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Desmond Whyte Memorial Lecture

Learning And Improvement In Hereditary Diseases

Altnagelvin Hospital, Londonderry, 3rd December 2015.

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Accepted

INTRODUCTION

Thank you for inviting me to give this year's Desmond Whyte lecture and for the President's very kind introduction. It is a great honour to give this lecture, and particularly in Altnagelvin hospital where I spent many Wednesdays in outpatients seeing families with genetic disorders, and regularly availed of the excellent radiology service that Desmond had set up some years ago. The title of the lecture fits in with Dr McBride's presidential theme of 'learning and improvement' and my specialty of Medical Genetics is an excellent example of this. 50 years ago, it hardly existed and has developed from virtually nothing to become a crucial part of everyday medical care in all branches of medicine. In fact it's getting so important to have a genetic test for patient care nowadays that our test budget is usually completely spent in the first quarter of the financial year - the demand for genetic tests from so many specialties other than our own, being so great.

I want to take a few areas of genetics and show where improvements have been made in recognition, testing, management and potentially treatment of genetic diseases, some of which used to be fatal in the first twenty years of life.

1. COULD YOU TEST YOUR NEIGHBOUR'S DNA JUST OUT OF CURIOSITY?

DNA testing only became commercially available in the 1980's. Before that, identity or paternity testing in that sense, used to be tedious. Testing last century relied on fairly inaccurate indirect laboratory techniques such as blood grouping, eye or skin colour, and until the late 1980's not much had changed. The invention of the polymerase chain reaction (PCR) allowed a technique where incredibly small samples of DNA could be detected and amplified in a test tube to give sufficient yield for DNA testing (fig 1). Lengths of DNA segments similar to supermarket 'bar codes' could be run out on agarose gels using techniques including Southern blotting and more recently microsatellites and single nucleotide polymorphisms ('SNiPs'), allowing comparison of the samples from (ideally) a child, the mother and the so called 'putative father'. Matching of the samples can now fairly confidently confirm or exclude paternity (fig 2). Older techniques where mismatch of blood groups made paternity less likely, did not allow reasonable proof of paternity to be achieved. Samples using forensic techniques can now be obtained from microscopic smears of DNA from crime scenes, dental floss, beer glasses and other personal items.

