shows that certain populations are in better agreement with HapMap than others and thus are likely to have more power in identifying disease susceptibility loci.

S7. The glutamatergic synapse protein HOMER2 is associated with schizophrenia in the Irish population

William P. Gilks[1], Emma Allott[1], Gary Donohoe[1], John L. Waddington[2], Michael Gill[1], Aiden P. Corvin[1], Derek W. Morris[1]

[1] Neuropsychiatric Genetics Research Group, IMM and Dept. of Psychiatry, Trinity College Dublin, Ireland. [2]Royal College of Surgeons in Ireland, Dublin, Ireland

Glutamatergic synapse dysfunction has been implicated in schizophrenia pathogenesis. To identify potential susceptibility

genes, we combined data from genome-wide linkage studies, the synaptic proteome and from keyword searches of genomic databases for glutamatergic genes. HOMER2 is located at chromosome 15q24, a region of significant linkage to schizophrenia. The HOMER2 protein forms a scaffold for post-synaptic glutamate receptors. To investigate its role in schizophrenia we selected HapMap-based tagging SNPs, integrating our own novel HapMap genotype data on five predicted functional SNPs into the SNP selection algorithm. We genotyped 12 tagging SNPs in our Irish sample of 375 cases and 812 controls. Single-marker association analysis showed disease association at rs869498, rs7174726 and rs12913501 (each SNP $p<0.05$, OR>1.3). These three SNPs are located in a 25kb region of intron 1 of the gene but are not in high linkage disequilibrium with each other ($r^2=0.02$). There was significant association of all two-marker haplotypes of the three SNPs, notably rs7174726-rs12913501 ($p<0.0005$). This 25kb region covers 4kb unique to higher primates strongly predicted to contain a transcription factor binding-site. HOMER2 is developmentally regulated, controlling synaptic plasticity and calcium homeostasis. This information, combined with our association results identifies HOMER2 as a putative susceptibility gene for schizophrenia.

S8. A linkage and association study of hip osteoarthritis.

D McGibbon, C Benson, G Meenagh, G Wright, M Doherty, A Hughes.

Queen's University Belfast.

Genetic and environmental risk factors affect risk of osteoarthritis (OA). Our study aims to identify susceptibility genes for hip OA.

A total of 426 Northern Ireland hip OA patients were genotyped using microsatellite markers and non-parametric linkage analysis carried out on affected sib-pairs. A peak LOD score of 1.64 (p=0.003) at 25cM on chromosome 19 indicated this region as potentially harbouring an osteoarthritis susceptibility gene. The best candidate gene in this region was *COL5A3*, which is flanked by genes *OLFM2* and *RDH8*. An association study using single nucleotide polymorphisms (SNPs) on unrelated Northern Ireland cases confirmed interest within this region with a significant p-value for SNP rs4804474 in *OLFM2* (p=0.016). This result was reproduced in a separate collection of 280 hip OA cases collected in Nottingham (p=0.03). The Northern Ireland and Nottingham cases combined gave the most significant p value of 0.009 for rs4804474. SNP's rs37455849(*COL5A3*) and rs889128(*RDH8*) give significant p-values in the Northern Ireland and Nottingham cases respectively of 0.037 and 0.025 but these values could not be reproduced. Further SNP genotyping across this region is required to fully elucidate the pattern of association and the location of the hip OA susceptibility gene.

S9. The Pathway to Breast Cancer Invasion.

Seona McErlean, Natalie Scott, Jenny Worthington, Gillian Brown, Adriana Falchi, Daniel Berrar, Anthony J Bjourson.

Institute of Biomedical Sciences, University of Ulster, Cromore Road Coleraine. N. Ireland BT52 1SA. aj.Bjourson@ulster.ac.uk.

Background: Considering the fact that the majority of breast cancer deaths are due to metastatic rather than the primary tumours, comparatively little consensus exists on the mechanisms of cancer invasion and spread to distant sites. In an attempt to better understand the invasive and metastatic processes we developed a model of breast cancer invasion and metastasis *in vitro* and subsequently validated this in a mouse model.

Methods: We developed a minimalist model of breast cancer invasion *in vitro* by converting a well known weakly-invasive breast cancer cell line into a series of progressively hyper-invasive sub-clones, ranging from non-invasive – to highly invasive. To identify the master regulators of invasion we performed micro-RNA (miRNA), messenger RNA (mRNA) transcriptional profiling and selected promoter methylation analyses on the non-invasive parental and selected hyper-invasive sub-clones. Systems biology pathway analyses was employed to identify genes that may represent key regulators of invasion. To validate the *in vitro* data, the parental (non-invasive) and hyper-invasive lines were stably transfected with a luciferase and their growth and metastatic spread was monitored in SCID mice using a Xenogen whole body imaging system.

Results: The pathway to invasion was clearly associated with an epithelial-mesenchymal transition (EMT), and a profound reduction in extracellular matrix (ECM) adhesion, altered cadherin expression, and silencing of interferon-γ (IFNγ) responsive genes consistent with archetypal immunoediting. Significantly however, this occurred independent of any Darwinian immune selective pressure and the simple process of selecting hyper-invasive cells concomitantly selected for a population that surprisingly, were also highly resistant to apoptosis (tolerant of hypoxia, more resistant to γ radiation, and more Trail-resistant). In addition, whole body imaging demonstrated that the *in vitro* selected cells were extremely invasive *in vivo* in SCID mice and rapidly metastasised to multiple organs within 3-4 weeks.

Conclusion: We have generated a useful model of invasion and metastasis. The genes identified appear to be directly related to the *primary cause*, rather than the *consequence* of cancer invasion & metastasis and may find utility as novel biomarkers and novel targets for therapeutic intervention.

S10. Sexual antagonism and autism susceptibility in the Xq/Yq pseudoautosomal region (PAR2)

Andrew McLellan, Freda Wynne, Melanie Ball, Tom Moore.

Department of Biochemistry, Biosciences Institute, University College Cork, College Road, Cork, Ireland. E: t.moore@ucc.ie

Autism is a neurobehavioural disorder associated with impaired language development, poor social interactions and stereotyped repetitive behaviours. We hypothesised that deregulation of the Xq/Yq pseudoautosomal region (PAR2) is involved in the profoundly male-biased affected sex ratio in autism. We therefore carried out TDT analysis on 95 autism multiplex families using 21 genetic markers in this region. In the proximal zone, which contains the brain-expressed imprinted *SPRY3* and *SYBL1* genes, we observed that multiple markers exhibited linkage / association with autism. In a further analysis involving datasets from which all male offspring or all female offspring were removed, we observed over-transmission of 'opposite' marker alleles to affected males and females for approximately half of the marker loci examined. We interpret these findings as evidence of sexually antagonistic selection operating at this locus. Our observations have general implications for human genetic studies and, more specifically, for the evolution and function of PAR2 genes.

POSTER PRESENTATIONS:

P1. EuroGentest: Quality Management and accreditation of genetic testing services

David E. Barton[1], Ros J. Hastings[2], Sarah Berwouts[3], Christine Brady[1], Philippe Corbisier[4], Anniek Corveleyn[3], Rob Elles[5], Brian Fowler[6], David Gancberg[4], Piotr Litynski[6], Milan Macek Jr[7], Ute Malburg[8], Gert Matthijs[3], Michael Morris[9], Clemens Mueller[8], Nick Nagels[3], Bettina Quellhorst-Pawley[2], Alexandra Stambergova[7], Jan Vermeesch[3], Kate Vickers[5], Elisabeth Dequeker[3].

1 National Centre for Medical Genetics, Dublin, Ireland,
2 John Radcliffe Hospital, Oxford, United Kingdom,
3 KU Leuven, Leuven, Belgium,
4 European Commission, Geel, Belgium,
5 St. Mary's Hospital, Manchester, United Kingdom,
6 University Children's Hospital, Basel, Switzerland,
7 Charles University, Prague, Czech Republic,
8 Universitaet Würzburg, Würzburg, Germany,
9 CMU, Geneva, Switzerland.

The EuroGentest network aims to improve and harmonize the quality of genetic services in Europe, from test development through to information for patients. The network encompasses Biochemical, Clinical, Cyto- and Molecular Genetics, Genetic Counselling and patient groups. Since January 2005, the EuroGentest Quality Management group has disseminated information on accreditation through five international workshops. A database on the current status of QAu in European genetic testing services will soon be publicly available. On the EuroGentest website, laboratories can find the EQA scheme most appropriate to their needs through discipline specific registers of schemes in Europe. All three laboratory disciplines have expanded their repertoire of EQA including a pilot pan-European cytogenetics scheme, CEQA. Minimum quality guidelines have been published for cytogenetics and some biochemical analytes. Draft guidelines for microarrays will be published later this year. In collaboration with EMQN, best practice meetings will be organised in 2007 for Familial Breast Cancer, Spinocerebellar Ataxias and Maturity Onset Diabetes of the Young to generate consensus guidelines. Finally QCMs for Prader-Willi/Angelman syndromes are being developed and validation of MLPA, diagnostic CF-testing kits and DNA extraction methods are in progress through a core group of accredited laboratories with reports due this year.

