Genética em Hansenologia / *Genetics in Leprosy*

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The corollary of the identification of *Mycobacterium leprae* as the etiologic agent of leprosy by Gerhard Henrik Armauer Hansen (1874) was the immediate rejection of the theory of hereditary transmission of this disease, which was supported until then by important leprologists as Danielssen & Boeck (1848). However, Hansen's discovery at no moment did affect the idea, based on empirical data, that M. *leprae* infection and leprosy manifestation strongly depend upon the degree of individual susceptibility to the development of this pathogenic agent, besides, of course, on environmental conditions. On the other hand, taking into account that no phenotypic manifestation can be produced without the commitment of some genetic entity, it was also clear that this susceptibility should depend upon inherited host factors. Notwithstanding this idea and the claims of some authors in the thirties for the need of genetic studies in leprosy (Rotberg, 1937; Tolentino, 1938; Aycock & McKinley, 1938; Aycock, 1940) such type of investigation was only undertaken by geneticists in the sixties. Curiously, the first geneticists who independently began such research (Spickett, prematurely deceased, in Great Britain, and Beiguelman, in Brazil) published their earlier papers in leprology in the same year (Spickett, 1962[ab]; Beiguelman, 1962[ab])

In the sixties, genetics was shy of a methodology for investigating human genetic involvement in the manifestation of infectious diseases, since medical genetic research was mostly concerned with constitutional and degenerative diseases. Of course, this situation contributed to the fact that investigations designed to evaluate the role of human genotype in determining susceptibility to leprosy infection, instead of pursuing a circumscribed line of research, adhered to different approaches, which will be commented here.

Genetic polymorphisms

During some time, genetic investigations in leprosy were mostly devoted to analyze polymorphisms in samples of patients with Hansen's disease, and almost forty polymorphic systems were studied, as shown in alphabetic order in Table 1.(For references see, for instance, Beiguelman, 1983).

Such polymorphisms were analyzed in the hope of finding associations between leprosy and some genetic markers, but an important fraction of these investigations provided negative results, another part showed weak associations, while the conclusions of other studies did not reach general agreement. The negative results are not surprising. They were indeed expected with great probability, since most of the genetic polymorphisms were chosen for study without a logical indication that susceptibility to leprosy might depend upon the polymorphic genes under investigation (HLA antigens are, of course, included among the rare exceptions). On the other hand, the conflicting results may be most probably attributable to large sampling fluctuations due to small samples, racial and geographical variations, inappropriate controls, and/or heterogeneous composition of the patients with respect to the clinical forms of leprosy.

Concerning these investigations, it seems important to stress that, in our opinion, the random choice of genetic polymorphisms for study in leprosy patients, although relevant to some geneticists, is useless for practical leprologists. Thus, when a weak association between leprosy and a polymorphic system randomly chosen is demonstrated beyond doubt, this association might supposedly detect an unbalanced linkage equilibrium or it may serve only to suggest that leprosy is one of the several forces that are maintaining the analyzed polymorphism, and reciprocally that this polymorphism may have some influence in the

Table 1. Genetic polymorphic systems studied in leprosy patients.

POLYMORPHIC SYSTEM	POLYMORPHIC SYSTEM
ABO blood groups	HLA antigens
Acid phosphatase	Inv antigens
Adenosine deaminase	Kell blood groups
Adenylate kinase	Kidd blood groups
Alpha 1 antitripsin	Lactate dehydrogenase
Beta 2 glycoprotein I	Lewis blood groups
Beta-lipoprotein Ag	MNSs blood groups
C3 (third component of complement)	P blood groups
Ceruloplasmin	Phosphoglucomutases 1, 2 and 3
Diego blood groups	6-Phosphogluconate dehydrogenase
Duffy blood groups	Properdin factor B
Esterase D	Pseudocholinesterase
Glucose-6-phosphate dehydrogenase	Rh blood groups
Glutamic pyruvic transaminase	S hemoglobin
Glyoxalase	Secretion of ABH substances
Gm antigens	Taste sensitivity to phenylthiourea
Group-specific protein	Thalassemia (beta)
Haptoglobins	Transferrins

manifestation of leprosy. However, practical leprologists, who are interested in the applications that genetics may provide to leprology, will make no use of such information, since it serves no diagnostic or prognostic purposes.

