Post-genome integrative biology: so that's what they call clinical science

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ABSTRACT – Medical science is increasingly dominated by slogans, a characteristic reflecting its growing bureaucratic and corporate structure. Chief amongst these slogans is the idea that genomics will transform the public health. I believe this view is mistaken. Using studies of the genetics of skin cancer and the genetics of skin pigmentation, I describe how recent discoveries have contributed to our understanding of these topics and of human evolution. I contrast these discoveries with insights gained from other approaches, particularly those based on clinical studies. The 'IKEA model of medical advance' – you just do the basic science in the laboratory and self-assemble in the clinic – is not only damaging to clinical advance, but reflects a widespread ignorance about the nature of disease and how clinical discovery arises. We need to think more about disease and less about genes; more in the clinic and less in the laboratory.

Science is an increasingly corporate affair and is becoming more bureaucratic, with more division of labour, more specialisation and more corporate strategies – in both the private and public sectors. The gap between basic science and clinical medicine has increased rather than – as many would believe – diminished. As a symptom of this gulf in understanding or loss of common culture, the power of slogans in medicine is increasing[1]. The chief claim or slogan of our time is that the medicine of the 21st century will belong to 'post-genomics'.

Genomics and post-genomics

If we are going to realise the potential of disease genetics, we must paradoxically spend less time thinking about genes and more time thinking about disease; and we must realise that the discoveries of the last ten to twenty years will remain marginal until we understand once again that discoveries made by those who care for patients, rather than advances in basic biological science, are what now limit the rate of medical advance.

Of sweat, hair and sun, and a few million years of evolution

Men sweat more than women[2] and have thicker skin, but the subcutaneous is thicker in women. Both those observations say something about thermoregulation and sexual dimorphism in man[3]. There is something quite particular about sweating humans compared to most other primates. We are designed to be able to lose heat very efficiently: we can sweat faster than our kidneys can produce urine. This evolutionary decision was made around two million years ago in Africa and it is the consequences of this decision that now occupy half a dermatologist's workload in the UK[3,4].

'Hair is nature's most effective sunblock[5].'

Dense body hair provides a more than adequate sunblock (Fig 1) but gets in the way of heat loss through sweating. If you lose that body hair, as we surmise happened during *Homo sapiens's* period in Africa, you have to invent some system to protect the interfollicular skin from the harmful effects of ultraviolet radiation or otherwise the skin burns and cancer develops[3,4,6].

The biological solution nature came up with was, of course, to adapt the functions of melanin. Melanin was not invented in order to prevent damage from ultraviolet radiation (UVR) but, as is common in biology, a new use was found for an old molecule. The scrotum of the blue vervet monkey (Fig 2) is blue due to the presence of melanin; it appears blue rather than brown because of light scattering[7]. Of course, the melanin of the scrotum is not there to be a sunscreen. Pigments in nature are used either to attract or to avoid other life forms; in this case, attraction for sexual purposes. The new role for melanin in protecting against UVR should not cause great surprise. Visible light is just one form of electromagnetic radiation, and ultraviolet just a few nanometers further down the scale from visibility.

A histological section of human epidermis demonstrates the caps of melanin predominantly on top of the nuclei of cells in the proliferative compartment –

