PERSPECTIVE

The genetics of complex ophthalmic disorders

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'Complex inheritance' is a relatively new term for multifactorial, polygenic, or quantitative inheritance that identifies characteristics, traits, or diseases that are determined by a number of genes. In some cases there may also be an environmental component to aetiology. Examples of such disorders are Alzheimer's disease, schizophrenia, bipolar affective disorder, hypertension, heart disease, and arthritis. In the 19th century this phenomenon was recognised as 'blending of inheritance' with progeny exhibiting phenotypes intermediate between parents. One of the great achievements of mendelian genetic (particulate) theory leading to its general acceptance was to explain this effect as the transmission of many different mendelian factors.¹

In developed countries the three most prevalent causes of blindness—age-related macular degeneration (ARMD), diabetic retinopathy, and glaucoma² are all thought to be aetiologically complex. Progress in molecular genetics now gives us sufficient understanding of the structure of the genome and the refined techniques to be able to begin to unravel their complexity with the promise of developing novel approaches to the management of patients with these conditions.

In this article an overview of the published work on aetiological influences in each of these diseases is presented with an outline of the molecular genetic methods that are most likely to lead to the identification of the responsible genetic abnormalities.

Age-related macular degeneration

In Great Britain, 50% of the 35 000 blind and partially sighted registrations per year are attributable to ARMD and recent reports suggest that the incidence of the disease is rising.³ The term ARMD applies to patients over 50 years of age and describes morphological changes at the macula which include soft drusen, abnormal pigmentation, geographic atrophy, and subretinal neovascularisation with subretinal scarring.⁴ Importantly, these features can be signs of other retinal dystrophies (for example, Sorsby's fundus dystrophy, Doyne's honeycomb dystrophy, malattia levantine) which can usually be differentiated from ARMD on the basis of establishing the appearance of these signs in other members of the patient's family before the sixth decade of life.⁵ It should be stressed that this definition does not assume that ARMD is a single aetiological entity.

GENETIC INFLUENCES

Many studies have suggested a role for genetics in the aetiology of ARMD and it seems likely that genetic predisposition is the strongest risk factor apart from advanced age. Hyman *et al*⁶ in a case-control study identified a significant increase in the incidence of ARMD in the siblings of affected individuals. This family association has been confirmed in twin studies^{7–9} and Silvestri *et al*¹⁰ have suggested a 19 times relative risk of the condition in family members

of affected individuals. Piguet *et al*¹¹ compared sibling pairs with spouses and demonstrated concordance of macular drusen between siblings but not with spouses sharing a common environment. This suggested that genetic background was the most important aetiological factor.

Further evidence suggesting a role for genetic influences has been the possible associations between sex¹² and race¹³ and ARMD. This latter association may involve the protective effect of ocular pigmentation as reflected in iris colour¹⁴ or changes in iris colour with age.¹⁵ Heiba *et al*¹⁶ undertook sibling correlation studies to quantify the role of genetic influences in ARMD in 564 families. A major genetic influence was confirmed in the aetiology of ARMD. Moreover, it was concluded, a single major gene could account for most of the variability of disease in this community. No specific gene or genomic locus has yet been positively associated with ARMD. The various genetic loci linked to monogenetic disorders that affect the macula,^{17–18} in particular those loci that include the retinal genes *peripherin/RDS*¹⁹ and *TIMP3*²⁰ would be appropriate starting points for a genome search for ARMD susceptibility genes.

ENVIRONMENTAL INFLUENCES

Weaker and inconsistent risks have been found with a wide range of other exposures. For example, cardiovascular disease,^{21–22} alcohol consumption (specifically beer),²³ reproductive history, and exposure to exogenous oestrogens in women,²⁴ smoking (most significantly associated with submacular neovascularisation),²⁵ and plasticisers contaminating ingested food²⁶ have all been associated with ARMD. This has led to the hypothesis that modification of environmental influences may lead to reduction of ARMD severity.

