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Eyes on Chromosomes

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Ophthalmological geneticists are already accustomed to being in the lead. Colour blindness was one of the first human conditions to be studied genetically, and the linkages of the colour blindness and haemophilia loci that were studied by Bell and Haldane (1937) were the first human linkages to be recognized. Congenital cataracts were among the first serious genetic conditions to be treated effectively and this possibility of therapy may well have been the stimulus for the great researches of Nettleship and Usher soon after the discovery of Mendel's genetic work. Professor Arnold Sorsby continued, in Britain, the fine tradition these men established, and it is fitting to discuss here, in his Festschrift, a more recent 'first' that his chosen field of endeavour can now claim and which owes a good deal to his help and encouragement.

This is the assignment of the first disease locus to a specific autosome. The total nuclear cataract (Fig.), afflicting a large pedigree well studied by Nettleship (1909), has its locus, appropriately enough, on the first chromosome. Part of the evidence bears on the linkage of this cataract locus to the Duffy blood group locus, *Fy*, which was the first linkage to be found with computer assistance (Renwick and Lawler, 1963). The same computer programme also found the linkage between *Fy* and the 'locus', *Un1*, that uncoils the secondary constriction of chromosome 1. This linkage placed *Fy* and hence the total-nuclear-cataract locus on chromosome 1 (Donahue *et al.*, 1968; Ying and Ives, 1968).

Before discussing this new development further, it will be useful to consider the present outlook for linkage studies and to discuss certain problems of heterogeneity that arise.

Current Chance of Success in a Linkage Study

With their strong tradition of primacy in genetics, ophthalmologists may well be the first group of clinicians to accept the challenge of tackling fairly systematically the mapping of the major disease loci in their field. But, before embarking on such a programme, many of them will wish to know what is the rate of pay-off in the search for new linkages or assignments.

It can be estimated that only 1 autosomal locus in 60 will lie close enough to a particular marker locus (such as a blood group locus) to show less than 30% recombination with it. This would be a distressing figure but for the fact that the same blood samples used to study linkage between the disease locus and one marker locus can give phenotypic information for many more markers. There is an even chance that at least one marker will be close enough if 20 are tested. The numbers of samples necessary to detect this closeness depend on allele frequencies in the population to a great extent, but the testing of samples from 200 suitable individuals for 20 markers will probably be adequate for picking up about 50% of the markers 'close enough' to the disease locus, in the above sense. The over-all success rate of such a pedigree study would therefore be about 1 in 4. The prospects are improving every year with the discovery of more markers. The Table lists some of the suitable ones, further details being given in Race and Sanger (1968) and in Giblett (1969), including the addendum.

Cell hybridization techniques have recently opened exciting possibilities of mapping autosomal loci for which the phenotype can be scored in cell cultures (Migeon and Miller, 1968). Unfortunately, few eye conditions can yet be scored in culture, and these methods will not be discussed here.

Problem of Classification

Within one pedigree, there is often no difficulty in being sure that all persons manifesting a rare heterozygous disease have one abnormal allele at one specific locus. However, the problem of heterogeneity arises when several pedigrees are available. A different disease allele in each pedigree would not confuse the linkage analysis if all were known to be at the same locus. But otherwise, in each pedigree of the group, the offending allele might be at a distinct locus with its own linkage relationships. The phenotypes might well be indistinguishable. The