P2. Osteopetrosis: clinical and skeletal findings in 2 early childhood cases.

Alex Magee[1], Sally Ann Lynch[2], Amaka Offiah[3].

1. Regional Genetics Service, Belfast City Hospital.
2. National Centre for Medical Genetics, Our Lady's Hospital, Dublin.
3. Department of Paediatric Radiology, Great Ormond Street Hospital.

Osteopetrosis is a rare, heterogeneous condition, characterised by osteoclast failure and classified into three forms: infantile malignant autosomal recessive osteopetrosis (ARO), intermediate autosomal recessive osteopetrosis (IRO) and autosomal dominant osteopetrosis (ADO). We present an unrelated 8 year-old girl and 5 year-old boy with a clinical and skeletal diagnosis of osteopetrosis and discuss the difficulties in determining recurrence risk in isolated cases.

Case 1. Elder of 2 sibs; non-consanguineous, clinically normal parents. Referred because of dental anomalies. Noted to have macrocephaly, short stature and prominent upper tibiae. No history of fractures. Skeletal survey showed findings consistent with IRO. No evidence of bone marrow compromise, abnormal renal function or cranial nerve compression.

Case 2. Youngest of 3 sibs; non-consanguineous, clinically normal parents. Fractures of both tibiae following trivial injuries age 2y. Normal stature with frontal bossing. Skeletal survey suggested type 1 ADO although IRO not out ruled. No evidence of bone marrow compromise or abnormal renal function.

Mutations in the ClCN7, ATP6i and other genes have been identified, but not all genes determining OP are known. In isolated cases of OP, diagnosis of type, and therefore recurrence risk, still relies on clinical and radiological findings. Some cases remain difficult to classify resulting in ambiguity over recurrence risks.

P3. The Genetic Basis Of Autosomal Recessive Osteogenesis Imperfecta In The Irish Traveller Population

J Walsh[1], D Lambert [1], DM Baldridge[2], R Morello[2], D Eyre[3], B Lee[2], AJ Green[1,4]

1. National Centre for Medical Genetics, Our Lady's Hospital, Crumlin, Dublin, Ireland,
2. Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas.
3. Department of Orthopaedic Surgery, University of Washington, Seattle, Washington, and School of Medicine and Medical Science, University College Dublin

Osteogenesis Imperfecta (OI) is usually an autosomal dominant disorder, and is clinically classified according to the Sillence classification of 1-IV. However, the Irish Traveller population has an autosomal recessive form of severe OI, which fits with type II/III in the Sillence classification. We have identified 16 patients in 5 extended Traveller families, where almost all the affected children are born with severe limb and thoracic deformities due to multiple fractures, including *in utero* fractures. Most have died within 6 months, of respiratory compromise. However, there are two surviving affected children at ages 5 years.

No type I collagen abnormality has been described in the Irish Traveller OI (Pope *et al.* 1989). Recently, the genetic basis of one form of autosomal recessive OI has been found, due to homozygous mutations in a gene CRTAP or cartilage associated protein (Morello *et al.* 2006). CRTAP is homologous to a family of prolyl 3-hydroxylases which modify collagen, and mutations affected the modification of collagen fibrils. A partner protein for CTRAP, LEPRE1 or prolyl 3-hydroxylase 1 (P3H1) has also found to be the basis of another autosomal recessive form of OI in people of African origin (Cabral *et. al.* 2007).

Samples from 3 affected Irish Traveller children were analysed for mutations in CRTAP and P3H1. No mutations were found in CTRAP, but all three were homozygous for a frameshift mutation c.232delC in exon 1 of the P3H1 gene. Cultured fibroblasts from one affected case were analysed by mass spectroscopy for prolyl 3-hydroxylation of type I collagen. The level of hydroxylation was markedly reduced, at a level of 15%, compared to 95-98% seen in normal controls.

These findings have now identified the genetic basis for autosomal recessive OI in the Irish travellers. This will now lead to improved OI diagnosis and genetic counselling for Travellers who have a family history of severe or lethal OI. In addition, these findings give further insights into the biology of bone collagen.

P4. Investigation of the impact of the 19bp Deletion polymorphism in Intron 1 of Dihydrofolate Reductase (DHFR) on gene expression.

Kirsty O'Brien,Anne Parle-McDermott.

Nutritional Genomics Group, School of Biotechnology, Dublin City University.

Dihydrofolate reductase (DHFR) is an important folate metabolising enzyme that is essential to cellular proliferation because of its role in regenerating tetrahydrofolate (THF) from dihydrofolate (DHF), which is formed during the folate-linked synthesis of thymidine. Folate genes are considered candidates for association with neural tube defects (NTDs) such as spina bifida due to the preventative effect of periconceptional maternal supplementation with folic acid. Investigation of an intronic 19bp deletion polymorphism within the *DHFR* gene found a significant protective effect in mothers of NTD cases when present in one (Relative Risk 0.59 (95%CI: 0.39-0.89), p= 0.01) or two copies (Relative Risk 0.52 (95%CI: 0.32-0.86), p= 0.01). Analysis of mRNA levels revealed a small increase in expression (~1.5 fold) associated with the 19bp intronic deletion polymorphism, but this was not significant (Parle-McDermott *et al*., *Am J Med Genet* 2007;**143(11)**:1174-1180).

We sought to further investigate the potential impact of the DHFR 19bp intronic deletion polymorphism on gene expression by employing a recombinant dual luciferase system. PCR products representative of DHFR intron 1 with and without the 19bp deletion were cloned into a Gateway® compatible pGl_3- promoter vector and verified by sequencing. Luciferase assays will be performed in HEK293 cells and the data presented.

P5. Disease frequency of Inborn Errors of Metabolism in the Irish Traveller Community

AM Murphy.

The National Centre for Inherited Metabolic Disorders (NCIMD) and National Centre for Medical Genetics (NCMG), Dublin

The frequency of Inherited Metabolic Disorders (IEMs) varies between ethnic groups, reflecting founder effect, genetic isolation, and the potential effects of consanguinity. These disorders are a major cause of morbidity and mortality in "Irish Travellers", an endogamous group of nomads who number 22,000 in Ireland and 15.000 in the UK.

We aimed to compare the birth prevalence of IEMs in Traveller with non-Traveller children attending a tertiary level metabolic centre and to examine possible genetic factors contributing to observed differences. A retrospective review of diagnoses in Travellers was performed for 5 years (2002-2006). Mean birth prevalence was calculated and compared with overall figures for IEM's in the total population.

Travellers constitute 9% of the total patient group, but only 0.5% of the Irish population. 21 IEMs were noted, Galactosaemia, MPS I, Mitochondrial cytopathies, Glutaric Aciduria Type I,GSD Type IIIa, Mucolipidosis Type II, Hyperprolinemia Type 11,progressive familial intrahepatic cholestasis Type I (PFIC1) and carbonic anhydrase deficiency being the commonest. The birth prevalence of IEMs in the Traveller group for this period was 12/1000. That for the total population was 2.45/1000. Common homozygous mutations in all cases of galactosemia (Q188R), MPS 1(W402X), GA1(E365K), Mucolipidosis type II (c.3502_3delCT), Hyperprolinemia Type II (G521fs(+1)) and PKU (R408W) confirm the homozygous nature of this ethnic group.

We propose that the high incidence of IEMs in Irish Travellers may reflect initial founder effects and the increased rate of consanguinity.

P6. Myoclonus Dystonia- Phenotype- Genotype Correlation in the Irish Paediatric Population

Langseth A[1], Doherty E[1], Nestor T [1], Lynch T[2], Asmus F[3], Mary MD[1]

1. Department of Neurology, The Children's University Hospital Temple Street.
2. Department of Neurology, The Mater Hospital Dublin 1.
3. Department of Genetics, Teubingen, Germany

Myoclonus dystonia (DYT 11) is characterised by proximal myoclonic jerks and dystonia which causes torticollis and writers cramp. It has been associated with mutations on the epsilon sarcoglycan gene on chromosome 7q21 and may be alcohol responsive. An audit of over 70 patients seen at a quaternary paediatric movement disorder clinic revealed 21 patients with myoclonus dystonia.

Aim: to investigate clinical phenotype genotype correlation in Irish children with myoclonus dystonia.

Methods: 21 children in 17 M-D families were evaluated using a standardized neurological examination and review of video material. SGCE mutation analysis was performed on all patients.

