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METHODOLOGY FOR FAMILY STUDIES OF GENETIC FACTORS

Report of a WHO Scientific Group

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WHO SCIENTIFIC GROUP ON METHODOLOGY FOR FAMILY STUDIES
OF GENETIC FACTORS

Geneva, 1-7 September 1970

Members :

- Dr W. F. Bodmer, Professor of Genetics, University of Oxford, England
Dr A. E. Boyo, Professor and Head, Department of Pathology, College of Medicine,
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Dr L. L. Cavalli-Sforza, Professor of Genetics, Institute of Genetics, University
of Pavia, Italy
Dr J. H. Edwards, Department of Human Genetics, University of Birmingham,
England (*Rapporteur*)
Dr G. R. Fraser, Associate Professor of Medicine, School of Medicine, University
of Washington, Seattle, Wash., USA (*Rapporteur*)
Dr O. Frota-Pessoa, Laboratory of Human Genetics, University of São Paulo,
Brazil
Dr A. Jacquard, Director of Research, National Institute for Demographic Studies,
Paris, France
Dr W. J. Schull, Professor of Human Genetics, Department of Human Genetics,
University of Michigan, Ann Arbor, Mich., USA (*Chairman*)

Secretariat :

- Dr I. Barrai, Chief, Human Genetics, WHO, Geneva, Switzerland (*Secretary*)
Dr N. E. Morton, Director, Population Genetics Laboratory, University of Hawaii,
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Dr K. N. Nazarov, Institute of Medical Genetics, Academy of Medical Sciences
of the USSR, Moscow, USSR (*Consultant*)

METHODOLOGY FOR FAMILY STUDIES OF GENETIC FACTORS

Report of a WHO Scientific Group

A WHO Scientific Group on Methodology for Family Studies of Genetic Factors met in Geneva from 1 to 7 September 1970.

The meeting was opened by Dr L. Bernard, Assistant Director-General, who welcomed the participants on behalf of the Director-General. He pointed out that the purpose of the meeting was to review the methodology for human genetic studies, which has developed greatly in the last decade. He noted the need in public health, particularly in efficient genetic counselling, for knowledge of all the factors that affect the transmission and expression of harmful genes. He pointed out the great importance, in view of the rapid growth of human populations, of developing methods for detecting any inherited susceptibility to disease, and for identifying differential fertility and mortality associated with specific genotypes. He hoped that the Group would review, in a wider and more critical way, the nature of the methods used for solving these problems.

1. INTRODUCTION

Several earlier reports¹ have dealt with the relationships and relevance of genetics to medicine and public health. To enhance the value of these reports and to provide proper perspective, it seemed appropriate to consider the methods in use for the analysis of family and population data for genetic purposes. The aim of the present report is to review the nature of these methods and to clarify some of the concepts and assumptions upon which they are based.

A preliminary requirement of any analysis is the evaluation of the type and format of data to be collected, although in human genetics collection is more frequently the result of opportunity than of design. In fact, data are collected more often on individuals than on sibs, and data on sibs are simpler to obtain than are data on the extended family. However,

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1964, No. 282; *Wld Hlth Org. techn. Rep. Ser.*, 1969, No. 416; *Wld Hlth Org. techn. Rep. Ser.*, 1970, No. 438.

collection of data with the family as the unit, or at least with the possibility of constructing family groupings, is desirable not only for family analysis but also for population genetics and demography.

2. SOME FUNDAMENTAL DEFINITIONS

Each gamete contains 22 autosomal chromosomes, or "autosomes", and one sex chromosome. The "zygote" (the product of fertilization) has, therefore, 22 pairs of autosomes and two sex chromosomes. The two autosomes forming a pair are termed "homologous". The genetic determinants of an individual consist of a large number of self-duplicating units, the "genes", which are distributed linearly on the chromosomes. Their positions are known as "loci". Genes that may replace one another at the same locus are called "alleles", an abbreviation for "allelomorphic genes". Loci on the same chromosome are termed "linked". Loci in the same place on homologous chromosomes are likewise termed "homologous".

The "phenotype" is what can be observed, as opposed to the "genotype", or set of genetic factors, which must usually be inferred, either directly from the phenotype or indirectly from patterns of inheritance of phenotypes in families. What appears as the phenotype is the result of the measurements and procedures used for observation. Refinements in these procedures, especially at the biochemical level, help to establish a closer link between phenotype and genotype, hence a clearer picture of the genetic determination of differences in phenotype. This clarification is especially important in the case of disorders that do not exhibit simple Mendelian inheritance. It is important also in distinguishing disorders that are clearly inherited from closely similar disorders that either are inherited in a complex way or not simply inherited or are the result of environmental factors. Those due to environmental factors have been called "phenocopies". Since, however, refinements in methods of observation should eventually lead to a distinction between inherited disorders and "phenocopies", we prefer not to use the term "phenocopy" in this report.

3. SEGREGATION ANALYSIS

The mechanisms of inheritance have as their end result the generation of phenotype probabilities, characteristic of a given mating type in a specified environment. These are called "segregation frequencies", and include not only classical Mendelian proportions, such as $1/4$ and $1/2$, but also those that have been altered by differential mortality or partial manifes-

tation, as well as empirical frequencies, the genetic basis of which may not be clear. In this report, "segregation" is taken to mean the presence of two or more phenotypes in a sibship.

Although segregation frequencies are specified either by genetic hypothesis or, empirically, from analysis of a series of matings, the actual proportions observed in any particular family depend on gene frequencies, chance, the way data are collected, and other factors. The statistical technique by which these factors are studied is called "segregation analysis".

Simple methods were developed by Weinberg (see Crow, 1965, for details) and other authors, assuming a uniform segregation frequency. These methods are useful for small numbers of families and for preliminary analysis of large masses of data. More powerful methods for detecting and interpreting genetic heterogeneity require a computer or tables of scores (Morton, 1969); they are useful primarily for large numbers of families.

3.1 Segregation frequency

It is convenient to consider only two mutually exclusive phenotypes, "normal" and "affected". This involves no loss of generality, since n phenotypes may be examined pairwise in $n - 1$ independent ways. "Sibship", "family", and "mating" are used interchangeably to denote sets of children. The number of affected children in a sibship of size s is conventionally designated by r , and a sibship in which $r = 0$ is called "non-segregating".

Within a random sample of sibships, the expected segregation frequency (p) may be constant, or there may be a proportion (h) of families that cannot segregate,¹ or a mixture of two or more segregation frequencies, or continuous variation as in multifactorial inheritance. By comparing the distribution that is observed with the distributions that are expected on the basis of these genetic models, it is theoretically possible to unravel even quite complex genetic patterns, and thereby to provide specific and reliable recurrence risks. In practice, the small size of human families makes it difficult to distinguish between the more complex patterns.

In some cases, the effect of a detrimental gene manifests itself more frequently as the birth order of the child increases (for example, erythroblastosis due to rhesus incompatibility). Such phenomena may lead to an ascertainment bias and to segregation frequencies that are difficult to interpret. In such complicated cases it is appropriate, where a sufficient

¹ Such an admixture of non-segregating families may be due to various mechanisms, which are fully discussed by Morton (1969).

number of families are studied, to consider each size of family separately. It should also be noted that limitation of reproduction after the birth of an affected child may cause biases in estimating the segregation frequency.

3.2 Frequency of sporadic cases

Genetic heterogeneity sometimes produces a mixture of sibships with two different segregation frequencies, one substantial (p) and the other low (m). For example, if acquired deaf-mutism, due to such factors as fetal infection, is not so diagnosed, it will be confounded with hereditary deaf-mutism due to a recessive gene (with $p = 1/4$ from normal carrier parents). Since the recurrence risk for acquired deaf-mutism (m) is very small, most cases will be isolated ($r = 1$). By chance, however, many cases of hereditary deaf-mutism in small sibships are also isolated. Therefore, a "multiplex" sibship (with two or more affected sibs) can with virtual certainty be assigned to the high-risk category, whose segregation frequency is given by p , whereas a "simplex" family (with only an isolated case) may be either low-risk or high-risk.

The frequency of sporadic cases (x) is defined as the proportion of cases in a population with a low recurrence risk (m), which can be estimated in random sibships. If only segregating families are ascertained, no reliable estimate of m can be made, but x can still be determined if m is negligible, i.e., if all low-risk cases are isolated. This model is applicable to many instances of genetic heterogeneity in which sporadic cases are due to primarily acquired causes of an unidentified nature, illegitimacy, or mutation.

