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Editorial

The Quick and The Dead

Humanity's accomplishments are many and varied, but one of mankind's most visceral drives, it would seem, is that for violence, war and the gratuitous felling and dismemberment of one's fellows. The meaningless and pernicious banality of a single unknown death has been movingly described by Wilfred Owen:

If you could hear, at every jolt, the blood
Come gargling from the froth-corrupted lungs,
Obscene as cancer, bitter as the cud
Of vile, incurable sores on innocent tongues[1],

The polar opposite, Medicine (*Primum non nocere* - First, do no harm), paradoxically, learned much, and in the case of surgery, probably almost everything, from conflict and war. Surgeons such as Ambroise Paré, Dominique Jean Larrey, William Beatty and Archibald McIndoe improved the lot of those for whom they cared, and left a legacy for those that would follow. So much has been written about The Great War, that it is difficult to imagine a perspective that might provide a fresh insight into the carnage. Professor Hedley-Whyte's historical review, 'Blood and War' achieves just that, and explores the relationship between the two, and the life-giving restorative of blood transfusions: liquid life among the haemorrhaging dead. The Journal wishes to thank Harvard University, the Rockefeller University, Johns Hopkins University, the Library of Congress, the Smithsonian Institution, and the Imperial War Museum for the hard work of their archivists and curators of art, as well as the trustees of the Evergreen Museum affiliated with Johns Hopkins University.

In Greek mythology, Death is personified as Thanatos, the twin brother of Hypnos, and the gods, respectively, of death and sleep. In her paper on Thanatophoric dysplasia, Dr Donnelly discuses the prevalence of this and other skeletal dysplasias in Northern Ireland. This range of disease too, spans the bridge between the living and the dead.

But is everything living or dead? Well, not quite. Vacillating between the two states is deoxyribonucleic acid, eternally reproducing but neither quick nor dead. In her masterful review, 'The Anatomy of a Chromosome,' Dr Stewart expounds on what can on occasion, seem that most non-intuitive of subjects. Read the review, and then turn to the proceedings of the Irish Society of Human Genetics. Hopefully it will have proved a personal enigma machine, decoding the code. It worked for me.

I am pleased to report the introduction of a new section entitled, "So you want to be a..". The Journal envisages this as a shop window, considering, issue by issue, a series of potential career pathways for the medical student or junior doctor. I am most grateful to Professor Peter Maxwell for providing the inaugural piece, on a career in Nephrology.

The Journal has also decided to embrace social networking sites, and now has accounts on Twitter (UMJ_Belfast) and Facebook. This initiative has been the result of Mary Crickard, our hard-working subeditor. My thanks to her. Please explore these new options, and let me have your verdict.

Barry Kelly
Honorary Editor

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Review

The Anatomy of a Chromosome

Fiona Stewart

Accepted 29 July 2010

A colleague of mine once said 'You know Fiona, this genetics is all very well but when we try and read a paper on genetics it's a bit like reading a car manual and then just when you get to the crucial bit, the language suddenly changes to Chinese and I can't make head nor tail of it'. I suspect he is not alone in his opinions and, if so, this is a great shame. It is well recognised in the genetics community that many health care professionals completed their training long before the great explosion in molecular genetics and so the role of the clinical geneticist includes that of being an educator. It is vital that we pass knowledge on to other clinicians so that they can make sense of all the new advances and use this to improve patient care.

Until recently cytogenetic and molecular genetics were two very separate disciplines. However as this review will show, molecular techniques are increasingly used in cytogenetics and the distinction between them is looking increasingly blurred and artificial.

Most of us are familiar with the standard karyotype obtained by looking at dividing cells under the microscope. For many years it has been a first line test in the investigation of learning disability, dysmorphic features and multiple congenital abnormalities. However, if you imagine your genetic material as being contained in a series of books then looking at a karyotype is a bit like looking at the bookshelf and seeing that all the books are there. It doesn't tell you if there are missing pages, missing chapters or indeed any spelling mistakes in any of the words on any of the pages. Newer molecular techniques are superseding it and its days as a front-line investigation are numbered.

Each of our cells should contain 46 chromosomes. Numbers 1 to 22 come in pairs and then there are the two sex chromosomes: XX in females and XY in males. Each chromosome is really a long tightly coiled string of DNA. Chromosomes are a method of organising the DNA into manageable units. It is estimated that each human cell contains about 2m of DNA. Donnai and Read[1] illustrate this nicely: if you were to enlarge an average 10μm cell to the size of a lecture room 10m across, then the DNA would be like a piece of string 2000km long. If it were not carefully organised, there would be a hopeless tangle and protein synthesis would be almost impossible.

When chromosomes are analysed we look not only at the actual number but also to see if there are missing pieces or extra pieces, so-called deletions and duplications. There is a standard method of nomenclature which can be very confusing. The short arm of the chromosome is referred to as the P arm and the long arm as the q arm. Each arm is then divided into numbered bands. Thus if the cytogenetic laboratory identifies an abnormality they can describe its precise location. The problem is that the description can lead to very complex formulae e.g. **46,XX,dup(2)(p13p22)** which means duplication of part of short arm of Chromosome 2. This is why sometimes it can be hard for non-genetic clinicians to make sense of the report. It can be helpful to have a clarifying sentence on the report to make it clear what is being described.

The situation can become more problematic when a translocation is being described. A translocation is a chromosomal rearrangement caused by the exchange of segments between two or more chromosomes. Such a rearrangement is described as **balanced** if there is no loss or gain of genetic material: the amount of DNA is correct but not all in the right place. If there is a pathogenic loss or gain of genetic material i.e. too much of one chromosome and not enough of another, it is said to be **unbalanced**. The word *pathogenic* is important because there are some parts of the genome that can be missing or duplicated with no ill effect - often referred to as normal variants or more correctly **large scale copy number polymorphisms**. Most people with a balanced translocation are clinically normal (unless one of the breakpoints goes through a particular gene and affects the function of that gene) whereas most individuals with an unbalanced rearrangement will have abnormal clinical features. Individuals with balanced translocations are at increased risk of miscarriage and also are at risk of having a child with an unbalanced translocation.

The standard nomenclature for translocations is often very confusing e.g.**45,XX,der(13;14)(p11;q11)** This describes a common Robertsonian translocation where one copy of chromosome 13 is attached to one copy of chromosome 14. Individuals with this balanced translocation are usually clinically completely normal. If you do receive a report, the meaning of which is not entirely clear to you, then it may

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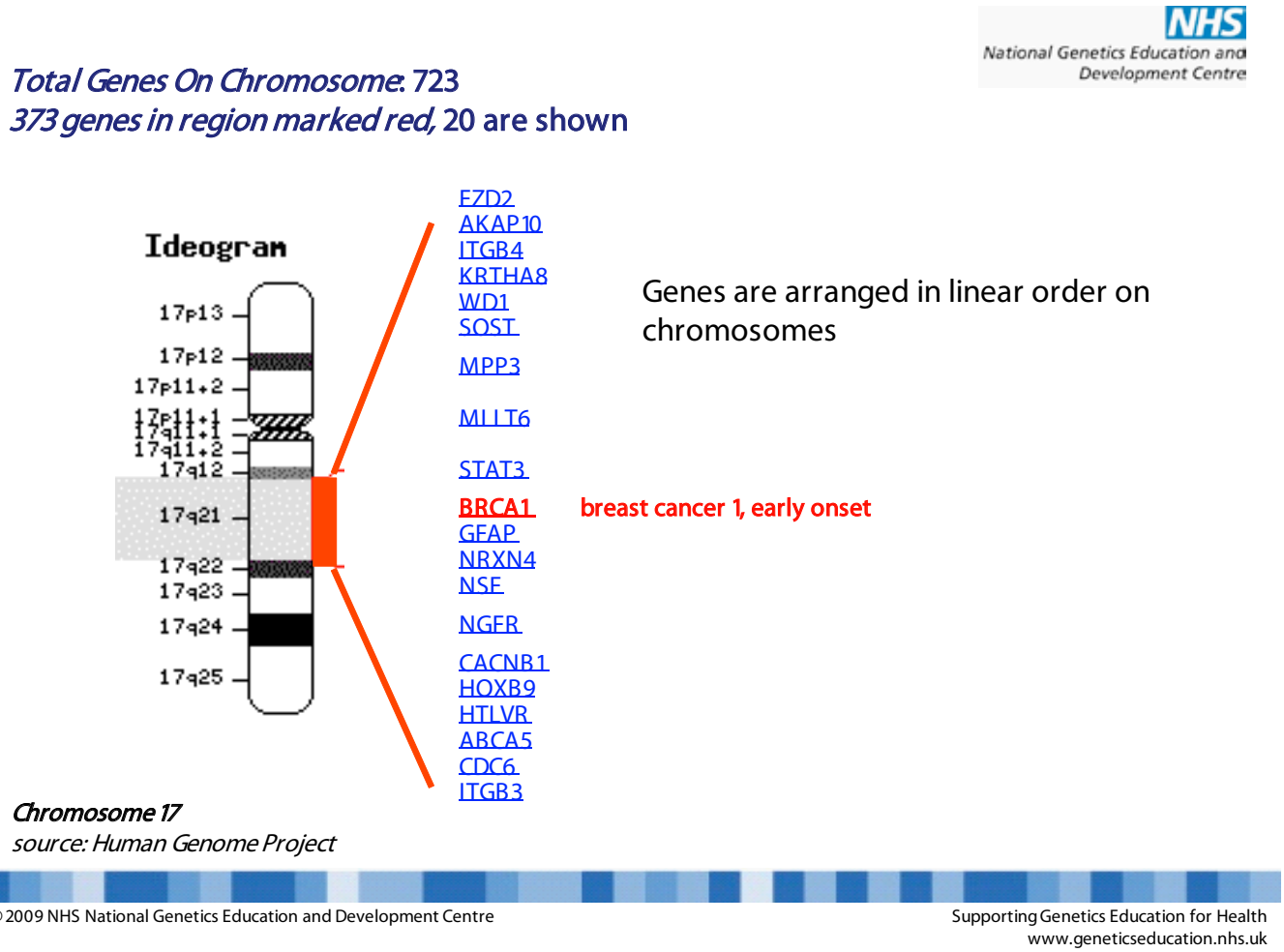

Fig 1. Illustration of the large number of genes contained within a small region of chromosome 17

be helpful to call the genetics department and either the cytogenetic staff or one of the clinicians will be able to help.

A technique for looking at small specific microdeletions is known as **Fluorescent in Situ Hybridisation** (FISH). In this technique, a DNA probe that is known to bind to a specific sequence on the chromosome has a fluorescent dye attached. If the sequence of interest is present on the chromosome, a fluorescent spot will be seen under UV light. If the sequence is missing, there is a microdeletion, and there will be no visible fluorescent spot. Paediatricians and cardiologists will be very familiar with the use of this technique to detect 22q11 deletion syndrome.

Microdeletions can also be detected using **multiplex ligation-dependent probe amplification** (MLPA). This is a useful technique that can enable us to look for a number of microdeletions or duplications at once.

As mentioned earlier, these techniques are being superseded by **genomic microarray techniques** (array CGH). These arrays use comparative genome hybridisation (CGH) of a mixture of test DNA from a diagnostic sample and normal control DNA. This technique will show if there is more or less DNA in any particular region and so will identify very small deletions and duplications not detectable by any other means. This is a major advance and in some parts of the UK, karyotyping as a first-line investigation for learning disability; multiple congenital malformations and dysmorphism has already been replaced by array CGH. The potential drawback of array CGH is that it may show variations, the significance of which is sometimes unclear.

The basic principles of DNA structure are quite simple. Chromosomes are really long strings of DNA. The structure of DNA is that of a double helix i.e. two polynucleotide chains wrapped round each other. Each nucleotide has three modules: the base which can be adenine (A) guanine (G), cytosine (C) or thymine (T); the sugar which is deoxyribose and a phosphate. The deoxyribose has five carbon atoms and links to the phosphate either by the 5' carbon or the 3' to make the sugar phosphate backbone to which the bases are attached. The RNA is also made up of four types of nucleotide. The bases are also A, G and C but thymine is replaced by uracil (U). DNA and RNA sequences therefore have a 5' end and a 3'end.

Genes are the functional units of the DNA and the function of a gene is to specify the structure of a protein. Protein synthesis involves transcription of the coding DNA (cDNA) into messenger RNA (mRNA) and then translation of the mRNA into a polypeptide. A triplet of three nucleotides in the messenger RNA (mRNA) known as a **codon**, codes for a particular amino acid. For example AGU codes for serine. There are 61 of these codons, so almost all amino acids are represented by more than one codon. There are also 3 stop

codons that tell the ribosome to dissociate and stop extending the protein. This part of the process from RNA to protein is usually referred to as **translation**.

A DNA sequence can therefore be written as a string of letters e.g. GTACACCTG. It is important to remember that sequences are written in a 5' to 3' direction. We also tend to use the term **upstream** to describe something that is closer to the 5' end than a particular area of interest and the term **downstream** if it's closer to the 3' end.

Chromosomes are a way of organising the DNA into manageable packages. Genes too are very organised. They consist of segments called **exons**, which code for proteins interrupted by non-coding sequences known as **introns**. Within the gene, there are also other regulatory elements e.g. promoters. It is estimated that the human genome contains approximately 21 000 genes (figure 1).

When a molecular test is ordered for a particular clinical condition, the laboratory are looking at the sequence of a particular piece of DNA to see if it is different from what would be expected. A simple comparison is to consider it as proof reading a piece of reading material to see if there are any mistakes. In the case of DNA the reading material is composed of only 4 letters. So what 'mistakes' can occur in DNA and lead to diseases?

There may be a deletion or duplication in the DNA, i.e. a piece of the gene is missing. If the DNA is regarded as being the code or the instructions for making a particular protein, then it is clear that a deletion in the DNA will have a consequent effect on the protein. It will either be abnormal, reduced or sometimes non-existent. As a triplet of three nucleotides – a codon – codes for a particular amino acid, if one or more of those nucleotides is missing there will be a change in the reading frame. It is therefore referred to as a **frameshift mutation**.

There may be a change in which one nucleotide is changed to a different one. This is referred to as a **point mutation**. It will alter the codon and may therefore change the amino acid. Sometimes it can be hard for families to comprehend that the change in one particular 'letter' of the genetic code can cause such severe abnormalities. I sometimes use the example of a 'gin and tonic' versus a 'gun and tonic'. One change in a letter, but two very different meanings ensue.

There are sometimes confusions about how mutations are described. A change that disturbs the reading frame is called a **frameshift mutation**, which is pretty straightforward. A point mutation that causes a change from one amino acid to another is called a **missense mutation**. If the mutation changes the codon for an amino acid to a stop codon, it is called a **nonsense mutation**. In the process of protein synthesis the non-coding introns are spliced out and the exons are joined together. If a mutation is likely to disrupt this process it is called a **splice site mutation**.

Rather like chromosome reports, molecular reports are written using internationally agreed standards. One of the most common tests carried out in any molecular genetics laboratory is mutation testing for cystic fibrosis. The commonest mutation is commonly known as ΔF508. The symbol delta was used to denote a deletion so this description means a deletion of phenylalanine at amino acid 508 resulting from a three nucleotide deletion. The correct current nomenclature is p.F508del. The prefix 'p.' refers to a numbered amino acid in the protein product and the prefix 'c.' refers to a numbered nucleotide in a gene sequence. If there is a point mutation or a substitution then the > symbol is used. Looking at another cystic fibrosis example; c.621+1G>T means that there is a change from a G to a T at position 621.

So, having ploughed through all this theory – how does genetic testing work in clinical practice?

Clinicians are aware that the time taken to get a molecular result varies widely. Why is this? Recalling the 'reading material' analogy, there is a huge variation in the gene size with some resembling a tabloid newspaper and others War and Peace or indeed all 24 volumes of the Encyclopaedia Britannica!

The other major time factor is whether the condition relates to common mutations that can be confirmed first, or whether the majority are private mutations. At the extremes of this spectrum, we could be looking for a spelling mistake that is always on line 6 of page 3 in your favourite tabloid or anywhere at all in the encyclopaedia. This can help account for the variation in reporting time and also the variation in cost. For some large genes that have a lot of private mutations genetic testing is rarely performed. The diagnosis should be clinically obvious and the cost to the NHS of large-scale testing would be prohibitive.

Let's look at the example of Duchenne Muscular Dystrophy. This is a life-limiting X-linked disorder. The diagnosis is suspected in boys who have muscle weakness and a significantly raised creatine kinase (CK). The dystrophin gene is found at Xp21, i.e. band 21 on the short arm of the X chromosome. It is an enormous gene comprising over 2 million DNA base pairs. Over 99% of the gene comprises introns which are non-coding. However this still leaves 79 exons. Looking for a mutation anywhere is this massive gene would be a very onerous undertaking. Approximately 75% of males will give a positive result on a dystrophin deletion/duplication analysis. If deletion/duplication analysis is negative, then further testing is carried out to look for point mutations. It can be a source of frustration for families and clinicians alike to go through this process, but this is the most logical and resource-efficient way to do it.

The other question that needs to be asked when ordering genetic testing is: 'why am I doing this?'. Professor Peter Farndon of the UKGTN (United Kingdom Genetic Testing Network) and NGEDC (National Genetics and Educational Centre) has described five good reasons for doing a genetic test:

- Diagnosis
- Treatment
- Prognosis and Management
- Pre-symptomatic Diagnostic Screening
- Genetic Risk Assessment

When is genetic testing helpful in making a diagnosis? Well

the good news is that history and examination is still the mainstay of clinical diagnosis, even for genetic disorders. A very good example of when genetic testing may or may not be helpful is to consider the two types of neurofibromatosis. Neurofibromatosis type I is an obvious clinical diagnosis and genetic testing is only rarely required. However neurofibromatosis type II is a very different condition. Skin changes are not obvious and there is a high risk of intracranial tumours. Genetic testing is very helpful for the at-risk relatives of an affected individual and will enable them to be appropriately investigated and followed up.

Genetic testing may not help make the diagnosis on an individual but may assist with genetic risk assessments for the wider family. In the example of the Duchenne family, identification of the mutation in the boy will enable us to see if his mother is a carrier (which would give a risk of ½ for future sons) or whether she is just at risk of gonadal mosaicism which give a significantly lower, though not zero, risk. Identification of mutations in other conditions can give an opportunity for prenatal diagnosis or even pre-implantation genetic diagnosis.

As technology advances, identification of particular mutations may have a greater influence on choices of treatment. Mucopolysaccharidosis type I is a lysosomal storage disorder caused by a deficiency of alpha iduronidase. Certain mutations such as W402X will lead to the Hurler form of the disease for which the treatment of choice is a bone marrow transplant. Other mutations cause the Scheie or Hurler Scheie forms which are treated with enzyme replacement therapy.

Genetic testing in the UK is organised through the UK Genetic Testing Network. If a laboratory wishes to offer a genetic test for other UK genetic centres the test undergoes a vigorous assessment to see what the benefits of performing the test might be for the patient and or the wider family. Technical methods, sensitivity and specificity are also examined. For any new test, a set of testing criteria is drawn up to ensure that these often expensive tests are being used on the appropriate target population. Other key questions are what impact performing a test will have on the NHS and also what impact not doing the test would have? If the test is approved, then a recommendation is made that commissioners should fund the test, although this is not mandatory. There is also a realisation that some genetic testing will move into mainstream medicine as not everyone with a genetic disorder will always see a clinical geneticist. Having this clear process in place should ensure that genetic testing is provided on an equitable basis throughout the whole of the UK and that the tests provided are fit for purpose.

This review is by no means comprehensive. Some of the more complex issues such as genomic imprinting or mitochondrial inheritance are beyond its scope. Further source material can be found at www.geneticseducation.nhs.uk and www.ukgtn.nhs.uk.

So, where does clinical genetics go next? We hope to see the widespread introduction of CGH microarray, although in these difficult financial times it may not be viewed as a priority by some. That said, failing to move to CGH microarray and persisting with conventional karyotyping, could be viewed as similar to the rejection of penicillin and continuing with poultices and other such treatments! Improvements in technology mean that whole genome sequencing will not be prohibitively expensive and its introduction into clinical practice may not be too far away. Such a development would be very exciting but would mean that we need to ensure that those ordering and using such tests have a thorough understanding of their uses and limitations.

The author has no conflict of interest.

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Paper

The prevalence of thanatophoric dysplasia and lethal osteogenesis imperfecta type II in Northern Ireland - a complete population study

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ABSTRACT

The minimum prevalence of lethal Osteogenesis imperfecta type II, thanatophoric dysplasia and achondroplasia were derived following detailed case note review of all perinatal lethal skeletal dysplasias (SD) in Northern Ireland over a 12 year period. Multiple sources of ascertainment, including genetic notes, radiological reports and post mortem findings, were used. 39 cases were identified. Thanatophoric dysplasia was the commonest diagnosis made (22), followed by osteogenesis imperfecta type II (four children) and achondroplasia (two children). Eleven other diagnoses each occurred once in the 12 year period. The minimum prevalence range, per live births, of each of the common skeletal dysplasias in Northern Ireland has been calculated; thanatophoric dysplasia 0.80/10,000, osteogenesis imperfecta type II 0.15/10,000 and achondroplasia 0.07/10,000. The prevalence range for thanatophoric dysplasia is much higher than reported in previous studies. We discuss reasons for the prevalence figures obtained.

Key words: Thanatophoric dysplasia, osteogenesis imperfecta type II, achondroplasia, prevalence, lethal skeletal dysplasias.

BACKGROUND

The genetic skeletal dysplasias are a large group of disorders that can present at any age and with a very varying phenotype. A proportion of these conditions are lethal due to significant dysplasia of the thoracic cavity, leading to pulmonary hypoplasia. Phenotypic variation remains, however, with some fetuses dying in the early second trimester, while some continue to full term and may live for a few months after birth. Lethal skeletal dysplasias can be picked up on antenatal ultrasound scan, with many having specific diagnostic features that can be detected from the second trimester onwards[1,2]. All have short ribs, with small chest circumference and abnormal chest to abdominal circumference ratios for gestational stage. Thanatophoric dysplasia (OMIM 187600 and 187601) is characterised by 'French telephone receiver' shaped femora in type I, and a cloverleaf-shaped skull in addition in type II. Osteogenesis imperfecta type II (OMIM 166210) causes multiple fractures, particularly in the long bones, which leads to bony deformity. Although some fetuses in our review had achondroplasia (OMIM 100800), it should be noted that the vast majority of infants with this diagnosis survive.

Accurate figures on incidence and prevalence of lethal skeletal dysplasias are difficult to estimate. Not many studies have published prevalence figures[3-5], and only one of these was published in the past 20 years. Our population of 1.75 million, is homogeneous, relatively static, with low immigration and emigration, has a defined geographical area and a health care system where our regional genetics service covers the entire geographical area, allowing prevalence to be calculated precisely. Ascertainment was from multiple sources to improve accuracy.

Table I

Breakdown of 'Other Diagnoses'

	Number
Undiagnosed SD, dysmorphic	1
Spondyloepiphyseal dysplasia congenita[a]	1
Hypophosphatasia[b]	1
Osteocraniostenosis / atenolol teratogenesis	1
Severe costovertebral dysplasia, Jarcho levin[c]	1
Weyer's syndrome, Acrofacial dysostosis[d]	1
Sirenomelia	1
Apert syndrome[e]	1
Pseudoachondroplasia, Hypochondroplasia[f]	1
Pacman dysplasia[g]	1
Larsen syndrome[h]	1

a - type II collagen disorder, b - bent bone dysplasias, c - dysostosis with predominantly costal or vertebral involvement, d - dysostosis with predominantly craniofacial involvement, e - craniosynostosis syndrome, f - FGFR3 group of dysplasias, g - Filamin group of dysplasias

METHODS

We carried out a review of all cases of lethal skeletal dysplasias, defined as perinatal deaths due to skeletal

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TABLE II

Karyotype results

	Thanatophoric Dysplasia	O.I. Type II	Achondroplasia	Other	Total
46XX	2	1	0	6	9
46XY	6	1	1	1	9
47XX	0	0	1	0	1
Normal, sex not reported	1	0	0	0	1
FISH T13/18/21 normal	3	0	0	0	3
Failed growth	5	0	0	1	6
Not tested	5	2	0	3	10

dysplasias occurring up to 6 months postnatally. The study period covered 12 years, from 1st January 1995 to 31st December 2006. Patients were identified from a variety of sources. A search was carried out using our departmental genetic database for all patients diagnosed with a skeletal dysplasia during this period. The records database from the radiology department in the Royal Belfast Hospital for Sick Children, where all regional and post-mortem paediatric radiographs are sent for reporting, provided a list of all children/fetuses with a diagnosis of a skeletal dysplasia within the study period. Post mortem reports were obtained for all patients with a lethal skeletal dysplasia. Genetics charts were reviewed retrospectively and data was collected on diagnosis, genetic and non-genetic test results, parental details and family history.

STATISTICS

Descriptive statistics were carried out on data stored in an Excel spreadsheet. The prevalence was calculated based on total population, and on numbers of total and live births, obtained from the Northern Ireland Statistics and Research Agency [www.nisra.gov.uk].

RESULTS

39 patients were identified who died from a skeletal dysplasia in the study period. Of these, 37 (94.9%) were an intra-uterine death or died in the immediate post-natal period. One child with achondroplasia survived to six months of age. Another, with suspected Pacman dysplasia, died at 11 weeks of age.

The diagnosis was thanatophoric dysplasia, type I or II, in the majority: 22 cases (56.4%), followed by osteogenesis imperfecta type II, 4 cases (10.3%) and achondroplasia, 2 cases (5.1%). There were 11 other fetuses that died from conditions with a significant skeletal component, only five of whom had a definite diagnosis (Table I).