A major area of interest has been the influence of oxidative metabolism within the retina, light exposure, and the supplementation of diet with antioxidants.²⁷ Normal metabolism with ultraviolet and high energy visible light exposure can trigger the formation of highly reactive free radicals leading to degradation of cellular proteins and polyunsaturated fatty acids. Cellular mechanisms designed to combat this may be particularly deficient in ARMD patients leading to accumulation of peroxidation products. Although histological changes similar to ARMD have been seen in animals exposed to light, no similar changes have been seen in human studies. Correlation of ARMD with antioxidant dietary content in humans has suggested possible protective roles for vitamins A, C, and E and selenium.^{28–29} Despite these positive associations between ARMD and environment, an almost equal number of studies find no associations.^{4–11}

Diabetic retinopathy

Chronic hyperglycaemia is considered by far the major determinant of diabetic retinopathy. Previous controversy regarding the benefit of 'tight' control of hyperglycaemia

has now been resolved with long term, strict control regimes resulting in significantly less progression of retinopathy.^{30–31} Other factors determining whether a patient with diabetes mellitus will develop retinopathy and its rate of progression probably exist. Prolonged hyperglycaemia secondary to other conditions—for example, pancreatitis, haemochromatosis, and acromegaly, are infrequently associated with retinopathy.³² The retinopathy seen in diabetes mellitus caused by mitochondrial mutations is atrophic rather than a vasculopathy.^{33–34} Proliferative disease is more prevalent for any given duration of time in insulin dependent compared with non-insulin dependent cases.^{35–36} In addition, proliferative retinopathy is seen significantly more often in diabetics with HLA-DR phenotypes 4/0, 3/0, and X/X when compared with control diabetic patients matched for age, sex, and duration of diabetes.³⁷ The role of other components in the aetiology of diabetic retinopathy may be small. This is in contrast with diabetic nephropathy in which a significant, second component (genetic) in addition to hyperglycaemia has been proposed based on the observation that the incidence of nephropathy in insulin dependent diabetes, rather than steadily increasing with the duration of hyperglycaemia, declines rapidly after a period of 15 years.³⁸ The underlying genetic abnormalities predisposing to systemic diabetes mellitus are therefore the most important factors in diabetic retinopathy aetiology. Whether one or more of the specific genetic abnormalities associated with systemic diabetes mellitus will be associated with an enhanced risk of retinopathy remains to be determined.

GENETIC INFLUENCES

Most work that has been undertaken on the aetiology of diabetes has related to type 1 or insulin dependent diabetes (IDDM). The importance of a genetic component has been established in many studies. The risk of IDDM in siblings of affected patients is on average 6% compared with a population frequency of only 0.4% in whites of European descent.³⁹ Non-parametric linkage analysis in humans has identified at least 12 genetic loci contributing to the aetiology of IDDM.⁴⁰ By far the most important (*IDDM1*) has been localised to the HLA region of chromosome 6p21 and relates to polymorphisms within the peptide binding sites of the class II molecules HLA-DQ and HLA-DR. *IDDM1* accounts for 35% of the clustering observed in families. The next most significant association is with *IDDM2* which localises to a variable number tandem repeat sequence in the insulin gene (*INS*) on chromosome 11p15.⁴⁰ Variation within the class I (~40 repeats) and class III (~150 repeats) alleles at this locus is thought to influence *INS* transcription,⁴¹ but a direct causal link between this effect and diabetes susceptibility has yet to be established. Other quantitatively less important loci are localised to chromosomes 15q, 11q13, 6q25, 18q, 2q31, 6q27, 3q21–q25, 10cen (*IDDM3-10*), the X chromosome (at locus *DXS1068*), and the glucokinase gene (*GCK*) on chromosome 7p.⁴⁰