If x is appreciable, and even when the recurrence risk is high after two or more affected children, the recurrence risk may be very small for sibships with only an isolated case, decreasing with the number of normal sibs. Analysis of this kind of heterogeneity is of obvious importance, therefore, both for genetic counselling and as a stimulus to the development of procedures for the differential diagnosis of sporadic cases.

3.3 Probability of ascertainment

Much of the complexity in analysis of rare phenotypes (and, therefore, in clinical genetics) is due to non-random sampling of families, with omission of those in which an affected individual was not present. This eliminates all non-segregating families with normal carrier parents. Among segregating families, the probability of detecting a family increases with the number of affected children.

Biases due to such non-random sampling must be removed if the genetic parameters are to be estimated correctly. In this way, the analysis will provide an estimate of the prevalence of a condition in the general

population, which, for a rare phenotype, it is not feasible to estimate otherwise (Barrai et al., 1965).

Random sampling of families through the parents, without consideration of the phenotypes of the children, is called "complete ascertainment". Selection of families through the children with exclusion of non-segregating families is "incomplete ascertainment", analysis of which is based on the concept of a "proband". A proband is defined as an affected person who, at any time, is detected independently of the other members of the family and whose detection should, therefore, be sufficient to ensure selection of the family in the absence of other probands. The term "propositus" is sometimes used as a synonym for proband, and is sometimes restricted to the proband first ascertained (the "index case"). Probands may be ascertained through hospital records, death certificates, inquiries to physicians, examination of a population sample, or other direct means. Affected individuals not represented in these primary sources, and detected only through family study of probands, are called "secondary cases". The number of probands in a family is designated by a , so that the number of secondary cases is $r - a$.

The concept of a proband leads directly to the "ascertainment probability". If there are R affected individuals in a population, A of whom are detected as probands, then the ascertainment probability is defined as $\pi = A/R$; it is the probability of an affected member of the population being detected as a proband. With incomplete ascertainment, an estimate of π is essential for a valid analysis of segregation and for determination of R . In the general case of "multiple ascertainment", there may be between 1 and r probands in an ascertained family with r affected, and each proband may have $t > 0$ ascertainment. The two limiting cases are "truncate ascertainment" ($\pi = 1$), in which segregating families are sampled at random, so that families with many affected children are no more likely to be selected than are families with only one affected child; and "single ascertainment" (π almost zero), in which the probability of ascertainment is so small that there is virtually no chance of having two probands in the same family, and the probability of a family being ascertained is proportional to the number of affected children. Information about sporadic cases increases with π . Analysis of the numbers of probands (a) and of ascertainment per proband (t) gives an estimate of π that is reliable if ascertainment are independent. Methods are available for estimating π when ascertainment are not independent, but the results are less reliable. The investigator should therefore make every effort to define probands and ascertainment appropriately and to use both sources of information in segregation analysis, while being aware of the ascertainment problem. The definition of probands and ascertainment may be difficult with certain methods of ascertainment, for example, through membership of societies for the handicapped. Fortunately, small departures from

the ascertainment model do not seriously disturb segregation analysis, but a gross error, such as assuming truncate ascertainment under conditions of single ascertainment, or *vice versa*, can lead to erroneous conclusions.

4. PARAMETERS OF POPULATION STRUCTURE

At least a third of the loci whose products are available for study in man have two or more frequently occurring alleles, which are said to be "polymorphic". The origin and maintenance of this variability is a basic problem of population genetics. The major issue is the extent to which contemporary selection pressures, particularly heterozygote advantage, are needed to maintain this high level of variability. If all alleles that are polymorphic in a population are independently subject to heterozygote advantage, an impossible overall selective burden accrues. However, much of the variability could, in principle, be due to neutral genes that have, by chance, achieved polymorphic proportions. The problem, in its simplest form, is to evaluate the relative contributions of these two factors, namely, selection and random genetic drift (chance).

It is presumed that the solution to this dilemma lies in the structure of populations. In this context, population structure may simply but broadly be defined as the array of all those factors that contribute to the persistence, loss, or spread of genetic variation. Three aspects of this structure appear particularly relevant to man and to the issue first stated; these are (a) the effect of migration and inbreeding on gene frequencies, (b) the relation between gene frequencies, on the one hand, and phenotype and mating frequencies, on the other, and (c) the statics and dynamics of genetic variability as revealed by inbreeding and outcrossing. These problems may be described in terms of a number of parameters, conspicuous among which are the following: coefficients of migration, kinship, and inbreeding; effective population size; selection coefficients; gene frequencies; and mutation rates.

4.1 Coefficients of inbreeding and kinship

Individuals who have one or more known common ancestors are said to be biologically related. Marriages between such persons are termed "consanguineous", and the offspring derived therefrom are said to be "inbred" with reference to an ideal population of infinite size in which gametes pair at random; such a population is said to be "panmictic". When not due to selection, departures from the random association of uniting gametes are measured by the coefficient of inbreeding, which Wright (1922) defined as the correlation between uniting gametes; this

coefficient can vary from -1 to $+1$. More recently, Malécot (1948) defined the coefficient of inbreeding (F) as the probability that, in an infinite population, a diploid individual will possess two genes at a given locus that are identical by descent, i.e., traceable to a single ancestral gene; he has shown that this probability is equal to the "coefficient of kinship" (sometimes termed the "coefficient of co-ancestry") of the individual's parents. The coefficient of kinship is a measure of the genetic resemblance between any two individuals, and is the probability that two alleles, one from each individual, are descended from the same gene. These definitions, despite interpretative differences, lead to identical results in calculating the coefficient of inbreeding based upon pedigrees, the so-called "pedigree F ".

If a representative array of individual coefficients of inbreeding in a population is averaged, the mean coefficient of inbreeding of the population is obtained. This mean can be used as a parameter of population structure (see Wright, 1965); as such it is, however, precise only with respect to those loci at which there are no significant selective differences among the possible genotypes. In practice, the simple averaging of a sample of inbreeding coefficients generally results in an underestimation of the true mean, because of the systematic under-reporting of remote relationships. Other methods of estimation, therefore, are commonly advocated (Li & Horvitz, 1953; Yasuda, 1968).

4.2 Determination of inbreeding coefficients

The estimation of an individual's coefficient of inbreeding ultimately involves some form of pedigree analysis. This may be explicit, in that a pedigree is actually drawn and analysed, or implicit, in that a statement of relationship is volunteered by the individual or individuals in question, presumably on the basis of family knowledge. Clearly, a variety of errors may occur. These range from acceptance of the remarks of a well-intentioned but misinformed person about non-maternity or non-paternity, to consistent under-reporting of remote relationships through ignorance or lack of interest. At all events, it is desirable to inquire into the actual position of each individual in a pedigree rather than to use terms for remote kinship, which are often used in a biologically imprecise way.

Efforts to appraise the reliability of consanguinity information obtained at interview suggest that facts of relationship are usually ascertained correctly whereas degrees of relationship sometimes are not. While it is clearly hazardous to extrapolate the findings in a few countries to the world at large, such findings suggest that, in large-scale studies in which economies can be made through the use of interviews, the data so obtained will be reasonably accurate if adequate care is exercised in their collection.

The effects of systematic under-reporting of remote relationships have been evaluated directly by estimating, from genealogies, the percentage contribution to the mean coefficient of inbreeding of a remote relationship (where "remote" is arbitrarily defined), and indirectly by comparing the mean coefficient of inbreeding based upon pedigrees with that derived from analysing population structure and the frequencies of certain blood group systems. If a remote relationship is defined as one in which the coefficient of inbreeding is less than $1/32$, then remote relationships contribute to the mean coefficient of inbreeding by 10% in Japan, 18–26% in Brazil, and 51% in Wisconsin. An example of indirect evaluation is the estimation that, in Switzerland, the mean coefficient of inbreeding based upon the ABO blood groups agrees closely with that derived from genealogical data; hence the amount of remote consanguinity that is not detected in pedigrees appears to be negligible. Again, the generality of these findings is unknown, and broader experience, based upon more countries and levels of social and economic organization, is obviously desirable.