Familial distribution

Studies on the familial distribution of leprosy were primarily concerned with the demonstration that types and groups of leprosy ought to be distinguished when dealing with intrafamilial risk of leprosy contagion. Thus, by investigating the contagion rate of leprosy in families in which the father or the mother or both parents were lepromatous, as well as in couples that included a lepromatous partner, Beiguelman (1971, 1972) observed that the consanguineous relatives of lepromatous patients are more prone to display the same polar type of leprosy than non-consanguineous relatives, all of them having at least five years of cohabitation with the lepromatous *focus.* In contrast, the non-consanguineous relatives of lepromatous patients exhibited a higher attack-rate of others forms of leprosy than the consanguineous relatives.

This conclusion was confirmed by Smith *et al.* (1978) who observed in Philippine families that lepromatous leprosy was about three times as prevalent when one parent had this type of leprosy than when neither parent had lepromatous or any form of leprosy. Moreover, this difference was not detected in families in which the affected parent did not exhibit the lepromatous type of leprosy.

The studies on the familial distribution of leprosy have also been concerned during some time with the demonstration that leprosy shows a family clustering. The demonstration that a communicable disease shows family clustering may not mean too much for genetic purposes, since this clustering may be more dependent on differential exposure conditions than on an inherited predisposition to its etiologic agent. Nevertheless, family clustering of a communicable disease is a necessary, though not sufficient, condition for supposing that some important inherited component of the host is involved in its manifestation.

Leprosy has been always admitted to be a familial disease, genealogical data with high concentration of leprosy patients being reported in ancient literature (Danielssen & Boeck, 1848). However, most of these referred to families who lived segregated from populations

where the prevalence of leprosy was usually low. Therefore, it seemed necessary to demonstrate the non-random occurrence of this disease even in populations in which leprosy prevalence is high, since the contagion risk through extra-familial *foci* increases among them. This type of investigation started in the late sixties, when it was demonstrated family clustering in a Brazilian population (Beiguelman *et at,* 1968°; Beiguelman, 1972). This conclusion could not be confirmed by Morton *et al.* (1972) for Micronesian sibships, but the propor-tion of lepromatous cases among leprosy patients in this population was half (22%) of that observed in Brazil (45%), in spite of the extremely higher global prevalence of leprosy in Micronesia as compared to Brazil (Mattos, 1964; Sloan, 1972). These discrepant results can be due to this difference, for it is known that the probability of finding multiple-case families is higher among those including a lepromatous patient than among those in which only non-lepromatous personls are included (Kapoor, 1963).

Any way, presently all papers show that leprosy exhibits family clustering, while the extraordinary development of the powerful methods of segregation analysis (Elston & Stewart, 1971; Morton & MacLean, 1974; Lalouel & Morton, 1981; Lalouel *et al.,* 1983) promoted the evolution of studies on familial distribution of leprosy to the investigation of the role of heredity in determining the family clustering. Nevertheless, as it will be seen, in spite of this evolution, the complex segregation analyses that have been made generated controversial results.

As a matter of fact, Serjeantson *et at* (1979) analyzed 340 families of leprosy patients from Papua New Guinea and concluded in favor of multifactorial inheritance for susceptibility to either lepromatous or non-lepromatous leprosy, while in the same year Smith (1979), after studying 91 Philippine families with at least a lepromatous or borderline patient concluded that his data could not distinguish between an autosomal recessive model or a multifactorial hypothesis for susceptibility to lepromatous leprosy. The analyses made by Haile *et al.* (1985) of data on 75 families of leprosy patients from South India suggested recessive inheritance specially for susceptibility to the tuberculoid type of leprosy, but the segregation analysis performed by Shields *et al.* (1987) on 269 kindreds including 552 leprosy patients from an isolate of Papua New Guinea was not able to differentiate between a Mendelian genetic and a purely environmental hypothesis for leprosy manifestation.

