PAPER: GENETIC MEDICINE – TODAY'S PERSPECTIVE*

AEH Emery, Emeritus Professor of Human Genetics, University of Edinburgh, Scotland

Everything is quite different in medicine nowadays.
Moliére, *Le Médecin malgré lui,* 1666.

SUMMARY

Over the last 50 years, developments in genetics have revolutionised our approach to understanding the nature and cause of much human disease. These advances include being able to diagnose and offer prevention through prenatal diagnosis for many serious single-gene disorders. Furthermore new treatments through gene and stem-cell therapy are being researched. It is also becoming possible to determine an individual's response to drug treatments and susceptibility to various infections. Recent evidence suggests that particular pathogens may affect the clinical manifestations of certain single-gene disorders. In fact understanding the role of pathogens in both rare unifactorial disorders and in more common multifactorial conditions will be one of the major challenges facing medicine in the future.

INTRODUCTION

Lord Rutherford, the father of nuclear physics who was awarded the Nobel Prize in 1908, is quoted as having said at the time 'All science is now either physics or stamp-collecting.' One could say, with a little imagination, that nowadays much of medicine is either genetics or stamp-collecting. Certainly developments in genetics have in recent years revolutionised our approach to the causes of many human diseases. Yet these developments have encompassed little more than one professional lifetime. In the late 1940s we were taught that protein was the basis of heredity, and in fact genetics as such was given very little consideration in the medical curriculum. But this all changed following the Watson and Crick demonstration of the DNA double helix in 1953 and the stimulus this gave to elucidating the molecular basis of many diseases. Further impetus came from the development of so-called genetic engineering or recombinant DNA technology in the 1970s. The last major step forward was the recent completion of the Human Genome Project with the DNA sequencing of the entire genome of some 3 billion (3×10^9) base pairs.

THE HUMAN GENOME

The Human Genome Project has revealed that two unrelated persons share over 99·9% of their DNA sequences. Furthermore over 98% of the genome does *not* code for protein. These non-coding regions include gene regulatory sequences and single nucleotide variants or polymorphisms (SNPs).[1] The latter occur about once every 1,000 base pairs and are proving important in helping to assess an individual's susceptibility (or resistance) to disease as well as response to various drug treatments.

The coding regions of the genome are estimated to account for some 30,000 genes. To date around 14,000 single-gene disorders have been recognised clinically. Of these, over 8,000 gene loci have been precisely identified and over 1,000 have been cloned. It has been further estimated that of the 2,000 or so clinically recognised dysmorphic syndromes, over 400 responsible genes have so far been identified.

Here a brief review is presented of some of the benefits to accrue from this new technology as well as some of the unexpected findings. My own particular field of interest, the muscular dystrophies and related conditions, has been particularly well researched by molecular biologists in recent times and will be used to provide a number of examples illustrating some of these developments.[2]

IMPLICATIONS FOR SINGLE-GENE DISORDERS

As more is learnt of gene activity, the original idea of 'one gene – one protein – one disease' is in certain cases having to be radically modified to 'one gene – several protein products – several diseases'. For example, different mutations of the *LMNA* gene, which codes for the nuclear membrane proteins, lamins A and C, have now been shown to account for no less than eight clinically very different disorders (Table 1).[3] This phenomenon of very different phenotypes being generated by different alleles has also been reported in several other genes but so far not to quite the same extent and variety of conditions as in the case of the *LMNA* gene. Admittedly this is a relatively uncommon event for which, at present, we have no satisfactory explanation, but the phenomenon nevertheless challenges our previous concepts concerning gene action.

It is now becoming clear that post-translational modifications of proteins are important in pathogenesis and that particular mutations can affect, for example, binding of the protein product. This can be important in determining the resultant phenotype.[4] This field of proteomics, and related structural genomics, is likely to prove even more challenging than genomics itself since

* Based on a public lecture presented at the Royal College of Physicians of Edinburgh on 11 February 2004.

TABLE 1
Clinical disorders resulting from different mutations of the LMNA gene.

Emery-Dreifuss MD	(AD, AR)
Limb girdle MD type 1B	(AD)
Dilated cardiomyopathy & conduction defects	(AD)
Atrial fibrillation ± dilated cardiomyopathy	(AD)
Partial lipodystrophy	(AD)
Charcot-Marie-Tooth type 2	(AR)
Mandibuloacral dysplasia	(AR)
Progeria (?Werner's syndrome)	(AR)

(MD: muscular dystrophy; AD: autosomal dominant; AR: autosomal recessive)

the number of protein variants considerably outnumbers the number of coding genes.