GENETIC TESTS

A karyotype result was available in 20 (51.3%) babies. Karyotype analysis failed in a further nine (23.1%), though three had trisomy 13, 18 and 21 ruled out by FISH (fluorescent in situ hybridization). Thus, a total of 29 (74.3%) had a karyotype result requested (tables II and III). There were equal numbers of males and females. There were two coincidental associations; one child with trisomy 21 (Down's syndrome, later confirmed by karyotype) and achondroplasia[6], and one with 22q11.2 deletion syndrome (Di George syndrome) and thanatophoric dysplasia. This was the only fetus with thanatophoric dysplasia that had a confirmed FGFR3 mutation as this diagnosis is commonly made radiologically. The two patients tested for 22q11.2 deletion syndrome both had characteristic dysmorphic facies, along with cardiac abnormalities; truncus arteriosus in the fetus who tested positive and dextroposition in the fetus who tested negative. The fetus tested for PTPN11 was thought clinically to have Noonan's syndrome, and had bilateral pleural effusions, polyhydramnios and widely spaced nipples.

NON-GENETIC TESTS

These consisted of antenatal ultrasound scans, radiographs (either ante or post-mortem), and post mortem studies (Table IV). Radiographs were the most accurate diagnostic test, and were carried out in 38 cases (97.4%). They confirmed the diagnosis in the majority (84.6% of total, 86.8% of those with radiology), while all remaining cases showed generalised

TABLE III

Results of other genetic tests

	Thanatophoric Dysplasia	O.I. Type II	Achondroplasia	Other
22q11.2 deletion detected	1	0	0	0
FISH 22q11.2 normal	0	0	0	1
FGFR3 mutation detected	0	0	1	0
PTPN11 normal		0	0	1

Table IV

Non-genetic diagnostic testing

	Thanatophoric Dysplasia	O.I. Type II	Achondroplasia	Other	Total
Total	22	4	2	11	39
Radiology:					
4 Confirmed diagnosis	21	4	2	6	33
Generalized abnormality	0	0	0	5	5
Not carried out	1	0	0	0	1
PM:					
Yes	20	3	1	9	33
No	2	1	1	2	6
Scan:					
Normal	0	0	0	1	1
Abnormal	22	4	2	10	38

abnormalities. One baby did not have radiology; a diagnosis of thanatophoric dysplasia was made on the basis of a characteristic antenatal ultrasound appearance, the parents declining further investigation.

There were 20 patients (84.6%) who had a post mortem. Dysmorphic features were frequently documented. Non-skeletal defects were rare. Cardiac defects occurred in three fetuses as follows: Fallot's tetralogy associated with trisomy 21 and achondroplasia, truncus arteriosus associated with 22q11.2 deletion syndrome and thanatophoric dysplasia, and dextroposition associated with possible Jarcho Levin syndrome. Hypospadias and an inguinal hernia occurred in a fetus with possible Pacman dysplasia. Hydrocephalus and cleft lip and palate were found in a fetus with thanatophoric dysplasia. These associations were considered to be coincidental. One child, who had a normal antenatal ultrasound scan, and died at 39 weeks gestation, had a diagnosis of severe costovertebral dysplasia, possibly due to Jarcho Levin syndrome.

Table V

Population details

Year	Total population	Total number of births	Total number of live births
95	1649100	23838	23693
96	1661800	24535	24382
97	1671300	24218	24087
98	1677800	23790	23668
99	1679000	23089	22957
00	1682900	21605	21512
01	1698300	22074	21962
02	1696600	21507	21385
03	1702600	21756	21648
04	1710300	22431	22318
05	1724400	22417	22328
06	1741600	23361	23272
Total	20295700	274621	273212

Data from Northern Ireland Statistics & Research Agency ref [www.nisra.gov.uk]

None of the fetuses were conceived by assisted reproductive techniques. Gestational ages at diagnosis and birth details were not accessible for every case. Although most abnormalities were picked up at the 20-week anomaly scan, some were seen as early as 15 weeks gestation. Most were diagnosed less than a week from birth (53.8%), though five fetuses (12.8% of total, 25% of those in whom gestation known) survived to full term (data not shown).

In total, 22 families (56.4%) were seen by our genetics service. There was no family history of skeletal dysplasia in any of these. Prevalence was calculated in three ways, based on total population, total number of births and total number of live births (Table V). As our numbers of cases of skeletal dysplasias fluctuate each year, a prevalence range was obtained, along with total prevalence for the whole study period (Tables VI, VII).

DISCUSSION

The relative frequencies of the three commonest dysplasias in our population study are as follows; 22 cases (56%) of thanatophoric dysplasia, four cases (10%) of osteogenesis imperfecta type II and two cases (5%) of achondroplasia. Overall these three diagnoses make up 72% of our population. This breakdown differs from that previously observed[1], and our proportion of babies with thanatophoric dysplasia is significantly higher.

Our prevalence rates were calculated according to three different population statistics. As expected, prevalence based on total population was much lower than prevalence based on total and live births, which were very similar. However, most studies in the literature state prevalence based on total births[3-5]. Our minimum prevalence rates per 10,000 total births are as follows; 0.80 (range 0-1.72) for thanatophoric dysplasia, 0.15 (range 0-0.46) for osteogenesis imperfecta type II and 0.073 (range 0-0.45) for achondroplasia. The range is higher than the overall figure for osteogenesis imperfecta type II and achondroplasia as the fact that, in most years, no children died

TABLE VI

Prevalence calculations per 10,000 population and total births

Year	Thanatophoric Dysplasia			O.I. Type II			Achondroplasia		
	No.	PTP	PTB	No.	PTP	PTB	No.	PTP	PTB
95	0	0	0	0	0	0	0	0	0
96	0	0	0	1	0.006	0.41	0	0	0
97	1	0.006	0.41	1	0.006	0.41	0	0	0
98	3	0.018	1.26	0	0	0	1	0.006	0.42
99	1	0.006	0.43	0	0	0	0	0	0
00	1	0.006	0.46	1	0.006	0.46	0	0	0
01	2	0.012	0.91	1	0.006	0.45	0	0	0
02	2	0.012	0.93	0	0	0	0	0	0
03	3	0.018	1.38	0	0	0	0	0	0
04	3	0.018	1.34	0	0	0	0	0	0
05	2	0.012	0.89	0	0	0	1	0.006	0.45
06	4	0.023	1.71	0	0	0	0	0	0
Total	22	0.011	0.80	4	0.002	0.15	2	0.001	0.073

Key: PTP = prevalence based on total population, PTB = prevalence based on total number of births

perinatally with these diagnoses, brings the overall average prevalence down. Our prevalence of thanatophoric dysplasia is higher than that previously reported[3], when our paternal age is taken into account; (average age 33.4 years, range 29 to 40 years). We expect our data to be more accurate than the prevalence rates previously published. Firstly, almost complete ascertainment should be possible in Northern Ireland due to the static nature of our population, contained in a well-defined geographical area, which is served by a single genetics unit. A central paediatric hospital reports on all X-rays and carries out all post mortems. Secondly, our figure for those in whom the diagnosis was uncertain, 20%, is much lower than in previous studies[4]. The prevalence of osteogenesis imperfecta type II and achondroplasia were much lower than previous reports. All of our cases represent new mutations. Unfortunately, detailed analysis of parental age was not possible as this information was not available in every case. Information on birth details, such as proportion of terminations, was also incomplete. However, this should not affect our prevalence rates as termination would have been carried out after the diagnosis of a lethal skeletal dysplasia had been made.

A karyotype was requested in almost 75% of cases. Six out of the 29 cultures (20%) failed to grow and this would not be unexpected from post mortem samples. It should be noted that amniocentesis is much more accurate[7]. Although the majority of karyotypes were normal, some interesting coincidental findings occurred. One child had trisomy 21 and achondroplasia, which has rarely been observed[6,8]. One had 22q11.2 deletion syndrome and thanatophoric dysplasia; this association does not appear to have been previously reported.

Radiology was the best investigative tool, confirming the diagnosis in the vast majority of cases (87%). There were 33

TABLE VII

Prevalence calculations per 10,000 births

	Thanatophoric Dysplasia	O.I. type II	Achondroplasia
Prevalence range	0 -1.71	0-0.46	0-0.45
Donnelly (this study)	0.80	0.15	0.07
Orioli[4]	0.09	0.43	0.46
Stoll[5]	0.28	0.64	0.64
Waller[3] - Atlanta	0.25		0.39
Waller[3] - Iowa	0.30		0.41
Waller[3] - Oklahoma	0.21		0.60
Waller[3] - Texas	0.21		0.39

(85%) fetuses that had a post mortem. Non-skeletal defects were extremely uncommon and were much more likely to occur in the setting of a second genetic diagnosis.

Here, we publish the most accurate prevalence figures to date for lethal skeletal dysplasias. We have shown that thanatophoric dysplasia is more common than previously thought. By raising awareness of this important group of disorders, we hope to improve antenatal diagnostic accuracy and genetic counselling.

ACKNOWLEDGEMENTS

With thanks to Drs Caroline Gannon and Claire Thornton, fetal pathologists at the Royal Belfast Hospital for Sick Children, who completed all the fetal post mortems and allowed us full access to their reports. The authors have no conflict of interest.

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Paper

Imprint Cytology Predicts Axillary Node Status in Sentinel Lymph Node Biopsy

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INTRODUCTION

Sentinel lymph node biopsy (SLNB) has gained widespread acceptance for axillary node staging in breast carcinoma. Results from the ALMANAC trial confirmed clear benefits for SLNB in terms of arm function and quality of life measures[1].

An accurate and reliable method of assessing sentinel lymph node (SLN) status at the time of primary surgery is desirable. It would avoid the need for a second operation in the significant number of patients who are node-negative at diagnosis.

Imprint cytology is a well-recognised simple technique for preparing a surgical specimen for pathological assessment. The excised SLN is sent fresh to the pathologist who processes it immediately. The cut surfaces are pressed onto a glass slide, which is then fixed and stained.

The aim of this study was to assess the use of imprint cytology as an intra-operative tool for evaluating sentinel lymph nodes in patients with clinically node-negative breast cancer.

MATERIALS AND METHODS

Data was collected prospectively in a specialist breast unit in a district general hospital serving a population of 300,000 and treating approximately 170 new breast cancers each year**.** Patients meeting the inclusion criteria stipulated by the 'New Start' programme[2] were studied consecutively.

Three consultant breast surgeons underwent training in SLNB (under the auspices of New Start/ Royal College of Surgeons of England). Each surgeon performed 5 cases proctored by a surgeon experienced in the technique, followed by a further 25 cases without supervision. After SLNB, a formal axillary node clearance (ANC) was performed.

SENTINEL NODE IDENTIFICATION

Sentinel nodes were identified using a combination of patent blue dye (Patent Blue V; Guerbet Laboratories Ltd, Birmingham, UK) and radioactive tracer (99mTechnecium-labelled human albumin nanocolloid particles; Nanocol; Nycomed Amersham PLC, UK). Depending on the scheduled time of surgery, 0.2mls of either 15mBq (same day) or 20mBq (following day) ^{99m}Tc nanocolloid was injected intra-dermally into the peri-areolar area of the breast. Uptake of radiocolloid was mapped and a skin mark placed at the level of the sentinel node, as identified by the static detector.

Following induction of general anaesthesia, 2mls of patent blue dye mixed with 2mls 0.9% saline was injected subdermally and gently massaged for 5 minutes. A small incision was made in the axilla at the point of maximum radioactivity as determined by the portable gamma probe (Europrobe, Bright Technologies Ltd, Sheffield, UK). Sentinel nodes were identified by tracing the blue dye (through direct visualisation of the blue-stained lymph channels) and the radio-isotope (using the gamma probe). All blue-stained nodes and/or radioactive foci were excised and sent to the pathology laboratory for immediate processing and analysis. The gamma probe was used to ensure that radioactivity levels had fallen to a point that would be consistent with removal of all sentinel nodes. The axilla was then examined to identify if there were any palpable nodes before it was cleared in the usual manner.

STAINING AND EXAMINATION OF NODES

Each sentinel lymph node was sectioned transversely into 2mm slices. Depending on the size of the node, 2 – 4 imprints were made from each slice by gently touching the cut surface of the node onto a glass slide. These were air-dried and stained with Rapi-Diff II stain (Triangle Biomedical Sciences Ltd, Lancashire, UK) before being reviewed by 2 or more pathologists using a multi-headed microscope. Analysis was performed during each operation with nodal status consistently determined within 45 minutes of the specimen leaving theatre. However, as these cases were carried out as part of the audit phase of SLNB training, results were not relayed intra-operatively and thus had no impact on the surgery performed.

In some cases, suspicious groups of cells were present on the imprints. However, a positive report was only given if the number and/or the morphological features of the cells were sufficient to give a diagnosis of definite metastasis. (Figure 1) All slices of the sentinel lymph node were formalin-fixed and embedded in paraffin. They were then examined after Haematoxylin and Eosin (H&E) staining. If negative on H&E, nodes underwent immunohistochemical staining with the monoclonal anticytokeratin antibody Clone MNF 116 (Dako, Glostrup, Denmark) using the avidin-biotin-peroxidase complex method. The pathologist who prepared and reported the imprint was also responsible for reporting the

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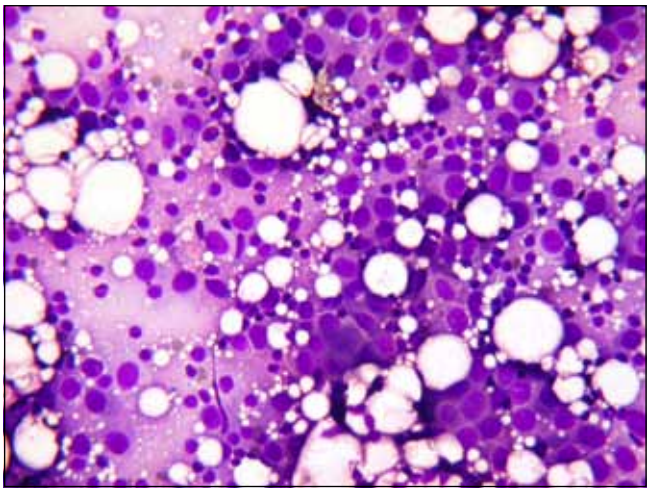

Fig 1. positive imprint cytology; Rapi-Diff II stain; x40 magnification

sentinel lymph node H&E / immunohistochemistry sections, the axillary nodes and the breast specimen.

RESULTS

Over an 11 month period, 102 consecutive patients with clinically node-negative disease had SLNB followed by axillary clearance. The mean age was 58.8 (28-89). The median tumour size was 20mm. Most patients (60%) had grade 2 tumours, 20% had grade 3 and the remainder had grade 1. Lymphovascular invasion was present in 33% of patients. (Table 1)

Table 1:

Primary Tumour Characteristics

Mean Age (years)	58.8	
Mean Tumour Size (mm)	20	
Lymphovascular Invasion (% patients)	33	
Histopathology (% patients)	Ductal	63
	Lobular	15
	Other	22
Tumour Grade (% patients)	1	20
	2	60
	3	20

An average of 2.3 nodes (1-9) were identified per patient. The identification rate was 100%. Sentinel node metastases were detected in 41 patients. Metastatic deposits >2mm were designated macrometastases (Figure 2) while those ranging between 0.2 and 2mm were considered micrometastases (Figure 3). Histopathological analysis of the axillary clearance nodes revealed metastatic disease in 44 patients, giving SLN biopsy a sensitivity of 93.2%.

Intra-operative imprint cytology identified 33 of the 41 patients with sentinel node positive disease. There were no false positives. There were 8 cases where imprint cytology of the sentinel node was negative but metastases were detected by H&E and immunohistochemistry. Of these false negatives, 4 were macrometastases and 4 were micrometastases. Immunohistochemistry did not detect any further metastases on the H&E negative sections. In total, there were 6 cases of micrometastases. Two of these were positive on imprint cytology, with one case having micrometastatic deposits in 2 out of the 4 detected sentinel nodes.

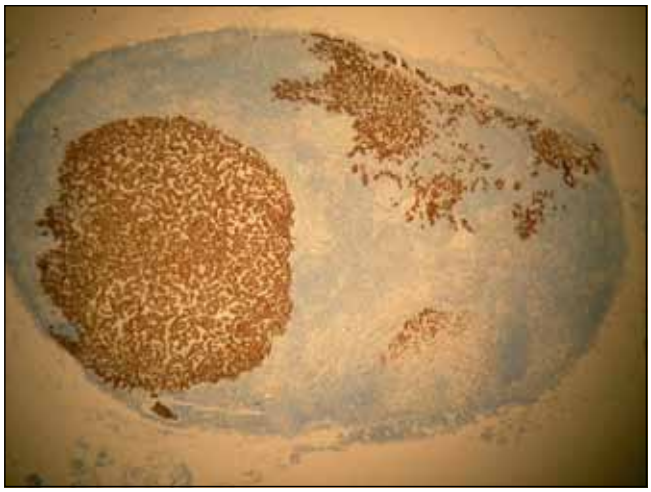

Fig 2. lymph node with macrometastasis; MNF 116 stain; x2.5 magnification

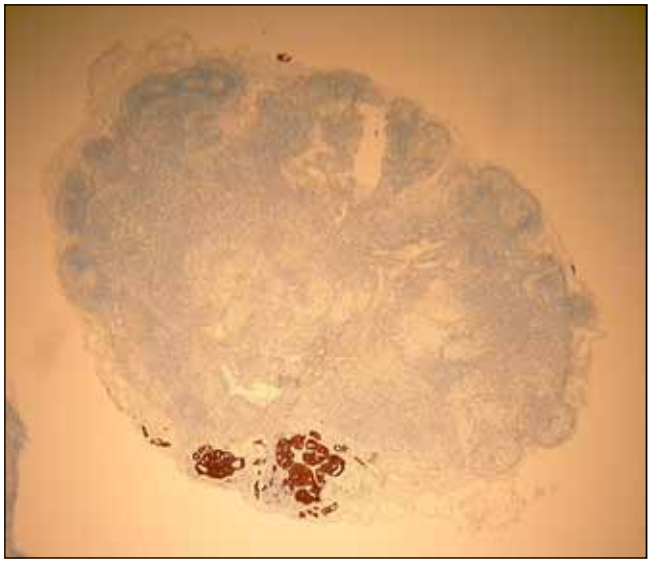

Fig 3. lymph node with micrometastasis; MNF 116 stain; x2.5 magnification

These figures give imprint cytology in this study an overall sensitivity of 80%, a specificity of 100% and a negative predictive value of 88%.

DISCUSSION

It is well accepted that axillary node status is the most important prognostic indicator in patients with invasive breast cancer. Knowing the nodal status is essential for correct cancer staging and helps determine the need for adjuvant therapies. However axillary lymph node dissection (ALND) is associated with significant morbidity, with up to 60% of women experiencing long-term side-effects. (3). Moreover ALND is unnecessary for women who have node-negative disease and studies have shown this can be as high as 70% in those with T1 and T2 tumours.[3]

Sentinel nodes have been shown to be representative of the presence or absence of metastases in the remainder of the nodal basin. Sentinel lymph node biopsy is increasingly

TABLE 2:

Sensitivity of imprint cytology

Sentinel node metastases	Final histopathology (no. of cases)	Imprint cytology (no. of cases)	Sensitivity of imprint cytology (%)
Macrometastases	41	33	80%
Micrometastases	6	2	33%

being used to predict axillary node status in breast cancer on a world-wide basis. It allows directed therapeutic node dissections and confines the morbidity of the procedure to patients who will potentially benefit from removal of involved nodes.[4] Data from the randomized controlled ALMANAC trial (Axillary Lymphatic Mapping Against Nodal Axillary Clearance) confirmed clear benefits for clinically node-negative patients undergoing SLNB, rather than conventional axillary treatment, in terms of arm function and quality of life measures.[1,5]

Various techniques for localization and assessment of the sentinel node have been employed by different centres over the past decade. Whilst there is no current "optimal" protocol[6], detection using a combination of radiotracer administration, preoperative nuclear medicine imaging, blue dye injection and intraooperative gamma counting has been advocated as this appears to increase the sentinel node yield and reduce the learning curve[6,7].

Once harvested, sentinel nodes undergo a thorough histopathological examination. Multisectioning rather than routine bisectioning is known to decrease the sampling error phenomenon and increase metastatic tumour detection[6]. Other studies have shown that cytokeratin immunohistochemistry staining also increases metastatic tumour detection when compared with H&E staining[6]. However immunohistochemical analysis of H&E negative sentinel nodes did not upstage any of the patients in our study.

The frequently reported methods of intraoperative assessment are frozen section histology and imprint, or touch-preparation, cytology. Reports of frozen section examination have described a sensitivity of 44-100% and a specificity close to 100%[8]. However, the procedure is time-consuming and the process of freezing, then thawing, the specimen can introduce artefacts. Furthermore, there is often significant tissue loss, potentially interfering with subsequent more detailed pathological examination with paraffin sectioning[8].

Imprint cytology proved to be a very efficient tool for intra-operative assessment of the sentinel nodes due to clear lines of communication between theatre and pathology staff. Although the time varied depending on the size of the node and the number of nodes requiring analysis, results were usually available within 45 minutes. This is comparable with the experience of other units carrying out intra-operative imprint cytology[9]. Whilst waiting for the result, surgery to the breast can be performed.

The literature suggests that imprint cytology is comparable in accuracy to frozen sectioning[10]. In this study the technique had a sensitivity of 80% and a specificity of 100%. It is important to remember that the definitive SLN status assessed by histopathological assessment of the node is the standard with which results of intra-operative evaluation are compared. Hence the detailed sampling carried out may indicate a less favourable intra-operative accuracy than if a limited sampling of the SLN had been performed.

There were only 8 false negative cases and 4 of these were micrometastases. The sensitivity of imprint cytology in detecting micrometastases in this study was 33% (Table 2). This proportion is higher than that found by other centres[8] and may be due to the relatively small numbers in the study. It is noteworthy that most subsequent cases of micrometastases in our unit have been imprint negative.

As SLNB becomes more widely used, detection of micrometastasis in sentinel nodes is increasingly proving a therapeutic dilemma. The prognostic significance and clinical relevance of these previously occult metastases is controversial[11]. Hansen et al examined the John Wayne Cancer Centre experience with 790 patients who had undergone SLNB[11]. They observed that, at 8 years, patients with micrometastases in their SLNs had better prognosis than patients with SLN macrometastases and had prognosis equal to those with SLN-negative disease.

In contrast, the International (Ludwig) Breast Cancer Study, one of the largest studies of patients with nodal micrometastases described to date, reported that 83 patients with micrometastases had a worse disease-free and overall survival after 5 years median follow-up than did patients who were node-negative on retrospective analysis and serial sectioning[12]. A large recently published retrospective review from the Netherlands also demonstrated that micrometastastes were associated with an absolute reduction in 5-year disease-free survival of nearly 10%[13].

Further data from larger studies with longer follow-up is thus required before definitive conclusions regarding the relevance and optimal management of micrometastases in sentinel lymph nodes can be made. Notwithstanding, even if micrometastases were regarded as positive nodal disease, in clinical practice 80% of patients in this series could have had their primary tumour and their axilla treated in one operation.

CONCLUSION

Sentinel lymph node biopsy is rapidly becoming the standard of care for patients with breast cancer. The technique can be learned quickly, but SLNB is a multidisciplinary process requiring continuous audit.

Imprint cytology has been shown to be reliable for predicting SLN status. This study has demonstrated that imprint cytology allows a one stage procedure in 80% of patients with node positive disease, and is to be commended as a useful and practical technique for those using SLNB for axillary staging in breast cancer.

The authors have no conflict of interest

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Case Report

Nicorandil induced penile ulceration

Michael Kinney, Declan O'Rourke, Hugh O'Kane, Patrick Keane, Thaigarajan Nambirajan

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Keywords: Nicorandil, Penile ulceration.

ABSTRACT

We report the unusual complication of penile ulceration caused by Nicorandil, a nicotinamide ester used in the treatment of symptomatic angina pectoris.

INTRODUCTION

Penile ulceration presenting in elderly patients most commonly will have either a neoplastic or infective aetiology. Biopsy is required to exclude a malignancy with subsequent treatment dependent on the histopathological diagnosis.

CASE REPORT

Two patients aged 87 and 80 presented with a 4-6 week history of painful penile ulceration on the dorsum of the penis (Fig 1). There was no associated palpable groin lymphadenopathy in keeping with malignant or infective disease. The patients had been taking nicorandil for 3 and 5 years respectively at a dose between 10-20mg twice a day. In addition to a history of cardiovascular disease both patients also were non insulin dependent diabetics and were previously smokers.

Biopsy of both lesions demonstrated inflammation with no evidence of malignancy (fig 2). On the advice of the cardiologist the nicorandil was discontinued in both cases and on follow-up six weeks later the pain had subsided but the large ulcer persisted necessitating a circumcision in both cases.

DISCUSSION

Nicorandil is a nicotinamide ester[1] which relaxes vascular smooth muscle of blood vessels. It has a duel mechanism of action, firstly by donation of a nitric oxide to activate guanylate cyclase and secondly through activation of ATP-sensitive potassium channels[2]. Arterial and venous vasodilation acts to reduce cardiac preload and afterload. It is a novel drug in its class which can be used alone or in combination with other anti-angina treatments[3].

In addition to well described side effects such as headache, facial flushing, dizziness and hypotension[1] recently cases of skin ulceration have been described. Ulcers of the oral mucosa[1], anal [4], perianal [5], parastomal cutaneous sites[6] have all been documented in the literature. Only one case of penile ulceration has been reported to date [7]. Classically the ulcers tend to be deep and well demarcated with histology revealing acute inflammation.