Estimates of the "mean" coefficient of inbreeding are generally either (a) the average of a series of presumably representative individual coefficients, with or without adjustment for unascertained remote relationships, or (b) derived from the departure of an observed distribution of phenotypes, or mating types, from that expected under random mating in a large population, i.e., from a consideration of the structure of the population (Li & Horvitz, 1953; Yasuda, 1968), or (c) based upon the frequency of isonymous marriages, i.e., those between individuals with the same surnames (Crow & Mange, 1965). The errors inherent in these various approaches differ. Those to which estimates of the first kind are subject have been described. Estimates based upon individual phenotype and mating type frequencies are sensitive to sampling biases, differential selection, illegitimacy, and phenotypic misclassification. Errors associated with estimates based upon isonymy are largely those related to the practices of giving surnames. These include changes of name or spelling, adoption, illegitimacy, and the possible polyphyletic origin of a given surname. The importance of each of these varies from culture to culture. An estimate based upon deviations from Hardy-Weinberg values,¹ whether based upon phenotypes or mating type frequencies, requires a substantial sample if the estimate is to have a small variance.

4.3 Use of inbreeding coefficients in genetic analysis

Coefficients of inbreeding are generally used either to provide some insight into population structure or to appraise the consequences of increas-

¹ If p be the frequency of allele A and q the frequency of allele a in a random mating population, and if $p + q = 1$, then the frequencies of genotypes AA , Aa , and aa will be p^2 , $2pq$, and q^2 , respectively. The values of these three genotype frequencies are known as the "Hardy-Weinberg values".

ing homozygosity upon mortality, morbidity, and fertility. In the first instance, the coefficient of inbreeding (or kinship) may be used, for example to determine the extent of isolation by distance in continuous populations, or to explore the rate of decay of heterozygosity in a population with a particular mating formula. Such uses do not necessarily involve the ascertainment of specific consanguineous matings; the coefficient of inbreeding is inferred from theoretical considerations, and represents a statement about a randomly selected pair of spouses, or a randomly selected pair of individuals in the case of the coefficient of kinship.

In appraising the effects of inbreeding upon mortality, morbidity, and fertility, it is necessary to ascertain a representative array of matings having different coefficients of inbreeding. Unions of related individuals have been ascertained in a multitude of ways. Often, the population sampled is unknown and possibly unknowable, or the randomness of the sampling is open to question. These inadequacies compromise, to a greater or lesser extent, the value of the various studies, irrespective of the method of selecting the comparison group of biologically unrelated spouses, i.e., those with a coefficient of inbreeding equal to zero. The selection of this latter group can be critical, since poor selection may obscure a true effect of inbreeding or create a spurious one.

Selection of the comparison group aims to eliminate or to minimize extraneous variability, particularly that which may be confounded with inbreeding. Three main comparison groups have been favoured: a random control, sibs, and close neighbours. The random control is commonly obtained from the unrelated group, which was identified by the process that generated the related group; since the former is usually much larger than the latter, systematic or simple random sampling occurs. There is, of course, no specific "one-to-one" matching involved. In the second comparison group, a married sib, unrelated to his or her spouse, is chosen for comparison, and in the third, residence proximity is the basis for selection (other factors may also be matched). Cogent arguments can be advanced for each of these methods of selection. For example, proximity of residence may reduce socio-economic variability; or the choice of sibs may minimize a variety of socio-economic differences. But each method has its disadvantages. Irrespective of the choice, it is highly unlikely that the investigator will be absolved from some form of covariance analysis; the form of the latter may be markedly simplified, however, by a wise choice of comparison group.

Analysis of data relevant to the effects of consanguinity and inbreeding upon mortality, morbidity, and fertility generally involves regression of the latter variables upon the coefficient of inbreeding, with or without allowance for extraneous variates. A variety of questions relevant to the form of this regression remain unanswered, for the simple reason that the range of F-values encountered in human populations is too small to reveal

nonlinear effects, unless the sample is astronomical in size. Countries with either high frequencies of inbreeding or unusually closely related marriages, or both, are particularly interesting in this regard.

4.4 The genetic load

The genetic load of a population is generally defined in two different but related ways. It may refer to the amount of deleterious mutations, at both the genic and the chromosomal level, in the genetic make-up of a population; or it may refer to the amount by which the adaptive value of a given population is decreased in comparison with the optimal genotypes, or to the excess of specific events responsible for this decrease (precocious mortality, morbidity, sterility, etc.). In both cases, any genetic variation that affects fitness affects the "load". The contribution, if any, of a given locus to the "load" of a population can be classified on the basis of the mechanism by which the load originates. A variety of alternative mechanisms are recognized; these include mutation (when the detrimental alleles are maintained in equilibrium in the population by mutation pressure, in spite of their continuous elimination by selection pressure), segregation (the heterozygote is presumed to have the highest selective advantage), and incompatibility (due to maternal-fetal incompatibility). Examples of these three types of load are those due to haemophilia, haemoglobins, and the Rh locus.

Genetic load theory is applicable only to intrapopulation studies. It provides a tool for comparisons between genotypes belonging to the same population, as well as for measuring their impact on the average adaptive value of that specific population. Since the homozygosity rate is higher among the offspring of consanguineous than of non-consanguineous marriages, inbreeding reveals a load that is largely concealed in panmictic populations. Morton, Crow & Muller (1956), and Crow (1958) developed a theory that, granted certain assumptions, permits the measurement and interpretation of this particular load. The measurement is generally made in terms of lethal or abnormal equivalents, depending on the variable that is being investigated. Although the validity of the model and the interpretation of the findings it produces have been questioned (see Schull & Neel, 1965), there remains a large element of support for these theoretical developments.

5. INBREEDING AND MIGRATION

As first shown by Malécot, kinship may also be predicted from the migration matrix, whose elements are the probabilities that an individual reproducing in one population was born in another (Bodmer & Cavalli-Sforza, 1968). There is a dearth of such demographic information for

many populations, especially pre-industrial ones. Knowledge must also be available of population sizes, which have to be corrected for the fact that more than one generation is represented in a living population. This requirement is met by calculating the "effective population size" (N_e), which is approximately one-third of the total population size. It can be estimated accurately on the basis of adequate genealogical and/or demographic data (Cavalli-Sforza & Bodmer, 1971).

In lieu of place of birth, other subdivisions may be considered for building the migration matrix, e.g., clans, tribes, or even socio-economic classes. Combinations of geographic and other classifications may have to be considered for a full description of the population, depending on its characteristics. Thus, for a highly mobile population birthplaces may be insufficient for a full description, but this is not necessarily so. For populations having strict marriage rules, sociological subdivisions may be adequate.

The comparison of kinship coefficients obtained by different methods can help greatly in understanding problems of population structure. Each method supplies a different type of information; that obtained from migration matrices or by simulation gives results that are expected to be close to those obtained from extended genealogies or isonymy—apart from the errors discussed before, which affect every method. When such estimates are close to those obtained from the distribution of a given genetic marker, the implication is that the latter is largely uninfluenced by selection. Work along these lines will help to solve the problem mentioned at the beginning of this section, namely, the evaluation of the relative contributions of selection and drift.

6. ESTIMATION OF LINKAGE IN HUMAN DATA

Gene loci situated on the same chromosome are said to be "linked" and to form a "linkage group". Detection of linkage involves the identification of loci forming such linkage groups and the subsequent assignment of a linkage group to the appropriate chromosome. Estimation of linkage involves measuring, in an indirect way, the relative distance between loci forming part of the same linkage group. This "distance" is related to the frequency of recombination between the loci in question during gamete formation; as the distance becomes larger, it becomes increasingly difficult to distinguish the behaviour of linked loci from that expected of loci situated on different chromosomes, i.e., there is independent segregation.¹ Both the identification and the estimation of linkage present considerable additional

¹ Independent segregation implies that the segregation of two alleles at one locus is unrelated to the segregation of two alleles at a second locus. A parent of genotype AaBb would thus produce AB, Ab, aB, and ab gametes in equal proportions.

methodological problems in man, as compared with other organisms, since experimental or directed breeding is impossible.

The first approach to the problem was based on the concept of scores, which measure, in family groups of appropriate types, deviations from independent segregation of pairs of alleles at two loci. Various types of such analyses have been used. The scores of choice at present are those developed by Morton (1955, 1956, 1957; see also Smith, Penrose & Smith, 1961). They express the probabilities of various family configurations, under the prior assumption of a range of recombination values; they are expressed as logarithms and thus are additive over multiple families. These tables can easily be used with two-generation material; they undoubtedly represent the best method of screening human data for the presence of linkage.