Another recent development is that, from expression profiling studies, the activity of *many* genes can be secondarily affected in some so-called single-gene (unifactorial) disorders. This is well illustrated in the case of Duchenne muscular dystrophy, which is caused by a deficiency of the muscle protein dystrophin due to mutations of the dystrophin gene on the X chromosome (Xp21).[5] However, it has now been shown that in this disorder the expression of some 327 other genes is also reduced, but it is increased in 77 others.[6] Some of these changes in gene activity might have been predicted from the known pathology of the disease (such as the increased expression of certain immune response genes), but not others. This phenomenon is again not restricted to muscular dystrophy but has recently been reported in several other single-gene disorders as well as in some more common conditions. For example in cancer, expression profiling is being used to possibly identify prognostic markers.[7]

It is clear that the more we learn about many single-gene disorders, the more complex the situation becomes. This could well have ramifications on how gene therapy might have to be approached in future.

THERAPEUTIC POSSIBILITIES

As more is understood of gene action and thereby pathophysiology, so it is more likely effective pharmacological approaches to treatment may be found. However, other approaches to therapy being explored also include gene and stem-cell therapy.

Gene therapy involves a number of different approaches (Table 2), which have exercised the ingenuity of some of the best molecular biologists of recent years.[8]

Replacing a mutant gene by a normal (or modified normal) gene carried in an appropriate viral vector has been the most researched technique so far. Apart from the technical problems of incorporating a large gene (such as the dystrophin gene with no less than 79 exons) into a vector, there can also be immunological and toxicity problems. Furthermore there is the added difficulty of ensuring effective delivery of the normal gene and its expression in all affected tissues which may have to include the heart and brain.

In gene therapy requiring a viral vector and gene insertion, it is now realised there can also be other serious problems, most importantly the possibility of 'insertional mutagenesis'. For example three years after apparent successful gene therapy for severe combined immunodeficiency, two of nine subjects died from T-cell acute lymphoblastic leukaemia due to activation of an adjacent oncogene. As a result, from January 2003 the US Food and Drug Administration placed a temporary halt on all such studies.[9]

Several other approaches however are possible. The use of naked (plasmid) DNA with expression limited in time and only to the site of injection could be an attractive alternative in certain diseases. For example the injection of *VEGF* (vascular endothelial growth factor) gene directly into the calf muscles to treat claudication is currently being assessed in several centres. An appropriate antisense oligonucleotide could be taken by mouth, though to date the therapeutic effectiveness of this approach has yet to be demonstrated. Another possible approach to therapy is the upregulation of a compensatory protein by an appropriate drug. In sickle cell disease treatment with hydroxyurea by inducing fetal haemoglobin synthesis has been shown to be therapeutically helpful.[10] In Duchenne muscular dystrophy upregulation of utrophin or ADAM-12 (a metalloprotease) may compensate for the deficiency of dystrophin[11] and a search is ongoing (5,000 candidate drugs have so far been screened) to find a possible drug which could effect this in patients. A further approach might be a

TABLE 2
Some approaches to gene therapy currently being explored.

Rationale	Method
Replace mutant gene by a normal gene or modified normal gene	Naked (plasmid) DNA, viral vector, etc.
Exon skipping ('read through')	Antisense oligonucleotides
Upregulation of a compensatory gene product	By a drug or by a vector carrying a strong promoter
Suppress STOP codon	Pharmacological agent

pharmacological agent to induce molecular changes which could be therapeutic, for example an aminoglycoside antibiotic (gentamicin or the less toxic negamicin) which results in read through of a stop codon in mice with muscular dystrophy.[12] Unfortunately in human muscular dystrophy gentamicin has so far not proved convincingly to be therapeutically effective in patients with a demonstrable stop codon in the dystrophin gene.

Some form of stem-cell therapy should avoid some of the problems of gene therapy. Here multipotent cells from an early embryo (in excess of IVF requirements) or umbilical cord or bone marrow can be used. Disorders currently being researched in this way include cardiac ischaemic damage (with bone marrow-derived cardiac myoblasts), Parkinsonism (with bone marrow-derived dopaminergic neuronal cells), muscular dystrophy (with bone marrow-derived skeletal myoblasts), diabetes and spinal cord injury. For example, the results of intramyocardial injections of autologous bone marrow cells as an approach to repairing ischaemic cardiac tissue are encouraging.[13] Somatic-cell nuclear transfer for therapeutic cloning is yet another possible approach. Stem-cell therapy could perhaps in future prove to be the most effective way of treating some conditions. However, if donor (not autologous) stem cells were to be used for treatment then some form of immunosuppression could be necessary.