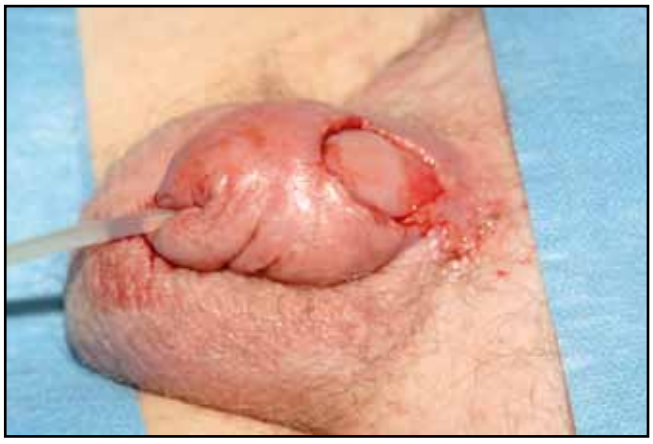

Fig 1. Catheterised penis with a well demarcated ulcer with surrounding erythema and oedema resulting in complete erosion through the prepuce exposing the glans beneath.

A number of theories for the pathogenesis of nicorandil-induced ulceration have been hypothesised including a direct toxic effect of nicorandil or one of its metabolites on the tissues[1]. A second theory is the "vascular steal hypothesis"[7]. Nicorandil due to its action on the local circulation causes alterations in arterial and venous flow, and the hypothesis is that this could have a profound effect on end arteries, such as the penis. Other risk factors for vascular disease such as diabetes and smoking as in these two patients could obviously contribute to any ischaemic phenomenon.

The differential diagnosis for patients presenting with penile ulceration should include malignancy, and infections such as syphilis. Serological testing was not performed in this group of two patients, for two reasons. Firstly the clinical risk was determined to be low based on the clinical history, and secondly the lesion we describe was painful in contrast to the painless chancre of primary syphilis. It would be important however in the sexually active population to determine the VDRL (Venereal Disease Research Laboratory) status of patients presenting with similar lesions. Nicorandil which has been introduced into clinical use within the last decade and it should be recognised in the differential diagnosis of these

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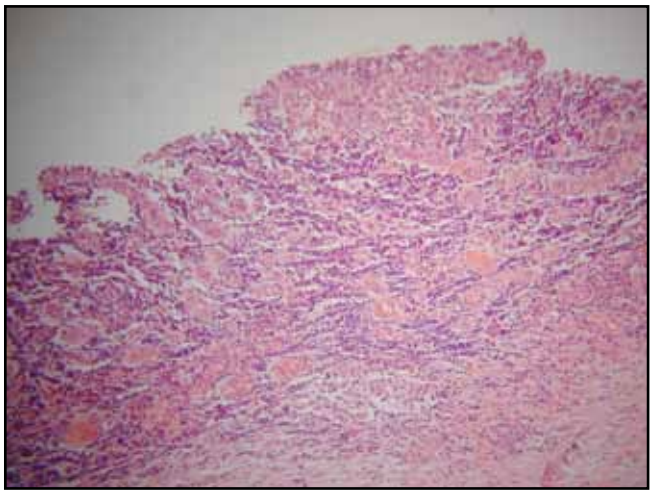

Fig 2. Photomicrograph of ulcer edge after hemotoxylin-eosin staining demonstrating acute inflammatory cell infiltration.

patients particularly in the setting of chronic administration. Many of the effects seem to be reversible on stopping the medication however if the ulcer is particularly large the defect may not heal requiring surgical intervention.

The authors have no conflict of interest.

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Medical History

Blood and War

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SUMMARY

In 1894 Ulsterman and pathologist Almroth Wright described the citration of blood. Twenty-one years later it was introduced into wartime and clinical practice. Harvard Medical School had a large part in providing Colonel Andrew Fullerton, later Professor of Surgery, Queen's Belfast, with the intellectual and practical help for the Allies to deploy blood on the post-Somme Western Front and in Salonika. The key investigators and clinicians were Americans and Canadians who with Fullerton and Wright instructed the Allies. The key enablers were two Harvard-trained surgeons surnamed Robertson—Oswald H. ("Robby") and L. Bruce (no relation). Physician Roger I. Lee of Harvard, surgeon George W. Crile of Cleveland, Peyton Rous of the Rockefeller Institute and Richard Lewisohn of Mount Sinai Hospital, both located in the Upper East Side of New York City, played key roles.

By Armistice in 1918, indirect citrated nutrient-enhanced blood transfusion was widely used by the Allies. Geoffrey Keynes was taught the techniques of blood transfusion by Dr. Benjamin Harrison Alton of Harvard at a Casualty Clearing Station near Albert at the time of the Battle of Passchendaele. Professor "Robby" Robertson, DSO, Sir Geoffrey Keynes and Sir Thomas Houston established blood banking.

INTRODUCTION

On July 14, 1894 the *British Medical Journal* published Ulsterman Almroth Wright's[1,2] description of the citration of blood. Development and deployment of blood transfusion was advanced by surgical scientists trained at Queen's Belfast, Harvard, and Johns Hopkins. We describe the cooperation between these institutions in the development of techniques for human blood transfusion. The use of citrates to allow storage of human blood for indirect transfusion took 21 years from 1894 to develop. Why so long?

PRE-WORLD WAR I

Almroth Wright visited William H. ("Poppy") Welch's Department of Pathology at Johns Hopkins in Baltimore at the time of Wright's publication on the citration of blood. Welch was later to become the éminence grise of American Medical Science, President of the U.S. National Academy of Sciences, and chairman of the Board of Directors of Research of the Rockefeller Institute (later University) in New York City, and plenipotentiary enabler of American Surgeon General William Crawford Gorgas in World War I[3].

Landsteiner in 1901 described human blood groups[4] (Fig. 1), Jansky different group incompatibility in 1907[8] and Moss isoagglutinins and isohaemolysis in 1910[9,10]. Their American

Fig 1. Collage of commemorative postage stamps and Austrian thousand-schilling note honoring Karl Landsteiner (1868-1943), by Lubush Stepanek, from the collection of Rockefeller University, New York, NY, and reproduced with the permission of the Trustees. Landsteiner was born in Vienna of Jewish parents, and graduated from the University of Vienna Medical School in 1891, already having published on the constitution of incinerated blood. He then trained under Hantsch of Zurich, Emil Fischer at Würzberg and Bamberger of Munich—five years as a chemist[5]. Landsteiner then became a pathologist and published extensively on the transmission of syphilis and poliomyelitis. He journeyed first-class by train with inoculated monkeys to the Pasteur Institute in Paris[5]. Peyton Rous, his colleague at the Rockefeller Institute, has written, "The fate of Landsteiner's effort to call attention to the practical bearing of the group differences in human bloods provides an exquisite instance of knowledge marking time on technique. Transfusion was still not done because (until at least 1915), the risk of clotting was too great"[6]. Between 1915 and 1921 Landsteiner's papers, many in German and some in Dutch, were at last frequently read and he accepted appointment as a member of the Rockefeller Institute. He built his family a house on Nantucket Island, Massachusetts, but after a few years "summer people" began to press near on their way to the lighthouse. The Landsteiners then moved to Newfane, Vermont, and Karl commuted by train to New York City. In 1939 Landsteiner became a Member Emeritus of the Rockefeller Institute, "an immaterial change"[6], and he went on to mentor Linus Pauling on "The Nature of the Chemical Bond"[6]. Pauling liked to say he was given by Landsteiner, "The best four day course in immunology in the history of the world"[6]. Just after having completed another edition of *The Specificity of Serological Reactions*[7], KL died in 1943 of a coronary obstruction; his beloved wife survived but a few months. Their son, a graduate of Harvard Medical School, became a renowned surgeon in Boston[5].

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Fig 2. George Richards Minot (1885-1950). Oil on canvas, dimensions: sight: 126 x 100.5 cm (49 5/8 x 39 9/16 in.), by Charles Sydney Hopkinson, 1943. Harvard Art Museum, Fogg Art Museum, Harvard University Portrait Collection, Gift of the former students and friends of Dr. Minot to the Medical School, 1948, HNA 70. Photo: Junius Beebe © President and Fellows of Harvard College.

G.R. Minot was born in Boston, to which his family had emigrated from Saffron Walden in 1630. His father, James Jackson Minot, was a physician on the staff of the Massachusetts General Hospital, and his mother a Whitney[23]. An early published lepodopterist, he graduated from Harvard College and thereafter its Medical School in 1912. Minot trained at the Massachusetts General Hospital and then at Johns Hopkins Hospital and Medical School under W.S. Thayer and W.H. Howell. In 1915 he was appointed as a Staff Physician at the Massachusetts General Hospital, and on June 29th, 1915 married Marian Linzer Weld. His Nobel Prize biography states "Minot early became, when he was a medical student, interested in disorders of the blood… Further he studied the coagulation of the blood, blood transfusion, the blood platelets, and the reticulocytes…"[23]. Minot's Nobel lecture of December 12, 1934 is a fascinating account of his work as a 1910 Harvard Medical School student, "In my father's wards at the Massachusetts General Hospital" that led to the development of liver therapy in Pernicious Anemia, " and concluded "Thus, upon the foundations, laid by previous investigators, do medical art and science build a structure which will, in its turn, be the foundation of future knowledge"[24].

contemporary, George W. Crile, described and investigated human direct artery to vein blood transfusion[11] and was soon after visited in Cleveland, Ohio by Berkeley Moynihan, later Lord Moynihan[12]. George W. Crile[11,13,14] Moynihan and Andrew Fullerton[15-20] of Belfast were excellent technical surgeons who mastered dissection of the radial artery of the donor who was laid alongside the isolated major vein of the patient, generally a cephalic at the elbow. The artery was connected to the vein. Measurement of transfusion volume was difficult. On adverse reaction the donor's artery was clamped[13].

On July 24, 1912 the first perioperative blood transfusion at the Massachusetts General Hospital was conducted by Drs. C. Alan Porter, Henry Marble and Adams Leland using a paraffinated collection vessel and delivery cannula. This transfusion was untoward as was the accompanying operation[21].* The previous month George R. Minot[22] (Fig. 2) had graduated from Harvard Medical School and been appointed House Officer at the MGH[24]. In 1913, Dr. William E. Ladd's team at Boston's Childrens' Hospital performed what was claimed to be the first truly therapeutic indirect blood transfusion, on a boy with generalized peritonitis and anaemia[25]. At this entirely successful procedure, Dr. L. Bruce Robertson, graduate of the University of Toronto and its Medical School was one of the two surgical house officers working under Dr. Ladd[26]. Contemporaneously, Oswald H. ("Robby") Robertson (Fig. 3) was a Harvard Medical School student who also worked in Roger I. Lee's (Fig 4) laboratory, and in the Homer Wright Pathology Laboratories of the Massachusetts General Hospital, where he became an intern in 1913[30,32]. Also working in this laboratory on blood coagulation and transfusion at this time were George R. Minot and Paul Dudley White, later to become President Eisenhower's cardiologist[33]. Oswald H. ("Robby") Robertson married a nursing colleague of L. Bruce Robertson's from Boston Children's Hospital[30].

ULSTER CONTRIBUTIONS

During mobilization of the British Empire for World War I the British War Office asked the Royal Colleges of Surgeons of England and of Ireland each to name chief consulting surgeons for the British Expeditionary Force to France. The English College named the Queen's professor Thomas Sinclair[34,35] and the Irish College nominated Andrew Fullerton, also of Queen's[15-20,34]. These distinguished surgeons were acceptable to the Canadians, French and the United States. P.T. Crymble[34,36,37], later to be Andrew Fullerton's successor as Queen's Professor of Surgery in 1933, was brought as Head of Chest Surgery for the British 13th General Hospital, which was visited by William H. Welch in August 1916[3], and later was merged with Harvard's U.S. Fifth General and based in Boulogne. Some histories refer to this base hospital, which also contained visiting Ulster pathologist Thomas Houston, and Norman R. Keith as Almroth Wright's hospital, others as Harvey Cushing's[31,38].

* An excerpt from the unpublished recollections of Dr. Henry Marble describes transfusion reactions before typing and cross-matching were in general use: "…That the transfusion of blood from one person to another could cause reactions was recognized and guarded against by giving the first 20 cc very slowly and watching for the symptoms of reaction. If the patient showed a flushed face, chills, swollen tongue, and pain in the kidney regions, the transfusion was stopped. Caution and judgment resulted in few severe reactions. If the artery to vein method was used one could only guess at the amount of blood transfused. With other methods the delay necessary to test for reaction often resulted in the clotting of blood in the flask, tube or syringe and the loss of the whole lot." [21]

Fig 3. Oswald Hope ("Robby") Robertson (1886-1966). Photograph from the U.S. National Archives, Washington D.C. , courtesy of the U.S. Department of the Army, Surgeon General, Armed Services Blood Program Office, Robertson Blood Center, Fort Hood Texas. O.H. Robertson was born in Woolwich, England but moved to California's San Joaquin Valley at the age of 18 months. He graduated from Polytechnic High School in San Francisco. After study in Germany he entered the premedical course at the University of California in 1906. In 1910 he obtained his M.Sc. before transferring to Harvard Medical School. Winner of the Dalton Scholarship at the Massachusetts General Hospital, he became an intern there in 1913-14. After his house officership he was appointed an Assistant in Bacteriology and Pathology at the Rockefeller Institute, in Peyton Rous' department[27]. He was the creator of the World's first blood bank in World War I in Belgium and France; later Professor of Medicine and Department Head at Peking Union Medical College, and from 1927 at the University of Chicago. Elected to the U.S. National Academy of Sciences in 1943[27], Oswald H. Robertson ended his career as Professor of Zoology at Stanford, specializing in the study of Californian habitats and especially the endocrinology of Pacific salmon[28,29]. King George V elected him, an American, a DSO in 1918[30].

After 1914, British authorities asked the United States to send qualified medical and surgical personnel to the Western Front. This was accomplished, first by Crile's Case Western Hospital, succeeded by Harvard and many other staffs formed from U.S. medical schools during the two and a half years before the United States entered World War I on April 6th, 1917[39,40].

Fig 4. Roger Irving Lee (1881-1965) Oil on canvas, dimensions: actual: 130.81x 107.95 cm (51 ½ x 42 ½ in.), by Robert Hale Ives Gammell, 1954. Harvard Art Museum, Fogg Art Museum, Harvard University Portrait Collection, Gift of Dr. Roger I. Lee to the Harvard School of Public Health, 1954, H547. Photo: Junius Beebe © President and Fellows of Harvard College.
Lee was a graduate of Harvard College and Medical School, trained at the Massachusetts General Hospital, where he became an attending physician. He there conducted research on blood with Paul Dudley White, George R. Minot and O.H. Robertson. Appointed Professor of Hygiene, Harvard University, he became Chief of Medical Service, U.S. Army Base Hospital Number 5. On February 28th, 1918, he succeeded Colonel Patterson as Commanding Officer of U.S. Army Base Hospital No. 5. On September 6th, 1918 he was detached for duty with the American Expeditionary Force as Senior Divisional Consultant in General Medicine, attached to the 3rd Corps[31].

ANTE-BELLUM UNITED STATES

Roger I. Lee and Richard Cabot of Harvard on April 5, 1915[41] suggested that with Welch's Carnegie funding, Oswald ("Robby") Robertson should proceed to work on further blood transfusion problems in Peyton Rous' (Fig. 5) laboratory at the Rockefeller Institute in New York[44-54]where William Welch was a prominent trustee[3]. Rous had been a student of Osler and Welch at Johns Hopkins[43]. The Rockefeller Institute is within easy walking distance of Mount Sinai Hospital where Richard Lewisohn's team were investigating and extending Crile and Moynihan's use of blood transfusion in human surgery[55-57]. Lewisohn had graduated as a physician from Freiburg in 1899 and emigrated to New York City in 1906, where he became a surgeon[58].

After the declaration of World War I on August 3, 1914, Roger I. Lee's MGH surgical colleague Beth Vincent (Fig. 6) was the instigator of blood transfusion of the wounded. This he did as a volunteer in Paris using the Harvard Kimpton paraffin-coated transfusion apparatus[21,61]. Alexis Carrel (Fig. 7), already a Nobel Laureate in 1912, followed Vincent's example, also in Paris, but used direct artery to vein anastomosis, as did fellow volunteer in Paris, George W. Crile[11,13,14].

Fig 5. Francis Peyton Rous (1879-1970), Oil on canvas by Gordon Stevenson (1892-1984), a student of John Singer Sargent. From the collections of Rockefeller University, and reproduced with the permission of the Trustees.

Born in Texas, his father who was a Baltimorean of English descent, died young. His mother was left with three young children and did not return to Texas. Peyton went as a scholar to Johns Hopkins where he scraped his finger during an autopsy, and a "corpse tubercle" formed. His axillary glands were removed and he returned to Texas. He was sent west from there as a ranchero; for a year he never had a bed. He returned, having lost a year, and graduated from Johns Hopkins Medical School in 1905 and was appointed an instructor in the University of Michigan Department of Pathology. He was sent to Dresden for 1907 and in 1909 joined the research team of the Rockefeller Institute. After only a few months he was asked by Dr. Simon Flexner to be his successor as director of the Laboratory for Cancer Research at the Rockefeller Institute, where he remained for 61 years[35]. Both Peyton Rous and his son-in-law Alan Hodgkin[42] received Nobel Prizes and were Fellows of the Royal Society[43]. A late addendum to his 1966 Nobel Biography states "Rous has not mentioned the pioneer research on blood transfusion with J.R. Turner and O.H. Robertson which led to the establishment in 1917 of the world's first blood bank near the front line in Belgium" [42]. Rous was a Fellow of Trinity Hall, Cambridge.

L. Bruce Robertson joined the Canadian Army in August 1914, and used indirect techniques developed by his teacher surgeon William E. Ladd of Boston Children's Hospital, and multiple syringe injections of blood[64-67]. By the early autumn of 1915, Harvard trained surgeons of the American Women's War Hospital in Oldway House, Paignton, S. Devon were routinely giving blood transfusions to soliders wounded in France[68]. In 1915 citrated blood techniques began to supercede the use of Kimpton paraffin-coated apparatus[46,48,49,55,56].

Harvey Cushing, having just succeeded his former MGH chief, Maurice Richardson, as Moseley Professor of Surgery, left Boston on Thursday, March 18th, 1915. He arrived in Paris, France on April 1, 1915, to take over the converted Lycée Pasteur from George W. Crile's Case Western Hospital, Cleveland. On April 11, 1915, Harvey Cushing went to the 2nd (French) Army and spent the next couple of days at Compiègne with "Alexis Carrel, an Americanized Frenchman [who] is not to be confused with a Gallicized Marylander, M. Charles Carroll de Carollton… who arrives driving his own militarized car…"[39]. Cushing writes that Carrel is "backed by Rockefeller money with an admirable staff" over which "Madame Carrel rules as "general tyrant", according to [Alexis] her husband."[39] Carrel and Cushing then visited a neighboring British hospital and were not impressed.

Having finished his Harvard Lycée Pasteur stint, Cushing went to the British Number 13 General Hospital during the Second Ypres battle; 720 to 900 wounded with hard-worked competent doctors and nurses. Dinner there, on May 3rd, 1915, was with Sir Almroth Wright, "as amusing and chatty as he was iconoclastic"[39]. On May 5th, 1915, Cushing visited Number 8 Field Ambulance at La Clytte, 3 miles from Ypres, where Captain Henry Bazett, Demonstrator of Pathology, Oxford University, was in charge[39]. Bazett co-authored with Colonel Andrew Fullerton, as Principal author, and Bazett's Oxford boss Professor Georges Dreyer, M.D., an influential article on techniques of direct blood transfusion[15].

On May 6th, 1915, Cushing went to England to stay with the Oslers at Oxford and meet Sir Walter Morley Fletcher, Secretary of the Medical Research Committee, (later Council (MRC)). They discussed publications, including on blood transfusion, and made further plans for the American University project to deploy personnel from 20-22 U.S. medical schools to France. On Saturday, May 8th, Cushing embarked on the steamer St. Paul which sailed through the wreckage and floating bodies from the *Lusitania,* sunk by U20, which were "strewn for some twenty miles or more"[39].

Roger I. Lee also went to France in 1915 but not with Cushing. The 1915 mission of this Harvard Medical Unit was to staff a British base hospital Number 22 at Dannes-Camiers, not far from British Number 13 at Boulogne. The Number 22 unit was under the professional leadership of Dr. Edward H. Nichols[31,69], a Harvard Professor of Surgery, later to command the U.S. Boston City Hospital Unit. Lee states "Thanks largely to Sir Almroth E. Wright and his typhoid inoculation, typhoid was not the terrible specter it had been in the United States Army in the Spanish-American War less than twenty years before" [31]. Dr. C. Allen Porter, a senior MGH surgeon, had now almost 3 years of experience of blood transfusion and was an old friend of Lee. Together they went to the front where Lee was wounded on the chin by a German with "a good eye"[31]. "By this time Porter and I were due home" at Harvard[31]. They returned with a conviction that blood transfusion had to be expanded and widely deployed up to the Allied front line[31].

USA NOW AT WAR

When Lee returned in June 1917 again to Dannes-Camiers, this time with Harvard's 5th U.S. Base Hospital (Fig. 8) , "We promptly got down to work. Due to the experience of some of us in the first unit, the new unit was much better prepared.

Fig 6. The Harvard Unit at the American Ambulance (World War I Hospital) at Lycée Pasteur Neuilly, Paris, April 1915. Standing: Wilson, Benet, Barton, Rogers, Coller, Cutler, Smith-Petersen. Nurses: Wilson, Cox, Martin, Parks. Seated: Boothby, Beth Vincent, Greenough, Harvey Cushing, Strong, Osgood[39]. Harvey Cushing's journal today fills nine bound volumes (one million words) in his library at Yale. Elliott Cutler succeeded Harvey Cushing as Moseley Professor of Surgery at Harvard, and during World War II was on the Allied Surgical Consultants committee with Sir Ian Fraser[59] and my father[60].

Fig 7. Alexis Carrel receives the Order of Leopold from King Leopold III of Belgium, June 19, 1937. From the Rockefeller Archive Center and reproduced with their permission.
Alexis Carrrel was born at Lyons, France on June 28th, 1873. His father died when he was very young and he was educated by his mother. In 1889 he became Bachelor of Letters at the University of Lyons, then B.Sc. (1890) and M.D. (1900). In 1902 Carrel began experimental work on transplantation at Lyons, but two years later he emigrated to the U.S. and worked in the Physiology Department of the University of Chicago for two years. In 1906 he was called to the Rockefeller Institute, where he remained for 33 years[62], apart from service as a Major in the French Army Medical Corps with command of a Rockefeller-supported Base Hospital at Compiègne[39]. Carrel died in Paris on November 5, 1944[62]. Carrel received the Nobel Prize in 1912 for his work on operations on blood-vessels and anastomoses in man[62]. In his Nobel Lecture, Carrel states ,"In his admirable method for the transfusion of the blood, Crile first used suture for the anastomosis of the blood vessels. Although the suture is difficult in very small vessels, it has, nevertheless, been used with success in the transfusion of the blood in infants. The study of the circulation of the blood through metallic tubes has led to a simpler technique… which will increase the efficiency of Crile's method"[63].

Oswald ("Robby") Robertson, Arlie V. Bock[21,70,72 †] and I (Roger Lee) tried to spread the gospel of the necessity of blood grouping to make blood transfusion safe"[31]. "…In a special meeting on transfusion, a 'well-known American' was pooh-poohing blood typing…He went on to demonstrate, and the poor devil of a patient died then and there"[31]. So the Oswald Robertson system of indirect transfusion of citrated Rous-Robertson-Turner nutrition enhanced stored cross-matched blood won out, later to be improved by the Geoffrey Keynes drip chamber[73]. Many, many medical personnel were trained in this system and questionnaires had to be completed. The experience and reputation of Fullerton, Sinclair, Carrel, Crile, Moynihan and Cushing supported Oswald (Robby) Robertson's bottled blood against the use of saline and the gum acacia supported by some English surgeons and Harvard's Professor of Physiology, Walter B. Cannon[31]. Lee writes, "Cannon and I had rather a tiff about it but his gum acacia solution was poisonous to humans"[31].

Fig 8. Some of the officers of U.S. Base Hospital Number 5 at Boulogne, 1917. The middle row: Capt. J.L. Stoddard, Capt. H.M. Clute, Capt. Arlie V. Bock, Capt. H. Lyman, Capt. A. Hepburn, Capt. Oswald ("Robby") Robertson, Capt. G.P. Denny, Capt. S.C. Haney, Capt. J.P. Wall, Capt. G.A. Horrax. Front Row: Capt. J.S. Forbes, Capt. Leonard Colebrook F.R.S., R.A.M.C., Maj. H. Binney, Maj. Prof. Harvey Cushing, Commanding Officer Designate Maj. Prof. Roger I. Lee, Maj. H. Binney, Maj. Albert E.B. Wood, R.A.M.C., Quartermaster Capt. C.E.T. Richmond, R.A.M.C., Capt. F.R. Ober, Chaplain Malcolm E. Peabody[31].

When Lee was made commanding officer of Harvard's 5th Base Hospital he "…expostulated vigorously, because this unit had been instigated by Harvey Cushing and Cushing had always referred to it as his child, his creation…I [Lee] must say that everyone was very nice about it. Harvey Cushing had a few squirms; otherwise he wouldn't have been Harvey Cushing"[31]. "He said it was too bad, that they ought to have picked out our top sergeant for the job" [31]. "To be sure, the sergeant is stupid, ill-mannered, and a bit of a drunk, but well suited for the job"[31]. Cushing and Lee continued as friends until Cushing's death. "But when he [Cushing] got up a sort of reunion of our unit, he just didn't send me an invitation," writes Lee[31].