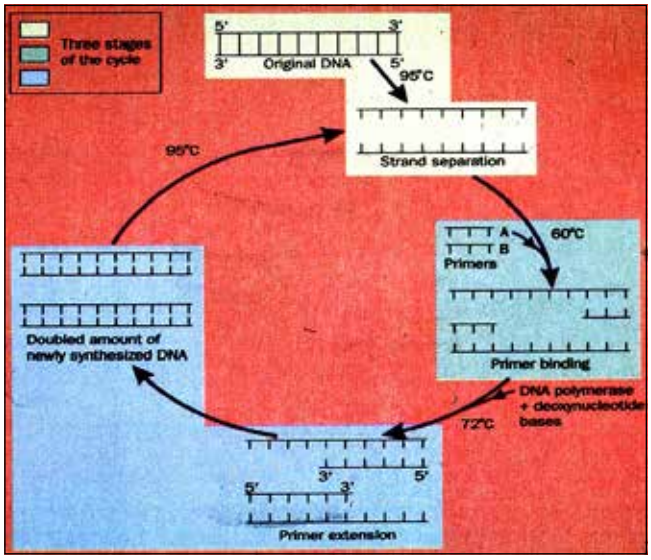

Fig 1. PCR amplification

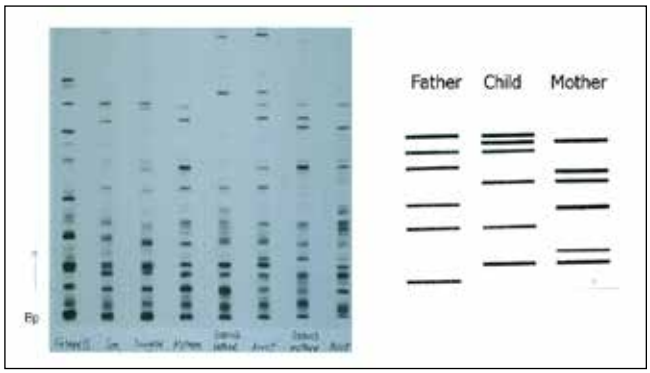

Fig 2. 'Bar code' paternity testing from the early 1990's

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The offence of unlawful DNA theft was introduced into legislation in 2004 and the law now prevents unlawful theft of DNA through the 2005 Human Tissue Act where it is an offence to remove DNA 'without permission' so if you want to test a neighbour's dental floss retrieved from their rubbish bin (for example) to see what genetic disorders they may have, you now should obtain their permission first. Employers cannot test employees for hereditary disorders either – they have a duty to make the workplace sufficiently safe, so if an employee has a genetic tendency to asthma, their working environment should have sufficient air quality to allow them to work adequately.

2. COULD BOYS WITH DUCHENNE MUSCULAR DYSTROPHY WALK WITH TREATMENT?

When I started working in genetics in the 1980's, Duchenne muscular dystrophy – a sex-linked recessive disorder - was uniformly fatal and diagnosed fairly late at around six years of age in boys. They had rapid progression to long leg bracing and death in adolescence from respiratory failure. Identification of the dystrophin gene in 1987 was the first major advance in allowing family screening and earlier diagnosis. In cases where the mother could be confirmed as a carrier, reproductive choice became possible, and accurate carrier testing became available for other family members. Especially those who could now be defined as having a low risk, who previously could not be reassured with certainty. Testing of affected boys now results in a molecular diagnosis in most cases, allowing confirmation without muscle biopsy. Biopsy in the latter part of last century was usually open and required an anaesthetic, neither of which parents of affected boys were particularly keen on. The dystrophin gene unfortunately is one of the largest genes in the human body (fig 3) with 79 exons, so testing was tedious and time consuming. Early studies suggested that frameshift mutations caused the severe Duchenne phenotype, and in-frame deletions caused a milder Becker phenotype. We now know that there is a continuum of mild to severe phenotype rather than two distinct ends of a spectrum. Numerous mutations occur and alter the dystrophin transcript and resulting protein expression in several ways. We now know that 'leakage' occurs where some patients with large gene deletions still express low levels of high molecular weight and semi functional dystrophin. Therapy with antisense oligomer induced exon skipping in patients with leaky mutations allows an increase of the dystrophin above baseline levels and helps restore muscle function to patients with a Duchenne phenotype [1].

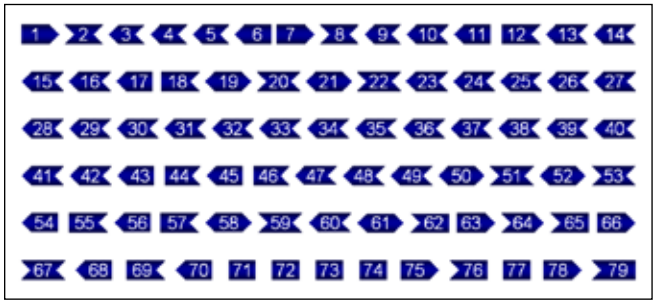

Fig 3. Dystrophin gene showing 79 exons.

Diagnosis is now possible in utero and using pre-implantation diagnosis can allow an unaffected embryo to be implanted into the mother. Cell free non-invasive DNA blood testing at around 8 weeks gestation can help a mother find out (by analysing small cell fractions of fetal material in the maternal venous circulation) if she is carrying a male or female fetus, and if female, she can relax for the rest of the pregnancy knowing she does not carry a male fetus that could be potentially affected.