Results: Age of onset ranged from 18 months to 14 years. A positive family history was seen in 11/21. Presenting symptoms were hand tremor, paroxysmal gait abnormality writing difficulties or a combination thereof. Clinical evaluation with pectoral muscles exposed showed irregular myoclonic jerks in all patients. SGCE mutation was found in 7/21 patients. All patients who had taken alcohol were alcohol responsive.

Conclusion: A typical adult M-D phenotype was rarely seen in children. Children were more likely to present with lower limb symptoms. Children with a positive mutation were more likely to present at an earlier age, were more likely to have a positive family history and were more likely to have lower limb symptoms.

P7. The IKBL protein inhibits TLR mediated activation of gene expression by NF kappa B

Arun Kumar Mankan, Jacqueline Daly, Emma Caraher, Dermot Kelleher, Ross McManus.

Department of Clinical Medicine, Trinity College Dublin and Institute of Molecular Medicine, Dublin Molecular Medicine Centre, Trinity Centre for Health Sciences, Trinity College Dublin, Dublin, Ireland, 8.

The Inhibitor of NF Kappa B like *(IKBL/NFkBIL1)* gene encodes a protein homologous to members of the IKB family. *IKBL* is situated in the MHC and a number of different polymorphisms in the gene have been associated with diseases such as Myocardial Infarction, Rheumatoid Arthritis, Diabetes Mellitus, Celiac Disease and Crohns Disease.

The function of IKBL protein has not yet been reported. We have demonstrated, by both EMSA and Luciferase Assays, that over-expression of IKBL inhibits the activity of NF kappa B.

We have further demonstrated that IKBL inhibits NF kappa B activation by both TLR 2 and TLR 4 pathways. mRNA and protein expression of IL8, a NF kappa B regulated pro-inflammatory cytokine, was also inhibited by IKBL.

We show that IKBL and HDAC3 may co-localise in the nucleus offering a possible mechanism since HDAC3 is a known regulator of transcription factors.

Our study suggests that IKBL is a member of the novel inhibitors of NF kappa B such as MAIL that are located in the nucleus and may inhibit the activity of NF kappa B by regulating the activity of other proteins that bind to NF kappa B within the nucleus.

P8. Use of the Promega Powerplex® 16 kit to exclude maternal cell contamination in prenatal testing

Brónagh Ó hIcí, Edith Gould, David E. Barton. National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin 12. E: bronagh.ohici@olhsc.ie

The exclusion of maternal cell contamination of prenatal samples is an important step in prenatal molecular genetic testing particularly when the prenatal sample shows the same genotype in the diagnostic test as the maternal sample. Traditionally, the exclusion of maternal cell contamination has been carried out by typing a number of linked or unlinked microsatellite markers from the maternal, paternal and foetal samples. This work becomes challenging when the microsatellite markers used are uninformative. If this occurs with several of the microsatellite markers selected for analysis the reporting of results may be delayed, as further microsatellite markers must be typed to complete the analysis. To assist in eliminating some of these problems, and with the additional aim of reducing the reporting time, we have been investigating the use of a commercial kit called Powerplex® 16 (Promega) for the exclusion of maternal cell contamination. This kit co-amplifies, in a single PCR reaction, 15 highly-informative microsatellite markers and the amelogenin locus. Here we describe the sensitivity of the Powerplex® 16 kit in detecting maternal cell contamination as low as 5% in prenatal samples.

P9. A systems approach to datamining association signals from whole-genome association studies

Colm Ó'Dúshláine, Derek Morris, Elaine Kenny, Carlos Pinto, Michael Gill, Aiden Corvin.

Trinity College Dublin, St James' Hospital, Dublin 8.

Whole-Genome Association (WGA) studies have the potential and power to detect a large number of significant associations with a disease phenotype. Understanding these findings can be difficult and replication is often an integral part of this type of study, but even this may only identify low hanging fruit. Published WGA data shows that, while novel genes have been found for the various phenotypes, a large number of genes still remain to be identified. For example, in a study of type 2 diabetes that found 5 genes for the disorder, Saxena *et al* (2007) commented that these 5 loci contribute only modestly to the overall variance in diabetes risk (~2.3%), indicating that many more genes remain to be found. We present a systems biology approach to mining WGA data. By availing of gene interaction data from KEGG HPRD BIND and Reactome, we integrate genes containing significantly associated SNPs into gene interaction networks. This provides a platform for inferring biological pathways enriched for disease associated genes. Our approach is flexible, taking account of the available interaction data and the number of significantly associated genes under investigation, and permits varying levels of intermediate interacting genes between the associated genes. We apply this method to the investigation of a number of existing WGA studies, highlighting heretofore unobserved pathway signals.

P10. Development of a *C.elegans* model system for genetic and molecular dissection of epigenetic mechanisms underlying trinucleotide expansion disorders.

Katherine Schouest[1], Avril Coghlan[2], Charles Spillane[1].

1. Dept Biochemistry & Biosciences Institute, University College Cork, Cork, Ireland. www.ucc.ie/spillane
2. Wellcome Trust Sanger Institute, Cambridge, U.K.

Model organisms are essential for the rapid advancement of genetic research on human diseases. *C. elegans* has already proven to be a powerful biomedical model in dissecting principles underlying diseases like Huntington's Disease and Myotonic Dystrophy, as well as in revealing potential targets for therapeutic treatment. We are using *C. elegans* as a model organism to investigate epigenetic phenomena associated with nucleotide repeat disorders and to identify modifiers that regulate the size and rate of the expansions. To identify candidate tester loci for our research we have screened the *C. elegans* genome for trinucleotide repeats that have at least 12 trinucleotide repeats and a high level of purity, as these are most likely to undergo expansion or contraction. We have identified 20 such repeats located in *C. elegans* exons and 17 repeats located within introns. To select one or more variable repeats for further analysis, the extent of polymorphism of these repeats is currently being examined over 48 wild isolates of *C. elegans*. The identification of polymorphic repeat loci will allow monitoring of these alleles through multiple generations in control animals and animals depleted of candidate epigenetic modifiers via RNAi. The project will ultimately facilitate the understanding of the epigenetic phenomena causing nucleotide expansion disorders.

P11. Analysis of SOD1 gene for IVS 2+50 del 7 genomic deletion in Northern Irish Keratoconus Patients

Dash DP[1], S George[3], Silvestri G[1,3], J Jackson[3], D Frazer[3], Hughes AE[2], Willoughby C[1,3],

Centre for Vision Sciences[1], Medical Genetics [2], Queen's University & Royal Victoria Hospital[3], Belfast, N. Ireland.

Purpose: Oxidative stress has been suspected as a major contributor to pathogenesis of keratoconus (KC) as accumulation of cytotoxic by-products from nitric oxide and lipid peroxidation, abnormal antioxidant enzymes found in KC cornea. Recently a heterozygous 7bp deletion in intron 2 (IVS 2+50 del 7) close to the 5' splice junction of the super oxide dismutase 1 (SOD1, MIM:147450) gene was reported in three KC patients (Udar *et al.,* IOVS; 2006). The purpose of this study was to screen for the IVS 2+50 del 7 of SOD1 gene in KC patients from Northern Ireland.

Methods: Blood samples were collected from 17 KC patients at RVH Eye Clinic and DNA was extracted from leucocytes. Conventional and FAM-labelled oligonucleotide primers were designed flanking the genomic deletion, IVS 2+50 del 7 of SOD1 gene. Screening for the intronic deletion was performed by PCR based direct cycle sequencing and fragment length analysis using ABI 3100 automated DNA sequencer.

Results: Seven out of 17 sporadic KC patients appeared to show a 2-3 bp genomic deletion within the previously published intronic region when sequencing in one direction, but there was no frameshift in the downstream sequence. Sequencing in the reverse direction and fragment length analysis failed to demonstrate any intronic deletion.

Conclusions: The genomic deletion (IVS 2+50 del 7) of SOD1 gene was not found in a subset of the NI population with KC. Sequencing results in this region should be interpreted with caution. Patient recruitment is ongoing and further analysis of the entire coding region of SOD1 is required to elucidate the role of this anti-oxidant

enzyme in KC. [Funding Authority: Research & Development Office, Northern Ireland].

P12. Predictive Testing for Carney Complex in a Child

Deirdre Donnelly, Shane McKee.

Department of Clinical Genetics, Belfast City Hospital, Belfast

This is a case of a 4 year old girl with a three-generation family history of Carney Complex. Carney Complex is an autosomal dominant condition, with around 400 patients reported worldwide. The phenotype is variable and there may be a degree of under-diagnosis. Manifestations include skin pigment abnormalities, myxomas, endocrine tumours and schwannomas. The family mutation has been found, delta FSC18 PRKAR1A. All affected family members have characteristic facial freckling. The child had a milder distribution of facial freckles and was considered to be affected by the family. Her mother requested predictive testing for confirmation. No intervention is recommended routinely until puberty in Carney Complex, although cardiac myxomas may present at any time from birth. These can cause embolic events or obstruct blood flow, leading to sudden death. Our case raises the question of whether to test an asymptomatic child who has similar facial features as affected family members. In this situation, the child may consider themselves affected. This child tested positive for the family mutation. Her mother was brought back to the clinic alone to be informed. The child has been referred to a paediatric cardiologist. She will be offered a genetics consultation when she is older.