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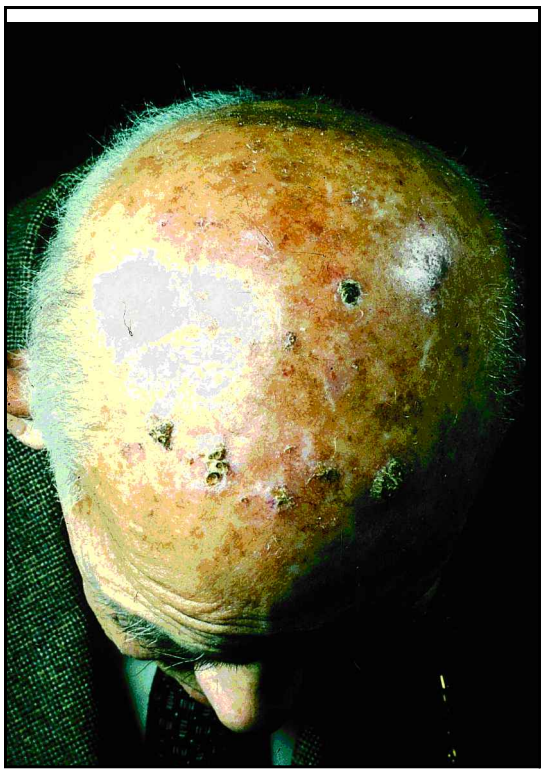
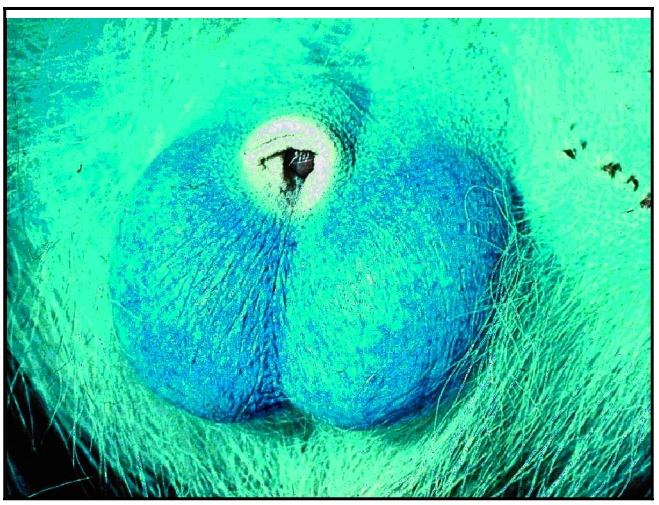


Fig 1 (left). Hair is an effective sunblock. The scalp is exposed to high levels of ambient ultraviolet radiation. In those individuals who lose hair, tumour rates rise considerably. Hair is therefore an effective sunblock.

Fig 2 (below). The scrotum of the blue vervet monkey is blue due to melanin and Tyndall light scattering.



protecting the DNA like a sun hat worn on a midsummer's day (Fig 3). Disease provides at least two clear examples that confirm this hypothesis: in patients with vitiligo, where there is focal loss of melanocytes, the resulting area burns more easily than the adjacent pigmented skin; a more extreme example is provided by albinism. Fig 4 shows a young albino from Tanzania who, like many, will in the absence of care die in his teens or early twenties from skin cancer. So, at the biological extremes, UVR will exert a major evolutionary drive on humans. Recent advances in genetics have enriched our understanding of these processes: a) at the level of the tumour, and b) at the level of the population.

Cancer as a genetic disease

The principal contribution of genetics to cancer over the last twenty years has been the realisation that the processes involved in hereditary cancer syndromes and sporadic cancer have much in common, that cancer can be viewed as a genetic disease, and that the successive accumulation of genetic abnormalities in a cell drives the malignant process[8]. Cancer is therefore an evolutionary disease, a race between the generation of somatic genetic diversity and host- or therapy-induced selection on the other[8]. It follows that there must be a relation between the pattern of genetic change in a tumour and clinical behaviour, and this argument leads to what I call the 'eppendorf test', the name chosen after those little test tubes beloved of molecular biologists. The test is simply stated: if you subject the DNA from a cancer to analysis you should be able to predict what happened to the patient. Skin cancer provides a fertile ground for testing this hypothesis[9].

Genotype versus phenotype and the eppendorf test

Non-melanoma skin cancer is common. In most Caucasian populations it is more common than all other cancers put together. There are many different tumour types with very different clinical behaviours but as far as we know they are all derived from the same cell, the keratinocyte. Basal cell carcinomas are slow growing, locally invasive tumours that do not metastasise; squamous cell carcinomas are more aggressive and metastasise; actinic keratoses are small clonal dysplastic lesions that can progress to squamous cancer but much more commonly spontaneously involute; and keratoacanthomas, lesions for all the world like squamous cell carcinomas, are exceedingly rapid growing but also invariably involute leaving a scar[9].