In contrast, Wagener *at al.* (1988), after analyzing 63 families with, at least, two leprosy patients, observed that their data favored a nearly dominant major gene with additive penetrance when they considered as affected individuals those who exhibited any form of leprosy. When only the tuberculoid patients were considered as affected, a recessive model was found to be the most likely, but the discrimination between models was poor. Abel & Demenais (1988) and Abel *at al.* (1989) analyzed 27 pedigrees from a Caribbean island (Desirade) and accepted the hypothesis that a Mendelian recessive major gene would control susceptibility to both leprosy *per se* and lepromatous leprosy. However, more recently, Abel *et al.* (1995), carrying out studies in 285 Vietnamese and 117 Chinese families living in Vietnam, concluded that the results were different according to the ethnic origin of the families. Thus, while in the Vietnamese families the hypothesis of a codominant major gene with residual familial dependence for leprosy *per se* could be accepted, in the Chinese families this hypothesis was strongly rejected. Concerning the distribution of non-lepromatous leprosy, a borderline rejection of the Mendelian transmission hypothesis was observed in the Vietnamese families, while the Chinese sample showed no evidence for a familial component. For lepromatous leprosy the discrimination between models was poor.

Finally, after analyzing 1,568 families of leprosy patients which were under the care of the late Dr. Reynaldo Quagliato, in Campinas, SP, Brazil, Feitosa *at al.* (1995) could not demonstrate Mendelian transmission for susceptibility either to lepromatous or tuberculoid leprosy. For the control of susceptibility to leprosy *per se* their data were compatible with the hypothesis of a recessive major gene, although with deviations from the expected Mendelian segregation proportions.

Obviously, the above mentioned discordant results of the segregation analyses may be a consequence of genetic heterogeneity.

However, it is also quite possible that the action of environmental factors or cultural variations, while influencing the manifestations of leprosy infection, may shadow the role of a major human genetic mechanism in determining susceptibility to such manifestations.

Leprosy prevalence and genetic distance

The application of genetic distance models to the distribution of leprosy prevalence by two groups of investigators (Bechelli *et al.,* 1973; Serjeantson *et.al,* 1979) have also produced discordant results. Bechelli *of al.* (1973) analyzed the prevalence of leprosy in 118 pairs of Burmese villages separated by different distances, under the hypothesis that in all villages biological and environmental factors, as well as socioeconomic conditions would be uniform. Since the distribution pattern of the correlation coefficients for the prevalence of leprosy has diverged from that known to occur for genetic markers under similar conditions, that is to say, since the distribution of these coefficients did not fit a monotonically decreasing function, as would be expected for genetic kinship, these authors concluded that the relation between prevalence rates and distance between villages would be primarily a function of the number of lepromatous and other infectious patients. However, it is possible that the hypothesis of uniformity of biological, environmental and socioeconomic conditions in the Burmese villages might not be valid.

In opposition, the study of Serjeantson *of al.* (1979) suggested the importance of genetic relationship between groups as a determinant of similarity in between-group susceptibility to leprosy. Thus, after analyzing the data on 183 villages from Papua New Guinea, they concluded that the epidemiological pattern of leprosy in these villages simulated the gene frequency distribution of 13 polymorphic systems in their dependence on geographic distances and linguistic differences.

Concordance rates of leprosy among twins

Studies in which the concordance rates of leprosy among monozygotic and dizygotic twins have been compared are scarce (Spickett, 1962[b]; Mohamed-Ali, 1965; Mohamed-Ali & Ramanujam, 1966; Chakravartti & Vogel, 1973). Moreover, unfortunately, they have not taken into consideration three criteria of the utmost importance in twin studies of an infectious disease such as leprosy, a disease with different clinical expressions (Beiguelman, 1972, 1974, 1978, 1983). These criteria may be summarized as follows: 1) both monozygotic and dizygotic pairs should have the same opportunities of exposure to *M. leprae;* 2) male and female monozygotic and dizygotic pairs should be compared separately, unlike-sex dizygotic twins being disregarded, since leprosy is more frequent among males, at least in age groups over 14 years (Doull *of al.,* 1942; Bechelli *et al.,* 1966; Beiguelman *of ah,* 1968[b]; 3) the twins should be composed strictly of informative cases concerning concordance or discordance for leprosy manifestation. Otherwise stated, pairs including an indeterminate or a borderline patient cannot be sampled, as a consequence of the instability of these groups and the low bacilloscopic index of the indeterminate group. Pairs composed of tuberculoid twins should also not be considered for comparison of concordance and discordance rates of leprosy, due to their low bacilloscopic index or the bias they may introduce, since sporadic cases of tuberculoid leprosy are less frequently detected than those occurring in families that include more than one leprosy individual. Therefore, due to sampling conditions, an excess of concordant tuberculoid pairs among monozygotic or dizygotic twins may distort the conclusions in any direction.