Another technique, which was developed by Penrose (1935, 1946, 1953) and which continues to be useful for screening more limited data involving only one generation, is the "sib-pair method". This depends on deviations, caused by linkage, from independence of association of alleles at two loci in pairs of sibs; the method is subject to misuse, for example, when attempts are made to analyse "linkage" involving such quantitative traits as eye colour and hair characteristics, which are not controlled by simple Mendelian inheritance.

It is interesting, as Haldane (1949) pointed out, that there is a special situation, involving persons with recessive conditions born of consanguineous parents, in which even single individuals can provide information on linkage. Clearly, in the extreme case of complete linkage (i.e., with a recombination frequency of zero) between the allele causing the recessive condition and a marker locus, such persons will always be homozygous at that marker locus, provided that both alleles causing the autosomal recessive condition in question are derived from a common ancestor. The method can be extended to the study of linkage between marker loci.

As in so many other fields where statistics and human genetics meet, the computer has revolutionized the study of linkage in man. Thus, generally applicable programmes are now available, which extend the scoring procedures discussed above to provide, for any specified linkage value, probabilities of configurations of alleles at two loci found in series of pedigrees of any degree of complexity; such calculations would have been impracticable before the computer era. Once these probabilities are calculated, the problems remain of whether they indicate that a recombination fraction lower than 0.5 is in fact present (detection of linkage) and, if so, what is the most probable value (estimation of linkage). In the case of X-linked loci, of course, only the second of these problems is relevant, since the fact that a gene locus is on the X chromosome will be obvious from simple pedigree considerations.

Despite this facilitation of linkage calculations by the use of scores and by the use of computer programmes, which were developed by various investigators, prominent among whom is Renwick (1969), many difficulties remain in the detection and estimation of linkage. Some are of a theoretical nature and include statistical problems concerning the most appropriate methods of analysis and significance testing; in particular, there is much controversy concerning the roles of sequential analysis and of Bayesian methods based on the prior probability distributions of linkage values. Both have their advocates, but both have disadvantages. Thus, it is somewhat doubtful whether sequential analysis is as appropriate in a situation involving the collection of human data as it is when the quality of an industrial product is in question. Again, the prior probability distribution, on which the application of Bayes' theorem depends, is at best uncertain in the case of linkage values. All these methods of estimation and detection of linkage are based on measuring likelihood and, in fact, such refinements as sequential analysis or the application of Bayes' theorem should not lead to any substantial discrepancy in the inferences that may be drawn from the data. Another theoretical problem that has been the subject of much discussion is the nature of the most appropriate function relating recombination fractions to chromosomal map lengths; again, the use of the various functions proposed should not introduce important discrepancies.

Other difficulties are of a more practical nature. At many loci they include the very widespread phenomena of variable expressivity and failure of penetrance, as a function of the level of observation, and the resultant difficulty in scoring the genotype. Other problems are caused by uncertainty of parentage and by technical difficulties that may lead to errors in genotyping.

Despite these obstacles, as knowledge of marker polymorphisms increases, there is every prospect that gaps in the present rather scanty linkage map of man will begin to be filled, and the rate of this process is likely to follow a geometric rather than an arithmetic progression. This rate will doubtless be further accelerated by the additional exploitation of karyotypic variation, normal and abnormal, and it may well be revolutionized by advances in the techniques of somatic cell genetics.

The potential use of karyotypic variation may be illustrated by the complete genotyping of persons with Down's syndrome (trisomy 21) and of their parents. Deviations of allele frequencies at a locus from those found in a control population may indicate that the locus in question is situated on chromosome 21; more substantial evidence may be afforded by inconsistencies between parents and affected children with respect to marker loci, due to simultaneous transmission of both alleles from a heterozygous parent. Although no assignment of a gene locus to chromosome 21 has been made by this method, a similar approach has led to the

probable assignment of the locus controlling haptoglobin production to chromosome 16. The Duffy blood group locus has been assigned to chromosome 1, because in a large family study a harmless morphological variant of that chromosome segregated together with alleles at that locus (Donoghue et al., 1968).

As regards methods involving somatic cell genetics, the locus determining the enzyme thymidine kinase has been localized by a method involving hybridization of human and mouse cells. In culture, such cells tend to expel the human chromosomes at replication. When a hybrid strain was created using mice that were genetically deficient for this enzyme, it was found that a human chromosome that was either 17 or 18 was obligatory for survival of the cell strain, suggesting that the locus in question is situated on this chromosome. Another recent use of somatic cell methods involves analysis of pairwise associations of enzyme electrophoretic differences, in similar hybrid cell strains between the mouse and man. This approach has led to the establishment of an autosomal linkage between the loci determining lactate dehydrogenase B and peptidase B.

6.1 The value of linkage in human genetics

As information accumulates concerning the nature of Mendelian variation, the logical next step from the point of view of the formal genetics of man is to consider the topography of the loci thus defined on the chromosomes, i.e., their linkage relationships. Once a substantial part of the linkage map has been filled in, this information has several possible applications. Some are theoretical, such as deductions that can be made from non-random arrangements of gene loci concerning evolutionary problems. These deductions are related to questions concerning the effects of selection on linked loci, common control mechanisms of linked loci, and the role of duplication of chromosomal DNA in evolution. Thus, for example, it is natural to expect that duplication of DNA would lead to clustering of loci with closely related functions. Such duplications would be exposed to two opposing tendencies, one towards random dispersion through chromosomal rearrangements and the other towards stability of clusters due, firstly, to selection acting on entire gene complexes and, secondly, to the development of common control mechanisms. Already, the fragmentary information available about the patterns of chromosomal distribution of gene loci in man is supplementing data from other species in throwing light on the above-mentioned evolutionary processes.

6.2 The recognition of the heterozygous state and other applications to genetic counselling

Other applications of increased knowledge of linkage relationships are more practical, being largely connected with possible improvements

in the scope of genetic counselling. Thus, help may be afforded in discriminating between multiple loci responsible for genetically heterogeneous clinical conditions such as deafness, blindness, and mental retardation. Information about heterozygosity at loci determining autosomal recessive diseases may be provided by linkage studies of this type of disease, where such heterozygosity is not detectable by more direct methods. Another potential use of linkage information is in the fetal diagnosis of genetically determined disorders, and in the detection of such disorders postnatally in cases where the age of onset of overt disease is late; Huntington's chorea is an example of this type of disease, in which the discovery of closely linked marker loci might be very valuable.

Lastly, gamete selection involving separation of X and Y sperm is likely to be possible in the near future, and is of very great relevance to the prevention of X-linked diseases. In the more distant future, linkage information may provide means of separating sperm formed by heterozygous males into those carrying the normal and those the pathological allele.

7. THE PROBLEM OF CARRIER DETECTION

An important prerequisite for genetic counselling is the detection of heterozygous carriers of detrimental genes. A simple example will illustrate this. A young woman, who has a haemophilic maternal uncle, may be a normal carrier of the X-linked gene that produces haemophilia when in the hemizygous state, i.e., when located on the single X-chromosome of a man. If she is a carrier, the risk of her first child being haemophilic is 25%, and the risk of her having at least one haemophilic child among three is greater than 50%. However, the young woman may be a non-carrier, in which case her offspring, barring mutation, are at no risk of being haemophilic. The above information is of little help to such a person, however, unless it can be determined whether she is a carrier or not. Her course of action will presumably reflect her degree of apprehension at the possibility of having an affected child. Ideally, the geneticist would be able to recognize carriers without failure. This is, however, seldom the case. On the other hand, for a number of diseases and defects it is possible to calculate the probability of a given person being a carrier. This probability, combined with the risk of a carrier giving birth to an affected child, leads to an overall probability that is useful as a basis for genetic counselling.

Two general complementary methods can conveniently be combined by mathematical manipulation to produce an estimate of the probability of a person being a carrier; these are (a) study of the pedigree of such a person, and (b) phenotypic study of the person by means of clinical examination plus biochemical and/or physiological tests. In the previous example, the pedigree approach leads to a probability of 25% that the woman is

a carrier, assuming, of course, that her haemophilic uncle does not represent a fresh mutation. This evaluation can be improved by taking into account other features of the pedigree. For example, if she has many non-affected brothers there is less probability that her mother, and hence she herself, is a carrier.