During the first half of the 1990s there was a torrent of activity trying to map genetic change to biological behaviour[9]. If genetics is predictive and genes are important in cancer, then examination of DNA should surely predict clinical behaviour. In this game of molecular snap, there is indeed some sort of broad relation between the pattern of genetic change and clinical behaviour: in general, more aggressive lesions had accumulated more mutations or genetic hits – at least between tumour types. For instance, we were able to show that basal cell carcinomas, squamous cell carcinomas and keratoacanthomas all have different patterns of genetic change[9].

Identification of the genes underlying cancer allowed new questions to be posed. Two major discoveries in skin cancer genetics came from the laboratory of Doug Brash at Yale, an engineer turned biologist. The first, in 1991, came when Brash

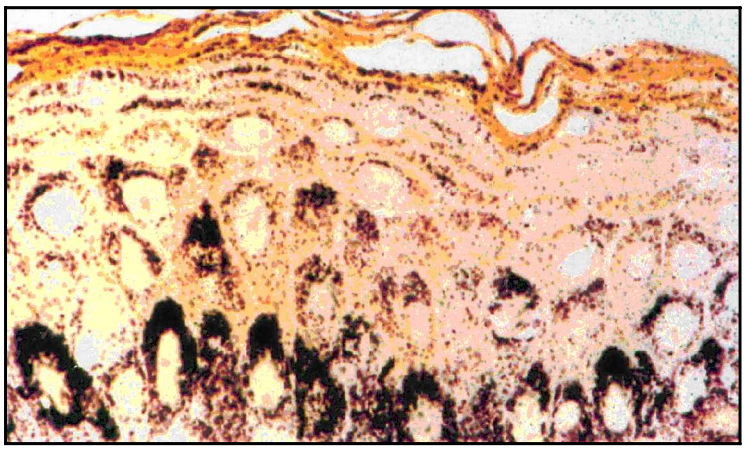


Fig 3 (above). Distribution of melanin in human epidermis. Melanin, or more correctly the collection of molecules that form melanin, appears on many sections to be capped over the nuclei of many keratinocytes. They are particularly prominent over the nuclei of basal keratinocytes.

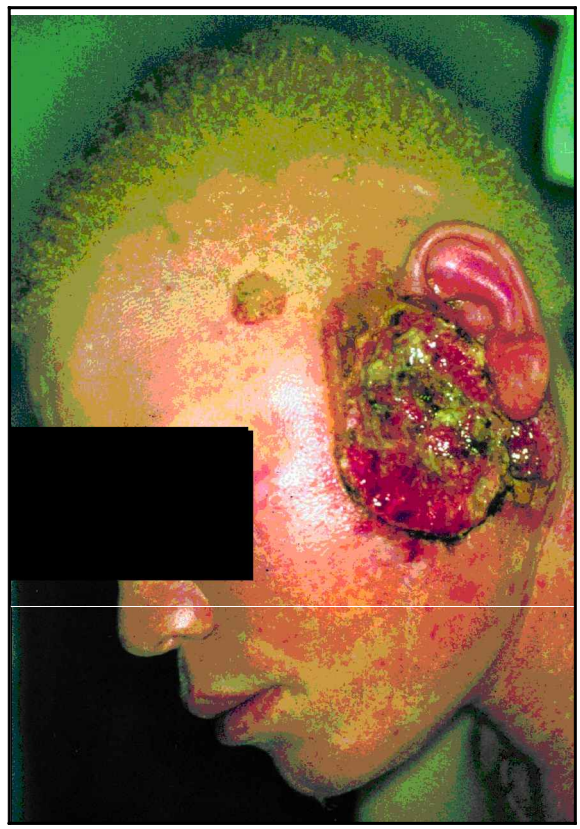


Fig 4. (right) The penalty for lack of pigmentation. A young type 2 albino in Tanzania shows one large squamous cell carcinoma and other dysplastic lesions. Without early treatment such individuals will die as young adults *(photo courtesy of Dr Sandy McBride)*.

and colleagues showed that the pattern of mutation in the p53 gene in squamous cell cancer showed the 'molecular footprints' of UVR induced mutagenesis[10]. The pattern of base changes observed was virtually pathognomic of UVR damage. UVR may be a tumour promoter; it may be an important suppressor of the cutaneous immune system; but in man mutagenesis is also critical. The converse, however, was also of interest. We subsequently showed that some cases of intraepithelial carcinoma had a different mutational spectrum[11]. Mutagens other than UVR may also be important in these cases. The pattern of mutation in a somatic cell may therefore act as an epidemiological tracer, marking historical events in the life of a cell.