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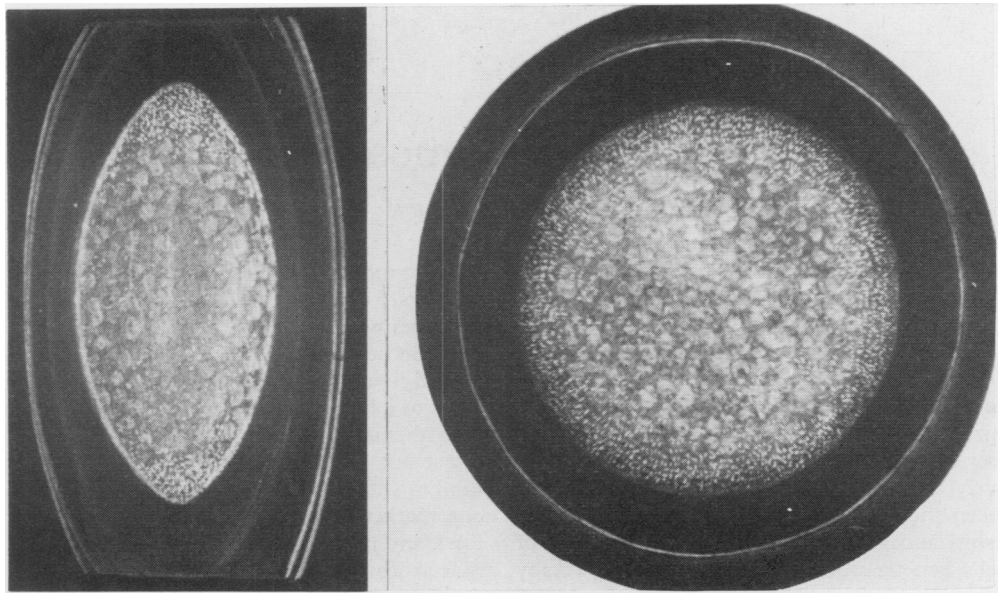

FIG. Painting of right lens of patient with total nuclear cataracts: slit-lamp microscopy. Reproduced from *Heredity in Ophthalmology* (François, 1961) by kind permission of Professor Jules François.

existence of such phenotypic mimicry by genotypes at two loci can be clear when one locus is on the X chromosome as proved to be the case in the mucopolysaccharidoses (gargoylism, etc.). Here it was only after this chromosomal heterogeneity of locus had been noticed that the clinical and biochemical delineation of the subtypes was undertaken. It was then appreciated that the X-borne (Hunter) allele (type II) usually produces no corneal opacities even in male hemizygotes, whereas the autosomal allele responsible in the homozygote for the type I form of the disease often does lead to corneal opacities.

From the point of view of research strategy in linkage work, this potential heterogeneity of locus puts a large premium on the choosing of those loci for which the heterozygous phenotype is distinguishable from homozygous phenotypes either directly (as the disease itself) or indirectly through some laboratory procedure. Such a strategy potentially allows a large number of offspring of heterozygotes in a single pedigree to be scored as recombinant or non-recombinant with respect to a marker locus. A clear-cut linkage of the relevant locus in *that* pedigree alone might therefore be established. Addition of another pedigree involves some risk that it might dilute the clear indications of linkage by adding data on a different locus producing a mimic disease condition. Unfortunately, even pedigrees of heterozygous conditions are often not large enough to stand alone in a linkage search, and full use has then to be made of the clinical phenotypes as criteria for a preliminary pooling of data from unrelated pedigrees.

Heterogeneity of locus is thus a ubiquitous difficulty in linkage work. But it is also a stimulus: the resolution of the heterogeneity (at a fundamental level) that can result from a linkage study constitutes, in itself, a valuable advance.

The difficulty and the stimulus are both well illustrated for the congenital cataracts. Reference has already been made to the assignment of the total-nuclear-cataract locus, *Cae*, to chromosome 1. The probability that *Cae* is linked to *Fy* is now 0·96, and the probability that *Fy* is on chromosome 1 is 0·98. The latter figure takes into account unpublished data of M. A. Ferguson-Smith, M. M. Izatt, and J. H. Renwick; M. Bobrow and R. A. Sanger; as well as earlier pedigrees (see Donahue *et al.*, 1968; Ying and Ives, 1968; Jacobs *et al.*, 1970).

It so happens that there is a slight hint that *Fy* may be linked also to the locus, *Caf*, of a more restricted cataract (a nuclear cataract). On one set of plausible prior probabilities, the probability that *Caf* is on No. 1, initially about 1 in 11, has been slightly increased, by data on two pedigrees, to about 1 in 4. (The lods for *Caf*:*Fy* are 0·769, 1·350, 0·685, $-3{\cdot}075$, $-\infty$ at 40, 30, 20, 10, 0% recombination, respectively.)