Family and twin studies have also confirmed a strong genetic component to type 2 non-insulin dependent diabetes (NIDDM)⁴² although contrary to previous work this is not as great as is seen in IDDM.⁴³ Again, mathematical modelling has pointed to the interaction of a number of genes.⁴⁴ Maturity onset disease of the young (MODY) represents 2% of NIDDM cases. It is transmitted in a simple autosomal dominant fashion with mutations in the glucokinase gene (*GCK*) and a locus on chromosome 20 accounting for 50% of cases and another locus on chromosome 12 accounting for a further 25%.⁴⁵ Missense mutations of the glucagon receptor gene (*GCG-R*) on chromosome 17q25 have been associated with a significant

number of French NIDDM cases⁴⁶ and two studies on the genetic basis of NIDDM in the GK rat have highlighted susceptibility loci on rat chromosomes 1, 2, 5, 10, and 17.^{47–48} The region linked to rat chromosome 1 is syntenic with human chromosome 11q15 where two genes, *SUR* and *BIR*, encoding constituents of pancreatic cell K⁺ channels (KAPT)⁴⁹ are found. Further work will determine whether the localisations identified in the GK rat will correspond to NIDDM loci in humans.

ENVIRONMENTAL INFLUENCES

Less emphasis has been placed on environment modulating diabetes susceptibility, although such influences are expected. The fact that only 10% of individuals with IDDM have a family history of the disorder, that epidemics of childhood diabetes have been reported,⁵⁰ and that spatial clustering of IDDM occurs⁵¹ all suggest that genetic predisposition is only part of the story.

Stronger evidence for the role of environment has been obtained from concordance rates in monozygotic twins. Olmos *et al*⁵² have looked at the occurrence of IDDM in identical twins over a 24 year period. In this study of 49 IDDM patients, only 15 of their identical twins developed IDDM (30%). Actuarial analysis further suggested that 94% of these identical twins who will develop IDDM will have done so within 30 years of its development in their sibling. The authors suggest that this implies genetic predisposition, with brief environmental exposure to a precipitating agent earlier in life. Infective agents seem the most likely.⁵³ Observations on diabetic (NOD) mice suggested that the situation is more complicated. Those reared in germ free environments are more likely to develop the disease and viral infection in fact seems to reduce disease frequency.⁵⁴ Advancing age, lack of physical activity, and obesity all contribute, along with genetic factors, in the aetiology of NIDDM. The specific risk of retinopathy in diabetes is also thought to be influenced by the presence of glaucoma, myopia and carotid occlusive disease (all thought to be protective), onset of puberty, pregnancy, and hypertension (all thought to adversely influence development and progression of retinopathy).⁵⁵

Genetic influences in glaucoma

Glaucoma is the consequence of many clinically distinguishable entities. The commoner primary glaucomas will be discussed here rather than the secondary conditions where glaucoma is associated with, or is an outcome of, other ocular abnormalities. Primary glaucoma may be subdivided by age of onset into congenital (onset <3 years of age), juvenile (adolescent onset), and maturity onset disease (POAG). A genetic influence has been established for each subtype and these subdivisions to some extent seem to correspond to different genetic entities. Mainly recessive⁵⁶ but also dominant⁵⁷ congenital glaucoma pedigrees have been reported. Dominant inheritance is found in juvenile disease.^{58–59} A history of simple mendelian inheritance is less often seen in POAG,^{60–61} although 13–47% of POAG patients have a positive family history⁶² and there is a seven to tenfold increase in prevalence of POAG in the first degree relatives of POAG patients.^{63–64} Twin studies have also shown a high concordance of POAG between monozygotic twins.⁶⁵ Few studies have identified significant environmental risk factors for glaucoma although isotonic exercise and limiting alcohol and tobacco consumption can help to reduce intraocular pressure, if only transiently.⁶⁶