Brash's second major discovery again took advantage of the important role p53 plays in cancer[12]. In the clinic, dermatologists get used to seeing patients with more than one cutaneous cancer, but Brash showed that if you were just to use a simple monoclonal antibody, you could take this process through several orders of magnitude. Fig 5 shows a small sample of epidermis stained with one of David Lane's p53 antibodies[13]. This epidermal sample is from the back of my hand, and shows

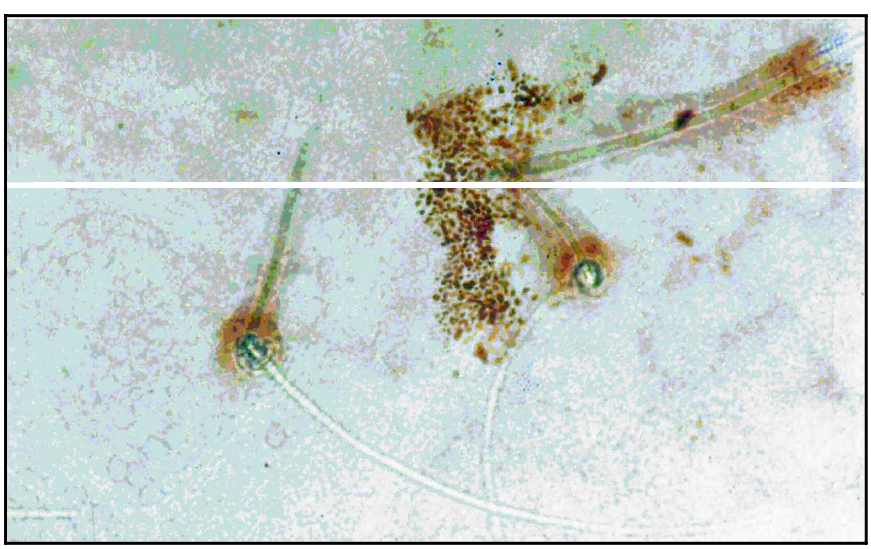


Fig 5. Wholemount staining of epidermis with a monoclonal antibody against P53. The clusters of immunostained nuclei represent cells with high levels of P53 immunoreactivity, in this case due to mutation of P53. The skin is from the back of the hand of the author.

a little cluster of p53 immunopositive cells that turn out more often than not to harbour p53 mutations. I might have several hundred of these clones, but so far no cancers. People with a history of skin cancer have, on average, many more of these lesions; they may exceed actual cancer numbers by several orders of magnitude[13]. For instance the older person in Fig 6 has a long history of skin cancers and, at a guess, perhaps 50% of the normal skin on light exposed areas harbours p53 mutations.

Nature, nurture and complexity

In many ways skin cancer may be regarded as an environmentally determined disease, the villain being UVR. However, UVR is only really hazardous in the context of pale skin: therefore cancer susceptibility is determined by pigment, which in turn is largely genetically determined, so it is a genetics problem, a point that the wise geneticist will emphasise in grant applications. Whilst albinos are predisposed to skin cancer (and there are other rare Mendelian disorders that also show grossly elevated risks of skin cancer), the attributable risk of these Mendelian disorders is perhaps less than 1%[9]. Far more important numerically are the genetically complex or non-Mendelian determinants of skin pigmentation. Sampling people from around the world, their sensitivity to UVR – judged by burning or cancer risk – varies greater than a hundred fold.