From 1931 to 1953, Roger I. Lee served on the Harvard Corporation—the other four fellows had all been trained in the law. The five Harvard fellows are self-perpetuating in perpetuity and everyone at Harvard is advisory to them—so

† John Hedley-Whyte worked with Prof. Bock in the Blood Gas Laboratory of the Anaesthesia Laboratories of the Harvard Medical School at the Massachusetts General Hospital[71].

Cushing may not have wished to draw attention to his friend Lee again becoming his 'commander'.

Harvey Cushing and Roger Lee were both extremely fond of Oswald H. Robertson whom they both knew as "Robby". Their diaries and letters constantly praise "Robby" and his accomplishments, and note that he was the nephew of Major General Sir James Murray Irwin, KCMG, CB, MD, (TCD), born in Manorcunningham, Co. Donegal[31,38,39,74]. Cushing, in particular, met many of the allied generals in France and England: he even talked á deux to Colonel Edward House, Woodrow Wilson's emissary and to King George V. Cushing was threatened with a British court-martial for his criticisms in his letters of many, chiefly English, not Irish, surgeons. He frequently mentions favourably Colonel Andrew Fullerton. "Black-Jack" Pershing, the American Commander in Europe squashed the threatened court-martial of Cushing, and the Royal College of Surgeons of Ireland summoned Cushing to Dublin and gave him an honorary fellowship[39]. Queen's Belfast followed with an honorary M.D. Cushing, Lee and Oswald H."Robby" Robertson liked and especially admired Andrew Fullerton and Professor P.T. Sinclair. O.H."Robby" Robertson praised them to his maternal uncle then the director of Medical Services, 3rd Army[39,74]. Irwin's KCMG was awarded for diplomatic service 1915-18 in France. Irwin and his sisters were children of the Raymoghy manse at Manorcunningham, Co. Donegal.

MORE CANADIAN CONTRIBUTIONS

The Boston and Toronto Children's Hospitals-trained Canadian Major Lawrence Bruce Robertson served in France in the Canadian Corps of the 3rd Army. He arrived in France in September 1915 and in June 1916 he published an article in the British Medical Journal, "The transfer of whole blood: a suggestion for its more frequent employment in war surgery"[66]. L. Bruce Robertson, while in France, did not cross-match. He was deployed as a transfusionist to the Second Canadian Casualty clearing station in early 1916. In the 36 cases, L. Bruce Robertson described in the BMJ he had three fatal transfusion reactions[66].

In December 1914, Edward Archibald of McGill had visited George W. Crile in Cleveland just before the latter's departure with his hospital for Paris. The Archibald family had lived in Londonderry, Ulster, from approximately 1640 to 1740 when they emigrated to Londonderry, New Hampshire, then to Nova Scotia, and finally to Montreal. Crile and Moynihan of Leeds were at that time the only surgeons with much experience of direct human blood transfusion. Archibald enlisted on April 7th, 1915. He was in France until October 1916. Of the eight patients he describes in advocating blood transfusion, 6 died, one benefitted and survived, the other survived despite a severe haemolytic reaction[75].

The main advance Archibald supported was the use of citrated blood. Archibald later became President of the American Surgical Association. There exists a warm obituary of Edward Archibald from Professor Wilder Penfield OM, a member of Professor Archibald's Department of Surgery at McGill[76].

Norman Guiou went to France on June 16th 1915 as a McGill medical student with the rank of private. Guiou, on October 27, 1915, saw Captain W.B. Howell, later a famous Canadian anaesthetist, give a pint of his own blood while he was giving an anaesthetic. Guiou soon returned to Montreal to finish his M.D.[77].

After O.H. "Robby" Robertson's urging, the Canadians moved from direct syringe or artery to vein anastomosis transfusion to using O.H. "Robby" Robertson bottles of stored citrated blood at Dressing Stations. Back at the ambulance units, Guiou and his men made compartmental wooden cases two feet long and fourteen inches high. To keep needles sharp, a glass blower designed a stone apparatus that led to standardization by the Allied Medical Research Committee. By Armistice both the American and Canadian forces were allowing the complete procedure of transfusion from Robertson bottles by dressers who were generally of the rank of acting sergeants, and by Harvard trained nurses who were widely deployed to other Allied Units[78,79] (Fig. 9).

Fig 9. Harvard's 5th Base Hospital Nurses' Group, Boulogne, June 1918[78]. Of the 65 nurses shown, 25 were temporarily detached individually to other units to teach the use of Robby Robertson's bottled blood transfusion. Many of the 25 had as many as ten different detachments to ten different Allied units as far forward as Dressing Stations. The U.S. awarded decorations other than Purple Hearts to two, the British decorated eleven. The British decorations included the M.M.[73,78].

By 1916 the Allies employed teams of surgical instructors to go forward from the base hospitals[31,39]. Geoffrey Keynes, educated at Rugby, Pembroke College, Cambridge, and Barts was trained by Major Benjamin Harrison Alton[73] of Harvard at his casualty Clearing Station near Albert[73,79]. Harvey Cushing was now under the command of Andrew Fullerton who was very supportive and with whom he became friendly[39]. According to Geoffrey Keynes, Cushing used to say he had had three chiefs, Maurice Richardson at the Massachusetts General Hospital, William S. Halsted at Johns Hopkins, and his friend Andrew Fullerton[79].

My‡ father's Lancet obituarist[80], Sir Ian Fraser, has written extensively of Fraser's experience of Sinclair, Fullerton and Crymble[34]. Colonel Sinclair was a much respected Consultant Surgeon to the 4th Army in France who, together with Captain N.C. Graham and Thomas Houston did the post-mortem (April 1918) on the German air ace, 80 'kills' 'Red' Baron Manfred von Richthofen, demonstrating that the Germans' leading ace was killed while flying[34]. Immediately thereafter, Sinclair joined General Allenby, previously commander of the 3rd Army in France, as Consulting Surgeon in Egypt, the Holy Land and Syria[34]. Sinclair and Moynihan have been described as "the pioneers of modern abdominal

‡ This and subsequent references to the first person are to the first author.

surgery[34]" and Fullerton as "The recognized master in genito-urinary surgery"[15-17,19-20, 34,81-82]. Crymble had an excellent record removing bullets and shrapnel from the chest[34,36,37].

By 1918 each base-hospital and casualty clearing station hospital was transfusing about 50 to 100 pints of blood to an average of 50 wounded each day on the Western Front (Fig 10). The Middle East usage now under Colonel Professor Sinclair's control was lower because Allenby's casualty rate was lower. Wounded Turkish prisoners were also transfused in significant numbers. Essentially, in 1918 all transfused blood was citrated. Some but not the majority of transfused blood, was typed and stored in blood banks designed by Dr. Oswald "Robby" Robertson[30,73,84]. Geoffrey Keynes did not in 1917 or 1918 transfuse any blood that had not been typed and cross-matched[73,79]. Fullerton, Keynes and the Robertsons' teams, including Harvard nurses, also carried blood in labeled portable wooden boxes generally carrying twelve [Oswald H.] Robertson glass bottles, each containing the Rous solution of citrate and enhanced glucose, which Keynes had improved by adding a glass drip chamber to improve calculation of infusion rate and as a check for possible emboli[73]. Pathologists such as Houston had become increasingly preoccupied with testing donors for blood transmissible diseases such as syphilis and malaria. Lightly wounded soldiers were added to the numbers of the fit soldiers and civilians who donated multiple times at monthly to six-week intervals[73,84].

Fig. 10. *The Interior of a Hospital Tent*, by John Singer Sargent, 1918, watercolour on paper (393 mm x 528 mm), IWM ART 1611; gift of the artist, 1919, © Imperial War Museum and reproduced with their permission."While gathering material for *Gassed* near Peronne, Sargent was struck down with influenza and taken to a hospital near Roisel. Here, he spent a week in a hospital bed next to the war wounded, which inspired this work"[83]. Roger I. Lee recounted his war time encounter in France with the artist, "an old friend in Boston"[31].

The increased organization and number of trauma teams made in 1918 less frequent scenes like those described in Oswald Robertson's diary for November 30th, 1917:

> "By noon, the wounded began to arrive, then more and more till there was a solid string of ambulances…We were simply deluged. We couldn't operate more than a small fraction…couldn't get rid of them as the ambulance trains were hung up…ammunition trains had the right of way…resuscitation ward was a veritable chamber of horrors…hemorrhage, hemorrhage, hemorrhage—blood everywhere—clothes soaked in the blood, pools of blood in the stretchers, streams of blood dripping from the stretchers to the floor. I was blood up to my elbows and my rubber apron was one solid red smear…The seven tables in the operating theatre were going every minute [for 21 hours]…we had taken in 1800 patients during the last 24 hours"[64].

The battle of Passchendaele finally wound down in the winter mud with half a million casualties. Shortly thereafter, L. Bruce Robertson's health deteriorated and he was invalided back to Canada and an early death in 1923 from influenza and pneumonia.

Like the Robertsons, Fullerton and Keynes developed during 'pushes' or battles a routine. Twenty-one hours continuously in the theatre, three hours sleep, then repeat 21 hours operating and three hours sleep, cycling up to four times[79]. Keynes writes:

> "The surgical teams had to work for an indefinite stretch of time…I was working for twenty-one hours. After three hours' sleep I had to operate continuously for another twenty-one hours. That night (as orderly officer) I was called up after only three more hours of sleep. One large tent [was] known as the 'moribund ward'…I made it my business during any lull in the work to steal in…choose a patient who was still breathing and had a perceptible pulse, transfuse him and operate…and transfuse blood again. I had the satisfaction of pulling many men back from the jaws of death"[79].

After Armistice on November 11th, 1918, the official history *Story of the Great War Based on Official Documents:Medical Services. Surgery of the War*[84] suggests in Volumes I and II that blood transfusion was the most important medical advance during 1914-1919[64]. The timely introduction of citrated-glucose-enhanced blood transfusion had halved abdominal surgery mortality and quartered extensive lower limb traumatic fatality rates. Colonel Fullerton was praised for his introduction to trauma management of copies of his parafinned cannulae and rubber tubing[84]. Canadian surgeon "Archibald had in September 1916 pointed out the easy technique of the citrate method[84] ". " …The use of preserved blood was introduced into France by Captain Oswald Robertson USA"[84]. "[His] plan of preparing beforehand bottles of citrated (Rous Turner glucose enhanced) blood ready for use met with great success during heavy fighting in 1917 and 1918"[84].

POST 1918

Professor Sauerbruch told my father in 1930 in Berlin that during World War I the Germans never had been able to employ blood banks but did Laurence O'Shaughnessy, his assistant and my father's great friend, know Moscow now had a blood bank? This was October 1930, my parents were on their honeymoon; the Adlon Hotel in Berlin was comfortable. My mother feared she might be taken to Moscow… but 'no' my mother was told. Geoffrey Keynes, Barts Assistant Surgeon and the London Red Cross had their own and superior blood bank founded in 1921[73,79]. By 1925, 500 patients could be transfused with cross-matched

citrated stored blood from this bank and now five years later, in 1930, the number had tripled and Barts and Guys together used 500 pints each year[79]. Belfast under the leadership of pathologist Thomas Houston, later knighted[85,86], set up a similar service to that of his war-time colleague in the Harvard 5th General Hospital, Oswald "Robby" Robertson[30]. Belfast thus became the second city in the world to have a blood bank. Cook County, Illinois' (Chicago) and New York City's were established in 1938 and 1939 respectively[74,79].

During World War II Sir Lionel Whitby[2] became head of Allied blood transfusion and banking with his wife Major Whitby, his de facto second-in-command[87]. They succeeded in keeping J.H. Biggart working to their wishes and his post World War II CBE was gazetted as being for blood-banking about which he was taught by Sir William W.D Thompson and Sir Thomas Houston[85].

On a late spring evening in 1959, Sir Geoffrey Keynes and his son Milo gave a dinner party at their London home to welcome Dr. Charles Huggins, Moseley Travelling Fellow from the Massachusetts General Hospital. Huggins had just described in a seminar the long-term deep freezing of human blood[88]. I was asked because I was senior house surgeon to the Pink Firm at Barts, Geoffrey's old firm. Milo was my senior registrar. We heard how in 1908, aged 21, Geoffrey Keynes established a friendship with Sir William Osler, a fellow bibliophile[79]. In 1917 Revere, the Osler's only child, died just after a blood transfusion given by George W. Crile and Harvey Cushing[79]. Keynes' bibliophilia led to friendships with Professors Harvey Cushing and John Fulton as well as the grieving Oslers.

Cushing, whether in war or peace was an early riser[38,39]. Milo, the Keynes' third son, we heard, was lucky enough to meet Cushing before breakfast in the Keynes' garden in Hampstead. This large garden has three steep terraces. His father said Milo had insisted on giving Cushing "a ride on a ramshackle go-cart assembled by himself and his elder brothers." Cushing was "deposited dangerously on his head at the bottom of the slope"[79]. Geoffrey Keynes said he knew Charlie Huggins's father (who was seven years later to share the 1966 Nobel Prize for Physiology or Medicine with Peyton Rous). The Nobel citation of Rous describes his epic work with Oswald H. Robertson on the preservation of blood –work done over a half-century before[42].

Geoffrey Keynes described how in the autumn of 1949, "When Margaret (née Darwin) and I were attending a surgical conference in Ireland, my mother….aided and abetted by my eldest son Richard, boldly bought the estate near Cambridge"[79]. We were warmly invited to call when next nearby.

CONCLUSIONS

The actual delay from Almroth Wright's 1894 description of citration of blood to its widespread deployment at the end of World War I was almost a quarter of a century. The biological and logistical problems as we have seen were not trivial. Blood groups had to be described and testing for them perfected. Surgeons had to learn how to perform arteriovenous anastomoses, isoagglutinins and haemolysins had to be characterized, donor response to phlebotomy quantitated, the conditions and timing to clotting determined and human response to blood transfusion studied. Gum had to be abandoned and saline relegated to a temporary role, and the route and dose of citrate to be elucidated. The dimensions of the apparatuses for phlebotomy and transfusion had to be described then optimized. The selection and care of donors had to be worked out. The performance of blood transfused after different time intervals and storage conditions had to be elucidated. The transmission of diseases such as syphilis, malaria and infectious hepatitis had to be prevented. Personnel, first surgeons, then other physicians, nurses and orderlies had to be trained. Techniques and equipment for transportation and storage of citrated blood had to be agreed and standardized.

Almroth Wright and his friend William H. Welch directed and steered funds to the three Nobel Laureates, Carrel, Landsteiner and Rous, who were all on the staff of the Rockefeller Institute. George R. Minot, the fourth Nobelist, was a quintessential Massachusetts General Hospital and Boston Brahmin. The surgeons chiefly involved: Crile of Cleveland, Moynihan of Leeds, Carrel of New York and Paris, Fullerton and Sinclair of Queen's Belfast, Keynes, Ladd and Oswald Robertson of Harvard, and his uncle, General Irwin, Director of Medical Services, 3rd Army and the Canadians Archibald and L. Bruce Robertson, supported by Harvey Cushing, were a pantheon of scholars. During and after the war, at the ceremonies and banquets honouring these surgical icons, who should be constantly sitting in the front row but the Ulster Son of the St. Mary's Manse, Crumlin Road, Belfast, Almroth Wright[1,2].

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Letters

RECURRENT VENOUS THROMBOSES AND ANTI-GLOMERULAR BASEMENT MEMBRANE ANTIBODY

Editor,

We wish to record an association between a severe thrombophilic state and the presence of anti-glomerular basement membrane (anti-GBM) antibody.

Case Report: An active man aged 41 noticed chest pain, haemoptysis and dark urine after a two-week lecture course late in March 2004. On 18 April he developed headache, dim vision, memory loss and epileptic seizures. Tests showed multiple cerebral haemorrhages and thrombosis of the anterior half of the superior sagittal sinus. Thromboses of the left brachial, axillary and subclavian veins and superior vena cava followed the insertion of a long line and he later developed a left femoral DVT and pulmonary emboli. He was anti-coagulated and transferred for convalescence on 28 May with an INR of 2.6 to Musgrave Park Hospital, Belfast. Despite continued anti-coagulation he developed a right below-knee DVT and pulmonary emboli in early June, extension of the right DVT to above the knee in mid June, and a recurrence of the left leg DVT in late July. His mother had multiple sclerosis and a cousin a lupus-like illness. A benign right adrenal phaeochromocytoma had been removed in 2000.

CRP was 38 mg/L (normal < 10) on 2nd April, 223 on 2nd May, and 111 on 28th June. Anti-GBM antibody titre was 193 (normal < 10) on 8th June, 108 on 17th June and 33 on 29th June. Plasma homocysteine was 21.3 micromol/L (normal 5.5–13.6). No other haematological, biochemical, or immunological abnormality was found, with thrombophilia screen, anti-cardiolipin antibody, convalescent urinary catecholamines, creatinine clearance and urinary protein in particular being normal. MIBG, CT and PET scans showed no phaeochromocytoma or other tumour, and OGD and colonoscopy were normal. He was given prednisolone from 1st July to 12th August. CRP was normal from 24th July onwards. Anti-GBM antibody titre was normal from 9th July onward apart from one reading of 27 on 27th July. He made an excellent recovery. He was advised to remain on warfarin for life.

Discussion: Anti-GBM antibody is not normally associated with recurrent venous thrombosis and its presence may simply have reflected an underlying immunological abnormality of unknown origin. Both it and the CRP responded promptly to steroid treatment. Only one thrombosis occurred after the prednisolone was started but the thrombotic tendency was probably already diminishing. Perhaps anti-GBM antibody should be looked for in other patients with severe thrombophilic states and high CRP.

We acknowledge the excellent care he received in the Lagan Valley Hospital, Lisburn, and the Royal Victoria Hospital, Belfast, and thank all who contributed to the good clinical outcome.

The authors have no conflict of interest.

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A CASE REPORT OF 5-FLUOROURACIL-INDUCED CORONARY ARTERY VASOSPASM

Editor,

5-Fluorouracil (5-FU) is a chemotherapeutic agent, frequently used in the treatment of solid tumours, including colorectal, breast and upper gastrointestinal cancers. 5-FU can cause angina (effort-related or at rest)[(1)], myocardial infarction, supraventricular and ventricular arrhythmias, acute pulmonary oedema, cardiogenic shock, cardiomyopathy, cardiac arrest, and sudden death, all recorded in association with intravenous (IV) infusion or bolus administration of the drug. We proceed to document a case of 5-Fluorouracil-induced coronary artery vasospasm.

Case History

A 76 year old gentleman diagnosed with adenocarcinoma of the low rectum and a coincidental left upper pole renal tumour, underwent abdomino-perineal excision of rectum (APER) and left nephrectomy. Pathology confirmed a PT1a papillary variant renal cell carcinoma requiring no further treatment and a rectal adenocarcinoma staged ypT3N1M0, for which adjuvant 5-Fluorouracil and Folinic acid (5-FU/FA) chemotherapy was planned.

The past medical history included supraventricular tachyarrhythmia (on verapamil), hypertension and hypercholesterolaemia. The adjuvant chemotherapy schedule comprised the administration of four cycles of weekly IV bolus 5-FU/FA chemotherapy.

After his first dose of 5-FU, oncology notes confirmed one episode of chest pain, with a history suggestive of angina. After cycle 3 IV bolus of 5-FU, he was admitted under the medical team with a history of two episodes of exertional ‘aching’ central chest pain, each lasting one hour, over a 24 hour period. Troponin ‘T’ at twelve hours was elevated at 0.07µg/l. However, the medical team conferred a diagnosis of ‘atypical chest pain’ and he was subsequently discharged within 24 hours.

In view of his persistent complaint of chest pain, 5-FU was discontinued and he was switched to oral Capecitabine at 25% dose reduction. Despite this, a further admission to the medical team occurred following an episode of central crushing chest pain on the day following Capecitabine treatment. 12-lead ECG confirmed transient antero-lateral ST-segment elevation and/or hyperacute T waves on presentation to Casualty, (figure 2 and 3). Cessation of chest pain was

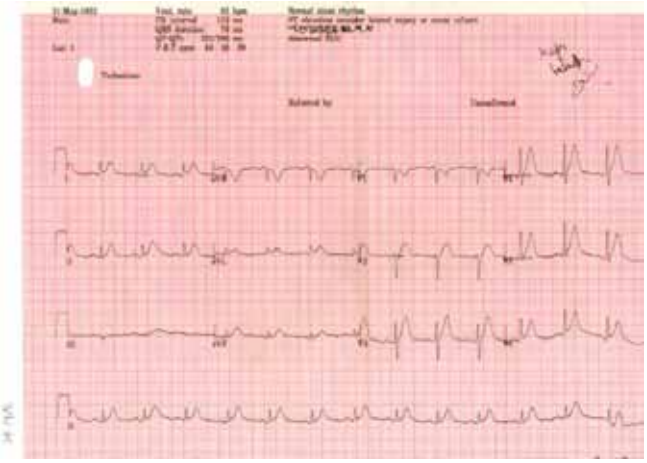

Fig 2. 12-lead Electrocardiogram demonstrating antero-lateral ST-segment elevation and/or hyperacute T wave changes.

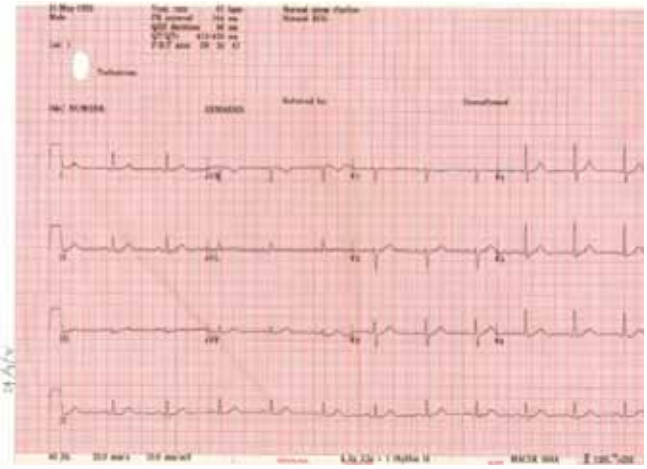

Fig 3. 12-lead Electrocardiogram showing normalised ST-segments/T waves after the administration of sublingual glyceryl trinitrate spray.

noted with sublingual nitrate. Troponin 'T' at twelve hours was 0.06µg/l and he was transferred to the coronary care unit. He was initially managed as an acute coronary syndrome (ACS) with aspirin, clopidogrel and therapeutic enoxaparin. An oral beta-blocker was commenced and buccal suscard, (sublingual nitrate), added as required. He had no further episodes of chest pain whilst an inpatient. He proceeded to coronary angiography. Angiography revealed non-obstructive coronary artery disease, with a minimally diseased small diagonal branch of the LAD artery and a small RCA, with minimal disease distally. Verapamil (a rate-limiting calcium antagonist) was discontinued in view of the instigation of beta-blocker therapy and replaced by amlodipine. A long acting oral nitrate was commenced. Following an uncomplicated inpatient stay, the patient was discharged. Capecitabine was discontinued and he continues to attend Oncology for review. Capecitabine was not replaced by another agent. He has had no recurrence of chest pain off Capecitabine. He recently sustained a traumatic fractured neck of femur but is otherwise in good health to date.

Discussion

5-FU is a chemotherapeutic agent used to treat many solid neoplasms. Cardiotoxicity, after 5-FU administration, has a reported incidence ranging from 1.27 to 18%[2]. The incidence of severe or life-threatening cardiotoxicity with 5-FU, i.e. the observation of electrocardiographic evidence of ST-segment elevation or the development of ventricular arrhythmias, appears to be much less frequent at 0.55%[3].

Many suppositions have been proposed to elucidate the mechanism of 5-FU cardiotoxicity, including direct myocardial toxicity, coronary vasospasm, autoimmune phenomena, thrombogenic effect, and increased levels of endothelin[4]. The commonest hypothesis suggests that coronary artery vasospasm is induced by 5-FU. In fact, *in vitro* studies propose that the activation of protein kinase C (PKC) modulates 5-FU-induced direct vasoconstriction of vascular smooth muscle[5]. Our patient's electrocardiogram demonstrated diffuse ST-segment elevation with hyperacute T wave changes, indicative of pervasive coronary artery vasospasm rather than isolated myocardial infarction secondary to a critical atherosclerotic lesion. This was supported by angiographic findings.

Clinical outcomes suggest that the vasospastic consequences of 5-FU are temporary and reversible. Support of the immediate discontinuation of 5-FU in the presence of cardiotoxicity has been advocated. Antivasospastic therapy should incorporate a full dose of calcium channel antagonist in addition to nitrate therapy.

In the majority of cases, 5-FU-induced cardiotoxicity is transient and reversible with supportive care. Nevertheless, in view of the potentially lethal profile seen with 5-FU cardiotoxicity and successful clinical outcomes associated with early detection and intervention, physicians should be aware of its existence.

The authors have no conflict of interest.

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Robert Harte, *Consultant Oncologist.*

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Ulster Med J 2010;**79(3):**137-145

Abstracts

13th Meeting of the Irish Society of Human Genetics, Friday 10th September 2010

Postgraduate Centre, Belfast Health and Social Care Trust, Belfast.

PROGRAMME:

10.00-10.55	Registration/ Tea and Coffee
10.55	Welcome
11.00-12.00	Plenary I: Clinical Research- 5 Spoken Presentations
12.10-13.00	**Keynote address:** "Advances in human genetics: what benefits for the patients?" **Dr. Arnold Munnich**, Hospital Necker-Enfants Malades and University Paris Descarte
13.00-14.00	Lunch and Poster viewing
13.45-14.00	Council meeting
14.00-15.30	Plenary II: Basic Research- 6 Spoken Presentations
15.15-15.45	Tea and coffee / Poster viewing
15.45-16.00	Business meeting
16.10-17.00	**Keynote address:** "A Singular view of the genome." **Prof. David Schwartz**, University of Wisconsin-Madison.
17.00-17.45	Wine Reception/ Presentation of Prizes / Meeting Close

SPOKEN PAPERS:

S01. An attenuated form of Morquio Disease seen in Northern Ireland.