P13. Association studies of SEMA6A, SEMA6B, PLXNA2 and PLXNA4 genes in an Irish schizophrenia case-control sample.

Elaine Kenny[1], Derek W. Morris[1], William P. Gilks[1], Emma H. Allott[1], Gary Donohoe[1], John L. Waddington[2], Kevin J. Mitchell[3], Michael Gill[1], Aiden P. Corvin[1].

[1] Neuropsychiatric Genetics Research Group, IMM and Dept. of Psychiatry, Trinity College Dublin, Ireland.

[2] Royal College of Surgeons in Ireland, Dublin, Ireland.

[3] Smurfit Institute of Genetics, Trinity College Dublin, Ireland.

Schizophrenia has a substantial genetic component. Finding genetic variants that alter risk may help in identifying pathways that are aetiologically important, both in predicting illness risk and developing treatments. Work by our group and collaborators have identified the Semaphorin 6 and Plexin A gene families as putative susceptibility genes for schizophrenia. Here we report analyses of SEMA6A, PLXNA2, SEMA6B and PLXNA4.

SNP maps were generated for all four genes. SNPs located within the gene region were ranked according to their location. Priority was given to SNPs in coding regions, UTR's, splice junctions, promoter regions, evolutionary conserved regions, regions containing clusters of transcription factor binding sites (TFBS) and conserved TFBS. Tag SNPs were chosen using HapMap CEU linkage disequilibrium data. Altogether 74 SNPs were genotyped across the four loci in a sample of 375 schizophrenia cases and 812 controls.

Three SNPs at SEMA6A, two SNPs at SEMA6B and three SNPs at PLXNA2 reached nominal levels of significance (p=0.01-0.05) prior to correction for multiple testing.

Given the limited power of our association sample and the number of SNPs tested, these findings require independent replication. However, the results may point to a role for abnormal axon guidance and cell migration in schizophrenia pathophysiology.

P14. Absence of linkage to known loci in an Irish RLS family

Skehan EB, Abdulrahim M, Parfrey NP, Hand CK.

Department of Pathology, University College Cork, Ireland.

Restless legs syndrome (RLS) is a neurological sleep disorder characterised by abnormal sensations in the legs associated with an irresistible urge to move. Symptoms occur predominantly at rest and worsen at night, resulting in nocturnal insomnia and chronic sleep deprivation.

RLS has an estimated prevalence of 5 to 15% in the general population. Familial aggregation has been widely reported and the condition is predominantly an autosomal dominant disorder. Molecular genetic approaches have identified five loci on chromosomes 12q, 14q, 9p, 2q, and 20p, in RLS-affected families from different populations. No disease-causing gene has yet been identified. The increase in symptom intensity at night has implicated the circadian system and response to treatment with dopamine agonists has suggested an abnormality in the dopaminergic pathway.

An Irish family with autosomal dominant RLS has been recruited, with the aim to localise and identify the gene responsible for the syndrome. The five described RLS loci were examined for linkage; however results indicate that the new Irish RLS pedigree is not linked to the currently described genetic loci. This provides further evidence of genetic heterogeneity in RLS. Future work includes a genome-wide scan to identify the novel locus in this Irish RLS family.

P15. The *CHEK2*1100delC* variant: present in the west of Ireland breast cancer population.

Colleran G, Rowan A, Miller N, Sawyer E, Curran C, Kerin M, Tomlinson I.

National University of Ireland, Galway, Ireland; Molecular and Population Genetics, Cancer Research UK, London, England.

Background: As part of a population-based approach to breast cancer genetics, a West of Ireland cohort is under study to elucidate inherited variation which predisposes women to developing breast cancer. *CHEK2* has been identified as a low-penetrance breast cancer susceptibility gene conferring a 2-fold elevated risk of breast cancer in women and 10-fold in men.

Materials and Methods: To evaluate the prevalence of the *CHEK2*1100delC* variant, DNA collected from 591 breast cancer cases and 572 healthy controls were analysed. FAM (carboxyfluorescein) labelled PCR products were capillary separated on the ABI 3700 and fragment analysis carried out using Genotyper v2.5. Normal PCR fragments measures 168bp and the *CHEK2*1100delC* could be clearly seen at 167bp. A sequenced control *CHEK2*1100delC* patient DNA was PCR amplified for each test reaction.

Results: The *CHEK2 *1100delC* mutation was found in three cases, one had a sibling who was affected with colorectal cancer. The mutation was not found in any control samples.

Discussion: We have established that the *CHEK2*1100 delC* variant is present in the Irish population and is in excess in cases over controls. Our data are consistent with effect on risk. Its role in the clinical setting has yet to be elucidated.

P16. Genetic interaction assessment of major Age Related Macular Degeneration (AMD) susceptibility loci within the Northern Ireland population.

GJ McKay[1], G Silvestri[1], N Orr[2], U Chakravarthy[1], AE Hughes[2].

[1] Centre for Vision Science and

[2] Department of Medical Genetics, Queens University Belfast, Belfast, United Kingdom.

Purpose: To assess the effect of Complement factor H (*CFH*), Factor B (*FB*), Component C2 (*C2*), *LOC387715/HTRA1* locus and the influence of Vascular Endothelial Growth Factor (*VEGF*) and Apolipoprotein E (*APOE*) within a Northern Ireland AMD cohort.

Methods: DNA samples (n=250) with end-stage wet AMD and an age and sex-matched control cohort (n=250) underwent ophthalmic examination with detailed medical and smoking history. Haplotype analysis was undertaken for *CFH*, *CFHR1*, *CFHR3*, *LOC387715*, *FB*, *C2*, *VEGF* and *APOE*. Haplotypic structure for each gene was determined from HapMap and tagged SNPs were multiplexed using SNaPshot technology (ABI).

Results: Results show a higher incidence of AMD risk haplotypes within the affected cohort with a decreasing incidence of protective haplotypes when compared to the controls for *CFH*, *C2/FB* and *LOC387715*. Genetic variation within *C2* and *FB* would appear to be less strongly associated with the disease cohort than previously reported. A significant role for *VEGF* in relation to wet AMD has not been shown.

Conclusions: It would appear that *CFH* and *LOC387715* remain the most strongly associated genetic factors with AMD and that *VEGF* is unlikely to have any significant involvement with disease manifestation within this population.

P17. C-banding analysis of a newborn with clinical features of Roberts syndrome

Helen Fitzpatrick, Sally Ann Lynch, Gillian Clarke, Aidan Doherty.

National Centre for Medical Genetics, Ireland.

Roberts syndrome (pseudothalidomide syndrome) is a rare autosomal recessive syndrome which presents with craniofacial abnormalities, limb, heart and renal defects. The condition has recently been mapped to 8p12, with mutations found in ESCO2, a gene essential for the establishment of sister chromatid cohesion responsible for clinical presentation (Vega *et al.,* 2005). A majority of affected individuals (about 80%) exhibit a chromosomal phenomenon known as "heterochromatin repulsion" (also referred to as premature centromere separation).

A newborn female infant of Polish origin was referred for cytogenetic investigation; her clinical features included multiple congenital anomalies, - hypertelorism, midline cleft lip and palate, severe symmetrical intra uterine growth retardation, absent radii and talipes.

Chromosome analysis revealed a female karyotype of 46,XX chromosomes. Approximately 50% of the cells examined exhibited a characteristic morphology with lack of a defined centromeric constriction with some pericentromeric regions being splayed out and appearing as "puffing". This phenomenon is characteristic of Roberts syndrome.

Limb defects, cleft lip and palate, multiple congenital anomalies; together with the characteristic heterochromatic repulsion are diagnostic of Roberts syndrome in this patient.

P18. Tuberous Sclerosis Complex – an audit of referrals to the Northern Ireland Genetic Service over a four period.

Crawford H, Shepherd CW, McKee S, Morrison PJ.

The Northern Ireland Tuberous Sclerosis Clinic, Northern Ireland Regional Genetics Centre, Belfast Health and Social Care Trust, A Floor, Belfast City Hospital, Lisburn Road, Belfast BT9 7AB

In the four years 1 June 2003 –1 June 2007, 19 patients were referred to the Northern Ireland Genetic Service for investigation of Tuberous Sclerosis Complex (TSC).

Results: 13 patients have a clinically confirmed diagnosis of TSC. Of these, 7 patients had seizures age <12 months as a first presentation of TSC. In these patients, 2 undiagnosed parents were identified as being TSC affected following the diagnosis in their child. 3 patients (age range 9 – 17) presented with angiofibromata and a diagnosis of TSC was made following referral by the Dermatologist. 1 TSC affected adult had a previously confirmed diagnosis of TSC. This patient came to Northern Ireland from Portugal.