The importance of being red

People with red hair burn readily in the sun and show a greatly increased risk of most of the major forms of skin cancer, including melanoma. Most of the quoted odds ratios are likely to be underestimates, as people with red hair reduce their exposure to UVR (in comparison with non-reds) because they recognise that they burn easily. Red hair is due to a relative preponderance of phaeomelanin, which is red or yellow, rather than eumelanin, which is brown or black. Their skin is also affected, in that there is considerable covariance between hair melanins and skin melanins. Phaeomelanin, for reasons still not completely clear, protects less effectively against UVR than eumelanin[6].

In 1992 Roger Cone in Oregon explained the genetic basis of the *extension* locus in mice. Mice with homozygous loss of function mutations at this locus have yellow hair[14]. It is an obvious model for human red hair: a Celtic mouse! Redheads frequently harbour mutations of the melanocortin 1 receptor. Following on from Cone's work we suggested that in man certain mutations of the melanocortin 1 receptor result in loss of function with consequent decreased cAMP signalling and a resulting shift to phaeomelanin rather than eumelanin production. A nice story, but as the accompanying commentary in *Nature Genetics* remarked, there were problems. First, we found over thirty sequence variants. Second, and perhaps even more worryingly, some alleles, some chromosomes, seem to have more than one change on them: some even three. Third, the endogenous ligand for this receptor is thought to be α-melanocyte stimulating hormone (α-MSH), a cleavage

Fig 6. The leather clad youth on the right side is the author, and the more distinguished gentleman on the left is James Watson. Watson wears a hat and even with this quality of picture can be clearly seen to have grossly photodamaged skin; he admits to a prior history of many skin cancers.

product of POMC. Yet sceptics had already decided that this peptide had little role in human pigmentary physiology. Whereas we were able to make the original claim and publish within twelve months[15], it has taken five years to dot the i's and cross the t's[16–20]. The problems are instructive and they are common to many areas of genetics involving complex traits.

First, there are the hazards of genetic case control studies or association studies. These biases are potentially large where there is a factor related to ethnicity or stratification in the population (or what epidemiologists would call confounding). Both skin colour and hair colour clearly have potential in this respect. We tackled this by carrying out family studies, which allow the alleles to be seen against a relatively homogeneous genetic background, and by conducting further genetic epidemiological surveys in different areas. Fortunately the story still held: red hair approximates to an autosomal recessive.

Second, we tested some of the putative mutant alleles in a functional assay based on *in vitro* transfection[21]. We could then be certain that the changes in the MC1R we had described were causative rather than being in linkage disequilibrium with other changes. Of the large number of sequence variants detected we can be certain that perhaps five or six are functionally significant. More recently, as an additional more physiological 'whole animal' assay, we have rescued mice null for MC1R using human MC1R mutation variants in order to define their function. So now, with experimental support, we can say that only a few of the MC1R variants are functionally significant, but that most single nucleotide polymorphisms (SNPs) in the MC1R coding region have no obvious phenotype and that red hair approximates to a Mendelian recessive. We have since moved away from the 'simple' Mendelian paradigm and shown that sun sensitivity is indeed genetically complex[18]. A Mendelian trait is in one sense just a complex trait with a high odds ratio. Skin burning is a response to UVR, the MC1R loss of function

mutations give an odds ratio of ~4 for belonging to a lighter/more sun sensitive skin type, and there is a clear dosage effect for both sun burn and skin cancer[22].

Finally, that this signalling pathway is important in man as well as mouse was confirmed by a subsequent report from Germany[23] of two sibs, both loss of function mutations of POMC, who in the absence of a family history of red hair had bright red hair in addition to a complex endocrine phenotype that could be predicted from the other known roles of POMC. Homozygous mutations of the MC1R receptor, or the ligand, POMC, lead to red hair.

The evolution of red hair and pale skin

The pattern of p53 mutation in a cancer cell may act as a historical tracer of a cell's life history. Similarly, human germline polymorphism can act as a marker of lineage – an organism's 'life history' over evolutionary time. The high degree of sequence diversity of the MC1R, whilst originally seeming a costly nuisance, offers us the possibility of investigating skin biology over the last few million years.