7.1 Ways of detecting carriers

Estimates vary of the proportion of normal persons who are carriers of at least one mutant allele that is potentially capable, when in homozygous form, of causing a severe recessive condition. However, such persons may well constitute a majority.

The classical definition of recessive demands that the heterozygote (Aa) and the dominant homozygote (AA) be indistinguishable in the phenotype. This is not always true, and the difference may be small or large. Phenotypic detection of carriers in such cases consists of applying techniques capable of revealing these differences, in order to recognize heterozygous individuals.

A number of recessive mutant genes lead to the production of no detectable structural enzyme or protein. If such a gene is located on an autosome, the heterozygote (Aa) differs from the normal non-carrier (AA) only in respect of the number of A genes. When no obvious difference is noticed between the AA and the Aa individuals, just one A must be sufficient to induce synthesis of the gene product in sufficient amounts to lead to normality. Under some stress conditions, however, the organism may be unable to function as efficiently with one A gene as with two; this is illustrated by the carrier of the phenylketonuria gene. On normal diets, the serum level of phenylalanine in the carrier is indistinguishable from that in the non-carrier. If a massive amount of this amino acid is administered to carriers and to non-carriers, a transitory rise in the phenylalanine level is observed in the blood of both, but the level returns to normal faster in non-carriers than in carriers. Through these loading tests carriers can, therefore, be detected.

In other instances, the carrier is recognized by a secondary effect of the gene. In Duchenne's progressive muscular dystrophy, an X-linked condition, the enzyme creatine phosphokinase is increased in the blood, not because the mutant gene produces an excess of the enzyme but probably because the membrane of dystrophic muscle fibres leaks proteins.

Loading tests can be as efficient in the case of X-linked genes as in the case of autosomal genes, and two other means of phenotypic detection of carriers exist because of the Lyon effect.¹ When a gene has a topogra-

¹ Random inactivation of one sex chromosome in female cells, so that approximately 50% of the cells have one X chromosome inactivated, 50% the other X chromosome.

phical kind of manifestation, it may be possible to observe directly a qualitative mosaic at the cellular level. Where no such topographical manifestation occurs, specific tests may be needed to demonstrate a mosaic at the cellular level. For example, in blood or fibroblast cultures obtained from carriers of the gene for G6PD deficiency, two types of cell can be distinguished through a specific colour reaction, some having the enzyme and others not.¹

In the case of a diffuse manifestation of the gene, the individual can often be recognized as a carrier as the result of a decreased amount of gene product. This is the case with some carrier females of the haemophilia gene.

7.2 Probability aspects of carrier detection

In a number of cases the detection of carriers is hampered by the fact that the quantitative distributions of carriers and normal homozygotes overlap. In carriers of the gene for haemophilia (A or B), levels of the anti-haemophilic factor vary from the normal range down to very near those of affected males; in the latter event, they can even present clinical symptoms of haemophilia. A similar situation obtains in Duchenne's progressive muscular dystrophy. Only about 70% of carriers have abnormally high serum levels of creatine phosphokinase; the others cannot be distinguished by this means from normal homozygotes.

This great variability of expression found among carriers of X-linked detrimental genes is explicable in terms of the Lyon effect. The differential inactivation of an X-chromosome appears to be random; some carriers thus happen to have more cells in which the paternal X-chromosome is inactivated, than others. This creates a difficulty in carrier detection, which can be met by ascribing to each person under study a probability of being a carrier, rather than a label of the "yes" or "no" type. With regard to counselling, this situation is not as inconvenient as it seems at first; after all, counselling is always based on probabilities. Even when the carrier state is verified without doubt, the transmission of the detrimental gene to an offspring is a matter of chance. When the carrier state is doubtful, a compound probability can be calculated, which offers to counselling the same type of support as does segregation frequency in those cases where the carrier state can be proved.

To increase accuracy in estimating the probability of an individual being a carrier, the results of any number of biochemical tests, phenotypic examinations, and pedigree data can conveniently be combined. An extended account of the technique is to be found in Murphy & Mutalik (1969).

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 366, p. 35.

There is, therefore, an urgent need to develop techniques for the detection of carriers. This detection is very important, for example in the case of female carriers of X-linked deleterious genes, who are at high risk of producing affected offspring, irrespective of the men they marry.

8. RECURRENCE RISKS

When one member of a family has a particular disorder, the probability that another member of the family, often a sib, will have the same disorder is the "recurrence risk". This probability provides one of the bases for counselling in genetic diseases.¹ In simple cases it is the same as the expected segregation frequency, which can easily be calculated when the genotypes of the individuals concerned are known. However, when the expression of the disorder is modified in part by the environment or by other genes, empirical risks have to be estimated from data on the incidence of the disorder among relatives of affected individuals. These sources of data are the same as those needed to assess whether a disorder has a simple genetic basis and, if not, how it may be genetically determined.

Undoubtedly, the most important initial step is diagnosis. Very similar disorders, such as the different forms of muscular dystrophy or of mucopolysaccharidoses, may have quite different modes of inheritance. Disorders such as schizophrenia, diabetes, and many forms of mental deficiency, which are not inherited in a simple Mendelian fashion, may be especially difficult to define clearly. Moreover, the recurrence risk may vary in such cases; it usually increases with the severity of the disorder. Clearly, a mistaken or inaccurate diagnosis is likely to lead to an incorrect assessment of the recurrence risk.

8.1 Estimation of the risk

There are three main categories of disorder, defined according to their mode of inheritance. They are (a) disorders that are determined by one or more recognizable genes, and that follow Mendelian patterns of inheritance; (b) disorders that are discrete but do not follow a simple pattern of inheritance; and (c) disorders that are due to readily identifiable chromosomal anomalies. Estimation of the recurrence risk varies with the type of disorder.

8.1.1 *Traits that are determined by one or more recognizable genes, and that follow Mendelian patterns of inheritance.*

In general, these provide *a priori* values for the recurrence risks in matings of a given genotype. When a genotype is not known, calcula-

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1969, No. 416.

tion of the recurrence risk depends on a knowledge of the appropriate gene frequency in the relevant population. In the case of a recessive trait, consanguinity will, of course, have to be taken into account in the determination of probable genotypes.

There are two important causes of departures from Mendelian expectations, namely, viability effects and incomplete penetrance. For disorders with a variable and comparatively late age of onset, such as Huntington's chorea, penetrance increases with age. In this case, therefore, the age of individuals must be taken into account in the determination of their probable genotype. Since penetrance is itself likely to be a function of the genotype, there may often be little distinction between a single gene with low penetrance and a multifactorial model of inheritance. In such cases, there is little point in forcing patterns of inheritance into single gene models by postulating incomplete penetrance.

As first emphasized by Haldane (1949), sibships containing only one affected individual should receive separate treatment from those containing two or more. This is because of the existence of "sporadic" cases due, for example, to new mutations. Many families requesting genetic counseling may have only one affected individual, in which case determination of the probability that this is a sporadic case is most important for the proper estimation of the recurrence risk. Clearly, the more extensive the available family history, the better are the chances of distinguishing a sporadic from a familial case. An obvious example is the fact that, for a disorder that is fully dominant, absence of the disease in the parents of an affected individual means that the individual must be a sporadic case. Even if the disorder is not fully penetrant, the more extensive the family history the higher the probability of finding an affected relative, if the case is familial rather than sporadic. Given the penetrance, one can obtain an estimate of the probability of not finding an affected individual in any given pedigree. For dominant disorders with very low reproductive values, new mutations are particularly important as a source of sporadic cases. They are less important for recessive disorders, because of the greater persistence of recessive genes in heterozygotes, which are likely to have almost normal reproductive values. Thus, for practical purposes, no autosomal recessive conditions are likely to be due to recent mutations.

Sporadic cases may also often be due to primarily acquired causes. Thus, thalidomide administered during early pregnancy has been identified as a cause of certain types of skeletal abnormality, associated with other defects, and maternal rubella as a cause of deafness and blindness. Advances in the clinical and laboratory definition of these embryopathies, coupled with the detection of other teratogenic agents, will in the future help to distinguish such sporadic cases from those primarily due to genetic mechanisms.

Although single gene models are often proposed on the grounds that, following detailed studies of a very few cases, there appears to be some missing or altered enzyme, segregation analysis is the key tool for the confirmation of such models, from which exact recurrence risks can be deduced.