Conclusion: In a stable population, the majority of patients with TSC are diagnosed in infancy or early childhood. A second group of previously undiagnosed, mildly affected adults was identified, following the diagnosis of TSC in their child. A third group of older children and adolescents was identified. An emerging trend is seen, where a proportion of new referrals for TSC are patients who have come to Northern Ireland from other parts of Europe.

P19. Audit Of Myelodysplastic Syndrome Cases Submitted For Cytogenetic Analysis Over A Two Year Period.

Johanna Kelly[1], Eibhlin Conneally[2], Elizabeth Vandenburgh[2], Sally Ann Lynch[1].

[1] National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin 12;

[2] St James's Hospital, Dublin 8.

Background: Myelodysplastic Syndromes (MDS) are a group of conditions of progressive bone marrow failure of normal maturation leading to peripheral cytopenias. More than one cell lineage is involved, with often up to three. It is typically a disease of the elderly. There is a 20-40% risk of transformation from MDS to Acute Myeloid Leukaemia (AML). Chromosome abnormalities would be expected in 40-50% of MDS cases. Evidence suggests that cytogenetic analysis can influence the clinical evaluation. The clinical boundaries between MDS and AML are indistinct and a similar overlap occurs cytogenetically. Cytogenetic analysis, therefore, does not prove informative for a differential diagnosis.

Aim: To minimise the number of borderline MDS cases submitted for cytogenetic analysis with a view to improve efficiency. This should allow the department to broaden the range of tests offered.

Results: 805 MDS samples were received over a two year period ranging from age 30-90. 164 of these samples were deemed not required on review of Bone Marrow morphology. Of the remaining 641 samples 83 of these samples had an abnormal cytogenetic karyotype. This gives a 12.9% abnormality rate for MDS cases referred to this facility. 80% of the abnormal karyotype cases were aged between 60 and 90.

Discussion: As the expected abnormality range in a cytogenetic

laboratory for MDS is 40-50% it is clear that a more stringent selection criteria needs to be implemented. It is also evident from the high percentage of abnormal karyotype cases in the age range 60-90 that MDS is a disease of the elderly. It is proposed that cytogenetic analysis for all MDS cases should only be completed on receipt of a diagnostic bone marrow morphology report, a complete clinical history or by personal communication with the requesting consultant.

P20. Detection of subtelomeric rearrangements in children with unexplained mental retardation using Multiplex Ligation-dependent Probe Amplification.

Karen Meaney[1], David E. Barton[1].

[1] National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin 12.

Mental retardation is a common lifelong disability affecting 1-3% of the general population. The cause of the disorder remains unknown in approximately half of all cases which can be a source of anxiety for the family of the affected patient. It has been reported that submicroscopic subtelomeric rearrangements may be responsible for 2.5-10% of all unexplained mental retardation cases.

Multiplex Ligation-dependent Probe Amplification (MLPA) can be used to detect cryptic subtelomeric imbalances. It has the advantage of being a cost effective, rapid and easy to use technique. We will present the results of 200 retrospective DNA samples tested for the presence of subtelomeric rearrangements using two different MLPA probe mixes. These samples are from children with unexplained mental retardation/developmental delay and normal karyotype and Fragile X results.

The objectives are to assess whether the introduction of a service in our laboratory to screen patients with idiopathic mental retardation/developmental delay for subtelomeric aberrations is practicable and of benefit to the Irish population and to determine whether MLPA is a suitable technique for the detection of these anomalies.

P21. There is no evidence of linkage or association between Parathyroid Hormone Receptor Type 1 polymorphisms or haplotypes with low bone mineral density in a Caucasian cohort

KG Hegarty[1,2], M Daly[1], F Shanahan[1], MG Molloy[1].

[1] Deptartment of Rheumatology and Sports Medicine, National University of Ireland, Cork, Ireland.

[2] Department of Medicine, National University of Ireland, Cork, Ireland.

[3] Alimentary Pharmabiotic Center, National University of Ireland, Cork, Ireland.

Previous studies have observed suggestive evidence of linkage between low bone mineral density (BMD) and the parathyroid hormone receptor type 1 (PTHR1) locus (3p22-21.1). In the present study, we tested for association between genetic variants in the PTHR1 gene and variation in BMD.

Four single nucleotide polymorphisms (SNPs), located throughout the PTHR1 gene, were tested for association with BMD in 278 nuclear families and 500 unrelated postmenopausal Caucasian women.

There was no evidence of linkage between the PTHR1 genotypes and BMD using Merlin (LOD scores < 1.0). The Family Based Association Test (FBAT) was used to test for association between the PTHR1 genotypes and haplotypes with BMD. There was significant association between SNP rs4683301 with variation in BMD at the femoral neck (P = 0.03) and lumbar spine (P = 0.02) using the genotype model in FBAT. However, following adjustment for covariates, there was no significant association (P >0.05). The PTHR1 haplotype, h3 (TC), was significantly associated with BMD at both skeletal sites (P < 0.00). However, after correction for covariates, there was no significant association.

Denser SNP genotyping may be necessary to better define the possible relationship between the PTHR1 gene and BMD variation in this cohort.

P22. Association of Methylenetetrahydrofolate reductase (MTHFR) polymorphism and the risk of Squamous Cell Carcinoma in renal transplant patients.

Mary E Laing[1], Patrick Dicker[2], Wen Lyn Ho[1], Fergal J Moloney[1], Gillian M Murphy[1], Peter Conlon[3], Alexander S Whitehead[4], Denis C Shields[5]

1. Department of Dermatology, Beaumont Hospital, Dublin 9.
2. Department of Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland.
3. Department of Nephrology, Beaumont Hospital, Dublin 9.
4. Department of Pharmacology and Center for Pharmacogenetics, University of Pennsylvania

School of Medicine, Pennsylvania, USA 5UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin 4, Ireland

Background: The relative risk of developing cutaneous squamous cell carcinoma (SCC) is significantly increased following organ transplantation.

Objective: We investigated genetic association with SCC in two pathways associated with cancer risks, with potential for modification by vitamin supplementation.

Methods: 367 renal transplant recipients (117 with SCC and 250 without any skin cancer) were genotyped for key polymorphisms in the folate pathway (MTHFR: C677T; methylene tetrahydrofolate reductase), and the vitamin D pathway (VDR: Intron 8 G/T; vitamin D receptor).

Results: Individuals carrying the MTHFR 677T allele had a marked increase in risk of SCC (adjusted OR= 2.54, p=0.002, after adjustment for age, sex, skin type, sun exposure score and immunosuppression duration; lower 95% confidence boundary OR of 1.41). In contrast, VDR polymorphisms were not significantly associated.

Conclusion: Folate-sensitive pathways may play a critical role in the elevated rate of SCC in renal transplant recipients.

P23. Study of the Knowledge of Inherited Metabolic Disorders among patients and their families in the Irish population

Rosie O'Shea[1], Eileen Treacy[2], Anne Marie Murphy[2], Sally Ann Lynch[3], Deborah Lambert[2,3].

[1] Department of Medical Genetics Cardiff University,

[2] The National Centre for Inherited Metabolic Disorders, Children's University Hospital, Temple Street,

[3] National Centre for Medical Genetics, Dublin.

Galactosaemia and Maple Syrup Urine Disease (MSUD) are recessively inherited conditions screened for by newborn screening in Ireland. Affected patients are followed at the National Centre for Inherited Metabolic Disease. We aimed to assess the degree of genetic knowledge imparted to families to determine if further formal genetic counselling would be beneficial. Adult patients and parents of affected children were interviewed in person using a questionnaire including 4 demographic, 8 knowledge, 2 information and 5 impact questions. To date, 8 adults (7 galactosaemia, 1 MSUD) and 18 parents (12 galactosaemia and 6 MSUD) have been interviewed. All parents of children with MSUD correctly answered questions on MSUD and recurrence, but did not know the risk or implications of carrier status. For galactosaemia; 9 of 12 parents scored 6/8 or better on knowledge, while all adults scored 3 to 5 of 8. 16/26 study participants requested more information about their condition and its transmission. Affected adults also identified a need to meet others with the same condition. Our study to date indicates that parents of children with these genetic disorders are well informed, however adult patients could benefit from further genetic counselling. This may reflect a reluctance to transmit genetic information within families.

P24. Determination of the most stable endogenous control gene using an *in vitro* model of folate deficiency.

Carroll N[1,2], Cahill S[1,] O'Brien K [1]Scott JM[2], & Parle-McDermott A[1].

[1] Nutritional Genomics Research Group, School of Biotechnology, Dublin City University, Ireland

[2] School of Biochemistry & Immunology, Trinity College Dublin, Ireland.