Why do humans vary in skin colour? We studied diversity in a range of human populations at the MC1R[17]. Fig 7 is a representation of allelic diversity within African and outwith African populations; allele frequencies are shown proportional to the area of the circles.

Have people with red hair or pale skin been selected for in Northern Europe – in other words, is there some advantage to the phenotype? Conversely, is the diversity in Europe due to loss of functional constraint in Africa? Functional constraint means that the gene is so critical that amino acid diversity is not tolerated (in evolutionary terms); but once out of Africa, the functional constraint is reduced, and nature becomes indifferent to mutation. To resolve this question fully will require even larger sample sizes than the six or seven hundred we have studied as well as probably a study of other genes. For the present, our data are quite compatible with constraint in Africa and loss of functional constraint in the rest of the world: ie we see no evidence for selection. Of course, given the power of testing, no evidence for selection is not the same thing as saying there is no selection. Our models suggest dates of origin of the common loss of function mutations of around fifty thousand years, compatible with what we know in terms of the 'Out of Africa' hypothesis[17].

Key Points

Genetics provides a powerful way of studying biology: however, medical science is not synonymous with biological science

Using studies of skin cancer and the genetics of susceptibility to ultraviolet radiation, I argue that the role of clinical discovery has been underplayed and that progress in patient orientated research is now rate limiting for advance

The hyping of genomics, and the delusions of some of its proponents, reflect not just fashion, but basic misunderstandings about the nature of disease, the difference between biology and medicine, and the history of how therapeutic advance occurs

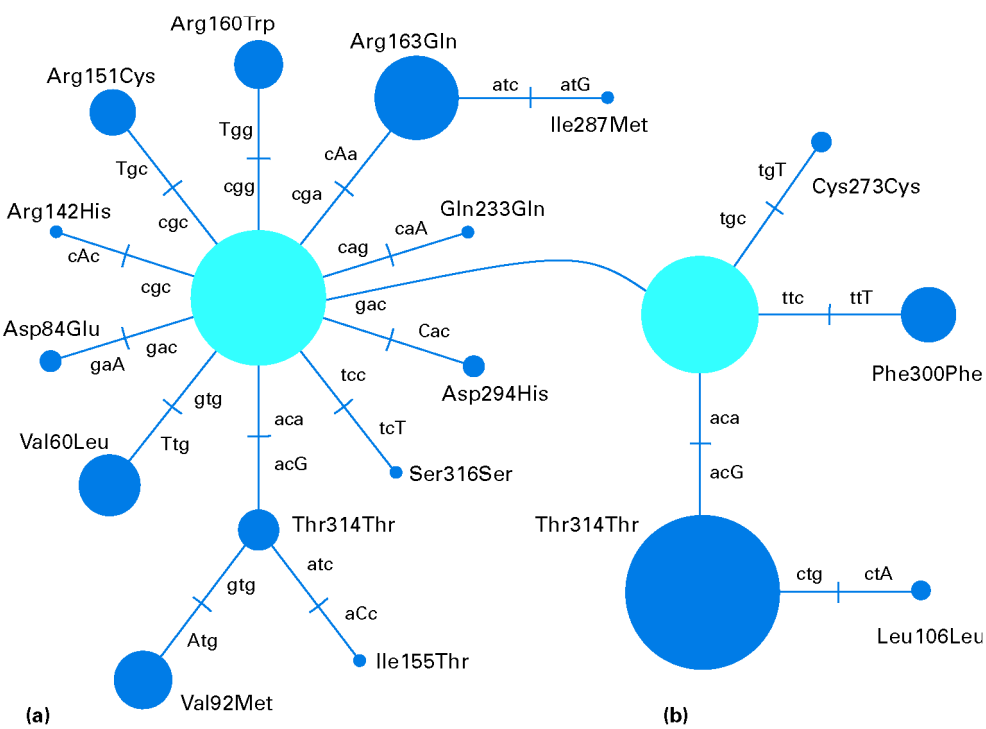


Fig 7. A gene tree representing a study of over 600 chromosomes from various world populations at the melanocortin 1 locus. The areas of the circle are proportional to allele frequencies. The root sequence is coloured grey and the consensus sequence black. The three letter codes refer to amino acid changes. In the African population all the nucleotide changes are silent (or synonymous) whereas in the other world populations the majority of changes change the predicted amino acid sequence.