8.1.2 *Disorders that are discrete but do not follow a simple pattern of inheritance*

These range from schizophrenia and diabetes, which are evidently to a large extent genetically determined, to many infectious diseases for which there is little or no evidence of any genetic component. Most of the common congenital malformations, which also come into this category, lie somewhere between these two extremes. Even for schizophrenia and diabetes, the segregation frequency is at the most 10–15% when only one parent is affected, so that very few sibships are found that include more than one affected individual. The main source of information for recurrence risk estimates, therefore, is a comparison of the incidence in the general population with that among relatives of given degree of affected individuals. Using the threshold model, this comparison provides an estimate of the heritability of the trait being considered. This heritability can, in turn, be used to predict the incidence of the trait in degrees of relatives for which no data are available. This prediction depends, however, on a comparison with the general population incidence. The heritability, by itself, provides no *a priori* basis for predicting recurrence risks; *a priori* values can only be obtained from specific gene models.

As already mentioned, variations in the definitions of disorders in this general category may have a major effect on the estimation of recurrence risks. Incidence estimates must then be related to, for example, the severity of the disease. The severity may, however, be difficult to quantify; in this case other measures, such as the age of onset (which is generally negatively correlated with the severity), can be used as a guide to the severity.

Another major problem in disorders of this category is the extent to which they are influenced by environmental factors. The incidence of anencephaly, for example, has been shown to vary markedly with socio-economic status, season, locality, and sex (Edwards, 1958). Clearly, in such cases recurrence risks must be calculated as a function of all such relevant parameters, and genetic counselling must be based on a knowledge of the values of these parameters for the family and for the individual in question.

Recurrence risks for many abnormalities other than those determined by single genes are generally low enough to have little effect on a couple's decision to have more children.

8.1.3 *Disorders that are due to readily identifiable chromosomal anomalies*

These can be further subdivided into those that are clearly familial, such as 15-21 translocations, and those that are not, such as trisomy 21, XO, and XXY. This is analogous to the distinction, for single gene disorders, between familial and sporadic cases. However, for chromosomal abnormalities this decision can usually be made on the basis of a careful examination of the karyotypes of members of the family being studied. Another special feature of some chromosomal anomalies, notably trisomy 21, is the striking dependence of the incidence of the abnormality on maternal age. There is some indication of an occasional tendency for chromosomal anomalies, other than translocations, to run in families. In this case the abnormalities can be considered as belonging to the previous category (see section 8.1.2). In general, however, the incidence of such abnormalities is not a function of the previous family history; counselling can therefore be based directly on incidence figures, which, in the case of trisomy 21, must of course be given as a function of maternal age.

The transmission of translocation follows simple Mendelian principles, with disturbed segregations and with a marked bias against unbalanced gametes, especially in the male. In general, if both parents have normal chromosomes, there is no serious recurrence risk.

A further complication is the occurrence of chromosomal mosaics. These could, if there were mosaicism in the gonads, lead to unpredictable but relatively high recurrence risks. Such questions can, however, at least in principle, be answered by a thorough cytogenetic investigation of the parents.

9. GENE FREQUENCIES

9.1 Nature and origin of genic variation

A large proportion of the genes of diploid organisms, including man, show variation; that is, two or more alleles of that gene co-exist in most populations. Existing alleles must have arisen by mutation, which may have occurred recently or long ago. In some cases, new alleles may have arisen by intragenic recombination. Mutation may be complex and may involve small or large chromosomal changes.

New alleles, freshly arisen by mutation, have a low chance of increasing in frequency, or even of persisting in the population over several generations, unless they have a selective advantage over pre-existing alleles. When they first appear, these new alleles will usually be in a heterozygous condition. If they are recessive, they will not be eliminated by selection and may accumulate in a population. Apart from cases of inbreeding, it is only when the frequency of these alleles has reached relatively high levels (usually still less than 1%) that the chance of two heterozygotes marrying

is not negligible, and homozygotes for the new allele may be observed. For recessive new alleles, only then does exposure to selection begin, whereas for dominant alleles it begins at once.

Recessive deleterious mutations can thus accumulate in populations up to certain levels, being eliminated by selection only when homozygous. A balance will be established between the production of fresh deleterious alleles by mutation and their elimination by selection. The same is true for dominant mutations, but a balance will be established, on average, at a much lower frequency of the deleterious alleles, since they are exposed sooner to selection. In comparing dominant and recessive mutations, however, the frequencies of individuals affected will differ less than will the frequencies of the genes.

Examples of deleterious mutations maintained at relatively low frequency by this mechanism of mutation-selection balance are, among dominants, achondroplasia and Huntington's chorea and, among recessives, phenylketonuria and several other types of mental deficiency.

When the intensity of selection against the mutants, due to decreased fertility, increased mortality, or both, is known, when a state of equilibrium can be assumed, and when the mode of inheritance as well as other data on population structure are known, the mutation rate can be estimated from the frequency of individuals affected. Mutation rates for some inherited pathological disorders have been estimated in man and are distributed over a wide range, but are mostly below one in 10 000 gametes per generation. A few chromosome changes seem to have higher rates. There are many difficulties in estimating mutation rates accurately and in recognizing the exact nature of the change involved in each case. The problem is an urgent one and deserves much further study.

Another important type of balance, which can lead to any level of allele frequency, occurs when heterozygotes have a selective advantage over both homozygotes. The classical example is sickle cell haemoglobin, which is due to replacement of the amino acid glutamic acid, in position 6 of the beta-chain of haemoglobin by another amino acid, valine. This mutant may have arisen only once or a few times in the history of man, but is now common in a large part of the Old World because heterozygotes are more resistant to *falciparum* malaria. Wherever malaria exists, or has been present for a sufficiently long time, the frequency of this gene may be so high that up to 30%, or even more, of individuals are heterozygotes. The frequency of the mutant gene has not become even higher, however, because homozygotes for it suffer from a serious disease, sickle cell anaemia, that greatly limits their capacity to survive to maturity and reproduce.

A very large number of human genes are "polymorphic"; polymorphism is defined as the presence of two or more alleles of one gene at substantial frequencies, i.e., above 1%. Many such polymorphisms

are probably due to a selection balance, of the type that occurs when heterozygotes are at an advantage over homozygotes. None, however, (except, perhaps, G6PD deficiency) is known in such detail as is sickle cell anaemia. Undoubtedly, many other types of selection balance exist. It is very likely that many other polymorphisms represent phases in the selection of new advantageous mutant alleles replacing the old ones, in which both the new and the old alleles are found at substantial frequencies in the population. These are named "transient" polymorphisms, because if they could be sought in earlier or later generations at a sufficient time interval, they would be found to differ quantitatively or qualitatively or to be absent altogether.

9.2 Gene frequency estimates

Gene frequencies are estimates of the relative frequencies of alleles. When all possible genotypes can be recognized and counted separately, gene frequencies can be estimated by simply "counting" genes.

When one or more alleles of one gene are dominant, some heterozygotes cannot be distinguished from homozygotes. Gene "counting" cannot, therefore, be applied directly. An estimate of gene frequencies can still be obtained, because it can be assumed that, except in special circumstances, marriages occur at random with respect to the genotypes considered. Under such conditions the well-known theorem of Hardy and Weinberg applies, which permits prediction of the phenotype frequencies and, conversely, the frequencies of genes from those of appropriate phenotypes. Thus, if it is known that a characteristic is fully recessive, the frequency of the recessive gene can be estimated as the square root of the frequency in the population of bearers of the characteristic.

With more than two alleles, at least one of which is recessive, calculations are more complicated. Methods are available, however, mostly based on a well-known general method of statistical estimation — the "method of maximum likelihood". They often involve heavy numerical work, which is best handled by computers. Programmes that use maximum likelihood and that demand a minimum of programming work are available, and can fit almost every particular case.

9.3 Relevance of genic variation to public health

Genic variation is of great relevance to public health in a variety of ways, depending on the variation considered :

(a) Polymorphisms that only rarely, if ever, involve known pathological consequences of their own, such as the ABO blood groups, are

of importance in the problems connected with making available the right kind of blood for transfusion.

(b) Many polymorphisms are probably connected with higher risk of specific diseases. Correlations found so far, e.g., for ABO blood groups, are not very striking, so that they are not useful, as yet, for the prognosis, diagnosis, or prevention of diseases such as gastric ulcers, with which they are correlated. More work along these lines may provide as yet unsuspected correlations that may prove of greater importance, especially for polymorphisms such as HLA and the Gm blood group system.