A number of candidate regions (for example, 6p21, 6p25, 11p, and 11q) have been proposed for congenital glaucoma based on chromosomal abnormalities identified cytogenetically where the condition was associated with

multisystem abnormalities. Sarfarazi *et al*⁶⁷ have shown tight linkage between autosomal recessive, simple, congenital glaucoma, and chromosome 2p21 in 11 of 17 Turkish families. This establishes the locus as important in the disease and suggests that recessive congenital glaucoma is genetically heterogeneous. However, confirmation of the quantitative significance of this result awaits studies in other ethnic groups. Linkage of autosomal dominant, juvenile glaucoma to chromosome 1q23-25 has been reported from a number of centres around the world.^{68,69} Morissette *et al*⁷⁰ have reported on a large French-Canadian pedigree, in which the disease was also linked to this chromosome 1q region. Interestingly, some family members with the disease haplotype had later onset glaucoma after 40 years of age. This implies that the disease gene mapping to this region may play a role in some cases of POAG. No other accounts of genetic abnormalities have been reported in other POAG groups although non-parametric linkage studies are under way.⁷¹ The possible roles of genes implicated in secondary glaucomas⁷² have not yet been fully assessed in POAG cases.

Molecular genetic methodology

Recombinant DNA technology and knowledge of the organisation of the human genome have become sufficiently refined over the past decade to make the task of identifying the genetic abnormalities leading to complex traits a practical possibility. Of particular importance has been the development of genetic markers (mapping to known genomic loci), based on the wealth of DNA polymorphisms that have been identified in the genome. These allow for the localisation of genetic abnormalities of interest to genomic regions sufficiently small to be screened using physical mapping technology.⁷³ The task of identifying predisposing genetic abnormalities in complex traits proceeds along the following sequence of events: non-parametric linkage analysis to identify important genomic regions, locus refinement—for example, using linkage disequilibrium mapping, the construction of a physical contig of the regions of interest, and the screening of candidate genes for disease related mutations.

LINKAGE ANALYSIS

Conventional linkage analysis requires the study of extended, affected pedigrees in which the disease status of each individual and the mode of inheritance are known, with the assumption of a single disease locus. Such pedigrees are rarely, if ever, available for complex traits and mode of inheritance—the relative contributions of dominant, recessive, X linked, and/or multifactorial inheritance, leading to the disease in the population of interest is often unknown. These assumptions do not apply to 'model free' non-parametric linkage analysis. Two statistical methods have been developed—affected sib pair and affected pedigree member analysis.⁷⁴ The former method will be described here since it has been the most successful; affected pedigree member methodology is the least statistically powerful and still requires the construction of (albeit small) pedigrees.

Affected sib pair analysis⁷⁵⁻⁷⁷ conventionally involves the study of two affected relatives (siblings) in a pedigree to determine whether they have inherited identical copies of a chromosomal region from a common ancestor. This to some extent will happen by chance, but if seen in a large number of affected sibships the association between the chromosomal location and the disease becomes more significant. It has recently been suggested that the most efficient sib pair studies are those where sibs are either concordant (both exhibiting near identical features of the disease) or extremely discordant (one affected, the

other normal).⁷⁸ Since the identification of such discordant pairs, especially in ARMD and POAG, can be prone to diagnostic error (for example, a normal examination may not guarantee an unaffected status), concordant sib pairs are usually the better study design.