The *Cae*:*Fy* and *Caf*:*Fy* linkage data do not differ sufficiently to indicate non-identity of the

TABLE

POLYMORPHIC MARKERS USEFUL FOR AUTOSOMAL LINKAGE STUDIES IN EUROPE (FROM RENWICK, 1969, WITH ADDITIONS AND CORRECTIONS)

Autosomal Loci		Commonest Allele in Europe	
		Allele	Approximate Frequency
Scored by difference in antigenic properties	*Erythrocytes*		
	ABO	O	0·66
	MNSs	Ns	0·39
	P	P_2	0·52
	Rhesus	r	0·40
	Lutheran	Lu^b	0·96
	Kell	k	0·95
	Lewis	L	0·75
	Duffy	Fy^b	0·59
	Kidd	Jk^a	0·52
	Dombrock	Do	0·60
	Cartwright	Yt^a	0·96
	Cost	Cs^a	0·84
	Leucocytes:		
	Histocompatibility	$HLA^{2;;5}_{LA;;4}$	0·10
	Platelets:		
	Ko*	Ko	0·92
	Zw*	Zw^a	0·85
	Serum:		
	Gm	$Gm^{-1,2,-3}$	0·61
	Inv	Inv^{-1}	0·92
	Ag lipoprotein	Ag^y	0·77
	Lp lipoprotein	Lp	0·81
	Erythrocytes:		
	Acid phosphatase	AcP^B	0·60
	Phosphoglucomutase$_1$	PGM^1_1	0·76
	Phosphoglucomutase$_2$	PGM^1_2	0·999
	6-Phosphogluconate dehydrogenase	PGD^A	0·98
	Adenylate kinase	AK^1	0·96
	Adenosine deaminase	ADA^1	0·94
	Leucocytes:		
	Phosphoglucomutase$_3$	PGM^1_3	0·75
	Serum:		
	Haptoglobin	Hp^2	0·60
	Transferrin	Tf^C	0·995
	Protease inhibitor	Pi^M	0·95
	Cholinesterase$_2$	E_2^{-5}	0·96
	Group-specific component	Gc^1	0·72
	Complement (3rd component)	$C'3^S$	0·78
	Amylase, pancreatic†	Amp^S	0·93
	Amylase, salivary†	Ams^F	0·96
Scored by difference in sensitivity to enzyme inhibitor	*Serum:*		
	Cholinesterase$_1$	E_1^u	0·97
Scored on secretory property	*Saliva:*		
	Secretor ABH	Se	0·52

* Weerdt *et al.* (1963).
† Kamarýt and Laxová (1966).

Cae and *Caf* loci. The phenotypes do not quite clinch this question either. In the nuclear cataract (known to many as Coppock cataract), the tiny dot-like white opacities are more uniform in size though they otherwise resemble those of the total nuclear cataract. The two phenotypes differ in extent. The Coppock type involves only the embryonic nucleus, whereas the total nuclear cataract involves the foetal nucleus as well. It is tempting to conjecture that the smaller (Coppock) cataract results from a variant form of a protein synthesized in early embryonic stages and the larger one from a variant form of a protein synthesized in the later foetus as well as in the embryo. This would be analogous to the way in which haemoglobin polypeptide chains are synthesized sequentially during intrauterine development. Gamma-chain synthesis succeeds epsilon-chain synthesis as development proceeds, but here also it is not yet known whether their loci are linked. Unless these conjectures and analogies are misleading, *Cae* and *Caf* may well be distinct loci (linked or otherwise) coding for different but related polypeptides.

If more linkage data on *Caf*:*Fy* follow the pattern already observed, it may be possible eventually to establish the presence of two or perhaps more cataract loci on chromosome No. 1. This raises the question of clustering on human chromosomes.