Fiona J Stewart[1], J Edmond Wraith[2], Karen Tylee[2], Alan Cooper[2]

[1] Northern Ireland Regional Genetics Unit Belfast city Hospital,

[2] Willink Biochemical Genetics Unit St Mary's Hospital Manchester

Morquio disease (mucopolysaccharidosis type IV) is an autosomal recessive lysosomal storage disorder causing predominantly skeletal manifestations. It is caused by a deficiency of galactose-6-sulphatase. In classical Morquio disease there is extreme short stature with height being between 90 and 120 cm. We have identified 10 individuals in Northern Ireland with an attenuated form of the disease. All were found to have glycosaminoglycans (GAG's) in their urine and reduced levels of galactose-6-sulphatase consistent with a diagnosis of MPS type IV. Height ranged from 142 cm to 160cm (5^{th} to 50^{th} centile). 6/10 patients have had at least one major joint replaced with two having had 3 joints replaced. Our patients have also shown evidence of osteoporosis with decreased bone density being seen in all cases tested so far. Mutations in the GALNS gene have been characterised in all cases and include p.I113F, p.T312S and p.A241A . We believe this diagnosis should be considered in young people presenting with epiphyseal dysplasia and also in young adults presenting with joint problems requiring joint replacement surgery at an early age. Urine should be screened for GAG's in the first instance followed up by enzyme studies if keratan sulphate is detected.

S02. Delineation of a recognisable phenotype of interstitial deletion 3 (q22.3q25.1) in a case with previously unreported truncus arteriosus.

Gillian Rea[a] , Simon McCullough[b] , Susan McNerlan S [b], Brian Craig[c] , Patrick J Morrison[a]

[a] Department of Clinical Genetics, Belfast City Hospital,

[b] Regional Cytogenetics Laboratory, Belfast City Hospital,

[c] Department of Paediatric Cardiology, Royal Belfast Hospital for Sick Children

Interstitial deletions of chromosome 3q22.3-25.1 are very rare. We describe a case of a female infant with a *de novo* deletion. Chromosome analysis showed an interstitial deletion with a female karyotype 46,XX,del (3)(q23q25.1)dn. Subsequent array CGH demonstrated the breakpoints as 3q22.3q25.1. We identify an emerging clinical phenotype which includes, congenital heart disease, slow feeding, skeletal abnormalities and developmental delay. Characteristic facial dysmorphism includes ear anomalies, short neck, and small chin. Eye features include short palpebral fissures (blepharophimosis), ptosis and epicanthus inversus which are in keeping with the well delineated phenotype of BPES-blepharophimosis, ptosis and epicanthis inversus, a rare autosomal dominant disorder characterised by an eyelid malformation. FOXL2 (a putative forkhead transcription factor gene) has been identified as the causative gene, it is located on chromosome 3 at q22.3 In this case, array CGH demonstrated that the FOXL2 gene was not deleted, this was unexpected given the classical BPES features demonstrated. It has previously been shown that a small proportion of the molecular defects within a cohort of BPES patients have extragenic microdeletions, including those found downstream of FOXL2, as in our case. This may be the result of potential long-range *cis*-regulatory elements regulating FOXL2 expression.

S03. Atypical 22q11 deletion detected by multiplex ligation-dependent probe amplification (MLPA) in patients referred for Prader-Willi Syndrome (PWS) testing.

Karen Meaney, Bronagh O'hIci, Sally Ann Lynch, David E. Barton.

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

PWS is caused by loss of a paternally-imprinted region on chromosome 15. It is characterized by hypotonia, short stature, hyperphagia, obesity, hypogonadism and mild mental retardation. We use MLPA to test for PWS. The MLPA kit contained a control probe located in the distal 22q11 region (SNAP29 gene). We detected reduced dosage of this probe in two patients referred for PWS testing. One was obese and developmentally delayed at age 24 years, the other was a neonate with hypotonia and feeding difficulties. Follow-up investigation confirmed 22q11 deletions in both cases. Chromosome 22q11 contains multiple low-copy repeats (LCRs) which can mediate non-allelic homologous recombination, making the region susceptible to rearrangements. The most common rearrangement is a 3Mb deletion from LCR22-A to LCR22-D which is associated with 22q11 deletion syndrome. FISH analysis is frequently used to detect 22q11 anomalies. Recent publications have described novel 22q11 deletions, some of which are distal to the commonly-deleted region and would not be detected during routine FISH analysis. Our results suggest an overlap exists in clinical features between PWS and 22q11 deletion syndrome, and demonstrate the importance of investigating MLPA control probe variations. Testing for atypical 22q11 rearrangements should be considered when PWS testing is negative.

S04. Taking On Passing On: Long term experiences and needs in affected BRCA1/2 mutation carriers

Lisa Jeffers[1,2] Dr Donna Fitzsimons[2], Dr Eilis McCaughan[2], Professor Patrick Morrison[1]

[1] Northern Ireland Regional Cancer Genetics Centre, Belfast Health & Social Care Trust,

[2] University of Ulster

Background: Research into the BRCA1/2 breast cancer susceptibility genes has yet to account for any long term psychosocial effects of genetic testing in gene carriers with a personal history of HBOC.

Aim of Study: This study was concerned with exploring the experience and needs of this group of women over a 2 year period.

Methodology: A grounded theory approach was taken using qualitative interviews (n= 49) and reflective diaries.

Analysis and Results: Taking On Passing On emerged as the basic social psychological process through which BRCA carriers with a personal history of HBOC respond to and resolve what is for them, a major concern – the passing on of a cancer gene to their offspring. Constant comparative analysis of the data traces the development of the process through the stages (1) Appraising Risk, (2) Formalising Risk (3) Minimising Risk and Maximising Survival and (4) Optimising Living. The theory contributes to the literature of transition theory, coping and adaption and psychological theories.

Implications for Clinical Practice: This prospective longitudinal study is relevant to clinical practice by contributing to our understanding of how women cope with learning their genetic status in the short and longer term and their ongoing needs once they leave a cancer genetic consultation.

S05. Diagnostic Gene Screening of Cardiac Disorders in the N. Irish Population

SV Heggarty*, W Wright*, FJ Stewart*, PJ Hart*, P McKeown§, CA Graham*

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

§ Cardiology, Royal Victoria Hospital, Belfast Health and Social Care Trust

The introduction of genetic diagnostic screening for cardiomyopathies and ion channelopathies in N. Ireland offers the prospect of early intervention and monitoring of asymptomatic patients at risk of developing fatal arrhythmias.

Hypertrophic Cardiomyopathy, a major cause of sudden death in young people is characterised by left ventricular hypertrophy in the absence of predisposing cardiovascular conditions. The sarcomere genes MYH7, MYBPC3, TNNT2 and TNNI3 of 52 patients were sequenced and pathogenic mutations identified in 29 patients (55.7%). In total 23 different mutations were detected, ~60% of patients had mutations in MYH7 (5 clustered in exon 23, 2 double mutations), and ~30% in MYBPC3 (1 compound heterozygote).

Long QT syndrome, a repolarization disorder of the heart, is identified by prolonged QT interval. It presents clinically in young people with episodes of syncope and potentially lethal *torsades de pointes* tachyarrhythmias. Screening of the KCNQ1, KCNH2, KCNE1 and KCNE2 genes has been carried out in 100 patients. 19 different mutations were identified in 54 patients, of which ~65% are found in KCNQ1 (5 compound heterozygotes) and a further ~30% in the KCNH2 gene.

Genetic screening in N. Ireland has proved an effective tool in detecting familial mutations for cascade screening of first degree relatives.

S06. Functional and Neuropsychological Assessment of S100B as a susceptibility gene for Schizophrenia and Bipolar Disorder

Elif Dagdan[1,4], Derek W. Morris[4], Matthew Hill[4], Matthias Rothermundt[5], Florian Kästner[5], Christa Hohoff[5], Jürgen Deckert[6], Christof von Eiff[7], Petra Krakowitzky[8], April Hargreaves[4], Emma Rose[4], Aiden P. Corvin[4], Gary Donohoe[4], Michael Gill[4], Patrick McKeon[2,3], Siobhan Roche[1,2]

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[6] Department of Psychiatry, University of Wuerzburg, Germany; [7]Institute of Medical Microbiology, University of Muenster, Germany; [8]Institute of Transfusion Medicine, University of Muenster, Germany.

The glial cell-derived neurotrophic factor, S100B, has been implicated in the pathology of bipolar affective disorder (BPAD) and schizophrenia (SZ). S100B protein levels are elevated in serum of patients with these disorders. We previously reported association of a S100B promoter SNP, rs3788266, with BPAD (P=0.0088). Here, we report that the disease-associated G allele of rs3788266 is associated with increased protein levels in serum of Irish BPAD probands (n=87), their first-degree relatives (n=67) and German controls (n=196). The G allele of rs3788266 is also associated with increased promoter activity in U373MG glioblastoma and in SH-SY5Y neuroblastoma cell lines as determined using the luciferase reporter system. Using an EMSA, the binding affinity of U373MG and SH-SY5Y protein complexes that bind to the S100B promoter were stronger on the G- compared to the A-allele promoter fragments. Finally, analysis of 433 SZ cases, 75 BPAD cases and 232 healthy controls identified a significant association between the G allele of rs3788266 and episodic memory, social cognition and verbal IQ. Overall, the data suggest that rs3788266 may represent a functional susceptibility variant that contributes to increased S100B serum levels observed in SZ and BPAD patients by increasing gene expression, which in turn impacts on cognitive performance.

S07. Investigation of RNA-seq as a method of SNP detection

Emma M. Quinn, Elaine M. Kenny, Paul Cormican, Amy S. Gates, Michael Gill, Aiden P. Corvin, Derek W. Morris

Trinity College Dublin

The development of Next Generation Sequencing offers the potential of new methods for mapping and quantifying transcriptomes. In particular, RNA sequencing (RNA-seq) has been used for measurement of transcript abundance, studying the diversity of splice isoforms and testing allelic influence on gene expression. Given that the majority of disease related SNPs are likely to be located in coding regions, we investigated RNA-seq as a method of identifying sequence variants (SNPs) in the transcribed regions of the genome. We performed RNA-seq using an Illumina Genome Analyzer II on lymphoblast cell line RNA samples from a trio of HapMap samples that have been whole-genome sequenced**.** We categorised genes and exons based on their overall X coverage and tested SNPs detected within these regions for concordance with the existing DNA sequence data. We found that at sufficient coverage (e.g. 20X), a high proportion of variants (80%) in a large number of genes/exons (~2,400/20,744 for 1 x lane 80bp) can be accurately detected using this method. We also detected a number of novel SNPs that Mendelised within the trio, but were not reported from the published whole-genome sequencing data. Whole-genome re-sequencing is the most comprehensive method of variation detection but it is costly. We demonstrate that in addition to transcriptome analysis, RNA-seq can detect a very high proportion of sequence variation in expressed genes potentially making it a useful technique for detecting coding SNPs in disease tissue samples.

S08. Gene-centric study identifies two novel genes, *CLCN2* (a voltage-gated chloride channel) and *KCNAB1* (a voltage-gated potassium channel) associated with blood pressure in two independent Irish populations.

Nina McCarthy[1], Ciara Vangjeli[1], Gianpiero Cavalleri[1], Kevin Shianna[2], Norman Delanty[1], Eoin O'Brien[3], Brian Harvey[1], Alice Stanton[1]

[1] Royal College of Surgeons in Ireland, Dublin,

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Hypertension is highly heritable. Loci identified by recent GWAS only explain a small proportion of total BP variation - identification of all genetic variants associated with BP will require complementary strategies. Here we report on a targeted candidate gene study with dense SNP coverage of multiple genes involved in electrolyte transport.

For the screening study 1860 SNPs in 81 genes were genotyped in healthy volunteers (n=358) with clinic BP measurements. For the replication study, 35 SNPs, in 21 genes, that were found to be associated with clinic systolic (SBP), diastolic (DBP) and/or pulse pressures (PP) in the screening study, were genotyped in a second independent population (n=380) for whom repeated ambulatory and clinic BP measurements were available. All association analyses were performed using an additive genetic model and adjusting for age and sex.

	CLCN2 SNP		*KCNAB1* SNP	
	Screening Population (MAF=0.21)	Replication Population (MAF=0.21)	Screening Population (MAF=0.04)	Replication Population (MAF=0.05)
Clinic SBP	**2.7** $^{0.02}$	**2.9** $^{0.003}$	**5.5** $^{0.04}$	**7.0** $^{0.0002*}$
Daytime SBP		**1.8** $^{0.009}$		**3.6** $^{0.006}$
Night-time SBP		**2.1** $^{0.001*}$		**3.5** $^{0.005}$
Clinic DBP	**2.1** $^{0.009}$	1.0 $^{0.1}$	0.3 $^{0.8}$	**3.0** $^{0.01}$
Daytime DBP		**2.0** $^{0.00003*}$		1.3 $^{0.2}$
Night-time DBP		**2.0** $^{0.00003*}$		0.9 $^{0.3}$
Clinic PP	0.7 $^{0.4}$	**1.9** $^{0.006}$	**5.9** $^{0.001}$	**4.0** $^{0.003}$
Daytime PP		-0.2 $^{0.6}$		**2.3** $^{0.009}$
Night-time PP		0.1 $^{0.8}$		**2.5** $^{0.001*}$

Table 1: Differences in clinic and ambulatory BP in mmHg (mean$^{p\text{-value}}$) per copy of minor allele for the two top signals in the screening and replication populations. Significant differences are in bold font. *P-values in the replication population which exceed the Bonferroni correction for multiple testing.

Table 1 summarizes the associations of the top two SNPs with BP - a synonymous SNP in a voltage-gated chloride channel gene (*CLCN2)* and an intronic SNP in a voltage-gated potassium channel gene (*KCNAB1).* These variants have not previously been implicated in the causation of hypertension. Our findings provide new insights into the pathophysiology of BP regulation, and may point to novel drug targets for hypertension treatment.

S09. CNV detection using Parallel Targeting and Sequencing of Multiple Genomic Regions.

Paul Cormican, Elizabeth A. Heron, Elaine M. Kenny, William P. Gilks, Aiden P. Corvin, Michael Gill, Derek W. Morris

Neuropsychiatric Genetics Research Group, Department of Psychiatry, Trinity College Dublin, Ireland

DNA copy number variation (CNV) has been recognized as an important source of genetic variation and in recent years extensive efforts have been undertaken to identify and correlate copy number variant genetic risk factors with disease phenotypes. Next Generation Sequencing (NGS) technologies have provided a potential new source of data for predicting CNVs and provide a feasible alternative to DNA microarrays for detecting such variants. In this study, we describe a sequencing-based strategy for high-throughput, cost-effective, targeted characterisation of structural variation including deletions, duplications and insertions. We have developed a method for CNV detection using read Depth Of Coverage (DOC) as a compliment to established Paired-End Mapping (PEM) strategies. We applied our algorithm to specifically targeted regions in HapMap individuals sequenced in-house, to varying levels of coverage. We tested the specificity of our method by examining the overlap between our predictions and regions of copy number variation which had previously been characterised in these HapMap individuals. Our method allows for the detection of copy number variable regions within targeted regions, quantification of copy number from the depth of read coverage as well as identification of genomic breakpoints at very high resolution.

S10. Genome Wide Association Study of Non-synonymous Single Nucleotide Polymorphisms for even common diseases.

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Associations of several single nucleotide polymorphisms (SNPs) with common diseases were identified in a study conducted by Wellcome Trust Case Control Consortium. In this study the effects of genetic variations in 14,000 cases and 3000 controls and identified 24 independent associations with seven common diseases in European population. We hypothesize that there are more chances of finding associations of rare SNPs with diseases by refined analysis of non synonymous SNPs (nsSNPs) in genome wide association studies. In the present study, we analyzed the association of 12,660 nsSNPs using a case control study in the WTCCC population. We simulated the genotypes at 10,798 nsSNP loci studied by the Stage 2 HapMap project using the genotype information from WTCCC for all 14,000 individuals studied for seven diseases and in 3000 controls. Subsequent case control association of 10,798 imputed nsSNPs and 1,862 genotyped nsSNPs was performed using an additive model and genotype model in a frequentist and bayesian framework. We have identified 4 nsSNPs associated with CD, 8 with RA, 5 with T1D and 1 with T2D. In total, 18 new associations with the seven diseases ($p < 5 \times 10^{6}$) studied by WTCCC. We also developed a pipeline which summarizes quality control measures which should be considered to minimize false associations in genome wide association studies.

S11. Screening of the NTD-associated *MTHFD1L* Gene Polymorphisms in an Irish Cleft Cohort

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Human gene *MTHFD1L* (methylenetetrahydrofolate dehydrogenase 1-like) produces 2 alternatively spliced mRNA transcripts; the short form lacking synthetase activity.[1] Previously, we demonstrated that polymorphisms adjacent to *MTHFD1L* alternative exon (8a) were associated with case risk of neural tube defects (NTDs) in the Irish population.[2] Similarly to NTDs; there is a link between folic acid and cleft prevention**.** In this study, we genotyped *MTHFD1L* DIP (Deletion/Insertion Polymorphism) rs3832406 and SNP (Single Nucleotide Polymorphism) rs17080476 in 981 Irish case-parent trios affected by clefting. Both assays were genotyped using Melting Curve analysis with HybProbes on a Roche LightCycler480® instrument. As this method is primarily designed for SNP genotyping where there are just 2 alleles, it was necessary to develop a novel assay in order to genotype the triallelic DIP rs3832406 polymorphism. Tests for association will be assessed by logistic regression and transmission disequilibrium test (TDT).

We demonstrate that Melting Curve analysis can be employed to successfully genotype challenging polymorphisms such as DIP rs3832406 and is a viable alternative to capillary electrophoresis. Results of this association analysis will be presented.

[1.] Prassannan P, *et al.,* 2003. *J Biol Chem* 278**:** 43178-43187. Parle-McDermott A, *et al*., 2009. *Hum Mut* 30 (12): 1650-1656.

POSTER PRESENTATIONS:

P01. Variable Phenotypic Consequences of a Terminal Deletion of Chromosome 8p

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

Chromosomal abnormalities are a frequent underlying reason for developmental delay in children. We report a family in which a brother and sister (aged 14 and 12 respectively) were referred for cytogenetic analysis due to similar phenotypes of moderate developmental delay and very mild dysmorphic features. Both siblings had an apparently identical terminal deletion of chromosome 8p, extending into 8p23. The presence of this event in both children would indicate parental inheritance, which in most instances is due to one parent being a balanced translocation carrier. However, on this occasion the children's mother also had an apparently identical 8p deletion. To support this, subtelomere FISH analysis with chromosome 8 probes was performed and demonstrated loss of the 8p subtelomere in all individuals, thereby excluding a semicryptic translocation in the mother. The mother displayed no obvious phenotypic anomalies, but did report a non-clinical VSD diagnosed when she was a child. This family illustrates the challenges faced in predicting phenotypic consequences of rare chromosomal abnormalities that are not directly associated with a well defined syndrome. Further, future genetic counselling of the children when they reach reproductive age is difficult due to the unpredictability of the potential implications of this 8p deletion.

P02. Optimisation and evaluation of MLPA for non-syndromic X-linked mental retardation

A Beckett, G Smith, S McCullough, M Humphreys, T Dabir, S McKee, A Magee, F Stewart, V McConnell

Northern Ireland Regional Genetics Centre, Belfast City Hospital, BT9 7AB

Mental retardation (MR) is characterised by a significant impairment of cognitive and adaptive functioning and is estimated to have an incidence of approximately 1-3% in the population.

X-linked forms of MR can be divided into syndromic and non-syndromic forms. Syndromic X-linked MR is present in combination with a specific pattern of physical, neurologic and/or metabolic abnormalities. Non-syndromic X-linked MR describes a condition which segregates in an X-linked manner in which male patients have no consistent phenotype manifestations other than MR.

The aim of the project was to optimise and evaluate multiplex ligation dependent probe amplification (MLPA) as a technique for identifying patients with non-syndromic X-linked MR. The kit used, SALSA MLPA kit P106 MRX, obtained from MRC Holland, can detect copy number

changes of 16 genes on the X chromosome that have been implicated in non-syndromic X-linked MR (RPS6KA3, ARX, IL1RAPL1, TSPAN7, PQBP1, HUWE1, OPHN1, ACSL4, PAK3, DCX, AGTR2, ARHGEF6, FMR1, AFF2 (FMR2), SLC6A8 and GDI1).

We have evaluated this MLPA test in a group of 52 patients referred with a broad phenotype of developmental delay and/or learning disability, together with a strong X-linked pattern of inheritance. No abnormalities were detected in the patients' samples. As a result of this project, it can be concluded that this test is not a practical technique to be implemented as a routine diagnostic screening method within the Belfast Genetics laboratory at the present time. A more appropriate first line test for patients in this referral category would be array comparative genomic hybridisation (aCGH).

P03. A novel mutation causing retinitis pigmentosa (RP) identified in the cyclic nucleotide gated channel beta 1 (*CNGB1*) gene using massively parallel sequencing

D.A. Simpson, L. Harwood, G.R. Clark, S. Alexander, G. Silvestri, C. Willoughby.

Centre for Vision & Vascular Science, Queens University Belfast, Belfast, United Kingdom.

Introduction: Screening the >40 genes implicated in RP is a challenge for molecular diagnostic screening. Targeted massively parallel DNA sequencing was used to develop a potential solution.

Methods: A custom sequence capture array was designed to target the coding regions of all known RP genes and used to enrich these sequences from five patients and a pool of 360 controls. Enriched DNA was subjected to sequencing (Genome Analyzer) and variants were identified by alignment of up to 10 million reads per sample to the normal reference sequence. Potential pathogenicity was assessed by functional predictions and frequency in controls.

Results: Known homozygous *PDE6B* and compound heterozygous *CRB1* mutations were detected in two patients. Common variants were identified in the pooled control sample. A novel homozygous missense mutation (c.2957A>T; p.N986I) in the *CNGB1* gene predicted to have a deleterious effect and absent in 720 control chromosomes was detected in one case in which conventional genetic screening had failed to detect mutations.

Conclusions: Sequence capture of a disease-specific cohort and subsequent high-throughput DNA sequencing can be used as a cost-effective genetic diagnostic tool, exemplified by the detection of a mutation in *CNGB1,* a rare cause of recessive RP.

P04. Infantile onset of complex I deficiency due to a m.10191T>C mutation in the mtND3 gene

Deirdre E Donnelly, Patrick J Morrison

Department of Clinical Genetics, Belfast City Hospital, Belfast, BT9 7AB

Complex I deficiency is a rare mitochondrial disorder. Mutations have been identified in at least nine nuclear genes and eight mutations have been found in mitochondrial DNA. We report a patient with a rare mutation, m.10191T>C, in the mtND3 gene who presented with macrocephaly, global developmental delay and seizures in infancy, and highlight the clinical phenotype of this rare disorder.

P05. The prevalence of thanatophoric dysplasia and lethal osteogenesis imperfecta type II in Northern Ireland – a complete population study

Deirdre E Donnelly, Vivienne McConnell, Anne Paterson*, Patrick J Morrison

Departments of Clinical Genetics and Paediatric Radiology*, Belfast HSC Trust, Belfast, BT9 7AB

The minimum prevalence of lethal achondroplasia, osteogenesis imperfecta type II and thanatophoric dysplasia were derived following detailed case note review of all perinatal lethal skeletal dysplasias in Northern Ireland over a 12-year period. Multiple sources of ascertainment, including genetic notes, radiological reports and post mortem findings, were used. 39 cases were identified. Thanatophoric dysplasia was the commonest diagnosis made (22 children), followed by osteogenesis imperfecta type II (four children) and achondroplasia (two children). Eleven other diagnoses each occurred once in the 12-year period. The minimum prevalence range, per live births, of each of the common skeletal dysplasias in Northern Ireland has been calculated; thanatophoric dysplasia 0.81/10,000 and osteogenesis imperfecta type II 0.15/10,000. The prevalence range for thanatophoric dysplasia is much higher than reported in previous studies. We discuss reasons for the prevalence figures obtained.

P06. Phenotypic variability in a three-generation Northern Irish family with Sotos Syndrome

Deirdre E Donnelly, Peter Turnpenny, Vivienne PM McConnell

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Sotos syndrome is a relatively common overgrowth disorder, following autosomal dominant inheritance, caused by mutations and deletions in the nuclear receptor Set domain containing protein-1, *NSD1* gene. In general almost all affected individuals have advanced bone age, macrocephaly, characteristic facial gestalt and learning difficulties. Other features include scoliosis, seizures, cardiac defects and genitourinary anomalies. Tumours are a rare occurrence. Genotype-phenotype correlations are unclear, though those with a deletion appear to have more severe mental retardation. Full penetrance is seen, although familial Sotos syndrome is extremely rare. The low vertical transmission rate, (not fully explained by cognitive impairment), is of great importance, particularly for mildly affected patients. We report a 3-generation pedigree with 7 affected individuals shown to harbour the *NSD1* missense mutation c. 6115C>T. To our knowledge this is the largest Sotos family to be reported. The observed phenotype is extremely variable, both physically and cognitively, thus highlighting the lack of precise genotype-phenotype correlations in Sotos syndrome, which previous extensive studies have highlighted. Our family provides further evidence that *NSD1* mutational analysis provides little prognostic information as many individuals with the same mutation have very different phenotypes. *NSD1* gene mutations should be considered in a wider phenotypic range.

P07. GLA mutation screening identified 17 cases of Fabry Disease

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Fabry disease, an X-linked recessive disorder caused by α-galactosidase A deficiency, is a multi-system disease with variable age-of-onset. A significant proportion of female carriers also present with symptoms, particularly later in life.