Genetics and clinical science

At the start I made some general comments about the organisation of science and set out to establish two claims:

- if we are to make progress, we need to spend less time thinking about genes and more time thinking about disease
- the rate-limiting factor for clinical discovery now lies in the clinic rather than the laboratory.

The relation between exposure to UVR and skin cancer was described by one of the giants of 19th century dermatology, Unna, under the name 'Seemannshaut' – sailor's skin. Although he suspected it was UVR rather than exposure to other aspects of the weather, formal proof had to await the invention of artificial UV sources in the early 19th century. The only Nobel Prize ever awarded to a dermatologist was given to Finsen for the use of UVR therapy to treat cutaneous tuberculosis. The widespread use of UVR by clinical dermatologists was associated with an increase in cancer risk, confirming Unna's suspicions that it was UVR rather than other aspects of the 'elements' that were causative. Not for the first, or the last time, machines and technology devised by physicists and engineers for other purposes have driven advance in medicine as much as 'basic' biology. The supposed model systems for UVR and skin cancer, both in mouse and other animals, and the formal epidemiology, came much later.

P53 mutations occur in sun-exposed skin. They occur after acute or chronic exposure, but as yet we have no idea of the relevant contributions of mutagenesis, tumour promotion or interference with the cutaneous immune system by UVR in man. In short, we have no quantitative model on which to base useful quantitative clinical predictions. By contrast, our 'guide of action' (William Clifford's definition of science) comes from the clinic and clinical epidemiology. This is not just some dated and avuncular defence of clinical observation or clinical serendipity, but reflects rather the nature of biological explanation. So from the perspective of the major discoveries that underpin clinical practice, discovery based in the clinic is not an optional extra. The role of immunosuppression in cutaneous carcinogenesis was a clinical discovery based on observations of higher cancer risks in those who had received systemic immunosuppression; the carcinogenic role of PUVA treatment was a clinical observation; the use of retinoids as chemotherapeutic or even therapeutic agents was again defined by clinical experimentation, and what we know about sunbeds or conversely, the efficacy of sunblocks, has relied on human studies and human experimentation.

Clinical prediction and molecular snap

What about the game of molecular snap? How useful is genetics as a predictive clinical tool? The evidence for the Mendelian disorders is clear cut. Genetic analysis offers the opportunities for prenatal diagnosis, for counselling based on understanding the mode of inheritance and, of course, in a wonderful heuristic bootstrap, has fed back to improve clinical skills – the apparent paradox that it is necessary to define a syndrome to be able to map the gene, but conversely, once the gene has been mapped it is possible to improve clinical diagnostics and redefine the syndrome.

Even with the apparently simple Mendelian disorders we are a long way from therapy based on the many promises made over the last twenty years. With respect to cancer, particularly skin cancer, molecular tools have as yet been extremely disappointing. Several years ago, we showed that actinic keratoses, those clinically banal lesions, harboured as many if not more mutations than squamous cell carcinoma[24]. Relying only on DNA to predict what happened to the patient was far more likely to be wrong than clinical observation. Similarly, a detailed molecular analysis of a large number of melanomas found that the pattern of mutation offered little prognostic information beyond tumour thickness measured with the histopathologist's ruler[25]. Of course, methods of analysis change: it would be churlish to imagine that an advance such as use of array technology may not allow clinically relevant progress, but it remains true that we have fallen in love with the slogan rather than reality. We have conflated one particular aspect of biology with medicine, when in reality the activities are far more diverse; and our intellectual approach has to mirror this. The fundamental error is not one of fashion, or training, but a logical flaw in our understanding of biological explanation.