(c) For polymorphisms clearly connected with specific diseases, such as sickle cell anaemia and thalassaemia, knowledge of gene frequencies is an essential prerequisite of any public health programme. Studies on a relatively small number of individuals to determine gene frequencies can predict very accurately the prevalence of disease, and therefore the magnitude of the public health problem due to the disease.

(d) Some polymorphisms determine differential sensitivity to drugs or differential capacity to metabolize them, e.g., the sensitivity of pseudo-cholinesterase mutants to some anaesthetic agents and of G6PD mutants to a great variety of drugs, and the insensitivity of some patients to isoniazid treatment of tuberculosis because of rapid drug metabolism. Knowledge of gene frequencies permits prediction of the magnitude of the problem involved for a given population.

(e) For rare defects, study of the mechanism of hereditary transmission, if any, and determination of gene frequencies are a basic step in determining ways to attack the disease.

9.4 Variation in phenotypic proportion under different mating systems

The relations between phenotype or genotype frequencies and gene frequencies, predicted by the Hardy-Weinberg theorem, assume random mating in infinite populations. Deviations are to be expected in real populations under certain conditions, the most important of which are the following :

(a) A substantial proportion of matings take place between relatives, as happens in some populations for social reasons or in small, highly isolated populations. Here, the proportion of homozygotes will be higher than that due to random mating by amounts that are predictable.

(b) There is assortative mating. This may occur in both directions : negative (marriage preferred between unlike individuals) and positive (marriage preferred between like individuals). The former determines a relative increase, and the latter a relative decrease, of heterozygotes. There is a potentially important application of negative assortative mating

to public health. If individuals with the sickle cell trait or with sickle cell anaemia married only homozygotes for the HbA gene, sickle cell anaemia would be entirely eradicated with hardly any disadvantage, immediate or remote. As this disease is responsible for a hundred thousand deaths per year in Africa, and many others elsewhere, the public health benefit could be enormous at very little cost. This procedure would not deny to anybody the right to marry or to reproduce, and would ensure progeny free from this disease. The restriction in the number of eligible mates would never be serious, and presumably would not reduce the chance of marriage and therefore the fertility of any genotype. It would, however, demand the testing of large numbers of individuals for sickle cell haemoglobin (a simple, reliable, and cheap procedure) and education of the population in this particular public health problem.

9.5 Variation with place

Gene frequencies are known to vary, for many genes, from population to population. The proportions of some genes, e.g., in the Gm, Rh, Fy, and Hp blood group systems, vary considerably between different ethnic groups. It is very likely that this is largely the result of different selection pressures in the different environments existing now or in earlier times. Other gene frequencies vary less from one ethnic group to another, and the variation that is encountered can, at least in part, be the outcome of chance. This phenomenon, also called "genetic drift", is the consequence of the sampling process that takes place at every generation, when gametes are sampled to form new zygotes. The statistical fluctuations thus arising at every generation may accumulate over generations and, given sufficient time, determine the disappearance or the fixation of alleles in the absence of selection. This phenomenon is more important the smaller the size of the population concerned. The extent of variation thus obtained is reduced by migration, and therefore the smaller and more isolated a population the larger are the differences expected because of drift.

Finally, a few gene frequencies show very little variation between ethnic groups. It is likely that this is the consequence of selection balance, which is more or less the same in all the environments of the populations studied.

Rare alleles, presumably maintained by a balance of mutation and selection, also show different gene frequencies in different populations. Thus, in some highly isolated populations a few rare defects, e.g., albinism, phenylketonuria, acatalasia, are found to be unusually frequent. Some hereditary diseases, e.g., Tay-Sachs disease, are more prevalent in certain ethnic groups. Most probably, many of these pockets of inherited defects are the consequence of genetic drift. For recessive diseases and in highly

isolated communities, a fraction of the observed increases may be the consequence of increased frequency of consanguineous marriages.

Differences in the frequencies of the HbS gene or thalassaemia reflect both the intensity of malarial infection in the area and the history of the population (principally, the duration of exposure to malaria).

9.6 Variation with time

Knowledge of the forces at play, which are mostly those of selection and drift, permits the accurate prediction of the variation of gene frequencies with time. Whilst known selective forces cause changes of predictable magnitude and direction, the magnitude of changes due to drift can be predicted only in terms of probability, and change in either direction (increase or decrease of gene frequencies) is equally probable. Data on gene frequencies in the past are practically unavailable. In general, changes in gene frequencies are exceedingly slow, a fact that greatly reduces the immediate effect of the classical eugenic approaches.

Mutation rates are of little importance in determining rates of change. However, an increase in mutation rates, even if small, is to be feared as a unequivocally deleterious event. As mutations are more often deleterious than not, any increase in mutation rate will cause an increase in deaths and disease. For this reason, the current interest in keeping the radioactive background low, and in detecting potential mutagens that could increase their concentration because of environmental pollution, is fully justified. An increase in mutation rate would be noticed almost immediately for dominant gene mutations and for chromosome mutations whilst that of recessives would require several generations, on the average, before being expressed. Monitoring, especially for the former two types of defect, could be a useful device for detecting a possible increase of environmental mutagens. Viral infections should be considered as potential inducers of chromosome mutations. Accurate epidemiological study of genetic defects should be considered an important method in controlling the quality of the environment.

10. THE ANALYSIS OF QUANTITATIVE INHERITANCE

Human beings differ qualitatively, e.g., in blood groups, and quantitatively, e.g., in blood pressure. Hereditary determinants are inferred from the phenotypes of relatives. Data used for this purpose consist of qualities, quantities, the time of observation, and the degree of consanguinity.

When the differences are qualitative and the pattern of inheritance can be explained by simple Mendelian models, analysis is straightforward, although difficulties can arise both in acquiring data and in making an

exhaustive analysis of complicated systems, even with computers. The problems of analysing single factor inheritance (segregation analysis) and multiple factor inheritance (linkage), both of which are instances of qualitative differences with an underlying simple, discrete distribution of genetic determinants, have largely been solved.

Where quantities are defined, as in weight, growth rate, or blood pressure, it is possible to apply algebraic methods that assume that the cumulative action of numerous determinants can be described by a linear function of these determinants. As Fisher (1918) demonstrated, the distribution of such measures in pairs of relatives can be summarized by correlation coefficients. The regression of response expected in an imaginary organism that is compounded of infinitely small, unlinked hereditary determinants, whose products act linearly in a uniform environment, is called the "heritability". Another interpretation of heritability, commonly used by plant and animal breeders, is that it is the proportion of the total variation in a population that can be attributed to genetic differences. The main problem in applying the concept of heritability to human populations is that it depends on assuming that all factors promoting similarity between members of a family are genetic. This is clearly not the case, as there is usually a strong correlation between the environmental factors influencing members of the same family.

Heritability can be useful in estimating the speed of response to selection, but provides no insight into the number or mode of action of the hereditary determinants in the absence of some prior knowledge, which is generally lacking.

It is important to point out that (a) if conventional views of gene action are accepted, all forms of human variation, both in health and disease, will to some extent be heritable, and tests of whether a condition or aptitude is heritable are unlikely to be fruitful; and (b) whilst estimates of heritability may be of value in predicting the effects of influencing, by genetic counselling, the fertility of some phenotypes or their future incidence, and may occasionally provide an indication of avenues of research directed at understanding the basis of disease, in general there is no reason to suppose that there is any connexion between a heritability estimate and the feasibility of reducing the frequency of a disease or disability by manipulating the environment. Therefore, studies of traits in man should be directed to understanding genetic mechanisms, detecting the role of specific environmental factors, and predicting phenotypes of relatives, rather than to estimating heritability *per se*.

Where qualitative data cannot be resolved satisfactorily by simple Mendelian explanations, quantitative methods may be used if the given quality is presumed to be determined by some underlying quantity. For example, we may have details of the proportions of individuals who are fair, or hypertensive, or talented, in which case the discontinuity is semantic

and quantitative data are present; or we may consider individuals who are sunburnt, or have had a stroke, or are literate, and infer that this is the result of some underlying, continuously distributed set of determinants.

A number of models have been proposed, of which the simplest is the threshold model. If the threshold is replaced by a continuously increasing exponential response, not only do relatives conserve the normality of their distribution, but the variance is unchanged. Attempts have been made to relate these models and to incorporate the concept of heritability within the second model. In man, however, neither model can resolve the contribution of genetic and other factors that make relatives similar.