An important determination before undertaking such a study is to decide on the size of the sib pair group to be investigated. This is dependent firstly upon the total contribution that susceptibility loci make to the risk of developing the disease. This is called the risk ratio, s , which may be calculated as the risk to a sibling of an affected proband versus the prevalence in the general population. For ARMD this is 10–30^{10,79} and for IDDM is 15.⁴⁰ The second important factor is the threshold to be accepted as a sign of significant linkage. This is determined by the maximum lod score statistic, T . For a monogenic disease $T=3$ corresponds to odds of 1:1000 in favour of linkage. During an initial total genome search (using marker loci 15 cM apart) susceptibility loci with <2 might be missed if the threshold used as suggestive of linkage were as high as this. Therefore at this stage $T=1.5$ may be more appropriate. This was the situation with diabetes where many of the lesser susceptibility loci would have been missed if a high threshold had been used initially.⁸⁰ In the case of ARMD however it has been suggested that a single genomic locus is associated with most of the genetic risk.¹⁶ A high threshold would still detect this locus. Many authors have proposed, and recent comparisons of concordance rates in monozygotic twins—100% versus dizygotic twins—28% to 42%⁹ suggest, that underlying susceptibility to ARMD may involve a number of genes.⁸¹ The best interpretation of these studies therefore would be that a number of susceptibility loci are involved but that one locus is associated with most risk. Therefore, during a first stage genome search in ARMD, a maximum lod score statistic of $T=1.5$ would be prudent to ensure that these minor susceptibility loci are not missed. Using this information, power graphs based on Risch *et al*,⁷⁶ 100 sib pairs and polymorphic microsatellite markers at ~ 15 cM intervals, the probability of detecting minor susceptibility loci with 1.5 would be $>90\%$.

The power statistic described above is based on haplotype data identical by descent (IBD). The power of affected sib pair studies in ARMD and POAG would be reduced to some extent since parental haplotypes would rarely be available and statistical analysis would be based on identical by state (IBS) data. In practice, this loss of power may be small especially if highly polymorphic microsatellite markers with polymorphism information contents (PIC) 0.8 were used.⁸²

Loci highlighted during a first stage genome scan are then studied intensively using microsatellite markers mapping more densely in the regions of interest. When each locus is looked at closely true susceptibility loci will then give higher lod scores. In an affected sib pair analysis, significant linkage is obtained when $T=3.6$ and highly significant linkage when $T=5.4$.⁸³ It has also been suggested that to confirm the significance of identified susceptibility loci a second complete genome scan or a 'replication study'⁸⁴ on an independent group of sib pairs be undertaken.⁸⁴ Failure to replicate a linkage result, however, may be due to population heterogeneity rather than suggesting error in the original study.

Simple affected sib pair analysis assumes that each locus identified contributes independently to disease susceptibility (genetic heterogeneity). As has been seen in diabetes, this may not be the case. Epistatic interaction occurs when the genotype at one locus has an effect on the contribution made by another locus. Such interactions suggest that the susceptibility genes at the two loci encode proteins that act

on the same or related biochemical pathways. Epistasis is seen with the diabetes susceptibility loci *IDDM1* and *IDDM2*⁸⁵ and *IDDM1*, *IDDM2*, and *IDDM7*.⁸⁶ Conversely, a genetic heterogeneity model best fits the interaction between *IDDM1* and *IDDM4*.⁸⁷ By undertaking such multilocus analysis⁸⁸ not only is the true significance of a susceptibility locus better quantified but also clues as to the biochemical function of the localised disease gene may be obtained.

LOCUS REFINEMENT

Linkage refinement to a region smaller than 1–2 cM is rarely achievable even when large, affected families are available. This is because recombination events rarely occur at this level. Such a refinement corresponds to approximately 1–2 megabases of DNA to search for mutations, a daunting task for the molecular geneticist. Linkage disequilibrium mapping is a technique that has been used successfully to help in such a situation.^{89–90} At the time a genetic mutation occurs the mutant allele will be completely linked—in linkage disequilibrium, with alleles of flanking markers. With time this association will decay. If it is assumed that this decay is due to recombination events only, a population based study of affected individuals and controls can be undertaken to compare the different frequencies of marker alleles in the two groups. The pattern or curve of disequilibrium between the disease and linked marker loci will exhibit a single maximum at the disease locus. The amount of linkage disequilibrium between a mutant allele and closely linked marker alleles can then be used to quantify the location of the disease relative to these marker alleles. In practice, however, other population genetic influences such as mutation, drift, breeding system, and selection influence this decay. The technique can therefore be imprecise depending on the statistical methodology employed.⁹¹ In part because of such influences, linkage disequilibrium mapping works best in genetically isolated populations.⁹²