The Northern Ireland Regional Genetics Centre offers a comprehensive screening service available through the UKGTN utilising sequencing and MLPA. Screening has been performed on 57 probands (21 male and 36 female) and mutations detected in 13 males (62%) and 4 females (11%). This resulted in cascade screening of 56 patients and the identification of 28 mutation carriers.

Fourteen mutations, all but two of which were private, were detected consisting of 6 missense, 3 nonsense, 2 splice site, 2 deletions and deletion of exon 1. Five mutations appear novel and of these, a nonsense mutation and deletion were considered pathogenic. The other missense mutations were detected in 5 patients all presenting with cardiac problems. Although the pathogenicity of one variant is uncertain, the others are thought to be consistent with a cardiac variant presentation.

In males with suspected Fabry disease, the pick-up rate is high but our experience suggests that Fabry disease is not a major contributor of disease in females with no family history of the condition.

P08. A case of Menkes disease due to a previously unreported variant.

Gillian Rea[a], Deirdre E Donnelly[a], Lisbeth Birk Møller, [b]Joanne Hughes[c], Patrick J Morrison[a]

[a] Department of Clinical Genetics, Belfast City Hospital,

[b] Kennedy Centret, Glostrup, Denmark,

[c] Paediatric Metabolic Medicine, Royal Belfast Hospital for Sick Children.

Menkes disease and occipital horn syndrome (OHS) are rare disorders of copper transport. Inheritance is in an X-linked recessive manner. Approximately one-third of affected males have no family history of Menkes disease/OHS. Infants with classic Menkes disease appear healthy until the age of two to three months, when loss of developmental milestones, hypotonia, seizures, and failure to thrive occur. There are characteristic changes of the hair (short, sparse, coarse, twisted, often lightly pigmented-pili torti).

ATP7A is the only gene known to be associated with Menkes disease and OHS. A multiplex protocol of targeted mutation analysis (MLPA), mutation scanning, and sequence analysis detects mutations in more than 95%

of affected individuals. We describe a case of Menkes disease where a previously unreported variant was identified in both the affected child and their mother. This variant, c.2781G>A (p.K927K) does not change the amino acid but might affect the splicing of exon 13. RNA analysis from the index case and fibroblast culture were undertaken to determine if the variant was disease causing.

P09. X marks the spot: Duchenne muscular dystrophy (DMD) presenting in a female child.

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The mechanism of chromosomal rearrangements involving translocations between the X chromosome and an autosome, one breakpoint involving the Dystrophin gene and resulting in a DMD phenotype in a female, have been recognised since the late 1970's.

Two such cases have been known to the Northern Ireland Regional Genetics Service throughout its history. We describe the presentation and clinical features of the most recent case, a 4 year old girl who was investigated for proximal muscle weakness. At presentation she had bilateral calve hypertrophy, a broad based gait and positive Gower's sign, early contractures were developing around her Achilles tendons. CK was 20-25,000 and a muscle biopsy showed a myopathic picture with fibrosis consistent with dystrophy. There was some dystrophin present but it was absent or substantially diminished in many fibres.

Chromosome analysis revealed a *de novo* balanced translocation; 46, X, t (X;17) (p21.2;q11.2). The breakpoint is at the site of the dystrophin gene. We also review the alternative mechanisms by which females may present with a DMD phenotype (skewed X-inactivation or females with a disease causing DMD mutation and a complete or partial absence of their second X chromosome (45X or uniparental disomy –UPD).

P10. A diagnosis of Tuberous Sclerosis Complex (TSC) in teenage years: the importance of follow-up.

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We present a twenty-year old female patient with TSC who was first referred to the genetic service in 2005 at the age of fourteen years by the dermatology service on the basis of facial rash. This patient was asymptomatic for the major signs of TSC such as learning difficulty and seizures. At the Tuberous Sclerosis (TS) clinic a diagnosis of TSC was confirmed. Subsequent mutation analysis identified a deletion in exons 28-31 of the TSC2 gene (probable mosaic). This patient found the diagnosis of TSC difficult to cope with. She failed to attend appointments at the TS clinic over a three-year period, and failed to attend for renal ultrasound screening as recommended in the clinical guidelines. She attended for renal ultrasound screening for the first time in 2010 when a 5cm mixed density nodule was identified in the left kidney. There was also a second moderately sized 2.5cm angiomyolipoma in the same kidney. Further imaging by CT scan has been arranged to further evaluate these lesions. This case highlights the importance of encouraging patients with TSC at all stages in life to attend for recommended screening, and identifies the issue and difficulties of non-compliance in teenage years.

P11. A Familial t(15;22)(q13;q11.1), Implications for Prenatal Genetic Testing

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Pregnancy in a familial balanced translocation carrier can pose a challenge to a genetics testing laboratory, particularly when the rearrangement is G-band subtle and involves chromosome 15. We report a t(15;22)(q13;q11.1) familial translocation that could appear to be subtle at lower banding resolutions. The mother, a phenotypically normal carrier of the translocation, reported a family history of a Prader-Willi like syndrome. FISH analyses were undertaken to define the translocation further and demonstrated that 15q breakpoint was distal to SNRPN, while the 22q breakpoint was in the alpha-satellite region of 22q11. Therefore the derivative chromosome 15 contained both SNRPN and the 22q11.2 region associated with Di George syndrome. At gestational week 11 a CVS biopsy was taken and sent to us for cytogenetic, MLPA and UPD studies. Due to the prior FISH testing, cytogenetic analysis could confidently define the foetal karyotype as: 46,XY,+der(15)t(15;22)(q13;q11.1),-22. This karyotype therefore demonstrates partial trisomy of 15pter-15q13 and insignificant monosomy of 22pter-22q11.1, with the MLPA and UPD studies also consistent with this finding. These results illustrate the importance of prior planning for prenatal testing of an individual with a family history of a balanced chromosomal rearrangement and the identification of the appropriate tests and markers.

P12. 17q21.31 microdeletion syndrome detected by Multiplex Ligation-dependent Probe Amplification (MLPA)

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High resolution aCGH has identified new genomic disorders in individuals with mental retardation and developmental delay. The 17q21.31 microdeletion syndrome is one of these disorders and is associated with a 500-650kb deletion encompassing the CRHR1 gene and the MAPT gene. The deletion mechanism is most likely non-allelic homologous recombination (NAHR) facilitated by a common 900kb inversion polymorphism.

Clinical features of the syndrome include developmental delay, hypotonia, facial dysmorphism, friendly/amiable behaviour, epilepsy, heart defects and kidney/urologic anomalies.

Our male patient, now 10 years old, first presented as a newborn with marked hypotonia, asymmetric IUGR and hypogonadism. He has thick curly hair, a short neck, a bulbous nasal tip, low set rotated ears, prominent forehead, irregular teeth, very hyperextensible joints and learning difficulties.

Microdeletion syndrome investigations using the MRC Holland MLPA kit, P245-A2, identified a microdeletion of the 17q21.31 region. This was further characterised using the MRC Holland MLPA kit, P371-A1. All 8 probes spanning the CRHR1 and MAPT genes were deleted.

P13. A qualitative prospective analysis of cancer genetic referrals to the Northern Ireland Regional Genetics Service (NIRGS)

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The NIRGS is a tertiary referral speciality, receiving all cancer genetic referrals within Northern Ireland. It is recognized that some of the referrals to the NIRGS are inappropriate or lack specific details in order to either determine appropriateness or to have a more structured and efficient approach to facilitate the patient care pathway. The broad aim of this audit was therefore to identify the a) appropriateness (according to departmental and NICAM guidelines) and b) deficits, in cancer genetic referrals, in order to improve the patient care pathway and departmental workload. This had not been formerly assessed previously to determine the extent of appropriateness or to develop a specific regional cancer genetics referral proforma. The results of a qualitative prospective analysis of the first 100 cancer genetic referral letters received from the 1st April 2009 are presented. These included the findings that over 50% of referral letters had no documented contact patient telephone numbers or details of other related family members who had previously attended the Genetic Service with 33% omitting the age of diagnosis of affected relatives or probands. Based on these results, a cancer genetics referral proforma was developed, which is currently being piloted prior to implementation with re-audit planned in the future.

P14. A diagnosis of Bardet-Biedl Syndrome despite unusual ophthalmic presentation

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The 13 year old male proband is the third child of non-consanguineous parents, born full term after an uneventful pregnancy. He was initially referred to the Northern Ireland Regional Genetic Service (NIRGS) with obesity, moderate learning difficulties, behavioural problems and dysmorphism. Additionally, there was a history of chronic, severe glue ear requiring repeated surgical intervention. Subsequent ophthalmic evaluation reported only 'some slight retinal pigment epithelial mottling' at approximately 9yrs of age but fundus and macular examinations were

normal, as were electroretinograms on two occasions.

A renal ultrasound, skeletal survey including bone age and initial genetic investigations were all normal. Despite clinically not meeting the diagnostic criteria of Bardet-Biedl Syndrome (BBS) suggested by Beales et al, 2001, subsequent BBS analysis was completed. This testing detected the proband to be homozygous for the missense mutation M390R in the BBS1 gene, confirming a BBS diagnosis. A review of this case and the literature is presented. This case highlights the need to consider the possibility of BBS even if suggested diagnostic criteria are not met.

P15. Cat Eye Syndrome: The Northern Ireland experience and review of the literature

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In 1965 Schmid in Zurich and Fraccaro in Pavia first reported on the association of coloboma and anal atresia with a small extra chromosome and proposed the term 'Cat Eye Syndrome (CES)'. It is now known that CES (estimated incidence between 1:50 000 and 1:150 000) is typically associated with a small supernumerary bisatellited marker chromosome (inv dup 22pter-22q11.2), resulting in four copies of this region. CES is known to exhibit extensive phenotypic variability (ranging from near normal to severe malformations). The cardinal features include coloboma, pre-auricular tags and/or pits, anal and cardiac defects and renal malformations.

A retrospective review of all CES cases presenting to the Northern Ireland Regional Genetics Service was undertaken. Cases were ascertained through chart, database and laboratory records with clinical features, phenotypic variability and genetic results being the factors assessed. A total of nine cases were identified, two of which were inherited, and 2 mosaic. Interesting only 1 had coloboma the feature from which this syndrome derived its name. The majority of our cases demonstrated developmental delay which is not one of the regarded cardinal features. Further delineation of our cohort with respect to clinical features, genetic aetiology and review of the literature will be presented.

P16. Familial Subtelomere Duplications or Deletions

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Duplications or deletions of subtelomere regions are detectable by either FISH or MLPA methods. These tests have been widely reported as valuable in detecting subtle chromosomal aberrations that have phenotypic consequences. Nevertheless familial duplications or deletions, the most common of which involve 10q and 4q, also exist. We focus on two cases that were subsequently referred to our laboratory following MLPA subtelomere investigations in an amniotic fluid sample, sent to exclude cystic fibrosis, and a boy with developmental delay. MLPA investigations performed by another institute had reported a duplication of 15q and 7q respectively. G-banding of the amniotic fluid sample had been performed at the NCMG and no visible abnormality seen. Subsequent parental FISH analysis showed the phenotypically normal father to have an enhanced 15q signal, consistent with a MLPA duplication result. Without family studies in the second case the subtelomere duplication of 7q (G-band was normal) could be have been interpreted as the explanation for this boy's developmental delay. However, again FISH showed inheritance from a phenotypically normal father. These and other cases illustrate potential serious consequences of reporting subtelomere anomalies in the absence of other abnormal test results or family studies.

P17. A Patient with AML and MDS-related changes, showing a complex karyotype including excessive telomere association.

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A 74 year old man presented initaially in April 2008 with mild asymptomatic anaemia. Bone marrow (BM) cytogenetic analysis showed a very complex hypodiploid karyotype. BM morphology at this time showed trilineage dysplasia with no excess blasts.

The patient showed progressive fall in platelets during the next nine months and developed fever, epistaxis and severe lethargy. BM morphology showed transformation to AML.

Follow up BM cytogenetics detected a near triploid clone with some of the abnormalities detected previously. However, on this occasion most of the abnormal metaphases included pairs of chromosomes involved in telomere associations. Twenty different pairings were seen, one of which, between 15p and 22p, occurred in three cells.

Telomere association is relatively rare but has been reported in several different solid tumour types, in lymphoproliferative disorders and in ataxia telangiectasia. To our knowledge this phenomenon has not been previously reported in myeloid malignancy. It's biologic and clinical significance is unclear.

P18. Uptake of Huntington disease predictive testing in a complete population and calculation of the prevalence.

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Introduction: Estimates of numbers of at risk patients availing of Huntington disease (HD) predictive testing are inaccurate as most patients who do not want testing simply do not attend clinics.

Methods: We used the Northern Ireland HD register to analyse the number of prospectively recorded predictive tests over a 20 year period and by calculating the prevalence and counting the number of at risk cases, have estimated the total uptake in a defined population.

Results: 212 patients completed predictive testing between 1990 and 2009. 92 (43%) received mutation positive results and 119 (56%) mutation negative. There was one intermediate allele result. There was no significant gender difference. 180 affected cases confirmed by molecular genetic testing were alive on the first of January 2001. The uptake of predictive testing in the entire HD 50% at risk population was calculated by three methods giving a range of 18.3-22.1%. Applying correction factors, the uptake after 10 years of testing was 29.2% and after 20 years was 22.1%. The prevalence of affected HD cases was 10.6 / 100,000 in 2001.

Conclusions: Total uptake of predictive testing has not previously been calculated and suggests that over two thirds of at risk patients do not come forward for testing until symptomatic. Presymptomatic testing for this late onset condition with no present treatment, and limited management options, still presents challenges for families.

P19. Multiplex MassARRAY spectrometry (iPLEX™) testing for Familial Hypercholesterolaemia

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An iPLEX™ assay testing for 55 LDLR mutations, ApoB p.R3527Q , and PCSK9 p.D374Y was developed in Belfast in 2007 as an inexpensive and rapid first-line test for Familial Hypercholesterolaemia.

A total of 864 patients with elevated cholesterol attending lipid clinics underwent FH testing via this assay. These include referrals from laboratories throughout the UK and from a variety of ethnic/genetic backgrounds. Mutations were identified in 117 (14%) of these patients and subsequently confirmed by direct sequencing. Following clinical review, a number of iPLEX-negative patients underwent fluorescent sequence analysis of the promoter and coding sequence (including exon-intron boundaries) of the LDLR gene, and of PCSK9 (exon 7) and ApoB (exon 26), and MLPA analysis. Of the 201 patients to date who have completed this, mutations have been identified in 62 (31%).

iPLEX™ analysis identified 43 different mutations in a total of 117 patients. The four most common mutations identified were ApoB p.R3527Q (12 patients), p.E101K (11 patients), c.2292delA and p.C231X (8 patients each). Neither of these last two mutations would have been detected by the Tepnel Elucigene assay. Tepnel analysis would have identified 18 different mutations in 73 patients (8.4%). iPLEX™ analysis therefore remains a cost-effective first-line screen for FH.

P20. Array CGH: Comparison of two commercially available platforms for the investigation of childhood developmental delay

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Array comparative genomic hybridisation is becoming an increasingly important and useful tool in the cytogenetic investigation of children with learning difficulties, dysmorphism and developmental delay. Currently there are a number of array platforms commercially available. We carried out a pilot study to compare the performance of 2 systems: a 4x44k oligonucleotide array (Agilent) and the Human CytoSNP 12 bead chip system (Illumina). Patients were children with apparently normal karyotypes, where previously performed microdeletion and subtelomere MLPA gave no diagnosis. The platforms were used to determine gains/losses of genetic material and also, in the case of the SNP arrays, regions of copy number neutral loss of heterozygosity. Forty-six patients were included in the study, alongside 16 abnormal controls, with 37 patients and controls run in parallel on both systems. Both platforms correctly identified all known abnormalities in 15 of the controls, however, one control with a small subtelomeric anomaly, run only on the oligonucleotide array, was not readily called. Both systems detected potentially clinically significant abnormalities in 5 patients (8%). Abnormalities ranged in size from approximately 0.4Mb to 2.5Mb and included a del(14q), del(3p) and del(16p). Both platforms were therefore found to be robust and showed high level of concordance.

P21. Non-multifactorial Neural Tube Defects in Northern Ireland

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Neural Tube Defects (NTDs) are generally considered to have multifactorial inheritance. However it has been reported that 2-16% of isolated NTDs and up to 25% of NTDs with other congenital malformations have a chromosomal abnormality. More than 50 %of spontaneous abortions with NTDs have abnormal karyotype. Syndromic NTDs are associated with other congenital malformations and show normal karyotype.

Our recent study of NTDs in Northern Ireland (NI) for the period of 2000-04 identified 125 cases with prevalence of 1 in 1000. Thirteen NTDs cases (10%) had multiple congenital malformations. Chromosome analysis was done in 10 and a cytogenetic abnormality was identified in 4 cases (40%) in this group. Chromosome analysis was done in total 24 cases of NTDs (12 amniocentesis and 12 postnatal) during the study period and abnormal karyotype was reported in 4 cases (17%). Non-multifactorial NTDs are relatively rare. However it is important to be aware of this group as the recurrence risk maybe higher than the common multifactorial NTDs. Chromosome analysis and detailed clinical or post-mortem examination should be done in all cases of NTDs. This may help in identifying causative loci/ genes and also to provide accurate genetic counselling to family members.

P22. Thirty-year trends in birth prevalence and antenatal diagnosis of Trisomy 18 and Trisomy 13 in Northern Ireland

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Trisomy 18 and Trisomy 13 are the second and third most common trisomies in live-born babies. The aim of this study were to determine the trends in birth prevalence and the antenatal diagnosis of trisomies 13 and 18 in Northern Ireland during a thirty year period by retrospective examination of clinical records. All trisomy 13 and 18 diagnoses during the period from 1979 to 2008 were identified from the Northern Ireland Regional Cytogenetic Laboratory and demographic details obtained. The prevalence at birth of live-born babies was 1/6508 for trisomy 18 and 1/14244 for trisomy 13 for the study period. Poisson regression analysis was used to compare the birth prevalence in three periods (1979-98, 1999-2003, 2004-08) and in the five maternal age categories. Increased maternal age (35 years and above) was noted to be an important factor in both trisomies. There was no significant change in the prevalence of trisomy 13 over the period of thirty year. However there was a significant rise in trisomy 18 cases for the last five year study period (2004-08) with the live birth prevalence of 1 /3105. This rise in prevalence was not totally explained by maternal age or termination of affected pregnancy ($Chi^2 = 7.39$, df =8, P =0.50) and is likely to be real. Similar trend was not noted in trisomy 13. There has been an increased in prenatal diagnosis of each trisomy and it is mainly related to abnormal scan findings.

P23. Management and Screening of VHL- Who's Responsibility is it?

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Von Hippel-Lindau (VHL) is an autosomal dominant disorder characterised by development of cancerous and noncancerous tumours of various organs. VHL patients need regular surveillance by various medical professionals. Early detection and intervention through surveillance prevents or minimizes deficits such as hearing loss, vision loss and neurologic symptoms related to these tumours and there are recognised recommended surveillance protocols for VHL and its clinical management. Due to the complexity of this condition many specialist clinics have been established in the UK, co-ordinated and managed by the local genetics department.

This audit aimed to assess the current screening arrangements for VHL patients in Northern Ireland by conducting a patient satisfaction survey. The objective was to assess the need for centralisation and co-ordination of their care by a regional multidisciplinary VHL clinic. Patients known to our service were identified and sent a postal questionnaire comprising ten questions related to current surveillance and their feedback. In view of limited numbers this was followed by a telephone call to maximise the response.

The results of this audit support the need for co-ordinated approach to VHL related surveillance and advocates the establishment of a regional VHL clinic led by the clinical genetics department.

P24. Hyperlipidemia, Hypertriglyceridemia contributing Premature Cardiovascular Disease in Type II Diabetes (T2D) Patients: The Prospective Hospital Based Study.

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Cardiovascular disease (CVD) is a leading cause of death and disability worldwide, with genetic and environmental predisposition. Majority of Cardiovascular disease occur in individuals >65 year old, but is being increasingly seen in individuals with age <50 years. In the present study APOC3 (C3238G), APOC3 promoter (C482T) and APOA5 (T1131C) genes involved in regulation of lipid metabolism and has recently been implicated in the pathogenesis of CVD in T2D patients. We investigated the effects of common variants of these genes on T2D patients as well as the association with CVD. Genomic DNA is extracted from blood samples obtained of 120 T2D subjects and 150 healthy volunteers following both inclusive and exclusive criteria. DNA samples were genotyped for C3238G, C482T variants using PCR-RFLP assay and then DNA samples followed by genotyping T1131C variant using ARMS-PCR assay. The 3238C>G, 482C>T SNP of APOCIII (S2 and TT allele) tend to have high plasma triglyceride concentrations was shown a risk factor in T2D subjects when compared with healthy volunteers. The rare allele 1131T>C (CC allele) is also associated with elevated lipids levels. The Haplotypes analysis performed for APOAV and APOCIII SNPs indicated significant differences ($P<0.001$) in distribution. Most significantly, a risk haplotype S2/T/C was obtained ($P<0.001$) and was associated with increased risk of CVD.

P25. Association of Variants in Candidate Genes Influencing Autonomic Nervous System Functionality with Blood Pressure Level

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Genomewide association studies have identified 13 novel loci associated

with blood pressure (BP). However, only a small proportion of total BP variation is explained by these findings. Here we report on a candidate gene study with dense SNP coverage of multiple genes involved in the autonomic nervous system (ANS), namely neurotransmitter receptors, metabolisers, and transporters. Using the Illumina GoldenGate platform, we genotyped 2364 SNPs in 168 genes in 358 healthy bank employees who had undergone clinic BP measurements (screening population, SP). Only the 58 SNPs in 33 genes, found to be associated with systolic, diastolic or pulse pressure within the SP ($p<0.01$), were genotyped (Illumina Veracode platform) in a second independent replication population (RP) – these 380 healthy bank employees had undergone repeated 24-hour ambulatory BP monitoring. Association analyses were performed using additive genetic models. Quantile-quantile plots showed enrichment for significant P-values in both populations. The top hits are in the SLC17A8 and the GABR1 genes. SLC17A8 encodes a sodium-dependent inorganic phosphate cotransporter and GABRR1 encodes the α subunit of the ionotrophic gamma-aminobutyric acid receptors. These genes have not previously been implicated in the causation of hypertension though the influence of the ANS on BP makes the observed associations biologically very plausible.

P26. Mutations in the Human Zinc Finger Transcription Factor 8 (*TCF8*) Gene in Keratoconus and Corneal Endothelial Dystrophies Support a Genotype-Phenotype Correlation.

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Purpose: Mutations in the human zinc finger transcription factor 8 (*TCF8*) gene have been reported in posterior polymorphous corneal dystrophy (PPCD) and Fuch's endothelial dystrophy (FECD). Although PPCD and keratoconus (KTCN) involve different layers of the eye, PPCD has been associated with KTCN in several reports. To investigate the role of *TCF8* mutations in the pathogenesis of keratoconus, mutational analysis was performed in 70 unrelated individuals with KTCN.

Methods: The coding regions of *TCF8* were PCR amplified, Sanger sequenced and analysed using Sequencher 4.7. Novel variants were screened in 100 unrelated population controls (200 chromosomes).

Results: A novel, heterozygous, missense mutation in *TCF8* was identified in a patient with familial KTCN and absent from 200 control chromosomes. The mutation, c.1920G>T, results in a non-conservative substitution of a highly conserved glutamine to histidine (p.Q640H). The mutation was detected in two siblings with KTCN and in their mother: a patient with FECD. The glutamine (G640) is an invariant residue in the homeodomain of TCF8.

Conclusions: This data supports a strong genotype-phenotype correlation within the *TCF8* mutational spectrum. Missense mutations in *TCF8* result in keratoconus and FECD while nonsense and frameshift mutations result in PPCD.

P27. Evaluation of eight candidate genes located within chromosome 6q22-q27 for association with glomerulonephritis in a UK population

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There is an inherited predisposition to glomerulonephritis (GN) which is a leading cause of end-stage renal disease. We examined eight biological and positional candidate genes located at 6q22-27, including *GJA1, IGF2R* and the *RAET1* family, for association with GN.

DNA was obtained from the national MRC-KRUK bank for GN (n_{max}=2,964) and genotyped using a case-control approach. Initially, individuals with GN (cases, n=583) were compared to individuals with no evidence of renal disease (controls, n=508). Potentially functional SNPs were identified from Ensembl. SNP genotype was downloaded from the International HapMap Project and tag SNPs selected using Haploview where SNPs were in Hardy-Weinberg equilibrium, $r^2>0.8$ and minor allele frequency >0.05. SNPs (n=53) were genotyped using TaqMan and MassARRAY® iPLEX Gold technology. Data were analysed in PLINK using the test for trend.

Investigating all common and potentially functional variants revealed statistically significant association for rs9397449 and rs9397070 in *RAET1G* (P=0.001 and P=0.04 respectively). RAET*1G* is an MHC class-1 related gene (6q24.2-q25.3) and SNPs in this region have recently been associated with diabetic nephropathy. Association was also suggested for *IGFR2* at rs3734181 (P=0.01).

This data suggests that genes within 6q22-27 influence glomerulonephritis and we are presently conducting independent replication studies to validate our findings.

P28. Identification of a novel locus for autosomal dominant Restless Legs Syndrome

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Introduction: Restless Legs Syndrome (RLS) is a common neurological sleep-related disorder, affecting up to 15% of the general population. The disorder is characterised by an irresistible urge to move the lower limbs, accompanied by uncomfortable sensations, occurrence at rest, improvement with activity and worsening of the symptoms in the evening or at night. Linkage analysis has identified six genomic regions. No causative gene has been identified. Association mapping has highlighted a further five areas of interest.

Aim: To map and identify the gene responsible for RLS in an Irish family (RLS3002).