3. SHOULD WE BE ABLE TO DIG UP GRAVES OR RAID MUSEUMS FOR OLD DNA SAMPLES?

Well why not if you have permission? We did just this and set the trend a couple of years ago. We had several cases of early onset pituitary disorders that Professor Brew Atkinson and his team referred in to us. We tested these for a recently described gene, AIP, that causes hereditary pituitary adenomas and found several had mutations within the gene. A mutation R304X was prevalent in several families living west of Lough Neagh. We documented the family histories and eventually found four large families with several affected generations from the Dungannon/ Magherafelt area. Some of the families mentioned that they might be related by legend, to the famous Irish giant Charles Byrne, who originated from the village of Littlebridge nearby. Byrne was 7'7" in height and left Littlebridge in 1781 for London to make his fortune. He retained an agent and was 'exhibited' for a fee sitting outside his house in a cane chair in Spring Gardens, near Trafalgar Square. Sadly, like several expatriate Northern Irishmen, he turned to drink and after his life savings of £700 were stolen, went into a despair and died. His skeleton was procured by unfair means, and exhibited by the anatomist John Hunter and is still on display in the Hunterian museum in the Royal College of Surgeons at Lincolns Inn fields at Holborn. My London colleague, Professor Marta Korbonits, was able to write and secure permission from the museum curator to extract a core of DNA from two of his teeth. Having carefully returned the teeth, sequencing of the archival DNA that we obtained, confirmed that he also carried the R304X mutation, and by careful haplotype analysis, we linked his DNA to that of the other four families confirming he was a relative. At the age of 251, this made him my oldest patient. His relatives were delighted that we were finally able to honour his memory by confirming his genetic lineage and his definitive diagnosis[2-4]. Finn McCool and other famous Irish giants lend evidence to the fact that there is a some truth behind the legends of the famous giants of Ulster. Historically evidence for hereditary giants elsewhere exists in the literature[5].

Amplification of archival DNA is now made possible by PCR of small amounts of DNA. Similar techniques have now been used to look at old unsolved criminal cases where DNA has been retained from samples and also for other historical events. Richard III's skeleton was recently exhumed and his royal lineage was famously confirmed in 2015. He was then

given a royal funeral in Leicester Cathedral[6]. My advice to those who don't want to be exhumed in the future and have their DNA chased by either a geneticist or a lawyer is to be cremated, as high temperatures in the furnace destroy DNA by denaturation if not evaporation! Budding artists should probably not try to emulate the great masters by signing their works by using their own blood as ink, for the same reasons.

4. WHY DO PATIENTS WITH DOWN SYNDROME HAVE WEAK MUSCLES?

Down syndrome was described by Dr John Langdon-Down in his article on the ethnic classification of idiots. I know some of you may still employ this classification for the grading of NHS managers. It is well recognised that patients with trisomy 21 have greater muscle fatigue than normal individuals. It has long been suspected that the extra genes on chromosome 21 interfere with regulation of muscle expression. Usually babies with trisomy 21 are tested at birth, not to confirm the diagnosis as it is usually fairly obvious clinically, but to check if they are the 95% of Down syndrome babies born due to non-dysjunction (which is more common with increased maternal age), the 2% that are mosaic with abnormal cell division occurring post-zygotically (a small proportion of certain cells may be Down syndrome and these children are typically very mild with few features), or most importantly, the 3% that are translocation Down syndrome where a balanced rearrangement of a parental chromosome may recombine to cause a familial type of Down syndrome with a risk of recurrence[7,8]. The parents of translocation Down syndrome have a high risk of recurrence – often around 10%, whereas mosaic cases usually have a negligible risk as they occur as a post-zygotic event. In all but the mosaic cases, muscle fatigue is well documented. Recent physiological studies confirm that there is a 40-70% decrement in knee extensor strength, typically giving a 20 year old with Down syndrome the equivalent muscle strength of a 70 year old[9]. Transgenic mice models have often given clues as to whether genes are downregulated or upregulated in certain genetic disorders and the Ts65Dn mouse has shown that of the 159 genes equivalent to the human, on chromosome 21, 106 are downregulated and 53 are upregulated[10]. When genes with known function regulating muscle physiology are looked at – several genes with a role in neuromuscular transmission are down regulated as are genes in skeletal muscle structure and function. Experimental muscle physiologists have concluded that overall in Down syndrome, there is normal muscle in the non-fatigued state, but abnormal post- exercise fatigue occurs and this is mediated by clear mitochondrial limitation. Evidence shows that exercise training including circuit training helps improve the recovery phase and reduces inflammatory cytokine activity so children with Down syndrome should be encouraged to lead as active a life as possible. In years gone by, often such children were put in an institution and suffered early death and intellectual deficiency as would we all if we were forbidden to exercise and given no formal education. The inference from mouse model studies suggests that if children with Down syndrome have good levels of fitness and are educated in the best schooling possible, then their educational attainment and life expectancy will considerably improve. We now find that lifespan has increased sufficiently that adult rheumatology clinics are utilised by 80% of adults with Down syndrome due to progressive arthritis and some often have earlier onset of dementia because of abnormal regulation of an extra amyloid beta precursor protein gene on chromosome 21.