Folate is an essential nutrient necessary for DNA synthesis, cellular proliferation and biological methylation reactions. Suboptimal folate status is a risk factor for several human diseases. An understanding of the molecular mechanisms linking folate status to these conditions is still incomplete. In a bid to dissect the molecular response to folate status we set up an *in vitro* model of folate depletion. RT-PCR is currently the method of choice to examine expression levels of a specific set of genes. Key to this method is normalisation of results to an appropriate endogenous control gene that is relatively unaffected by the experimental conditions. Inaccurate normalization can lead to findings that do not reflect the true experimental variation. We sought to identify the most appropriate endogenous control gene for normalisation of gene expression data in our *in vitro* model of folate depletion in HEK293 cells to ensure only true gene-specific variation in response to folate levels will be reported. This was undertaken using the TaqMan® Human Endogenous Control Plate that enables the evaluation of 11 endogenous control genes. HEK293 cells were cultured for 14 days in conditions of depleting folate. Decline in cellular folate levels was confirmed by intracellular folate assay. Duplicate cDNA samples from Day0, 3, 8 and 14, representing different degrees of folate depletion, were used. RT-PCR was performed on an ABI 7500 PCR System. GUS control gene was shown to be the endogenous control gene that displayed the least amount of variation across samples and therefore is the most accurate choice as a single normalisation gene for HEK-293 cells under conditions of depleting folate.

P25. Down syndrome and Achondroplasia: A rare combination

Tabib Dabir[1], Brian McCrossan[2], A Sands[2], L Sweeney[3] and A Magee[1]

[1] Northern Ireland regional genetics Service, Belfast City Hospital, Belfast BT9 7AB,

[2] Department of Paediatric Cardiology,

[3] Department of Paediatric Radiology, Royal Belfast Hospital for Sick Children, Belfast, BT12 6BA tabib.dabir@belfasttrust.hscni.net

Achondroplasia and Down syndrome are the commonest genetic conditions within their respective categories. Presence of these two genetic entities in the same patient is a rare event and has been reported only thrice in medical literature. We report the fourth case with this rare combination.

The proband was a female infant born at term to a Caucasian couple with maternal and paternal age of 41 and 43 years respectively. The clinical features included frontal bossing, flat nasal bridge, down slanting palpebral fissures, long philtrum, thin lips and bilateral simian creases and Tetralogy of Fallot. The clinical diagnosis of Down syndrome was confirmed by karyotyping. She also had relatively large head, depressed nasal bridge, rhizomelic shortening of all limbs, protuberant abdomen and trident configuration of both hands. These features were suggestive of achondroplasia and the radiological features were consistent with this diagnostic possibility. FGFR3 gene mutation analysis showed G380R G > A mutation.

The combination of Down syndrome and achondroplasia in our patient is likely to be a chance event because of the advanced parental ages. Molecular confirmation of achondroplasia is not routinely requested. However it was extremely useful in this case from diagnosis and counselling point of view.

P26. Natural history of Williams Syndrome: a report of 2 cases

Tabib Dabir[1], Alex Magee[1] , Alistair N Graham[2]

[1] Northern Ireland Regional Genetics Service, Belfast City Hospital, Lisburn Road, Belfast BT9 7AB,

[2] Department of Cardiothoracic Surgery, Royal Hospitals, Grosvenor Rd, Belfast BT12 6BA. tabib.dabir@belfasttrust.hscni.net

Williams syndrome (WS) is a well described genetic syndrome affecting 1 in 20,000. However there is relative paucity of medical literature devoted to adults with this condition. We report 2 adults with WS diagnosed in their late 50s.

Case 1: 57 year old lady referred with clinical suspicion of Turner syndrome. She has learning difficulties, short stature, kyphoscoliosis, joint stiffness, cardiac pacemaker for complete heart block, abnormal glucose tolerance test, hypertension and constipation. She lives in a residential home with her older sister who also has learning difficulties and short stature.

Case 2: 57 year old man referred to genetics department with clinical features, mild learning difficulties, diabetes, sensorineural hearing loss, constipation, and was operated for inguinal hernia, bladder diverticulae, aortic valve replacement and aneurysm of ascending aorta. He also had stroke and has never lived independently.

These are probably the oldest reported cases of WS. Their clinical features and the associated medical complications delineate the natural history of this condition. It also highlights the need for better understanding/ awareness of this condition among professionals working in adult services.

P27. Implementation of a Luminex-based CF Assay at NCMG – A Validation Experience

Trudi McDevitt, Caitriona King, Melissa Rogers, Solvig Roring, Shirley McQuaid, David E Barton.

National Centre for Medical Genetics, Our Lady's Children's Hospital, Dublin, Ireland. Email: trudi.mcdevitt@olhsc.ie

With the aim of improving the efficiency of the NCMG cystic fibrosis (CF) service, we looked for a multiplexed CF assay which could be adapted to the mutation spectrum of the Irish population.

Using Luminex™ Liquid Bead Array Platform (Applied Cytometry), we evaluated the Signature™ CF 2.0 ASR from Asuragen which tests for 25 of the CF mutations included in the ACMG/ACOG recommended CF panel.

We evaluated this assay on a variety of sample types and on a large cohort (n=468) of DNA samples of known genotypes to examine sensitivity and specificity. All samples except one were genotyped correctly during this initial validation, indicating that the Signature™ CF 2.0 ASR was a sensitive and robust assay for CF diagnostics. We observed a discordant result between our ARMS assay and the Signature™ CF 2.0 ASR for one sample. Subsequent investigations revealed this discrepancy to be due to the presence of CF mutation V520F, which resulted in non-amplification of the mutant allele due to its position under the exon 10 forward primer in the Signature™ CF 2.0 ASR.

We describe collaborative efforts by NCMG and Asuragen to address the issue of SNPs under primers in commercial ASRs.

P28. Partial trisomy 13: A case report, verification of the phenotype and review of the literature

G Smith, D McManus, V McConnell.

NI. Regional Genetics Service, Belfast City Hospital, Belfast Health and Social Care Trust

Partial trisomy for distal chromosome13 has previously been described in 20 patients. The reported phenotype consists of microcephaly, distinctive facies, high arched palate, postaxial polydactyly, genitourinary anomalies, 2/3 toe syndactyly, moderate mental retardation and relatively few major malformations.

We describe a further case in a male infant born by emergency C/S to a primagravida due to failed induction at T+3, after an uneventful antenatal history. Birth weight was on 75th centile. On examination he presented with striking dysmorphic features, a high arched palate, long fingers and toes with bilateral postaxial polydactyly, bilateral 2/3 soft tissue toe syndactyly and hypospadias.

Cytogenetic analysis revealed additional chromosome material at 9p24.1 which was confirmed as 13q22.3->qter by FISH studies. Subsequent parental chromosome analysis indicated that the unbalanced rearrangement had arisen from an adjacent I segregation of a maternal t(9;13)(p24.1;q22.3).

This case provides further evidence that trisomy 13q22.3->qter presents with a characteristic spectrum of abnormalities. A review of the literature indicates that, of the features of full trisomy 13, congenital heart defects, clinodactyly and frontal bossing appear to be associated with proximal 13q trisomy, while genitourinary anomalies, micropthalmia, cleft palate and polydactyly are more prevalent in trisomy for distal 13q.

P29. A Genotype-Phenotype Correlation Study In An Extended Irish Kindred With Variegate Porphyria Caused By *PPOX* Q435X

[1]VEF Crowley, [1]K Lim, [1]C Darby, [1]C Collison, [1]A Balfe, [2]S Heggarty

1. Biochemistry Department, Central Pathology Laboratory, St James's Hospital, Dublin 8.
2. Genomics Core Facility, Regional Genetics Centre, Belfast City Hospital

Variegate porphyria (VP) is genetically heterogeneous, and demonstrates variable penetrance and expressivity of clinical and biochemical phenotype within affected families. Phenotypic variability may be related to the nature of the underlying pathogenic mutation. Q435X is the most common VP-causing mutation encountered in the UK (7%) and we report a genotype-phenotype correlation study of Q435X in an extended Irish kindred

Molecular genetic scanning of *PPOX* identified a nonsense mutation Q435X in two subjects with confirmed VP. A further twenty-two adult members of this extended family were screened for Q435X. In total 67% (16 out of 22) were mutation positive. Plasma fluorescent emission spectroscopy (PFS) screening was also undertaken in all subjects, and had a specificity of 100% but sensitivity of only 80%. A clinical questionnaire revealed that only 19% (3 out of 16) of mutation positive subjects had clinically overt cutaneous manifestations of VP and13% (2 out of 16) had experienced acute episodes.

The results of this genotype-phenotype study suggests that Q435X demonstrates a less penetrant cutaneous phenotype but greater penetrance of acute neurovisceral attacks than a well characterised South African founder mutation R59W. Furthermore, PFS was only 80% sensitive, thus confirming that mutation analysis is diagnostically superior in the detection of presymptomatic carrier status.