It seems clear that twins sampled to compare the concordance rates of leprosy among monozygotic and dizygotic pairs should be ascertained starting from lepromatous patients. Only the pairs who have a like-sex co-twin affected by the lepromatous or the tuberculoid type of this disease should be considered for comparison of concordance rates of leprosy. Healthy co-twins of lepromatous patients who have cohabited for more than five years after the beginning of the disease may also be informative depending on both the degree of severity of the disease and the regularity of treatment of the affected co-twin.

Of course, the consideration of these mandatory sampling criteria pose great difficulties for obtaining an appreciable number of

monozygotic and dizygotic pairs in a short time. However, it is also true that these obstacles could be easily circumvented by a collaborative multinational program (Beiguelman, 1974, 1983). At any rate, in spite of the criticisms to leprosy twin studies here expressed, all of them suggest that monozygotic pairs are more prone than dizygotic twin not only to manifest leprosy but also the same leprosy form.

Mitsuda reaction

The Mitsuda reaction is a consequence of events that follow the phagocytosis of the leprosy bacilli contained in lepromin by the macrophages of the skin (histiocytes). A positive reaction occurs when these bacilli are destroyed by the macrophages that transform themselves into epithelioid cells. That is why a positive Mitsuda reaction is histologically defined by the presence of epithelioid elements usually assuming a tuberculoid or tuberculoid-like structure, where acid-fast bacilli are absent or scarcely found. In the negative reaction neither the phagocytized acid-fast bacilli are destroyed nor a tendency to a tuberculoid structure is seen (Bechelli *et al.,* 1959; Azulay *et al.,* 1960; Petri *et al.,* 1985). Therefore, the Mitsuda reaction evaluates the macrophages' ability to digest the leprosy bacilli contained in lepromin, but the association between this ability microscopically observed and the macroscopic expression of this reaction is not complete. Thus, in spite of less probable, the absence of histologic correspondence may be found either among positive or negative Mitsuda reactions (Bechelli *et al.,* 1959; Azulay *et* al., 1960; Petri *et al.,* 1985).

Taking into account that the bacilli contained in lepromin are always heat-killed, Mitsuda reaction cannot be, of course, considered as a replica of leprosy infection. However, Mitsuda reaction has a high prognostic value, since Mitsuda-positive contacts of leprosy patients are free from the risk of manifesting lepromatous leprosy (Darmendra & Chatterjee, 1955; Quagliato, 1962). Otherwise stated, the positive Mitsuda reaction indicates that the macrophages are able to destroy either dead or living leprosy bacilli. In contrast, contacts who persistently exhibit a negative Mitsuda reaction are under the risk of contagion and to manifest lepromatous leprosy, in spite of the possibility that the macrophages' ability to destroy leprosy bacilli might not be the exclusive organic factor that inhibit the proliferation of *M. leprae.*

Rotberg (1937) was the first author to suggest that Mitsuda reaction might be genetically controlled. According to his hypothesis, positive Mitsuda reactors would have a *natural factor,* perhaps inherited, for resistance to lepromatous leprosy. The fraction of the population deprived of this factor, which enabled resistance to *M. leprae* proliferation, would compose an *anergic margin.* This hypothesis anticipated 25 years the investigation of the possibility of Mitsuda reaction being a genetic polymorphism, when 220 Brazilian families with individuals of North-Italian origin free of leprosy were analyzed (Beiguelman, 1962', 1963, 1971). In these families it was demonstrated beyond any doubt that the distribution of the Mitsuda reaction macroscopically analyzed in the offspring and in the parental generation are closely associated. Otherwise stated, a higher proportion of Mitsuda-negative individuals is bom to Mitsuda negative parents, the opposite being observed in the offspring of Mitsuda positive parents. This distribution lead Beiguelman (1962[b] to suppose that an autosomal gene pair could be responsible for Mitsuda reaction, the negative response being a recessive trait. Nevertheless, according to this hypothesis a variable proportion of the recessive homozygotes might manifest the opposite phenotype as a consequence of environmental influences.

The parent-offspring association of the Mitsuda reaction macroscopically examined has been confirmed in another sample of 100 Brazilian families free of leprosy (Beiguelman & Quagliato, 1965), as well as in families of leprosy patients from Brazil and from India (Beiguelman, 1965; Saha & Agarwal, 1979; Kundu *et al.,* 1979). Moreover, when the Mitsuda reaction was quantitatively analyzed by Botasso *et al.* (1984) in families free of leprosy as well as of leprosy patients, it was observed a significant parent-offspring correlation of the mean responses.