THE FUTURE

We have seen great advances in genetic testing from early karyotyping to the latest DNA technology. Whole genome screening is already possible and the technology available and the costs to utilise it are improving in a similar way to the speed of computer chips over the last decade. Several famous individuals have had their genome analysed including Black Sabbath singer John Michael 'Ozzy' Osbourne whose ADH4 gene allows him to metabolise alcohol very speedily and combined with his CLTCL1 gene giving him the propensity for addictive behaviour, and his increased proportion of Neanderthal DNA, means he can still sing and provide entertainment for the public for many more years to come. He presumably also has a gene for musicality and this may someday be identified on a further analysis of his DNA.

Commercial companies such as 23andMe can provide over the counter or web based mouth swab tests for all sorts of curious genes including telling you if your lower or upper back is hairy or not, and if your ear wax is wet (European ancestry) or dry (South East Asian ancestry). You may not necessarily impress your friends with such test reports on yourself as they may say you could have saved your money by either looking at the reflection of your back in the mirror or sticking a finger in your ear. Whole genome technology does open the exciting prospect of having your personal genome checked on an admission to hospital, if not at birth, and the attending clinician can then check your predisposition to genetic diseases, microbiological and pharmaceutical sensitivities and responses, and personally tailor your treatment with what we now call 'precision medicine'.

Whether being told what genetic predispositions you might die of, will adjust individual behaviour any more than the advice we give today, I think is still unlikely. In twenty years' time we will be much better at the diagnosis and treatment of patients admitted to this hospital, but prevention may still remain very much an individual choice as it is today.

ACKNOWLEDGEMENTS

This is a condensed version of the lecture. The author has no conflict of interest to declare.

REFERENCES

1. Fletcher S, Adkin CF, Meloni P, Wong B, Muntoni F, Kole R, *et al*. Targeted exon skipping to address "leaky" mutations in the dystrophin gene. *Mol Ther Nucleic Acids*. 2012;**1:**e48.
2. Chahal HS, Stals K, Unterlander M, Balding DJ, Thomas MG, Kumar AV, *et al*. AIP mutation in pituitary adenomas in the 18th century and today. *N Engl J Med*. 2011;**364(1)**:43-50.

3. Bradley L, Morrison PJ**.** Giants of the British Isles. *Ulster Med J*. 2011;**80(1):**31-2.

4. Williams F, Hunter S, Bradley L, Chahal HS, Storr HL, Akker SA, *et al*. Clinical experience in the screening and management of a large kindred with familial isolated pituitary adenoma due to an aryl hydrocarbon receptor interacting protein (AIP) mutation. *J Clin Endocrinol Metab*. 2014;**99(4):**1122-31.

5. Donnelly DE, Morrison PJ. Hereditary Gigantism – the biblical giant Goliath and his brothers. *Ulster Med J*. 2014;**83(2):**86-8.

6. King TE, Fortes GG, Balaresque P, Thomas MG, Balding D, Maisano Delser P, *et al*. Identification of the remains of King Richard III. *Nat Commun*. 2014;**5:**5631.

7. Devlin L, Morrison PJ. Accuracy of the clinical diagnosis of Down syndrome. *Ulster Med J*. 2004;**73(1)**: 4-12.

8. Devlin L, Morrison PJ. Mosaic Down's syndrome prevalence in a complete population study. *Arch Dis Child*. 2004;**89(12)**:1177-8.

9. Mendonca GV, Pereira FD, Fernhall B. Reduced exercise capacity in persons with Down syndrome: cause, effect, and management. *Ther Clin Risk Manag*. 2010;**6:**601-10.

10. Cowley PM, Keslacy S, Middleton FA, DeRuisseau LR, Fernhall B, Kanaley JA, *et al*. Functional and biochemical characterization of soleus muscle in Down syndrome mice: insight into the muscle dysfunction seen in the human condition. *Am J Physiol Regul Integr Comp Physiol*. 2012;**303(12):**R1251-60.