POLYMORPHIC MARKERS: DRUG RESPONSE AND DISEASE SUSCEPTIBILITY

One of the most exciting developments resulting largely from the Human Genome Project is the potential of using polymorphic markers (single nucleotide polymorphisms (SNPs)) as indicators of genetic factors which can affect an individual's response to particular drugs (Table 3) thus paving the way for what is now being referred to as 'personalised medicine'. That is, it might indicate the likely therapeutic effectiveness (or toxicity) of a particular drug in an individual, an area of study referred to as 'pharmacogenomics'. The *CYP* gene locus is particularly polymorphic with over 70 variant alleles at the *CYP2D6* locus alone and is the best studied of pharmacological interest to date.[14] A recent and important application of this technology relates to the drug tamoxifen widely used in treating breast cancer and its prevention in women at high risk. The drug is converted to active metabolites by *CYP2D6* enzymes but this activity is inhibited by certain antidepressants and some *CYP2D6* alleles are inhibited more than others. These antidepressants are often used to treat the side effects of tamoxifen (such as hot flushes) and therefore these findings clearly have important therapeutic implications.[15]

But of equal interest are those polymorphisms associated with disease susceptibility because understanding the molecular basis of susceptibility (or resistance) could one day lead to more effective treatments or preventive strategies. Some examples are given in Table 4. A particularly interesting example is the *CCR5* polymorphism. The *CCR5* (chemokine receptor gene) protein is used by HIV to gain entry into the cell and individuals with a 32-bp deletion of the gene are particularly resistant to infection.[16] Furthermore, particularly of contemporary interest, is a polymorphism within the prion protein gene, namely methionine homozygosity at codon 129, which has now been found to be responsible for susceptibility to variant Creutzfeldt-Jakob disease (CJD).[17]

THE UK BIOBANK

A number of countries are involved in developing gene databases or 'gene banks', the idea being to store genetic samples from blood or tissue to be linked over time with medical and lifestyle information.[18] The UK Biobank plans to gain such information from recruiting 500,000 individuals aged 45–69 years who will be followed up for at least ten years.[19] The project raises many ethical problems particularly relating to patient confidentiality. However, if medical data over time could be linked to say genetic polymorphisms associated with therapeutic responses and disease susceptibilities, the results could prove extremely valuable.

WHAT OF THE ENVIRONMENT?

In the past, studies of populations, families and especially twins have clearly demonstrated that both genetic and environmental factors are involved in the aetiology of many common so-called multifactorial conditions. In some such disorders the nature of these environmental

TABLE 3
Examples of gene loci associated with response to particular drugs.

Drug	Locus
Warfarin, phenytoin, anti-arrhythmics, anti-depressants, etc.	*CYP* (cytochrome p450)
Succinylcholine	*PPC* (plasma pseudo-cholinesterase)
Sulphonamides, procainamide, isoniazid	*NAT* (N-acetyl transferase)
Mercaptopurine	*TMT* (thiopurine methyl transferase)
Digoxin	*MDR* (multidrug resistance)
Tolbutamide	*SUR* (sulphonyl receptor gene)

factors is fairly clear, as for example in the case of diet-related cardiovascular disease. But in many other instances the specific environmental factors causing the disease in those believed to be genetically predisposed, for example multiple sclerosis and motor neurone disease, are as yet unknown.