In the Brazilian families of leprosy patients the parent-offspring association of the Mitsuda reaction was closer than among the families

free of leprosy, probably because the former were more exposed to sensitizing agents (repeated lepromin injections, BCG vaccination and primary infection with *M. leprae)* than people in the general population. The higher exposure to sensitizing agents is considered to strength the correspondence between the macroscopic and microscopic reactions induced by lepromin injection. Thus, when the distribution of the Mitsuda reaction macroscopically examined was analyzed in Brazilian families including at least a strong positive positive Mitsuda reactor (in 41 couples one spouse was a tuberculoid patient, while in 65 couples one of the spouses was a lepromatous patient) the parent-offspring association was so striking, that the familial distribution fitted well the hypothesis of an autosomal gene pair being responsible for the Mitsuda reaction, the positive response being the dominant trait (Beiguelman, 1965). However, this monogenic interpretation encountered an apparently strong obstacle in the data on 81 individuals born to undoubtedly Mitsuda negative parents (24 lepromatous couples), among whom 30.9% showed a macroscopically strong positive Mitsuda reaction.

This result lead Beiguelman (1967, 1968, 1971, 1983) to suppose that the lysing ability of the macrophages for the phagocytized *M. leprae* might be a *threshold phenomenon.* Thus, the macrophages of individuals with both *lyser* and *non-lyser* phenotypes would exhibit different degrees of lysing ability for *M. leprae* (lysing *thresholds).* Otherwise stated, among the lysers there would be individuals whose macrophages would express their lysing ability for phagocytized leprosy bacilli more strongly than the macrophages of others. However, the non-lyser phenotype would also be a heterogeneous group, since it would include individuals with no activity at all for phagocytized *M. leprae,* as well as individuals whose macrophages would disclose various degrees of an incipient lysing activity for leprosy bacilli. This possibility was later confirmed by Convit *et al.* (1979), who observed that the macrophages of some lepromatous patients are able to destroy the bacilli of a concentrated lepromin after 90 to 120 days, while the macrophages of others preserve the bacilli undestroyed after 120 days.

If it is assumed that either the lysers or the non-lysers are unimodally distributed according to the lysing thresholds, one can also accept that the lysing capacity of the human macrophages for phagocytized *M. leprae* is bimodal. This lysing ability would, then, be a semidescontinuous trait, the lysers and non-lysers being discriminated by the threshold corresponding to the antimodal area. Therefore, it would be assumed that the lyser and non-lyser phenotypes would be controlled by a *major gene,* i.*e.,* by alleles whose expressivity is highly dependent upon both genetic and environmental modifying factors, but the clinical expression of the lysing ability for phagocytized leprosy bacilli would depend on environmental factors. Thus, in spite of the high probability of finding a histologically positive Mitsuda reaction in individuals who are also positive at clinical examination, that is to say, in spite of the close association between the histologic and macroscopic expressions, the former would be inherited while the second would be environmental. This theory does not exclude the possibility that the non-lyser phenotype may be determined by more than one allelic pair in homozygosis *(genocopies).*

The lysing threshold theory offers arguments to explain why Beiguelman (1965) found 30.9% strong positive Mitsuda reactors among the individuals born to undoubtedly non-lyser (lepromatous) couples, since it may be supposed that:

1. Some lepromatous couples might be genocopies. As a consequence of their different genotypes, they could generate children with a lyser (heterozygous)genotype, that could be more probably associated with a positive macroscopic Mitsuda reaction.

2. In spite of having the non-lyser phenotype, some individuals born to lepromatous couples could be transformed into lysers *(phenocopies)* by BCG vaccination or repeated lepromin injections, chiefly when their lysing threshold would be near the antimodal value.

3. Some macroscopic Mitsuda positive reactors born to lepromatous parents might exhibit no histologic correspondence, i. *e.,* no active macrophage participation.

4. Some Mitsuda positive children of lepromatous couples would be illegitimate, since the prevalence of illegitimacy was not

uncommon (Beiguelman & Pinto Jr., 1967).

The lysing threshold theory provided elements for explaining the occurrence of the different leprosy forms, the epidemiological data on lepromatous and tuberculoid leprosy in the world, as well as the results of a quantitative analysis of Mitsuda reaction in twins (Beiguelman, 1983). