Population studies: Potential and limitations for analysis of genetic and non-genetic factors in leprosy

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Human populations

The diversity of human populations has arisen in the course of evolutionary history, as the result in part of the interplay between genetic, environmental and social factors. Migration patterns in early history set initial limits on particular gene pools, which were moulded by elements of chance, cataclysmic events in nature and by selection pressures both environmental and social. Because of the tenuous nature of much of the evidence, often viewed through narrow frames of the movie of historical time, the relative influences of culture and natural selection are in most cases indeterminate (1). The influence of infectious disease in the population equation is, however, certain in principle, if not in precise detail, probably being more significant than armed conflict (2). The influence of infectious disease on the human gene pool has been documented in a number of major studies this century, one of the most well-known examples being that of the relationship between haemoglobin S and malaria (3).

In broad perspective it is apparent that in at least some cases the genetics of the host can be important in respect of susceptibility or of resistance to particular infectious agents. These may operate as selection pressures on large or small population groups, having some influence on the gene pools of these groups. Even where such a relationship between infection and host genetics is strongly suspected, the added complexity of socio-cultural forces may be considerable (4, 5). Thus, in leprosy, host factors may be genetic or socio-cultural (6). The frequently uncertain weighting of the two has led at times to considerable debate as to whether small, isolate populations or larger, polyvalent populations represent the best milieu for analysis. This argument may be a stale-mate : both have advantages and disadvantages.

Clearly, if major, well defined disease susceptibility or resistance genes exist with respect to the liability of individuals to develop clinical signs following exposure to a specific infection, greater confidence may be engendered if the relationship

holds in several large outbred populations. On the other hand, studies on a series of isolates living in geographical contiguity permit the comparison of differing gene pools in a common environment. In reality there is a continuum from isolates such as exist in some island populations to marked polyvalency which exists for example in the United States. Because the chance of occurrence of unusual mutants in clusters is likely to be higher in isolates, there may in some cases be greater opportunity to dissect the complex relationships between infection, genetics and environment in isolates, where some features may become exaggerated. In this context the Pacific region has been fascinating, revealing a number of foci of high incidence of unusual diseases. Some such as kuru are associated with demonstrable infectious agents yet are subject to extreme geographical constraint in the absence of clearly defined, relevant host genetics. As in this case, socio-cultural phenomena may play a dominant role yet do not explain the whole disease picture. Isolates in the Pacific have provided classic examples of the results of sudden spread of viruses common elsewhere, whether such isolates be located on physical islands (2) or in population islands (7).

Population studies of leprosy

It is not surprising, perhaps, that population studies of leprosy have led to controversial interpretations of the relative importance of environmental, socio-cultural and genetic factors in explaining distribution and changing prevalence patterns. There is an overwhelmingly large environmental component in leprosy, which has clouded many attempts to identify genetic factors by epidemiological, pedigree or genetic marker analysis (8, 9). The inexorable decline of leprosy rates in economically successful countries has led to a minimizing of emphasis on genetic factors (6) when there are so many socio-economic explanations. At the same time familial susceptibility is a well described phenomenon (10).

Familial clustering per se of any infectious disease may simply reflect close contact, an interpretation not infrequently supported by greater correlation of the incidence in mothers and their children than in fathers and their children. This can be true for infectious diseases as disparate as leprosy and kuru. Such patterns do not exclude genetic factors governing susceptibility of individuals within such family groups: HLA restriction of susceptibility to the common Epstein-Barr (11) or influenza (12) viruses attests to this.

Studies of population distribution of prevalence rates in a series of geographically close villages in Burma led to the conclusion (13) that similarity in leprosy rates was not inversely related to distance as would be expected for genetic kinship. However, reanalysis of these data has suggested (14) that there is

indeed a consistent decrease in leprosy similarity when distance classes are pooled to reduce sampling effects. Population studies in Papua New Guinea have provided evidence for multifactorial rather than single gene inheritance by incorporating information on assortative mating for leprosy (14).

Immunological studies on leprosy in the past decade have been carried out in a number of populations in different regions of the world. These have led to a descriptive picture of differing immunological deficiencies in the polar forms of leprosy (15). Both cell mediated and humoral immunity appear to be important though their relative importance in all phases of the disease process still requires clarification. The inference (16) that depressed T-cell responsiveness to *M. leprae* in lepromatous leprosy is secondary to the infection and is thus an acquired rather than an inherited characteristic is open to challenge. This is an important point, since the alternative possibility of a genetically defective T cell response as a basis for the lepromatous response could have practical implications in relation to the likely success of vaccines. The very differentiation of the disease into separate types, and the high infection rate but low rate of clinical expression, are both manifestations which are consistent with a spectrum of immunogenetic controls. There is no reason why a genetic basis of this kind should not be greatly modified by environmental and socio-cultural factors : such modification would be expected.