Clustering

The task of making some sense out of the sequence of loci on human chromosomes will, some day, have to be undertaken. Many loci (or even most loci) are believed to have their origin in tandem duplications and multiplications that have become established features of the karyotype, with subsequent partial divergence in the functions of the locus and of its copies in the course of evolution. Thus, there would be a 'memorial' reason for expecting a clustering of loci with related function. A second reason stems from the work of Fisher (1930), Lewontin (1965), and other population geneticists, who have shown that there are evolutionary forces that tend to preserve such groupings and even build up others containing loci that interact in selection. This interaction has to be such that the selective advantage of genotypes in conjunction is not merely the product of the selective advantages of the genotypes at the various loci individually (Arunachalam, 1970). The selective interactions between cataract loci might well qualify if the loci carry polymorphisms of certain types. In these circumstances, even a loose linkage could confer an advantage over independence (non-linkage).

From considerations of this nature, and from the apparent paucity of forces that might neutralize clustering tendencies, it is probably safe to assume that clustering exists in man. Indeed, tight clustering definitely exists (e.g. $Hb_{\beta;\delta}$, $Gm_{2;1;3;4}$, $HLA_{LA;;4}$, $cb_{p;;a}$), and since there is no objective way of demarcating a boundary between tight and loose clustering, logical difficulties would arise in any attempt to deny the existence of loose clustering. At best, the attempt might place an upper limit on the magnitude of the loose clustering tendency

when defined in some arbitrary way. Tests of significance are therefore unlikely to be useful, in the sense of testing data for a clustering phenomenon—they will mainly indicate whether the sample size was adequate for its detection. In a more restricted context, however, significant testing does have value. Despite the presumed existence of true clustering, an appearance of clustering of a particular class of loci on a particular chromosome might still be spurious and due to chance sampling of loci. (For analogy, a random page from a London telephone directory could, by chance, point to an illusory excess of bookshops in Oxford Street rather than to the real cluster of departmental stores to be found in that street.) Here, statistical tests, to assess the possible influence of chance, might avoid a wrong conclusion that a real cluster had been observed.

(Despite the above comments on the presumed existence of clustering, the level of clustering in man may not be conspicuous. Unless it turns out to be much higher than in the mouse, it is unlikely to cause a big departure from the simple model of uniform distribution of autosomal loci, employed in most linkage analyses.)

The majority of loci will have selective interactions not merely with their antecedents but with many other loci. The present sequence of human loci, if known, would, therefore, represent the compromise complex of linkage relationships so far achieved in human evolution by a series of chromosomal rearrangements on the evolutionary road towards the 'optimum' for man. Since the fixation of a chromosomal rearrangement is slow, even on an evolutionary time scale, the 'optimum' must be in relation to all the changing environments man has met or will meet in the relevant epochs during which each rearrangement is 'under test'.

In many ways, the sequences of loci on the chromosomes are akin to the sequences of shops and businesses on the streets of a large city: all are the resultants of series of past accidents interacting with continuing economic forces. Certain businesses such as insurance offices and restaurants show considerable clustering; others, such as grocers much less. Some streets might seem to have a roughly random sequence; others to specialize to a high degree on one function. We can expect comparable inequalities in the degree of clustering of loci concerned with different functions and also in the degree to which clustering of any nature shows in different chromosomes. Only tentative attempts at measuring the intensity of clustering for any class of loci have yet been made, even for well-mapped organisms such as drosophila.