P30. The Molecular Basis of Acute Porphyria in the Republic of Ireland.

[1]Vivion EF Crowley, [1]Cindy Darby, [2]S Heggarty, [3]Sharon Whatley, [3]Mike Badminton.

1. Biochemistry Department, Central Pathology Laboratory, St James's Hospital, Dublin 8,
2. Genomics Core Facility, Regional Genetics Centre, Belfast City Hospital.
3. Cardiff Porphyria Centre, Department of Medical Biochemistry, University Hospital of Wales, Heath Park, Cardiff

Acute porphyrias, which include acute intermittent porphyria (AIP), variegate porphyria (VP) and hereditary coproporphyria (HP), are autosomal dominantly inherited disorders affecting key enzymes in the haem biosynthetic pathway, and demonstrate variable penetrance (20%) and expressivity. Clinically these disorders may manifest with photosensitive skin lesions (VP and HP) and/or acute neurovisceral episodes (AIP, VP and HP), the latter being potentially associated with significant morbidity. While biochemical investigations, including blood, urine and faecal porphyrin analysis, are critical for the diagnosis of active porphyric disease, these investigations may not be sensitive enough to identify presymptomatic mutation carriers. Hence molecular genetic analysis has become an important component in kindred follow-up for identifying porphyria susceptibility.

The Biochemistry Department, St James's Hospital, Dublin, in collaboration with Cardiff Porphyria Centre, have recently established a biochemical genetic service for the acute porphyrias. Mutation scanning using PCR and direct nucleotide sequencing has identified 11 different mutations in 12 porphyria kindred within the Republic of Ireland. This includes mutations in *HMBS* (R26C, R26H, IVS4+1G>A), *PPOX* (IVS4-1G>A, Q435X, W427X, A150D, Q375X) and *CPO* (R332Q, R332W, c.1291-1292 ins TG), causing AIP, VP and HP respectively.

This unique insight into the molecular basis of porphyria in the ROI population clearly indicates that acute porphyrias are genetically heterogenous within this cohort.

P31. Genetic variants of Complement factor H gene are not associated with premature coronary heart disease: a family-based study in the Irish population

Meng W[1], Hughes A[1], Patterson CC [1], Belton C[1], Kamaruddin MS[2], Horan PG[2], Kee F[1], McKeown PP[1,2].

1. Centre for Clinical and Population Sciences, Queen's University Belfast, Institute of Clinical Science, Grosvenor Road, Belfast, BT12 6BJ, Northern Ireland, UK.

2. Regional Medical Cardiology Centre, Royal Victoria Hospital, Grosvenor Road, Belfast, BT12 6BA, Northern Ireland, UK.

Background: The complement factor H (CFH) gene has been recently confirmed to play an essential role in the development of age-related macular degeneration (AMD). There are conflicting reports of its role in coronary heart disease. This study was designed to investigate if, using a family-based approach, there was an association between genetic variants of the CFH gene and risk of early-onset coronary heart disease.

Methods: We evaluated 6 SNPs and 5 common haplotypes in the CFH gene amongst 1494 individuals in 580 Irish families with at least one member prematurely affected with coronary heart disease. Genotypes were determined by multiplex SNaPshot technology.

Results: Using the TDT/S-TDT test, we did not find an association between any of the individual SNPs or any of the 5 haplotypes and early-onset coronary heart disease.

Conclusion: In this family-based study, we found no association between the CFH gene and early-onset coronary heart disease.

P32. Identifying potential candidate genes in an Irish bipolar disorder sample linked to 14q21-32.

Cassidy F, Zhao C, Badger J, Delaney C, Mooney L, Roche S, McKeon P, Dobrin SE.

Trinity College Dublin, Dublin, Ireland.

Bipolar affective disorder (BPAD) is a severe and debilitating psychiatric illness. Family, twin and adoption studies have established a substantial genetic component to the illness but the genes involved have yet to be fully elucidated. A 10cM genome-wide linkage scan (WGS) was performed in a collection of 60 Irish BPAD affected sib pairs to locate chromosomal regions that may harbour susceptibility genes. The most significant result was on chromosome 14 at 75cM (14q24). Since the region of the chromosome containing significant *P* values was substantial, we undertook a fine-mapping analysis to refine the linkage peak. 144 SNP markers (400kb resolution) were analysed in an extended sample of 88 ASPs. Linkage analysis resolved our original linkage peak into 4 separate peaks, two of which overlap with published linkage peaks for related psychiatric disorders, such as anxiety and alcoholism. The most significant NPL score of 2.71 was at 67.84Mb, remarkably close to the original WGS peak score at 68.2Mb. In an additional analysis, two SNPs were found to be associated with BPAD (rs24166076 at 46.97Mb and rs4902942 at 71.21Mb). This project has substantially refined the region of chromosome 14 predicted to contain a candidate susceptibility gene for BPAD.

P33. Estimating carrier risks by linkage in a Duchenne Muscular Dystrophy family with a triple X female

Aileen Butler, David E Barton.

National Centre for Medical Genetics. Our Lady's Childrens' Hospital, Crumlin, Dublin 12.

We received a referral for carrier status on a female with a brother deceased with a clinical diagnosis of DMD, and no genetic testing done. There was no other family history of DMD. The consultand's mother had a CK of 480, and a number of other children (2 daughters and 1 unaffected son), all of whom had different fathers. The two eldest daughters had been adopted separately at an early age, and were also requesting information regarding carrier status. DNA samples from their fathers were unavailable, but we did receive a sample from their unaffected half-brother. Thus, the request was for linkage analysis in a very unusual and complicated pedigree, where samples were unavailable from many significant family members, including the index case. Linkage analysis commenced, yielding unexpected results which provided evidence of 3 distinct alleles at 5', intergenic, and 3' Dystrophin polymorphic markers in the index case's mother. Subsequent cytogenetic analysis confirmed a 47, XXX karyotype. Despite the presence of three Dystrophin haplotypes in her, and the complex pedigree, we were successful in haplotyping the family. Furthermore, it was possible to assign carrier risks to all at-risk females, two of whom were estimated to be at a <1% risk.

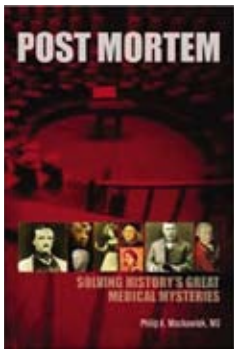
P34. Distal Duplication 10q: a case of gonadal mosaicism?

David McManus, June Jones, Moya Clarkson, Alex Magee

Regional Genetics Service, Belfast City Hospital, Belfast BT9 7AB.

Partial duplication of chromosome 10q is a rare abnormality usually associated with significant dysmorphism and intellectual deficit. The majority of published reports relate to segments encompassing up to one third of the long arm. Typically another chromosome is involved, which is likely to influence the phenotype. We present two sibs with a pure duplication of the most distal bands of chromosome 10q who show a relatively mild phenotype. TM and EM are sisters, in a sibship of five, who present with developmental delay but no significant dysmorphic features. Cytogenetic analysis demonstrated a small distal duplication comprising bands 10q26.13 to 10q26.3. Parental karyotypes were normal, suggesting gonadal mosaicism. Phenotypic expression is more developed in TM, the elder sister, who is now 10 years old. She presents with learning and behavioural difficulties with minor stereotypic movements, low muscle tone, hyperextensible joints, slight bilateral clinodactyly and exaggerated lumbar lordosis. The mild phenotype is clearly a function of the small size of 10q duplication in contrast to the severe phenotype normally observed.

Book Reviews



Post Mortem - Solving History's Great Medical Mysteries. Philip A. Mackowiak. American College of Physicians, Philadelphia, June 2007. Hardback, 350pp. £19.95. ISBN 978-1-93051-389-1.

We are fascinated by other people's problems; we are fascinated by celebrity - and when celebrities have problems, the effect is synergistic, rather than additive. This is not merely the province of tabloids and day-time television - serious scholars crave their fix of morbid gossip too. Historical diagnosis has a long and juicy history, and in "Post Mortem", Mackowiak revisits some of the most controversial diagnostic conundra from ancient times to the more recent past. In many cases we have only documentary accounts or artistic representations, and the reports of the times may be coloured by hearsay, political spin or mistaken superstition. Be that as it may, we like to feel in touch with our forebears, and maybe there is no better way to do it than to pick over their symptoms, real or imagined. This volume arises from a selection of cases from a series of historical Clinicopathological Conferences (CPCs; analogous to the Grand Rounds or clinical meetings that we in the UK know and love) held by the author in Baltimore, where he is a distinguished professor of Medicine.