Although studies in Mexico showed no association between leprosy and HLA antigens (17), the positive association observed in India between HLA-DRW2 and tuberculoid leprosy (18) is impressive. Such evidence is not only consistent with a recessive gene conferring susceptibility to one polar form of leprosy but also suggest this is linked to a region of interest with respect to immune response genes. While the generality or otherwise of this association remains to be tested in other populations, the data clearly imply the possibility that in some populations genetic factors may be substantive, at least in determining the type of leprosy which develops. No convincing genetic marker associations have yet been described for lepromatous leprosy and the possibility of genetically-based susceptibility to develop leprosy as a disease class remains uncertain.

Studies in the Pacific region

Leprosy is widespread in the Pacific region. Some of the most intensive studies have been undertaken in Melanesia, especially Papua New Guinea, which represents the largest concentration of indigenous peoples in the region. This country has more than 150 language groups in a total population of about 3 million. It represents great geographical diversity, from fertile tropical coast and swampy river deltas to very high mountains, adjacent to

the equator. Such geographical and population diversity exists in many other regions of the world but Papua New Guinea offers one of the most concentrated and accessible areas for study. There is a great deal of data available on the genetics of population subgroups which in general demonstrate an association between genetic isolation and language separation.

The distribution of leprosy in Papua New Guinea has been well documented over a substantial period (19), with intensive studies being carried out in the Karimui region. The general pattern is of great disparity of prevalence rates among different language groups and among different villages or village clusters within a single language group, even over long periods of observation. Rates range from nil to over 100 per 1000 population. In a recent study in the Bogia subprovince (14) the distribution was found to be variable, from a rate of 0 per 1000 in three language groups to 60.9 per 1000 in the Moresada language group. Twenty-five languages comprise this region of 32,964 people. The average lepromatous rate was 4 per 1000, the tuberculoid rate was 15.3 per 1000, with the average total rate of 22.9 per 1000 and a male:female ratio of about one. To some extent the differing leprosy rates may reflect subpopulation group size, geography or cultural variation reflected in language isolation. It is difficult, however, to attribute it to major environmental or health care divergence.

While there are insufficient data for total genetic analysis of all language groups, information from twelve genetic systems permitted Serjeantson (14) to draw interesting conclusions concerning average leprosy, genetic and cognate kinship values for some languages and to derive a partial correlation between kinship, cognates and distance. Of particular interest was the finding that pleiotropy with erythrocyte 6PGD phenotypes was restricted to tuberculoid leprosy and that pedigree data fitted a multifactorial rather than a single-gene model of inheritance. More recently it has been found (20) that there is a significant association between HLA-DRw2 and tuberculoid leprosy, so that markers on both chromosomes 1 and 6 appear to be in genetic disequilibrium with respect to this polar form. The HLA data are consonant with those from India (18). Together they underscore the impression that host genetic susceptibility is important in determining the type of pathology which results from infection with *M. leprae* and provide evidence for the hypothesis of multigenic inheritance of susceptibility to the tuberculoid form.

New markers

Association of tuberculoid leprosy with the HLA-DR locus suggests the possibility that the susceptibility gene could be concerned with immunological function. That genes concerned with DNA processing can also be associated with immune functions is inferred

from the association of ionizing radiation sensitivity and immunological deficiency in the autosomal recessive disorder ataxia telangiectasia (21). The observation that ataxia telangiectasia heterozygotes are radiation sensitive (22) indicates that DNA processing mutants are common, as indeed do studies on a range of associated diseases (23, 24). In multiple sclerosis (24) DNA sensitivity and HLA appear to be in linkage disequilibrium with disease susceptibility but there is no evident linkage between the two gene classes (Gipps and Kidson, unpublished observations). Indeed, so far there is no evidence for genetic linkage between radiation sensitivity genes and the HLA system (25).

Studies on DNA sensitivity genetics and leprosy in the Bogia region have recently been carried out in collaboration with Chen of this laboratory, in conjunction with the studies of Serjeantson (14) and Dry (20) on other genetic markers in the same population. These studies have used radiation induced chromosome aberrations in lymphoblastoid cell lines (26) as the primary assay. Preliminary results are shown in Table 1.

Table 1. Total chromosome aberrations induced by gamma radiation in lymphoblastoid cells from leprosy patients and controls in Bogia Subprovince, Madang Province, Papua New Guinea.