Genes and environment revisited

How do genes and environment interact? Genes are not just there at the beginning of life: they exert their effect throughout life. Genes are no more primary than the environment is primary. We remain confused about our concepts linking genes with disease. All diseases are 100% genetic and 100% environmental[26]. What makes this statement seem counterintuitive is that we have taken narrow technical definitions of terms like heritability and conflated them with how we should gain mechanistic and therapeutic insight. Skin cancer provides a pertinent example. Earlier, I used the usual grantsmanship to argue that if you are interested in understanding skin cancer you should study genes. The danger is that people end up confusing peer review with reality. Consider the concept of heritability: an example may be more helpful than a textbook definition[27]. In Scandinavian twin studies of basal cell carcinoma it is not necessary to include genetic factors to explain the incidence of the disease in twins[28,29]. Environment appears to be more important. But heritability informs about variation in a particular population, not whether the genes are important in pathogenesis. So for any population the heritability of the disease may be low but particular genes and their products may be important rate limiting steps in pathogenesis. Imagine transferring the population of Sweden to equatorial Africa and then conducting the same study looking for genetic components of basal cell carcinoma risk. It would be bizarre now if a large heritable component were not found. Conversely, looking only at the population of blacks there would be low heritability. Low heritability therefore does not mean that melanin is not a key

step in preventing sun induced skin cancer – just look at albinos. Heritability informs only about allelic variation in a particular population[28]. It says nothing about whether a gene and its product is a rate limiting step in a disease nor, importantly, is it congruent with pathways that might be rate limiting and useful for therapeutic attack.

Prediction of disease risk

The study of genetic determinants of disease help in understanding particular causal pathways and may also allow identification of particular high risk groups. There is a widespread prejudice that such strategies will be clinically useful and that newer technologies such as single nuclear type polymorphisms (SNPs) will be important in this respect. However, these arguments remain unconvincing[30,31]. First, there is always the temptation to imagine that technology will somehow solve what are not technological, but rather biological problems – in this case, the difficulties of separating selection from neutral variation and human demographic history and diversity[31]. The MC1R illustrates the problems of assigning function even to coding region SNPs in this respect. Second, whilst Mendelian disorders can be viewed as complex diseases with high odds ratios which consequently allow secure clinical prediction, for the majority of the common diseases affecting humans (including cancer and inflammatory diseases) it is unlikely that genotypic investigation will offer much useful clinical prediction[30]. The temptation to imagine that large heterogeneous datasets will somehow overcome the uncertainties and dubious clinical utility of low odds ratios should be resisted. For instance, from what we know about psoriasis it is unlikely that current attempts to identify 'disease genes' will produce useful predictions of clinical behaviour in terms of future risk, disease course or therapeutic attack[28]. Ironically, phenotypic measures, although currently out of vogue, would seem to offer much more. Asking individuals whether they have got a family history of a disease and how they responded to treatment previously may still be more pertinent.

Conclusion

Genetics is a powerful way of doing biology. It not only provides insights into disease but also underpins attempts to understand human evolution. The danger is that the tractable approaches genetics has offered have been turned into a slogan based on a view that genetics, aka genomics, is a way of solving all public health problems. Any branch of biology, it seems, now tags on the ending 'omics' (metabolomics, proteomics etc) and shouts for special funding because it can 'end disease'. This view is mistaken (moromic, perhaps?) – mistaken because of an ignorance about the nature of clinical discovery and the nature of complex biological systems. Nobody ever seems to mention clinicomics. Just as one cannot map a physiological response like fear to a synapse, it should cause no surprise if measurement of tumour thickness predicts outcome better than sequencing a handful of candidate genes. Nor is it surprising if asking somebody about their history of skin burning episodes predicts better than sequencing of the melanocortin 1 gene. It may or may not. Treatment of psoriasis with UVR is no less logical a therapy than identifying the mantra of the genetic causes of this disease. Just because a patient has a Mendelian cancer syndrome doesn't mean the tumour should not be cut out.

Medicine is one peculiar and particular branch of applied biology which draws on many disciplines, from medicinal chemistry to physics and engineering. Rather than wed itself to one technology, to one slogan by a particularly well funded and vocal interest group, it should remain intellectually promiscuous. Post genomics is simply what you do after you realise the game has moved on.

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