10.1 Association with Mendelian traits

If most Mendelian markers are assumed to have selective value, a difference in susceptibility to disease or in some variable may be expected. The collection of data relating either states (e.g., leprosy) or variables (e.g., weight) to markers is of great interest, although only large surveys can be expected to be definitive. It is important, therefore, that data should be presented in a form that allows addition of series, and should not be subjected to selective publication on the basis of significance tests.

11. DEMOGRAPHY AND HUMAN POPULATION GENETICS

Human genetics, particularly human population genetics, can neither evolve independently of all other biological disciplines, nor contribute to man's ultimate betterment, without a concern for, and an integration of its findings with, other population-oriented sciences, especially demography. Human population genetics has too frequently ignored the potential contributions of demography, although these two disciplines have numerous areas in common. This undoubtedly reflects, in part, a lack of awareness of the demographer's tools, and the knowledge that age-specific birth and death rates for different genotypes are difficult and costly to obtain.

The description of human populations calls for concepts and methods that have gradually been developed by demographers. Demography makes an important contribution to research in genetics through :

(a) better definition of the subject being studied, whether this is a group of populations or a group of families;

(b) the revealing of certain parameters, which themselves constitute genetic characters, connected with the population as a whole or with certain sub-populations.

Only a few examples will be given, in which co-operation between the geneticist and the demographer is fundamental.

The populations to which geneticists refer are usually groups of individuals of all ages. Demographic data are necessary to pass from one group to the other; without such data, certain misinterpretations may arise, particularly with respect to the size of the population.

The frequency and the effect on the death rate of a given disease in a family may be influenced by its socio-cultural level; a harmful gene can result in child-deaths more often in certain environments than in others where more effective care is given. Family size, also, may have an influence on the frequency of certain diseases.

In all these cases, only a precise demographic description of the population studied makes it possible to determine the part played by demographic factors in the variations observed, and thereby to isolate purely genetic effects and to appraise properly the public health burden involved. It follows, also, that comparison of observations made on various populations can result in valid conclusions in the field of genetics only if the effect of any demographic inequalities has been corrected.

12. RECORD LINKING

The only method of studying total populations in detail is by the automatic linking of records, so that relationships between any two individuals can be defined, the depth of the relationship depending on the duration of collection of the records.

Once a framework of reliable civic records has been established, it can be used to define parameters relating to migration, consanguineous marriages, fertility, and death. By linking with medical records, it will be possible to define accurately familial concentrations of diseases, including malformations, familial associations of different diseases, and the relative fertility of persons who have, or whose relatives have, various disorders.

This will allow accurate recurrence risks to be assessed and suspected familial concentrations of disease to be exposed to expert medical scrutiny; it will also provide projections of the future incidence of disease that is determined by simple genetic mechanisms. Recurrence risk figures could be provided routinely, on the basis of local evidence, at antenatal clinics, at post-natal clinics, or on demand.

Further research work, e.g., on blood group and disease associations, and on segregation of single loci and pairs of loci (genetic linkage), could easily be accomplished by linking blood donor and recipient records; this has been done in Iceland.

In addition, it may be worth while storing cord and donor blood, blood taken at antenatal clinics, and placental tissue in liquid nitrogen, in the hope that systematic procedures will be developed for the long-term mapping of the human genome and for estimating the mutation rate by analysis of specific proteins.

The problems are large, but there are great potential benefits both to public health and to our knowledge of genetics.

Meanwhile, it should be appreciated that retrospective recording of population data is difficult and inaccurate, and that the type of data collected by established administrative departments in the larger countries are often inadequate in content, or, owing to legislative difficulties, are not available for full use.

The essential basis for an efficient system is the accurate designation of parentage of every birth, with full dates of birth and places of birth of both parents and child, and the availability of such data for research purposes, subject to suitable safeguards of confidentiality.

13. MEDICO-LEGAL APPLICATIONS OF GENETIC DATA

The medico-legal situations in which genetic data are often applied include criminal offences, especially against persons, the identification of individuals, disputed paternity, problems relating to organ transplantation, and the reported effect of certain karyotypes on criminality. These applications should, in general, be restricted to regular Mendelian systems that require the use of specific laboratory methods.

The increasing application of genetics to medico-legal practice, and the desirability of defining its implications, value, and limitations, strongly underline the need for future deliberation in this area.

14. PROCESSING OF HUMAN GENETICS DATA

While relatively simple techniques are available for performing the types of analyses discussed in previous sections, there have been very considerable recent developments in the application of computer technology to such problems in human genetics (Morton, 1969). Apart from enabling laborious calculations to be avoided, the computer allows the introduction of methods that would otherwise be intractable. Such methods can be applied to extensive data collected in a standardized manner, and have a considerable advantage in terms of statistical efficiency. This means that more precise estimates of genetic parameters can be obtained. Such efficient analyses imply a saving in the cost of collecting data, in that a

smaller survey will provide the same amount of information as a larger one that is analysed in a statistically less efficient manner.

15. RECOMMENDATIONS

Developments in population genetics have led to increasingly powerful and refined methods of extracting health-related information about human genetics. Continued development of such methods can be expected to yield further benefits, and should be pursued.

The following topics are among those needing special attention, as emphasized in the report :

(a) Further research is needed on the problem of detecting carriers of genetic disorders.

(b) In spite of much work there is, so far, little precise information on mutation rates in man. Such work needs re-emphasis, particularly with regard to the study of biochemically defined gene products, and also in view of the possible increase in new environmental mutagens.

(c) With regard to thalassaemia and sickle cell anaemia, it is worth investigating, at a pilot level in appropriate populations or sections of populations, whether a premarital counselling procedure, carried out along the lines of section 9.4 as part of public health education, would be of real benefit.

(d) The Group noted the difficult legal problems related to some advances in knowledge, and to their application to such problems as transplantation and genetic counselling, and suggested that this subject might require special consideration.

Many opportunities for obtaining information of value in human genetics are missed, because of a lack of attention to the fact that data should be collected with the family as the unit. Every effort should be made to encourage the collection of data grouped by family, or at least with the potentiality of constructing family groupings. This may often involve the application of record linking procedures, as discussed in section 12.

In spite of their power, the statistical methods at present being used in human genetics, especially at the population level, do not, in general, lead to an understanding of the underlying genetic mechanisms of disorders that do not follow simple patterns of Mendelian inheritance. For such disorders, especially, it seems important to emphasize the need for refinement in the measurements and procedures used for observation of the phenotype of the disorder, at the biochemical and cellular levels. In addition to resolving previously undetected heterogeneity, such refinements

should help greatly in establishing a closer relationship between phenotype and genotype, and so in providing a clearer picture of the genetic determination of disorders whose inheritance is not clear-cut, as well as in helping in the discovery of new methods of therapy and disease prevention.

There has been insufficient comparison of alternative methods, especially of the newer ones, which are executed by computer programmes. Comparison of laboratory diagnostic standards, by submitting the same data to different methods and to different investigators, who do not know how the data were generated, has proved of great value in other health-related fields as diverse as psychiatry and histocompatibility-testing. An additional benefit of such efforts is that they bring active investigators together to discuss the results of applying different methods to the same data. This is valuable both in matters of interpretation and opinion, as in medico-legal problems or genetic counselling, and in matters of inference, as in segregation and linkage analysis. In the latter case, programmes could be tested by artificial data. The Group recommends that such studies should be encouraged.

The Group considered the question of reference centres. It drew attention to the existence of the International Reference Centre for Processing of Human Genetics Data, and to its willingness to have visiting investigators and to process, by any of its computer programmes, data and control cards submitted according to its specifications (Morton, 1969).

The Group noted that the extensive data on genetic variants in populations were widely dispersed, and pointed to the great advantages that would ensue if the data were available at some centre in a computer-compatible format.

In addition, the Group suggested that the possibility be investigated of establishing systematic contacts between scientists engaged in collecting samples from population groups and those interested in studying specific types of variation, with a view to achieving optimal utilization of the samples in question.

The Group records with profound regret the death shortly before the meeting of Dr J. Sutter, of the National Institute for Demographic Studies, Paris, who had been invited as a participant. He played an active part in the preparatory work of the meeting and his skilful and comprehensive approach was much appreciated.

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