Method: Eighteen members of the RLS3002 family participated in the study; eleven affected and seven unaffected members. All known RLS loci and associated regions were examined for linkage. A genome wide linkage analysis scan was conducted.

Result: Linkage was excluded from published loci. The genome-wide scan identified a region of linkage with a maximum LOD score of 3.59, (α=0.00). A genetic region of 2.5 Mb was defined by haplotype analysis. Candidate genes have been identified and are the subject of further study.

Conclusion: We have successfully identified a novel locus for Restless Legs Syndrome, which will enable us to attempt to identify the causative gene in this family.

P29. Implantation and Pregnancy Outcome In Relation to Inherited Coagulopathties

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Thrombosis interfere with feto-maternal interaction in the site of fetus implantation, progression of fetal growth and pregnancy termination . These situations are seen in relation to spontaneous pregnancy or artificial reproductive technology (ART). .Many inherited mutations and polymorphisms are attributed to this status. Some of these mutations are seen in factor V Leiden, prothrombin, and methylenetetrahydrofolate reductase. We selected 48 women with pregnancy complications included recurrent abortion, IUFD, and ART failure. Among 13 women were investigated for factor Leiden, 5 patients were positive (38.46%), 75% were positive for MTHFR (12/16), four cases are homozygote for these gene, and 8 cases are heterozygote. None of the 19 women tested for prothrombin was positive for mutation of this gene.

Although our samples are very small, our results are in Hardy-Weinberg equilibrium for MTHFR gene mutation. This study is continued with large population and in comparison with control group to confirm effects of these mutations in implantation and pregnancy outcome in our population.

P30. Identification of novel *STRA6* variants in patients with anophthalmia-microphthalmia-coloboma

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Anophthalmia (absence of an eye), and the related condition microphthalmia (small eye), are relatively rare disorders estimated at 3 and 14 in 100,000 births respectively. The aetiology of anophthalmia/microphthalmia (AM) is not well understood but observed familial clustering suggests a significant genetic component. Several genes have been implicated in AM but, to date, mutations in these genes account for less than 25% of cases.

We report studies of an extended Irish family from an endogamous nomadic group presenting with various forms of AM. We employed homozygosity mapping to search for causative mutations in the affected members of the pedigree. Previous analysis excluded any causative mutation in known AM genes. Genotyping was undertaken using the high density Illumina 1M SNP platform. Homozygosity mapping identified a 0.9Mb homozygous segment on chromosome 15q22.32 shared by all 6 affected individuals but not shared by unaffected relatives. The candidate region was isolated by NimbleGen's target enrichment service and sequenced on the Illumina Genome Analyser.

We identified 3 homozygous variants (2 non-synonymous and 1 utr) in *STRA6*, a membrane protein involved in the metabolism of retinol. One of the NS variants is a double nucleotide polymorphism (DNP), a newly recognized source of genetic variation that is predicted to play a major role in disease predisposition.

P31. Targeted Sequence Capture and Next Generation Sequencing of a 5Mb Region on Chromosome 15q Previously Linked to Keratoconus

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Purpose: Our group previously mapped a large Northern Irish family affected by autosomal dominant, clinically severe keratoconus and anterior polar cataract to a 5.5Mb region on chromosome 15q22. The linkage region contains 85 candidate genes and in 28 no pathogenic mutation was identified by Sanger sequencing. To identify the molecular genetic defect in this family targeted sequence capture and next generation sequencing (NGS) was performed.

Methods: A custom Nimblegen sequence capture array was designed to capture 5Mb of the 5.5Mb region (a 0.5Mb repetitive region was excluded). NGS of the enriched region was performed (Genome Analyzer) and reads aligned to a reference sequence using 'Genomics Workbench' software (CLC bio).

Results: Twenty-five potentially-pathogenic coding sequence variants were identified in affected family members. These variants were present in eleven known and predicted genes within the linkage region. Variants in seven genes were excluded following Sanger sequencing on the basis of non-segregation, presence in population controls and failure to replicate NGS data. Analysis of the remaining four genes is ongoing, in addition to intronic variants surrounding splice sites.

Conclusion: Custom targeted sequence capture followed by NGS of a linkage region is an effective strategy for disease gene discovery.

P32. ADAR RNA editing in neurodegenerative diseases.

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In humans ADAR RNA editing controls key properties of excitatory glutamate receptors. ADARs convert specific adenosines to inosines in transcripts and inosine is read as guanosine during translation. Loss of RNA editing is implicated in neurodegeneration and glutamate excitotoxic neuron death in stroke and ALS. I will present our work on RNA editing loss in ALS motor neurons and on human mutations in ADAR proteins.

Drosophila also provides an excellent study model; in Drosophila ADAR RNA edits more than fifty known neuronal transcripts. Adar mutants are locomotion-defective with age-dependent vacuolisation in brain and retina. There is no extensive neuronal apoptosis. Instead intracellular membrane structures resembling those seen in autophagy mutants and in human lysosomal storage diseases appear and large fluid-filled vacuoles develop. The reduced viability at eclosion in an Adar null mutant is rescued by heterozygous Tor mutants which lead to increased autophagy. Overexpression of Atg5 also rescues the reduced viability as well as the locomotion defects, neurodegeneration and the reduced longevity of the Adar null.

Increased autophagy is clearly protective in the Drosophila Adar neurodegeneration and possibly also in human neurodegenerations associated with loss of ADAR RNA editing if underlying molecular mechanisms are conserved.

P33. The impact of the MTHFR 677C>T polymorphism on RUNX1 DNA methylation patterns

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Folate/riboflavin status in combination with the MTHFR 677C>T genotype have previously been identified as important factors for consideration in health and disease. We identified RUNX1 as a responder to changes in folate/riboflavin status and considered DNA methylation as the mediator of this response. In the present study we assessed whether DNA methylation in the proximal promoter of RUNX1 correlated with MTHFR 677C>T genotype. DNA methylation within a CpG island of the proximal promoter of RUNX 1 was assessed by Methylation-sensitive high resolution melting (MS-HRM) in a panel of DNA samples from the Coriell lymphoblast collection. Comparison of the DNA methylation profiles of each genotype group shows that the CC and CT groups have a broadly similar pattern. The TT group, however, shows a dramatic enrichment of samples with 0% DNA methylation of their RUNX1 proximal promoter. The methylation profile of the TT group was compared to a combined CC/CT profile by Mann-Whitney test using SPSS yielding a P-value of 0.06 i.e, not significant. In conclusion, TT individuals may tend to exhibit 0% DNA methylation of their proximal RUNX1 promoter compared to CC or CT individuals particularly in the context of nutritional status. However, this requires further investigation.

P34. No correlation between unmetabolised folic acid levels and folate gene polymorphisms in an Irish population.

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Folic acid is an essential nutrient required for growth and development with many health benefits, particularly the prevention of neural tube defects. This has led to liberal voluntary fortification of a wide range of foods within Ireland. The increase in folic acid consumption has resulted in the appearance of circulating unmetabolised folic acid in the blood stream of the majority of the population. The risks and/or benefits associated with this are unclear. We wished to investigate whether circulating unmetabolised folic acid levels are influenced by genetic factors. We examined three functional and/or disease associated polymorphisms within genes involved in folate metabolism. These included the MTHFR (methylenetetrahydrofolate reductase) 677C>T, the DHFR (dihydrofolate reductase) intron A 19bp deletion/insertion polymorphism (DIP) and another DIP within intron 7 of MTHFD1L (mitochondrial 10-formyltetrahydrolate synthetase) c.781-6823ATT[7-9]. The polymorphisms were genotyped in 138 individuals from an elderly Irish cohort (aged 60-86 years recruited from the Lifeways study, a longitudinal study commissioned by the Health Research Board in 1999). Although trends were apparent, statistical analysis indicated that there was no significant correlation between polymorphism genotypes and unmetabolised folic acid. Further investigation is required in order to reach a definitive conclusion.

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Abstracts

Ulster Medical Society Junior Doctors' Prize Evening 5th November 2009

Ulster Medical Society Rooms

PLATFORM PRESENTATIONS

Successful eradication of Methicillin-resistant *Staphylococcus aureus* in Adults with Cystic Fibrosis

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People with Cystic Fibrosis have a high risk of MRSA infection. Prevalence has increased from 7% of patients in 2001 to 17.2% of patients in 2005 in the USA (1). It is unclear whether MRSA respiratory infection influences lung function, which antibiotics (if any) should be used to treat MRSA infection and for how long. This study investigates the prevalence and antimicrobial resistance of MRSA isolates in the Northern Ireland adult cystic fibrosis population, lung function changes associated with MRSA positive sputum and antibiotic treatment, antibiotic choices, the length of treatment and the efficacy of treatment. A retrospective study was carried out on all patients with Cystic Fibrosis (CF) attending the NI Regional adult CF centre. Belfast City Hospital, with a history of MRSA between 1999 and February 2009. Case notes were used to ascertain FEV_1 three months prior to infection, at date of initial sputum culture positive MRSA, at the end of antibiotic treatment, and three months post infection. Information on the antibiotics used and duration of treatment were also obtained. Twenty eight adult patients (mean age 25 (7) years) were included in the study. FEV_1 (%predicted) at initial positive sputum result was [64(24)].The majority of infections (82%) were treated with 6 weeks of rifampicin dose 300mg po bd and fusidic acid 500mg po bd and the remainder with combination therapy based on antimicrobial sensitivity and reported side effects. FEV_1 (%predicted) showed a small but significant improvement after antibiotics for first MRSA infection only [7(15)%], p=0.04, (95% CI 0-13%). Overall 26 of the 28 patients remained MRSA sputum culture negative for six or more months following antibiotic treatment. 23 patients were treated with rifampicin and fusidic acid, of whom 21 had successful eradication of MRSA. Linezolid was used in 5 infections involving four patients with recurrent MRSA infections and proved ineffective in 4 of these.This study demonstrates that FEV_1 improves significantly following antibiotic treatment for first isolation of MRSA from sputum culture in CF. Rifampicin and fusidic acid for six weeks are effective first line agents.

Heart fatty acid binding protein (H-FABP) in combination with the 80-lead body surface map (BSM) improves early detection of acute myocardial infarction

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Despite modern high sensitivity assays for cardiac troponin T (cTnT) there remains a sub-group of patients who present with ischemic-type chest pain and have a negative cTnT at first medical contact. This group will contain patients at a very early stage of the infarction process. We aim to assess the usefulness of H-FABP in combination with the 80-lead BSM for improving early diagnosis of acute myocardial infarction (AMI).Enrolled were 407 patients (age 62 ± 13 yrs; 70% male). Of these 407, 180 had cTnT < 0.03µg/L at presentation. AMI (peak cTnT ≥0.03µg/L) occurred in 52/180 (29%). Of those 180 patients, 27 had ST elevation (STE) on ECG and 104 had STE on BSM. H-FABP elevation (≥5ng/ml) occurred in 95/180, with a significant proportion in the AMI group (42/52 *v* 53/128, p<0.005). BSM STE was significantly associated with H-FABP elevation (p<0.001). Of those with initial cTnT < 0.03µg/L, the c-statistic distinguishing AMI from non-AMI using H-FABP alone was 0.644 and BSM alone was 0.716. Using the combination of BSM and H-FABP the c-statistic was 0.812 (p<0.001). In patients with acute ischemic-type chest pain who have a normal cTnT at presentation the combination of H-FABP and BSM identifies those with early AMI.

BRCA1 is a predictive marker of response to chemotherapy in sporadic epithelial ovarian cancer

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Reduced expression of the BRCA1 tumour suppressor gene

occurs in a substantial proportion of sporadic epithelial ovarian cancers (EOC). Treatment of EOC includes both platinum and taxane chemotherapy, however, no predictive markers exist to guide treatment decisions. A reduction in BRCA1 expression leads to enhanced sensitivity to platinum but relative resistance to taxane based chemotherapy *in vitro*. We therefore investigated the relationship between BRCA1 protein expression by immunohistochemistry (IHC) and survival in EOC, correlating outcome with chemotherapy received. We identified 292 archival tumour samples from two UK ovarian cancer databases. BRCA1 protein expression was assessed and correlated with overall survival (OS) and response to chemotherapy. Patients with detectable BRCA1 staining had a significantly improved median OS (41.7 *v* 19.8 months, p=0.0004) and response rates (84.5% v 55.8% p=0.003) following platinum/taxane as compared to platinum only chemotherapy. In contrast, patients with no detectable BRCA1 displayed no differences in median OS (41.5 *v* 38.9 months p=0.74) or response rates (72.7% *v* 77.8%, p=0.555) whether treated with platinum/taxane or single agent platinum regimens. This study provides evidence that BRCA1 protein expression is a useful predictive marker of response following chemotherapy in sporadic EOC. Further validation of these findings in independent clinical trial populations is under investigation.

Training basic life support to school children using medical students and teachers in a 'peer training' model – results of the 'ABC for life' programme.

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The 'ABC for life' programme is a specifically designed course of instruction to teach 10-12 year old children basic life support (BLS) skills in Northern Ireland using a three tier 'peer training' model of medical students, primary school teachers and P7 children. Medical students instructed small groups of teachers from WELB, who then taught pupils in their schools. Five pupils from each school were selected randomly and given a questionnaire to assess knowledge of BLS immediately before and after a teacher led training session. 38 teachers were trained (190 pupils). Mean age was 10.7 years and 35.4% were male. Baseline CPR knowledge or change following training were not affected by sex, positive family history of heart disease or previous BLS training. Scores improved markedly following training with a mean increase from 57.2% to 77.7% (t-test, p<0.001). There is good transfer of knowledge down the teaching chain from medical student to teacher to P7 pupil. By using this method, large numbers of children are being taught BLS. 350 schools have currently been trained and received manikins / resources. We are currently researching practical skills using recordable manikins.

Patients' Perception of Doctors' Workplace Attire

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The DHSS at present enforce a dress code policy for healthcare professionals and have introduced plans for a single uniform for doctors. Their current dress code policy advises short sleeves and no loose clothing as this is expected reduce the number of hospital acquired infections. There is very little scientific evidence to justify the guidance. With little clinical evidence to support the introduction of uniforms into the workplace, patient opinion should be considered an important factor. This study aims to demonstrate how doctors' attire affects inpatient perception of infection control, professionalism, clinical ability and accessibility. Data was collected in June and July 2009 in the Ulster Hospital surgical wards and statistics analysed using SPSS. A Surgical SHO was photographed in three sets of clothing –traditional shirt and tie, policy advised clothing and surgical scrubs. A set questionnaire was given to participants with four questions – How would you rate the pictured doctor's ability to PREVENT the spread of infection, their clinical ability, their professionalism and accessibility? 194 patients scored the photographs out of 10 for each question. The results were analysed using the Friedman test and Wilcoxin signed ranks test – each question had highly significant results. Scrubs scored significantly higher than the policy advised clothing and traditional shirt and tie for perceived prevention of spread of infection. The traditional shirt and tie scored significantly higher with regards to professionalism, clinical ability and accessibility. With no clinical evidence to indicate that work wear is related to the number of hospital acquired infections, and patients otherwise significantly preferring the traditional shirt and tie, it seems there is no indication for introduction of uniforms into the workplace, or in fact a dress code policy at all.

Wavelet transform analysis of blood velocity waveforms may identify very early microvascular disease in type 1 diabetes mellitus

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Diabetic microvascular disease markedly increases the risk of a future cardiovascular event. Current techniques for identifying microvascular disease rely on identification of structural abnormalities of retinal vessels or the detection of albuminuria. The ability to detect dysfunction at an even earlier stage would permit preventative treatment of those at highest risk. Blood velocity waveforms are composed of incident waves generated by cardiac contraction and reflected waves from downstream vessels. The wavelet transform is a mathematical tool that facilitates the comprehensive analysis of waveforms. Its utility remains unknown for the study of blood flow waveforms. 39 subjects with well controlled type 1 diabetes (median age 33 years) and 39 well matched control subjects (median age 30 years) were studied. Maximum blood velocity waveforms were recorded from the common carotid, ophthalmic, central retinal and interlobular arteries using Doppler ultrasound. Wavelet analysis of waveforms identified abnormalities in all vascular territories. These were not apparent when traditional waveform analysis parameters were used. Significant correlations were present between measures of urinary albumin excretion and wavelet-derived indices from the interlobular renal arteries. Wavelet analysis of blood flow velocity waveforms is a powerful technique which appears to be capable of detecting microvascular

disease at a pre-clinical stage.

POSTER PRESENTATIONS

Radiation exposure during EVAR is significant and not influenced by aortic neck morphology

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Endovascular aneurysm repair (EVAR) is a routine vascular procedure, with widely recognised benefits, but exposes patients to significant radiation. The aim was to assess exposure and determine the influence of neck morphology. All EVAR procedures in a prospective database were included. Neck measurements, sac diameter, radiation dose, screening time and contrast volume were recorded, along with subsequent radiation. Results are expressed as mean (±standard deviation). 320 elective patients from October 1998 to October 2008 underwent EVAR. Mean screening time was 29.4minutes (±23.3), radiation dose was 468.8Gycm^2 (±283.6). 64 patients underwent emergency EVAR. The mean screening time was 22.9minutes (±18.2), radiation dose was 538.4Gycm^2 (±345.7). During the first post-operative year CT exposed patients to 15.0Gycm with 5.0Gycm in subsequent years. The neck diameters were smaller and sac diameter ($p<0.0001$) greater in emergencies. Screening time ($p=0.053$) and contrast volume ($p=0.04$) were lower in emergencies, with higher radiation dose ($p=0.12$). There was no correlation between anatomical and radiological parameters. EVAR procedures and follow-up involve large amounts of radiation, determined by mode of presentation and not anatomy. Radiation exposure poses risk to patients, and health care professionals. Alternative follow-up imaging modalities should be considered and regular audit encouraged.

How accurate is PET scanning in the detection of colorectal hepatic metastases?

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Liver metastases occur in approximately 50% of colorectal cancer patients, for which Positron Emission Tomography (PET), first introduced in Belfast in 2002, is an essential pre-operative investigation. The study aim was to compare the characteristics of hepatic metastases on PET with histology. A retrospective review of all colorectal hepatic metastases patients who underwent surgical intervention from August 2002 to December 2008, was performed. Patient demographics, colorectal staging, number of metastases and their maximum diameter from both PET and pathology reports, were recorded. Values are expressed as mean (±SD).

141 patients were identified (28 excluded – no PET). The maximum diameter on PET (4.2cm±2.6) was similar to pathology (4.8cm±3.6; $p=0.39$), with significant correlation ($r=0.72$, $p<0.0001$). The number of lesions on PET (1.6±1.0) was similar to pathology (1.7±1.3; $p=0.43$) with significant correlation ($r=0.80$, $p<0.0001$). Overall, PET accurately predicted the number of lesions in 76 out of 113 patients (67.3%). Mean SUV max was 9.22 (±4.39), with no correlation to lesion diameter ($r=0.25$, $p=0.045$), but significantly increased with decreasing differentiation ($p=0.01$). PET scanning accurately detected the number and size of lesions, with radiological evidence of poorer differentiation. Further studies of non-surgical patients are required to assess its overall accuracy.

Pathways of Oxaliplatin/5-Fluorouracil Resistance in Colorectal Cancer

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The development of drug resistance limits the effectiveness of current chemotherapeutic agents used to treat colorectal cancer and the discovery of these underlying mechanisms of resistance is a priority. Transcriptional profiling of pre-treatment metastatic colorectal cancer liver biopsies and HCT116 parental, oxaliplatin and 5-Fluorouracil resistant cell lines was performed using the Affymetrix HGU133 Plus 2.0 array and Almac Diagnostics Colorectal Cancer Disease Specific Arrayä (DSAä). Pathway analysis of the microarray data was performed using Metacore and Gene Set Enrichment Analysis (GSEA) was employed. Data analysis identified panels of in vitro and clinical genes whose expression is acutely altered in the parental setting following drug treatment and also basally deregulated in the resistant cells. Significant pathways involved in these panels of genes were compared with the results of the GSEA to produce a final ranked gene list of pathways. This list included groups of Cell Cycle, Focal Adhesion, Insulin and MAPK signalling genes. A candidate gene approach was used to select individual genes from these pathways for incorporation into siRNA screens. This study demonstrates the utility of microarray expression data analyzed by pathway and Gene Set Enrichment Analysis to identify pathways of Oxaliplatin/5-Fluorouracil resistance in colorectal cancer.

Therapeutic Impact of Radiation Exposure in Acute Surgical Patients

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To examine the use of radiological investigations in acute surgical patients and assess whether a guideline-based radiation exposure risk/benefit analysis can aid in the choice of investigation used. A prospective observational study was completed from April to July 2008 for all acute surgical admissions and the use of radiological investigations was then evaluated against The Royal College of Radiologists (RCR) guidelines. 380 acute surgical admissions (M=174,

F=185, Children=21) were assessed and 734 radiological investigations performed (mean = 1.93 investigations/patient). 680 (92.6%) were warranted which included 142 CT scans (19.3%), 129 chest x-rays (17.6%), and 85 abdominal x-rays (11.6%). Clinically, radiological imaging complemented surgical management in 326 patients (85.8%). The average radiation dose was 4.18 millisievert (mSv) per patient or 626 days of background radiation exposure. CT imaging was responsible for the majority of radiation exposure, with a total of 1,310 mSv (82.6%) being attributed to CT imaging in 20.8% of acute admissions. Subgroup analysis demonstrated that 92.8% of the CT scans performed were appropriate.

Radiation exposure was generally low for the majority of acute surgical admissions. However, we recommend carefully evaluating CT imaging requests particularly in patients with clinically confirmed pathologies and in younger women.

Fahr's disease – a case series from the Irish Traveller Community

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Fahr's disease (or idiopathic basal ganglia calcification) is a rare neurodegenerative condition characterised by pyramidal and extrapyramidal signs, cognitive dysfunction, neuropsychiatric manifestations with basal ganglia and extrastriate calcinosis radiologically. Although genetic linkage studies to the IBGC1 locus on chromosome 14q have been carried out, no specific gene has been identified in this condition. We describe an extensive Fahr's disease kindred in a consanguineous family from the Irish Traveller Community. Index cases presented aged 30 and 40 with depression, buccolingual dyskinesia, dysarthria, blepharospasm and segmental dystonic posturing. Examination revealed evidence of hypokinetic and hyperkinetic movement disorders with associated cognitive and psychiatric dysfunction. Imaging demonstrated bilateral striatopallidodentate calcification in all the affected patients. Investigations revealed no abnormality of calcium or iron metabolism nor intracranial vascular pathology. A third similarly symptomatic case and a number of possibly pre- or sub-symptomatic cases were identified on further examination of this kindred. Clinical, para-clinical and radiological data is presented. This is one of the largest kindreds with Fahr's disease described to date, suggesting an autosomal dominant inheritance with variable penetrance.

The epidemiology of congenital myasthenic syndromes in Northern Ireland

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Congenital myasthenic syndromes (CMS) are a heterogeneous group of disorders due to inherited abnormalities in neuromuscular transmission. To date information on the frequency of these conditions comes from specialist centre case series reports. Population based data is lacking. Cases were ascertained from systematic review of patients attending the regional neuromuscular clinic over the past 30 years and from those identified and excluded from an epidemiological study of autoimmune myasthenia gravis in the area. Cases were confirmed clinically, genetically and with neurophysiological examination. 14 cases of CMS were identified in the region giving a prevalence rate of 8.2 per million (95%C.I.:6.0, 10.4) and an estimated incidence of 0.2 (95%C.I.: 0.06, 0.62) per million person-years. Age at diagnosis: 0-60 years (mean: 15.2 years); 4 females, 10 males. Dok-7 syndromes were the most common (43.9%) followed by slow channel (21.4%) and acetylcholine receptor deficiency syndromes (14.3%). One case was fatal: mortality rate 0.1 (95%C.I.: 0.01, 0.71) per million person years. All treated cases responded well to specific treatments with functional improvement. This data suggests that CMS is relatively common among the heritable neuromuscular disorders. Their treatability makes recognition and genetic diagnosis vital.

Triple A syndrome: Multiple evolving clinical features.

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Triple A syndrome is a rare autosomal recessive disorder characterised by primary adrenal insufficiency, alacrima and achalasia. Neurological features may also be present. Various combinations of these features may be present which evolve over time. Triple A syndrome is caused by mutations in the AAAS gene, whose function is incompletely understood. An 8 year old girl presented with a hypoglycaemic seizure following an overnight fast. Examination revealed palmar skin crease pigmentation. A Synacthen test confirmed primary adrenal insufficiency (basal cortisol 174 nmol/l, stimulated cortisol 173 nmol/l (NR >500nmol/l), ACTH 2980 ng/l (NR <55 ng/l)). Mineralocorticoid replacement was discontinued due to normal electrolytes, renin and aldosterone concentrations. Alacrima was noted at age 9 years. Barium meal demonstrates delayed oesophao-gastric transit in keeping with achalasia. Neurological examination is unremarkable. Genetic testing revealed a homozygous mutation (1144_1147delTCTG) in exon 12 of AAAS gene which causes a frameshift with a premature stop codon (p.Ser382ArgfsX33). We present a rare case of isolated glucocorticoid deficiency due to Triple A syndrome. Marked variability in clinical features is noted even within same kindreds. Diagnosis allows screening and symptomatic treatment for the subsequent development of associated features and reduces the risk of presentation with potentially life-threatening adrenal failure.

Outcomes of individuals with acute lymphoblastic leukaemia treated according to the UKALL 12 protocol in Ireland.