However, evidence is now emerging which suggests that particular micro-organisms may target proteins known to be defective in at least some unifactorial disorders. In this way these pathogens could play a role in affecting the clinical manifestations of these disorders. For example, protease 2A of Coxsackievirus B3 specifically cleaves cardiac muscle dystrophin. This would explain the cardiomyopathy which can result from such infections.[20] But most importantly from the point of view of the present discussion, infections with this virus may contribute to the severity of the disease in some cases of Duchenne or Becker dystrophy in which the dystrophin protein is already significantly reduced. Some other examples of muscle proteins known to be primarily or secondarily defective in certain dystrophies and which are also targets for damage by various micro-organisms[21,22] are given in Table 5. The molecular and biochemical evidence of the possible effects of certain micro-organisms in these diseases is accumulating and there is now a need to examine this relationship from a clinical and population perspective. It is doubtful however that the phenomenon is restricted to the muscular dystrophies. It could be of importance in some other single-gene disorders such as for example the so-called actin myopathies,[23] and even in some multifactorial conditions in which there is a genetic susceptibility. For example *Chlamydia pneumoniae* is an emerging risk factor in cardiovascular disease[24] and cytomegalovirus in atherosclerosis.[25]

Human genetic variation is likely to transform our understanding of medical biology and the practice of medicine over the next few years.[26] Our detailed understanding of the interaction of genetic factors with specific environmental factors in common disorders will be the most important challenge facing medicine in the future.

TABLE 4

Examples of gene loci associated with susceptibility to various common diseases. (Some have yet to be confirmed.)

Disease	Locus
Alzheimer's disease	*ApoE* (apolipoprotein)
Asthma and allergy	*GST* (glutathione S-transferase)
Parkinson's disease	*MAPT* (microtubule-associated protein tau)
Stroke	*ACE* (angiotensin converting enzyme)
Schizophrenia	*DRD* (dopamine receptor)
	MAG (myelin-associated glycoprotein)
Manic-depression	*MAO* (monoamine oxidase)
Cancers	
Colon and bladder	*NAT* (N-acetyl transferase)
Lung	*CYP* (cytochrome p450)
Infections	
HIV (resistance)	*CCR5* (chemokine receptor)
Variant CJD (susceptibility)	*PRNP* (prion protein)

TABLE 5

Muscle proteins defective in genetic disorders but which are also involved in the pathogenicity of acquired infections. Adapted with updating from Emery[21]

Disorder	Protein	Infection	Action	Result
Duchenne & Becker MD	Dystrophin (& Sarcoglycans)	Coxsackie B3	Protease 2A	Cardiomyopathy
Oculopharyngeal MD	PAB 2	Influenza A	NS 1 (blocks host cell metabolism)	Myositis
Congenital MD	Lamin α 2	Parvovirus B19	Receptor	Cardiomyopathy
Actin myopathies	Actin	Yersinia Sp.,	Protein kinase	Inhibits phagocytosis
		Clostridial Sp.	Protein kinase	Inhibits phagocytosis
		Pasteurella haemolytica	Polymerisation & binding, etc.	Toxicity Virulence
		Candida albicans	Polymerisation & binding, etc.	Toxicity Virulence
		Various bacteria	Polymerisation & binding, etc.	Toxicity Virulence

CONCLUSIONS

Over the last 50 years developments in genetics at both the clinical and molecular level have revolutionised our approach to human disease. These developments now offer the possibility of prevention of many serious single-gene disorders through reliable prenatal diagnosis for those found to be at risk. But we are now beginning to see how developments in genetics may also lead to possible new approaches to treatment and hopefully to ways of assessing an individual's response to drug treatment and susceptibility to various common diseases.

Recent developments have also revealed that at the molecular level the situation can be much more complex than was previously envisaged. Different mutations of a single gene can generate different protein products resulting in very different diseases. This new field of proteomics may now take over problems generated by genomics.

Finally with all the current emphasis on genetic mechanisms, the environment must not be ignored. The role of various micro-organisms in affecting the manifestations of certain genetic disorders is only now beginning to be understood at the molecular and biochemical level. Professor A Chakravarti at the Johns Hopkins Institute of Genetic Medicine has recently said:

> To some there is a danger of genomania with all differences (or similarities) being laid at the altar of genetics. But I hope this does not happen. Genes and genomes do not act in a vacuum and the environment is equally important in human biology.

The next challenge in genetic medicine will be unravelling the complex relationships between genes and environmental factors, most importantly the possible role of pathogens in both rare unifactorial disorders as well as in some more common multifactorial disorders.