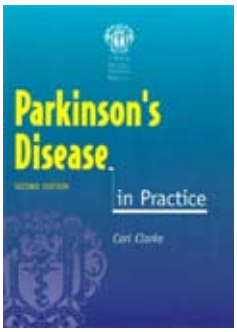
The first case on Mackowiak's cold analytic slab is Egypt's heretic sun king Akhenaten, who ruled in the glorious heyday of the New Kingdom in the 14th century BCE. Abandoning the ways of his forefathers, Akhenaten launched a radical programme in which he overturned the cults of the traditional Egyptian gods, and promoted the monotheistic worship of his one true god, represented by the solar disc, the Aten. Even the traditional representational art of Egypt was re-defined, and his depictions show him with elongated limbs, a pot belly, a serpentine neck, and other features previously unseen - and unthinkable - in the normally austere and idealised Egyptian artistic canon.

There has been a lively debate over whether these were depictions of Akhenaten in his true likeness, or an affected artistic style encapsulating new Atenist ideas of other-worldly royal divinity. Taking the former approach, many scholars have proposed diagnoses that might account for this etiolated phenotype. By far the most plausible proposition in my opinion is the connective tissue disorder Marfan syndrome, although Mackowiak ditches this on relatively flimsy grounds in favour of Klinefelter syndrome. Akhenaten and his wife, the legendary beauty Nefertiti, had at least six children who were represented in a similar style to Akhenaten himself, which would seem to exclude Klinefelter at a stroke, as Klinefelter syndrome causes infertility (and it is not even a particularly good match for the phenotype). Mackowiak largely glosses over this important objection, and sticks with what I feel is the wrong conclusion. However, whatever hypotheses we construct, without DNA confirmation we are not going to be able to resolve the issue. Akhenaten's mummy has never been firmly identified, so this may remain an open question, although recent work in Egypt suggests that the genetics of the New Kingdom rulers (including Akhenaten's probable son, Tutankhamun) might be a soluble problem after all. Watch this space.

Other cases from antiquity range from Herod the Great, whose gangrenous penis and worm-ridden demise were recounted with evident relish by the Jewish historian Josephus, to the emperor Claudius of Rome, who was plagued by movement and personality difficulties. We then advance a millennium or so, and run across Joan of Arc, Christopher Columbus and Florence Nightingale, among others. The cases are presented in a modified clinical format that is immediately familar, although such terminology as "the patient" (when referring to the case in question) feels a little contrived when we are discussing such matters from the dim and distant past. Each chapter ends with a very welcome set of references, which will undoubtedly prompt many readers to delve a little deeper.

This volume lends itself well to dipping, as each chapter is pretty much self-contained. It is a pleasant read, and stimulates and informs in equal measure. It is unfortunately let down a little by several typographical errors that seem to have crept in at the editorial stage. I am left thinking that "Differential Diagnosis" might have been a more appropriate title (with the format re-structured accordingly), and that a rather more argumentative discourse might have appealed more to a medical audience – generally a cantankerous bunch. Nevertheless, we get a strong feeling of "what might have been", had things turned out differently for our patients, or had their ailments been diagnosed correctly at the time. Perhaps it is indeed best that we have them at the remove of several centuries - for one thing, it makes the relatives less likely to sue.

Shane McKee



Parkinson's Disease in Practice (2nd edition). Carl Clarke. Royal Society of Medicine Press, London, December 2006. Paperback, 100pp. £18.95. ISBN 978-1-85315-745-5.

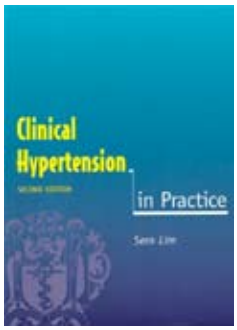
The first edition of this small book won a first prize in the British Medical Association's Medical Book Competition in 2002 and was favourably received by reviewers. The management of Parkinson's Disease has seen recent changes, including last year's NICE guidelines, warranting a second edition. In his preface, the author notes that the previous edition of his book was popular with "general practitioners, Parkinson's Disease Nurse Specialists, allied health professionals, pharmacists and even patients." This is a medical textbook which can be read by people with quite different levels of background knowledge, which is a testament to the author's comprehensible writing style. The chapters on epidemiology, aetiology and pathophysiology are brief and accessible, but feel comprehensive. The level

of detail is certainly adequate for the intended audience of non-specialists.

The book describes recent advances in diagnostic radiology which are improving discrimination in difficult cases and advancing understanding of the disease. Each of the different groups of medical therapies is appointed a chapter. What seemed (to this medical SHO) a mildly bewildering array of pharmaceuticals for the treatment of this disease is reduced to four groups with clear indications and remarkably uncomplicated pharmacology. In the spirit of Evidence Based Medicine, the author often provides brief descriptions and analyses of trials and illustrates these with graphs and confidence intervals. The reader is therefore acquainted with some of the controversies and background knowledge to confidently interpret the new NICE guidelines for the diagnosis and management of Parkinson's Disease. The final chapter concerns these new guidelines, and this book is harmonious with the recommendations. Parkinson's Disease in Practice is an accessible, authoritative introduction to the current knowledge in this debilitating illness which should be of interest to any doctor working on medical wards.

Declan Bradley

Clinical Hypertension in Practice (2nd edition). Sern Lim. Royal Society of Medicine Press, London, September 2006. Paperback, 114pp. £18.95. ISBN 978-1-85315-659-5.



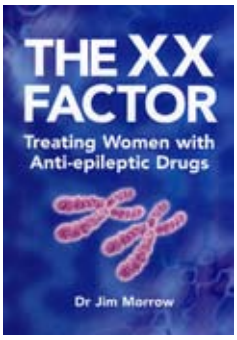
The invisible health hazard of hypertension is the focus of this short volume. It aims to be accessible to GPs, hospital doctors, students and nurses and is intended to be a summary of the current best practice, evidence and guidelines. The opening chapters describing the current understanding of the mechanisms of hypertension provide a background necessary to understanding the basis for treating hypertension. Where the book shines, though, is in the subject of clinical assessment. There is practical guidance for initial and further investigations, with comprehensive information about the interpretation of results and steering towards certain differential diagnoses. This, above all, makes this book a valuable resource for anyone treating hypertension.

With recent changes to the NICE guidelines for the treatment of Hypertension and so many trials of the newer pharmaceutical therapies, the long treatment chapter might warrant division into separate chapters for each class of drug in a future edition. Amidst the many drug trials, the lifestyle changes which lower blood pressure are not lost. The reader can confidently inform patients of the benefit of these non-pharmacological interventions.

The author offers further guidance in treating hypertension in the elderly, in those taking other medications and in pregnancy as well as giving clear guidance for the treatment of hypertensive emergencies. Clinical Hypertension in Practice provides a welcome refresher and update. Of its intended audience, junior medical staff and General Practitioners might find it most useful.

Declan Bradley

The XX Factor. Treating Women with Anti-epileptic Drugs. Jim Morrow. National Services for Health Improvement, Dartford, Kent, 2007. 80pp. £5.99. ISBN 978-0-9554803-2-4



There is increasing awareness and knowledge as to the risks of anti-epileptic drugs, particularly when used in pregnancy. Yet managing the care of women with epilepsy is even more complicated and difficult. Retrospective pregnancy registers are providing more reliable information regarding teratogenicity but it takes time to acquire information for many of the newer anti-epileptic drugs. Retrospective and small prospective studies continue to raise concerns regarding developmental outcome. Sodium valproate is of particular concern in both regards, but it remains one of the most effective treatments for specific epilepsies. Finally, increasing numbers of women are treated with anti-epileptic drugs for conditions other than epilepsy such as bipolar effective disorders, migraine, and chronic pain.

This small book with its eye-catching title attempts to address the evidence that is currently available in a no-nonsense, easy-to-read format. Although it recognises the lack of information for women taking anti-epileptic drugs for medical conditions other than epilepsy, the focus is on women with epilepsy. The book is divided into 10 chapters. The first 2 cover general issues of diagnosis and of the anti-epileptic drugs themselves. The remaining chapters are women specific, covering adolescence, fertility and sexuality, contraception, pregnancy, motherhood, and the menopause. The format is clear and easy to read with useful key points at the end of each chapter.

A wide range of health professionals should find this book helpful, including General Practitioners, Neurologists who see patients with epilepsy but would not consider themselves Epileptologists, Specialist Epilepsy Nurses, Practice Nurses, and Midwives. Psychiatrists and those who work in headache and pain clinics should also read this book if only to alert themselves to the uncertain risks they are exposing their patients to when prescribing anti-epileptic drugs.

This book does not of course provide all the answers but it does comprehensively go through the issues and meets its promise to present the evidence currently available. For those who need more detail, it directs the reader to a comprehensive list of over ninety references. This is a helpful book for those who need a quick update on this important area.

Aline Russell (Glasgow)

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