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Acute Lymphoblastic Leukaemia in adults is a disorder which poses important challenges with 5 year survival rate in adults of only 30-40%. There are currently no official guidelines on the treatment of ALL. In NorthernIreland, all patients with ALL were enrolled in the UKALL 12 Trial or treated according to its protocol. To assess the outcomes of patients treated for ALL in Belfast City Hospital in comparison to the preliminary results of the UKALL 12 Trial. Retrospective, proforma based case note review of a sample of 33 patients in Belfast City Hospital, including those who were ineligible to be enrolled in the UKALL 12 trial but were treated according to its protocol. The results of this analysis revealed that patients in this sample performed at least as well as the initial UKALL 12 Trial results. 97% of the sample from BCH achieved remisson and 100% survived remission induction, in comparison to the 91% in the preliminary UKALL 12 results who achieved remission and 95% of patients who survived its induction. 5 year survival in our sample was 70%, in comparison to 38% in the UKALL preliminary results. These results are encouraging; difference in standards could be partly attributable to difference in sample size, and also due to difficulty in obtaining some patients' notes.

Carotid Body Tumours – A Northern Ireland Experience

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Carotid body tumours (CBTs) are rare vascular neoplasms originating in paraganglionic cells of the carotid bifurcation. Symptomatology often relates to an overall pressure effect while their rich vascularity and invasive nature contribute to technical difficulties during removal and associated co-morbidity. The aim of this study was to review all patients diagnosed with CBTs by our institutions and compare our experience will all published evidence. We completed a retrospective review of all patients who had CBTs managed in our institutions between 1987 and 2009. Patient demographics, clinical symptomatology, investigative modality, therapeutic intervention, pathological analysis and long-term outcomes were assessed. Twenty-nine patients were identified with 33 CBTs and 3 glomus intravagale tumours (GITs). 6 patients had bilateral CBTs (21%), one patient had a synchronous GIT while 4 familial cases (15%) were identified. There were 14 men and 15 women with a mean age of 49 years (range 16-85). Surgery was not performed in 3 patients. 26 patients underwent a total 30 operative procedures for the resection of 28 CBTs and 3 GITs. Pre-operative embolisation was performed in 2 patients (7%). Conventional operative treatment included subadventitial tumour excision. A vascular shunt was inserted to facilitate vascular reconstruction in 6 (19%) cases. Five patients (16%) required en-bloc resection of the carotid bifurcation with continuity restored with an interposition vein graft. For access the external carotid artery (ECA) was ligated in a further 4 patients (13%) with the ECA being utilised for reconstruction of the internal carotid artery in one patient. Shamblin classification demonstrated 6 grade I, 5 grade II, 9 grade III and 12 unclassified tumours. Mean tumour size was 3.72cm (range 1.8- 8cm). No peri-operative mortalities were recorded. Immediate complications included peri-operative stroke secondary to an occluded vein graft (n=1), tracheostomy (n=2), emergency haematoma drainage (n=2), transient and permanent cranial nerve damage (n=9 & 10) and Horner's syndrome (n=1). Late complications included pseudoaneurysm of vein graft with subsequent stoke (n=1) and an asymptomatic vein graft occlusion (n=1). Post-operative radiotherapy was required in 3 patients. There were two malignant tumours and there was one case of tumour recurrence and death secondary to pulmonary metastases at 4-years. Two other patients died of unrelated causes. Other patients remain well with no evidence of recurrence (mean follow up of 1801 days, range 159 -9208 days). Management of carotid body tumours remains within the remit of the vascular surgeon who uniquely possess the operative skills to manage these technically challenging tumours. Our experience is comparable with other modern case series reports where surgical intervention conferred a long-term survival advantage.

Mortality within 30 days in patients over 70 years receiving chemotherapy: a single institution retrospective analysis.

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Life-expectancy in western populations is increasing, as is incidence of malignancy in older persons. As most chemotherapy trials routinely exclude those over 70, information on complications and outcomes is sparse. We examine mortality rates in patients over 70 within 30 days of administration of chemotherapy in routine clinical practice. We retrospectively reviewed case notes of patients over 70 at date of first chemotherapy cycle, receiving chemotherapy at the Northern Ireland Cancer Centre during 2006. Baseline demographics, patient characteristics, treatment received, treatment-related complications, death within 30 days of chemotherapy and overall survival were recorded. Actuarial Survival was estimated using the Kaplan-Meier method. 284 patients were identified, median age 74 years (range 70-88). The most frequent tumour sites were colorectum (25.0%), lung (22.2%) and ovary (12.0%). Median survival was 17.7 months for all patients (95% C.I. 14.4-20.9), 12.1 months for palliative patients (95% C.I. 9.7-14.5, n=184) and had not been reached for those receiving radical or adjuvant chemotherapy (n=102). Mortality within 30 days was 3.5% (n=10). All deaths occurred in those receiving palliative chemotherapy (5.4% of all palliative patients). One death was treatment related. There was no excessive mortality in patients aged over 80. Our results compare favourably with previously published non-clinical trial outcome data for similar age-groups. Further investigation is required into assessment and management of elderly patients receiving chemotherapy.

A review of breast cancer in women under 40-years of age in Northern Ireland

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There are few studies examining breast cancer in women under the age of 40-years, particularly in Western European populations. Such tumours are reported to be more aggressive, possibly due to a different pathophysiology compared to older patients. We performed a retrospective review of all women less than 40-years of age, diagnosed or treated with breast cancer, from June 2001 to June 2007 to assess pathophysiological factors that may influence clinical outcome and prognosis. All clinical records were reviewed for data regarding patient demographics, clinical presentation, pre-operative investigations, surgical and pathological findings, treatment and outcome. 58 women (mean age 34.9 years, range 27– 39 years) were identified. One patient was excluded as her data was incomplete due to treatment outside Northern Ireland. 98.2% (n=56) patients presented directly to our symptomatic clinic following concern upon self-examination. 89.5% (n=51) patients had a palpable lump on clinical examination. 71.9% (n=41) patients had no family history while 10.5% (n=6) had an affected first degree relative. Mammography was less sensitive than ultrasound (64.3% vs. 82.4%) while fine needle aspiration cytology was 92.5% sensitive for malignancy. 29 (50.9%) patients underwent breast conserving surgery (BCS) of which 7 proceeded subsequently to completion mastectomy due to involved margins. 26 (45.6%) patients required total mastectomy primarily while 2 (3.5%) patients were treated palliatively due to metastatic disease. The mean size of invasive tumour in BCS was 2.13cm and in mastectomy was 3.95cm. The mean nearest resection margins for BCS and mastectomy specimens were 2.58mm and 6.38mm respectively. From a total of 55 primary resections, 85.5% (n=47) of tumours were invasive ductal carcinoma. 57.4% (n=31) and 40.7% (n=22) were grade II and III tumours respectively. Lymphovascular invasion was identified in 50.9% (n=28) while 40.0% (n=22) were lymph node positive for metastatic disease. 76.8% (n=43) and 39.3% (n=22) were oestrogen (ER) and progesterone (PR) receptor positive respectively. 30.2% (n=16) were Human epidermal growth factor receptor-2 (HER-2) positive. The mean Nottingham prognostic index was 4.37 (range 2.2–8.4). Neo-adjuvant and adjuvant chemotherapy was administered to 9.3% (n=5) and 80.0% (n=44) of surgically treated patients respectively while 76.4% (n=42) patients received adjuvant radiotherapy. 76.4% (n=42) of patients were treated with tamoxifen. 4 patients received Herceptin® therapy. Statistically significant univariate factors adversely associated with overall survival were time from referral to out-patient department attendance ($p=0.038$), administration of neoadjuvant treatment ($p=0.019$), surgical intervention ($p<0.001$), progesterone receptor positivity ($p=0.018$) and tumour recurrence ($p<0.001$). 86.0% (n=49) patients were alive at mean follow-up of 52 months. 82.5% (n=47) remain disease free. Our study reports a low familial trait rate combined with a high proportion of hormonally active tumours less than grade III which suggests that breast cancer in this series of young women from Northern Ireland may be less aggressive and more hormonally responsive than anticipated.

The Effect of Sporting Events on Emergency Department Attendance Rates in a District General Hospital in Northern Ireland

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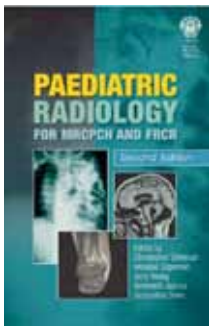
Previous studies have reported a conflicting relationship between the effect of live and televised sporting events on attendance rates to emergency departments (ED). The objectives of this study were to investigate the relationship of major sporting events on emergency department attendance rates and to determine the potential effects of such events on service provision. A retrospective analysis of ED attendances to a district general hospital (DGH) and subsequent admissions over a 24-hour period following live and televised sporting activities was performed over a 5-year period. Data was compiled from the hospital's emergency record books including number of attendances, patient demographics, clinical complaint and outcome. Review patients were excluded. Analysis of sporting events was compiled for live local, regional and national events as well as world-wide televised sporting broadcasts. 137668 (80445 male) patients attended from April 2002 to July 2007. Mean attendance rate per day was 80 patients (Male=47). 6.9 patient episodes per day were related to participation in a sporting activity or a consequence of a sporting injury. Mean admission rate was 13.6 patients per day. Major sporting events during the study period included; Soccer: 4 FA Cup and 1 World Cup (WC) finals; Rugby: 47 Six Nations, 25 Six Nations games involving Ireland, 1 WC Final, 2 WC semi-finals, 2 WC quarter-finals and 4 WC games involving Ireland; and Gaelic Football (GAA): 5 All-Ireland finals, 11 semi-finals, 11 quarter-finals and 5 provincial finals. There was no correlation identified between any of these sporting events and total emergency department attendance, sporting injury and non-sporting injury rates ($r<0.15$, $p>0.07$). However, multinomial logistic regression demonstrated that FA Cup final ($p=0.001$), Rugby Six Nations ($p=0.019$), Rugby WC games involving Ireland ($p=0.003$), GAA All-Ireland semi- and quarter-finals ($p=0.016$ & $p=0.016$) were predictors of patient admission rates. This study suggests that live or televised sporting events do not significantly affect ED attendances to a DGH. However, some events appeared to be predictors of patient admission rates. Although it may be beneficial to consider the effect of sporting events on service stratification during these periods, the overall effect is probably minimal and should not create a major concern for future service provision despite the implementation of the European Working Time Directive.

Book Reviews

PAEDIATRIC RADIOLOGY FOR MRCPCH AND FRCR SECOND EDITION



Chris Schelvan, Annabel Copeman, Jacky Davis, Annmarie Jeanes and Jane Young. The Royal Society of Medicine Press. December 2009. Paperback 304pp. 27.50. ISBN: 978-1-85315-702-8.

This book is aimed at trainee paediatricians and radiologists preparing for membership and fellowship examinations. It is written by 4 consultant radiologists and 1 consultant paediatrician.

It opens with 8 short chapters outlining a system of image interpretation for the chest radiograph, a brief explanation of renal nuclear medicine, an example of normal fluoroscopy of the upper GI tract and lower male urinary tract, a few key points on the physics of CT and MRI with some examples of normal anatomy of the chest, abdomen and brain, a reminder of the importance of radiation protection and patient safety and a few words on the importance of non-accidental injury to both paediatricians and radiologists. This is followed by 106 radiology cases in a random order which keeps interest levels and could be used as viva practice.

The initial image for each case is allocated a single page with a short clinical history and several key questions related to pertinent imaging findings and relevant clinical associations or important facts. The majority of cases are plain radiographs, however there are also a few examples of other imaging modalities including fluoroscopy, CT, ultrasound, MRI and nuclear medicine. The answer to the questions on each case is provided on the following page with several key radiology and clinical points along with a suggested further reading section.

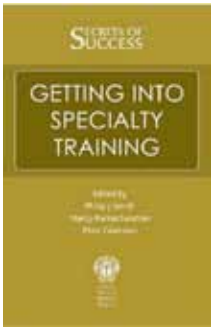
The images used are of good quality and cover the most important areas of paediatric imaging. The book is well laid out and well indexed. It is easy and enjoyable to read and covers a broad range of classic paediatric and neonatal diseases as well as a few rarer cases.

Although imaging is a small part of the MRCPCH examination, this book will be useful to consolidate clinical knowledge and the 'clinical hot list' with every case will be helpful revision in the months prior to the examination. It also provides an understanding of important radiograph findings for day to day practice which will be useful for paediatricians in training and for image interpretation on call.

The cases presented are not exhaustive but are basic examination material and should be core knowledge for radiologists in training, especially those preparing for FRCR vivas. The book is also a good summary for general radiologists who wish to have a quick refresher of the classic images of important neonatal and paediatric conditions.

Julie Yarr

GETTING INTO SPECIALTY TRAINING



Edited by PJ Smith, M Ramachandran, MA Gladman, The Royal Society of Medicine Press, 259 pages ISBN 978-1-85315-893-3, £ 18.95

This book is written for junior doctors approaching the change from Foundation Programme to Specialty Training. It gives a useful background to the changes in Specialty Training brought in by Modernising Medical Careers and the subsequent modifications that followed the Tooke report. It goes on to describe the process of application for Specialty Training and gives practical, clear, easy to read, and step by step advice on how to fill in an application form for Specialty Training.

A section on the Specialty Training Interviews gives simple, brief and helpful outlines regarding the organisation of the National Health Service and approaches that could be taken when asked questions about audit, teaching and research.

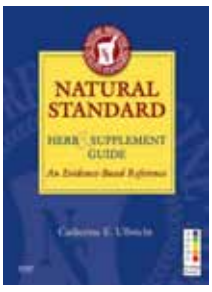
The section on Specialty-specific questions is very good and is comprised of eleven Specialty-specific chapters. Each of these chapters gives an introduction to the Specialty; followed by examples of the skills and knowledge applicants would need to consider in order to demonstrate their commitment to that Specialty when completing the application form. Each of these chapters also give examples of the type of Specialty-specific questions a candidate for appointment might face at interview and provides candidates with a structured approach that they could use to formulate their answers.

The book finishes with two chapters on what happens next after the interviews. The first of these which gives a very brief introduction to the steps a successful candidate might expect ahead after entry into Specialty Training (workplace-based assessments, postgraduate examinations and Annual Reviews of Competence Progression). The second gives sensible steps an unsuccessful candidate can take to gain employment, review their options and to re-apply as well as suggesting other options for an unsuccessful candidate outside the National Health Service.

Overall the book is very well written and easy to read and gives a comprehensive and practical guide to the type of preparation necessary for success at interviews to enter Specialty Training.

Keith Gardiner,

NATURAL STANDARD HERB AND SUPPLEMENT GUIDE: AN EVIDENCE-BASED REFERENCE



Catherine E. Ulbricht. Mosby Elsevier, Missouri, USA; 2010. Hardback. 871pp £48.99 ISBN: 978-0-323-07295-3

This reference text is a comprehensive exploration of over 360 herbs and supplements used in the treatment of a variety of clinical disorders. The

book is organized alphabetically and each herb/supplement is consistently subdivided into six sub-sectors that include related terms, background, evidence, dosing, safety and interactions. It has two appendices and full contents and index sections to help the reader quickly and efficiently find desired information.

As the title indicates, this book grades the evidence in a system that ranges from A (strong scientific evidence) through F (strong negative scientific evidence), employing the Natural Standard evidence-based criteria (www.naturalstandard.com). The preface section details how the research methodology, the systematic aggregation analysis and review of the literature have been performed. No peer-reviewed references are cited in the book, but there is a link to the Natural Standard's website, where there are comprehensive lists of peer-reviewed literature relating to each herb/supplement.

Overall, this book is an excellent reference text and a good compendium of information on herbs/supplements, which duly deserves a place on your personal bookshelf.

John E. Moore

OXFORD HANDBOOK OF GENETICS

Guy Bradley-smith Sally Hope Helen V firth Jane A. Hurst., Oxford University Press, 494 pages, ISBN 978-0-19-954536-0 £27.95

This book is described a guide for the non-specialist and is aimed at the primary care clinician. It begins with a ten-page glossary of abbreviations that is perhaps a little off-putting but then gets down to business and starts by outlining the concept of family. I was a little surprised that they discuss adoption before consent and confidentiality but maybe this reflects the authors' experiences of genetic enquiries in General Practice. Chapter 2 discusses genetic inheritance very clearly and chapter 3, genetic investigations. The information is very helpful but perhaps not always in what I would consider a very logical order. The bulk of the book discusses a selection of genetic disorders. These are described very clearly and give a quick overview of conditions such as myotonic dystrophy and tuberous sclerosis. The choice of conditions described in the book is perhaps a little inconsistent. There is quite a lot of information about Noonan syndrome whereas other conditions such as Prader Willi or Williams get the briefest of mentions and my own interest, Fabry disease, is not mentioned at all. However I appreciate that it is difficult to select conditions and those that are covered, are covered very well. There is an extensive section on inherited cancers. This describes the need to take a detailed three generation pedigree. It also gives examples of the surveillance programmes recommended for at risk individuals. There is a helpful section on the interface between primary care and genetics. The authors emphasize the paramount importance of accurate diagnosis in order to provide appropriate genetic advice to the extended family. Anything that encourages referrers to provide as much information as possible to the genetics service is to be welcomed.

So is this is a useful book? Well, yes I think it is. My concern is how it will actually be used in practice. I don't think it is a book that is intended to be read cover to cover but more to be consulted as needed. Professor Peter Farndon talks about 'just in time 'information i.e. genetic information instantly available if one is seeing a patient with a genetic disorder. There are a lot of excellent on-line genetics resources. Would someone lift this book off the shelf and look up the information or would it be easier to hit a button on the PC and get on-line information? For those of us who still prefer our information in the printed form then I would recommend this book. If I were one of the authors I would be considering an electronic 'App' version of the book.

Fiona Stewart

Ulster Med J 2010;**79(3):**154-155

So you want to be a **Nephrologist**

Accepted 30 June 2010

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WHAT IS NEPHROLOGY?

Nephrology (literally the study of the kidney) has evolved over the last 60 years to become a major medical specialty. Early pioneering renal physiology experiments yielded insights into blood pressure regulation, electrolyte and acid-base balance. This physiological research, coupled with advances in immunology, was translated into effective life sustaining therapy for chronic kidney failure (dialysis and kidney transplantation). Nephrology now encompasses a wide range of clinical treatments for acute kidney injury, chronic kidney disease, end-stage renal failure, bone mineral metabolism, renal anaemia and prevention of kidney transplant rejection.

WHERE IS NEPHROLOGY GOING AS A SPECIALTY?

Renal medicine has experienced unprecedented growth over the past 20 years driven in part by the rising number of persons with end-stage kidney failure needing dialysis and/or renal transplantation. Visible evidence of the increased numbers of patients requiring renal replacement therapy is the development of multiple new renal units both in teaching hospitals and district general hospitals.

Fig 1. Haemodialysis (an expensive half-way technology that is long overdue a paradigm shift in clinical application)

HOW ARE NEPHROLOGISTS TRAINED?

Training in nephrology follows the nationally agreed renal medicine curriculum and is competency based. Entry to training is by competitive multi-station interview and from 2010 applicants will need to have attained MRCP (UK) PACES or equivalent prior to application. Typically it will take a total of three years of clinical nephrology training to attain a CCT in Renal Medicine. During this period the specialist trainee will rotate between the sub-disciplines of nephrology including transplantation, dialysis, general nephrology and consult services with a minimum of 3 months acute kidney transplant experience (managing patients in the peri-operative and early phases of kidney transplantation). Procedural skill in placing ultrasound-guided central venous catheters for dialysis (Figure 1) is a mandatory component of training. Developing expertise in native and transplant kidney biopsy (Figure 2) is encouraged but is no longer an essential requirement of training. During training the Specialty Certificate Examination (SCE), organised by the Royal College of Physicians, must be passed. This is an MCQ exam based on the Renal Medicine Curriculum approved by PMETB

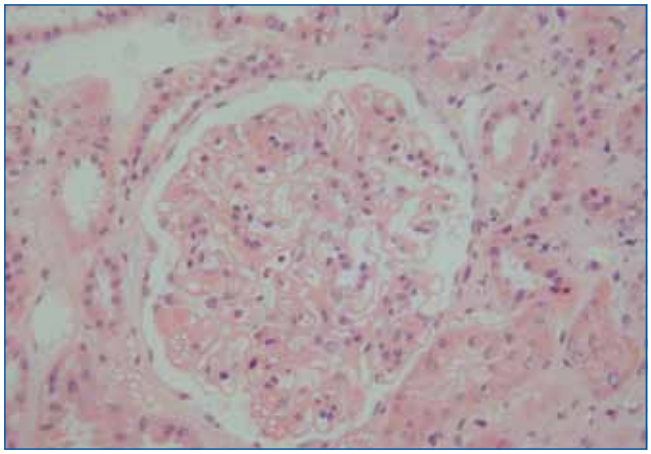

Fig 2. Membranous glomerulonephritis (diagnosed following ultrasound-guided percutaneous renal biopsy and assessment by renal pathologist)

CAN I COMBINE RENAL MEDICINE WITH OTHER INTERESTS?

A majority of trainees also enrol for training in general internal medicine to attain a dual CCT in medicine and nephrology. This is a pragmatic choice recognising that many nephrology posts are in hospitals where the consultant nephrologist contributes to the acute medical take in. Some nephrology trainees, and particularly those with a special interest in acute kidney injury, have combined renal medicine and intensive care medicine training and forge a career in the interface areas between critical care and nephrology. A small number of trainees have entered a clinical academic training pathway e.g. academic clinical fellow (ACF) or academic clinical lecturer (ACL) and combine nephrology training with extended periods of research and teaching. An academic pathway will lead to a postgraduate degree e.g. MD or PhD and for some particularly tenacious individuals, who secure additional peer-reviewed funding, it can lead to the first steps on a clinical academic career ladder.

WHAT PERSONAL ATTRIBUTES ARE USEFUL TO BE A NEPHROLOGIST?

A wide range of interests in medicine and an aptitude for applying physiological principles to management of some complex clinical problems is desirable. Nephrology provides an intellectually stimulating and challenging working environment.

Nephrologists have many patients who they will care for over very long periods of time. This continuity of care is important for the quality of care provided (particularly to patients with end-stage renal failure). An interest in managing acutely unwell patients is also necessary and ability to escalate care when appropriate.

A willingness to learn some procedural skills is necessary to practice nephrology e.g. central vein catheterisation and renal biopsy. Good communication skills are essential to liaise effectively with patients and relatives; doctors in other medical specialties, general practitioners, surgeons, anaesthetists, intensivists and allied health professionals

A combination of determination, attention to detail and team spirit will help sustain a lifelong interest in the practice of renal medicine. On many occasions, a sense of humour also helps!

WHAT ARE THE BEST AND WORST ASPECTS OF NEPHROLOGY?

The best aspects are learning to apply knowledge, skills and aptitudes to solve challenging problems. There are excellent teaching and research opportunities. The specialty is growing and a career in nephrology is secure and rewarding.

The worst aspects are arguably the frustration of not being able to provide optimal care to some patients e.g. shortage of donor organs for kidney transplantation

WHAT ARE THE UNSOLVED CHALLENGES IN NEPHROLOGY?

Acute kidney injury (AKI) is common, expensive to manage and associated with a high mortality. Surprisingly there is still limited evidence for any therapeutic regimen to prevent or treat AKI apart from ensuring optimal fluid resuscitation and avoidance of obvious nephrotoxins. There is an urgent need for new strategies to reduce the risk of AKI and accelerate recovery of injured kidneys. ***Chronic kidney disease*** (CKD), defined as an estimated glomerular filtration rate (eGFR) of <60mL/min/1.73m^2 is present in up to 5% of the adult population. CKD is associated with an increased risk of cardiovascular events and premature death, particularly when the eGFR is <30mL/min/1.73m^2. The pathophysiology of accelerated cardiovascular risk associated with kidney disease is incompletely understood and is currently a very active research field. ***Polycystic kidney disease*** (PKD) is the commonest monogenic cause of kidney disease and hypertension. The molecular and cell biology of cyst growth in PKD is now much better understood and an exciting era of clinical trials is now underway with drugs for prevention of kidney failure secondary to PKD. ***Renal transplantation*** is the optimal form of renal replacement therapy for many patients with end-stage renal failure. The holy grail of transplant research is the achievement of immune tolerance that would permit organ transplantation without exposing patients to the long-term risks of immunosuppressant drugs. There is still a wide range of interesting clinical and basic science research questions to answer in renal medicine. The future, for trainees and consultants, will be full of opportunities to translate research findings into better care for persons with kidney disease.

I WANT TO KNOW MORE – WHERE SHOULD I GO NEXT?

Nationally, information relevant to prospective UK-based renal trainees is available on the Renal Association website (www.renal.org). The Renal Medicine curriculum is published on the PMETB website (www.pmetb.org.uk) and located in approved curricula section.

THE ULSTER MEDICAL JOURNAL

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1. Articles submitted for consideration should be typewritten in single spacing, with wide margins, preferably in Times Roman 12pt font. They should be fully corrected and alterations in proof may be disallowed or charged to the authors. Please submit electronic files on CD or memory stick along with **ONE** hard copy of each article (including tables and figures). Those unable to submit an electronic version should contact the editorial office. Colour images and tables are encouraged and there is currently no charge for colour reproduction. **Electronic copies of images and tables should be included as separate high resolution .jpg or .tif files and NOT embedded in the Word manuscript file.**

2. For full or short papers, the text should indicate the purpose of the paper, and should include an introduction, sections on materials and methods, results, and a discussion relevant to the findings. A brief factual summary / abstract should be provided at the beginning of the paper along with up to six key words. For case reports, these should be novel or particularly important cases and not just good teaching points, with a maximum of 10 references and appropriate patient consent for publication of photographs. Letters to the editor should be less than 500 words with up to 5 references and 1 table and / or figure.

3. Scientific measurements should be in SI units (DN Baron. *Units, Symbols and Abbreviations. A Guide for Medical and Scientific Authors*. 5th ed. London: Royal Society of Medicine, 1994). Blood pressure may be expressed in mmHg and haemoglobin concentration as g/dl.

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