REFERENCES

1 Guttmacher AE, Collins FS. Genomic medicine – a primer. *New Engl J Med* 2002; **347:**1512–20.
2 Emery AEH. The muscular dystrophies. *Lancet* 2002; **359:**687–95.
3 Bonne G, Yaou RB, Béroud C *et al.* 108th ENMC International Workshop, 3rd Workshop of the MYO-CLUSTER project: EUROMEN, 7th International Emery-Dreifuss Muscular Dystrophy (EDMD) Workshop, 13–15 September 2002, Naarden The Netherlands. *Neuromusc Dis* 2003; **13:**508–15.
4 Norwood FLM, Sutherland-Smith AJ, Keep NH *et al.* The structure of the N-terminal actin-binding domain of human dystrophin and how mutations in this domain may cause Duchenne or Becker muscular dystrophy. *Structure* 2000; **8:**481–91.
5 Emery AEH, Muntoni F. *Duchenne muscular dystrophy*. 3rd ed. Oxford: Oxford University Press; 2003.
6 Noguchi S, Tsukahara T, Fujita M *et al.* cDNA microarray analysis of individual Duchenne muscular dystrophy patients. *Hum Mol Genet* 2003; **12:**595–600.
7 Ciro M, Bracken AP, Helin K. Profiling cancer. *Curr Opin Cell Biol* 2003; **15:**213–20.
8 van Deutekom JCT, van Ommen G-J B. Advances in Duchenne muscular dystrophy gene therapy. *Nat Rev Genet* 2003; **4:**774–83.
9 Juengst ET. What next for human gene therapy? *BMJ* 2003; **326:**1410–11.
10 Old J. Hemoglobinopathies and thalassemias. In: Rimoin DL, Connor JM, Pyeritz RE *et al.* editors. *Emery & Rimoin's Principles and Practice of Medical Genetics*. 4th ed. Edinburgh, London & New York: Churchill Livingstone; 2002;1861–98.
11 Moghadaszadeh B, Albrechtsen R, Guo LT *et al.* Compensation for dystrophin-deficiency: ADAM-12 overexpression in skeletal muscle results in increased α 7 integrin, utrophin and associated glycoproteins. *Hum Mol Biol* 2003; **12:**2467–79.
12 Barton-Davis ER, Cordier L, Shoturma DI, *et al.* Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of mdx mice. *J Clin Invest* 1999; **104(4):**375–81.
13 Perin EC, Dohmann HF, Borojevic R *et al.* Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischaemic heart failure. *Circulation* 2003; **107:**2294–302.
14 Meyer UA. Pharmacogenetics and adverse drug reactions. *Lancet* 2000; **356:**1667–71.
15 Stearns V, Johnson MD, Rae JM *et al.* Active Tamoxifen metabolite plasma concentrations after coadministration of Tamoxifen and the selective serotonin reuptake inhibitor Paroxetine. *J Nat Cancer Inst* 2003; **95:**1758–64.
16 Dean M, Carrington M, Winkler C, *et al.* Genetic restriction of HIV-1 infection and progression to AIDS by a deletion of the *CCR5* structural gene. *Science* 1996; **273:**1856–62; **274:**1069.
17 Collins SJ, Lawson VA, Masters CL. Transmissible spongiform encephalopathies. *Lancet* 2004; **363:**51–61.
18 Austin MA, Harding S, McElroy C. Gene banks: a comparison of eight proposed international genetic databases. *Community Genet* 2003; **6:**37–45.
19 Barbour V. UK Biobank: a project in search of a protocol. *Lancet* 2003; **361:**1734–8.
20 Lee GH, Badorff C, Knowlton KU. Dissociation of sarcloglycans and the dystrophin carboxyl terminus from the sarcolemma in enteroviral cardiomyopathy. *Circ Res* 2000; **87:**489–95.
21 Emery AEH. Muscular dystrophy – an evolving concept. In: Emery AEH, editor. *The Muscular Dystrophies*. Oxford: Oxford University Press; 2001; 1–10.
22 Emery AEH. Muscular dystrophy into the new millennium. *Neuromusc Dis* 2002; **12:**343–49.
23 Sparrow JC, Nowak KJ, Durling HJ *et al.* Muscle disease caused by mutations in the skeletal muscle alpha-actin gene (*ACTA* 1). *Neuromusc Dis* 2003; **13:**519–31.
24 Kalayoglu MV, Libby P, Byrne GI. *Chlamydia pneumoniae* as an emerging risk factor in cardiovascular disease. *JAMA* 2002; **288:**2724–31.
25 Bason C, Corrocher R, Lunardi C *et al.* Interaction of antibodies against cytomegalovirus with heat-shock protein 60 in pathogenesis of atherosclerosis. *Lancet* 2003; **362:**1971–7.
26 Meyerson M. Human genetic variation and disease. *Lancet* 2003; **362:**259–60.