Group	Total aberrations per cell $\pm$ SD	P
Controls (n=10)	0.27 $\pm$ 0.05	
Lepromatous leprosy (n=11)	0.57 $\pm$ 0.22	<0.001
Tuberculoid leprosy (n=18)	0.47 $\pm$ 0.17	<0.001

By the Wilcoxon-Mann-Whitney rank-sum test both lepromatous and tuberculoid leprosy patients were significantly more sensitive than unrelated healthy controls. Lepromatous leprosy patients as a group were more sensitive than tuberculoid patients but the range of individual sensitivities overlapped and in both groups some patients' values were in the control range.

Family analysis showed that sensitive siblings of affected parents in both polar leprosy groups may not necessarily develop leprosy at any age. About half the children of one sensitive parent were sensitive but most of these children were less than 15 years of age, so that potential leprosy status may not be apparent. The results of these studies will be published in detail elsewhere, but the data are consistent with dominant inheritance of DNA sensitivity in at least some families.

These findings point to the probability that a genetic system(s) monitored by cellular radiosensitivity is associated with susceptibility to leprosy as a disease class. Just how heterogeneous is genetic predisposition of this type remains to be

ascertained. Indeed, on theoretical grounds, the number of gene loci concerned with DNA processing functions in man is perhaps of the order of 100, possibly a complexity not dissimilar to that of the HLA system. It is likely that the functions of some genes in this class are associated with immunodifferentiation (27) and thus it is tempting to suggest that the mutants associated with susceptibility to leprosy may be of this type. Perhaps some of the T-cell abnormalities observed in lepromatous leprosy may have an underlying genetic basis of this kind even though they require *M. leprae* infection for overt expression. On the other hand, associations with genes in this class may simply reflect linkage disequilibrium, as is also possible with respect to the HLA system and tuberculoid leprosy. There is no particular reason at this time to believe that the cellular radiosensitivity in all sensitive individuals in this population group reflects a single mutant, nor indeed that they represent mutations at a single locus.

What is important at this juncture is that there is now a glimmering of a genetic basis for susceptibility to lepromatous as well as tuberculoid leprosy. Clearly similar studies need to be done in other populations, especially those for which HLA data are already available. In the present studies definitive conclusions about possible linkage between HLA and DNA sensitivity require data from a larger population sample than has been examined thus far.

Most of the gene products coded by genes in the DNA processing (sensitivity) class in man are undefined, although clearly many are concerned with functions such as replication, repair and recombination. From the viewpoint of readily assayable genetic markers for leprosy they are thus somewhat remote at this time. The methods available which monitor cellular or DNA radiosensitivity are analogous to defining specific T cell functions by a mitogen response, or HLA by a mixed leucocyte response, i.e. relatively crude and polygenic in nature. Hopefully gene or gene product probes will become available in the not too distant future.

Practical implications of population studies on leprosy

Despite the limited pertinent genetic data it is thus now possible to have some confidence that genetic factors do play a role in susceptibility to leprosy and to the particular type of pathologic process which occurs in the human host. From the nature of the immune dysfunction in lepromatous leprosy it would not be surprising if more than one class of genes controlling T-cell responses were involved. While gene transposition and DNA deletion are essential steps in B-cell differentiation (28) we do not yet know whether DNA manipulation is required for the genesis of discrete T-cell subsets. The possibility is attractive in that it might conceivably link DNA sensitivity to disease susceptibility involving critical or semi-stochastic events in T-cell

differentiation (26). Such an association is quite hypothetical at this time.

Given that susceptibility to leprosy has a genetic basis, at least in part, does this affect proposals for intervention in the natural history of the disease in human populations? If genetic susceptibility exists in population groups in Europe, this has not precluded the disappearance of leprosy from most countries in that region (6). Clearly the environmental and socio-economic factors are of great importance in determining the outcome. Despite the problem of drug resistance, chemotherapy is relatively successful, although not always in those populations at greatest risk - for logistic or socio-cultural reasons.

The main uncertainty surely lies in the realm of the possible relevance of the type of genetics concerned to the response to a vaccine of those individuals at greatest genetic risk. If, indeed, genetic susceptibility is related to genes controlling immunodifferentiation and immune function, then especially in lepromatous leprosy, response to vaccination may also be under the control of those same genes. It is thus possible that a vaccine would not protect the most important susceptibles, namely those likely to provide a continuing reservoir of infection. While this suggestion is purely conjectural, it does have important practical, including economic implications.

This is perhaps the most compelling argument in a pragmatic world for further selected population studies on leprosy, since those areas at highest risk are least likely to be fortunate enough to experience economic development of sufficient rapidity to render a potential vaccine irrelevant. Such studies, carried out prospectively, could most usefully be done in populations at high risk which are being considered for vaccine trials. Quite apart from genetic analysis, the other useful output from population studies is the simple one of selection of population groups in which vaccines might be best tested. Here the clear evidence of differing leprosy incidence in contiguous isolate populations is salutary.

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