Linkage disequilibrium can also be used to investigate weak linkage reports. A good example is the *IDDM2* localisation for which there is little evidence for linkage but strong evidence of linkage disequilibrium. This is because the locus has a true small effect but relatively few alleles in the population.⁹³

PHYSICAL MAPPING AND SCREENING CANDIDATE GENES

Once sufficiently small genomic regions (for example, 1 megabase) have been identified using linkage analysis, with or without disequilibrium mapping, conventional studies have proceeded to the construction of physical contigs of each region of interest. Libraries of cloned genomic DNA fragments of various sizes are available. These fragments are amplified within various vectors—for example, yeast YACs (yeast artificial chromosomes) or bacteria cosmids, PACs (P1 artificial chromosomes), and BACs (bacterial artificial chromosomes). Such YACs or cosmids mapping to the region of interest are isolated from libraries by probing with microsatellite markers linked to the disease locus. An overlapping, ordered arrangement of clones is then constructed by sequencing the terminal ends of each fragment and then using this sequence to 'pull out' the next clone from the library.⁹⁴ Once the contig is complete, various methods such as 'CpG island' mapping⁹⁵ and cDNA selection⁹⁶ have been used to identify coding sequence which can then be screened—for example, using direct sequencing to identify polymorphisms or mutations associated with disease status.

Such physical mapping strategies are laborious, costly, and often inefficient. With the growing wealth of information on mapping and gene structure now available, a more

fruitful strategy has often been to identify genes already known to map to the region of interest and screen the most likely candidates based on the known biochemistry of the condition of interest. Such a strategy is illustrated by the success of identifying *TIMP3* as the disease gene in Sorsby fundus dystrophy within a large genomic region.²⁰ Missense and nonsense mutations may be identified using (i) direct sequencing; (ii) the altered banding patterns of single stranded DNA in non-denaturing gels (SSCP)⁹⁷; (iii) resolution of heteroduplex molecules by virtue of their instability in denaturing gradient gels (DGGE)⁹⁸; (iv) altered heteroduplex mobility in non-denaturing gels⁹⁹; (v) chemical¹⁰⁰ or RNaseA cleavage methods¹⁰¹; (vi) by enzymic mismatch cleavage (EMC) using bacteriophage resolvase which recognises mismatch (mutant) bases and cuts the DNA at that site¹⁰²; or (vii) oligonucleotide ligation assay (OLA)¹⁰³ a fully automatable genotyping technique using oligonucleotides that end at a variable site to distinguish alleles. The latter two methods are particularly suited to the large scale identification of the intragenic polymorphisms most often associated with complex trait susceptibility.⁴¹

Conclusions

As is being shown in diabetes, in different groups of patients, different mutations and combinations of susceptibility loci may lead to disease.¹⁰⁴ This makes the assessment of the true significance of environmental influences extremely difficult. One possible explanation for the conflicting reports on environmental influences in ARMD may be that particular external agents are only important to a subgroup of ARMD patients who have inherited one particular combination of disease susceptibility genes. With such an unsteady base to work from, progress towards a better understanding of ARMD, diabetic retinopathy, and glaucoma is best directed initially to determining the underlying genetic influences that predispose to these diseases. This is achievable with current molecular genetic technology and refined statistical methodology. Such information in itself would have practical benefits. Many therapeutic options are already available for diabetic retinopathy and glaucoma, which benefit from being applied early in the disease. Current clinical screening methods could be optimised by directing resources to those patients with proved genetic predispositions. In addition clinical treatment trials could be undertaken on genetically homogeneous groups of sufferers resulting in more meaningful and definitive conclusions.

Detailed genetic information on ARMD, diabetic retinopathy, and glaucoma will emerge over the next few years. It is to be expected that this will lead to dramatic changes in the way these important ocular diseases are perceived, undoubtedly resulting in improved clinical management.

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