Genetic Classification of Heterozygously-determined Cataract using Linkage Relationships

Heterogeneity of locus has already been mentioned as a hindrance in linkage studies. It can also be considered as an important aspect of genotypic diagnosis itself. The problem can be stated in terms of the classification of early cataracts, excluding for the moment those ('recessive' cataracts) that reflect homozygous or hemizygous genotypes. It will be assumed that at one autosomal cataract locus there is, in the total human population, a series of rare alleles, any one of which, in heterozygous combination with a high-frequency (normal) allele, will produce some form of cataract; and that, at each one of an unknown number of other autosomal cataract loci, there is another series of alleles. We have, as yet, no simple way of deciding whether the allele or even the locus involved in one cataract pedigree is the same as that in another, except perhaps in a trivial case—where the two pedigrees are really one and can be traced to a common ancestor in historical times. We know from other organisms that only in a very general sense does similarity of cataract or other phenotype speak in favour of identity of allele or even identity of locus. Implication of fundamentally *different* polypeptides in the two pedigrees, if it should ever become feasible, would almost prove that two loci were involved. The opposite biochemical finding —implication of the *same* polypeptide in the two pedigrees—would be an almost reliable indication of one locus (unless true duplicate loci are to be reckoned with). These biochemical approaches should be pursued but meanwhile linkage relations offer some information. The critical *direct* test of linkage between the two cannot be undertaken because of the extreme rarity of the combined genotype—the double heterozygote. But *indirect* tests, involving linkage with a marker locus, are practicable. Convincing diversity of linkage relationships in the two pedigrees would connote two loci: similarity would somewhat favour one locus but never conclusively.

In short, genotypic diagnosis in man is usually incomplete, and linkage studies have a part to play in remedying this incompleteness. Such genotypic diagnosis has more than academic importance. In some of the early cataracts, for example, we are perhaps sampling alleles at some of the loci that are also concerned with senile cataracts. As soon as geneticists can arm themselves with knowledge of the linkage relationships of some of the loci for these early cataracts (that are relatively simple to study), they may begin to find it rewarding to study the

cataract loci in pedigrees in which presenile cataract is common. And later, even the high-frequency senile cataracts may become accessible to similar methods. For example, it may turn out that some of the common alleles, at several of these early cataract loci, can predispose, in certain combinations and certain environments, to senile cataract. The unlikeliness of this eventuality cannot be readily judged merely from dissimilarities in phenotype.

Several large pedigrees of early cataract studied in Glasgow give positive linkage findings (standardized likelihood reaching 10 or more) with some of the many marker loci tested, particularly with *Rh*, *Hp*, *Gm*, E_2, *ABO*, and *AK*. Unfortunately, apart from the *Cae*:*Fy* linkage, none of these linkage hints is convincing in any pedigree by itself, and a large arbitrary factor is introduced if resort is made to a combining of data from different pedigrees particularly if they have phenotypes that differ macroscopically or microscopically or in age of onset. On the clinical side, this arbitrary factor must be minimized by improvement in the discrimination of clinical phenotypes and it will probably be necessary to improve the techniques for the transfer of such information between research workers. For rather trivial examples, information might be conveyed by reference to specific illustrations in standard atlases such as Vogt (1931) or Waardenburg, Franceschetti, and Klein (1961); and by reproductions from direct photography or slit-lamp microphotography (whatever its limitations) as well as by verbal description of intrafamilial variation, and by tabulations of rate of progression (if any) and of age of onset.

Neither the clinical approaches alone nor the linkage findings alone are likely to reveal the number of loci and the range of phenotypes that each locus, through its series of genotypes, accounts for. Both approaches will be required and, because there is no lack of promising leads for several linkages involving cataract loci, the time seems ripe for this concerted attack on the genetical and clinical classification of this group of conditions. The mapping of some locus untroubled by mimics would be easier, but the mapping of cataract loci, by potentially contributing to a resolution of the cataract classification problem, might well be more rewarding. Finally and rather importantly, if suitable linkages can be found with markers testable on the amniotic cells or fluid, the first steps in fundamental control of early genetic cataracts can be made by enabling parents to choose termination of a high-risk pregnancy (Edwards, 1956).

Summary

An outline is given of some of the methods, the progress, and the prospects of mapping the loci of ophthalmic conditions on the human autosomes. The recent first assignment of a disease locus—that for total nuclear cataract—to a specific autosome (chromosome 1) is among the topics discussed. More general points are made about the variety of cataract loci that must be anticipated and about the possibility that these loci might be clustered on particular chromosomes.

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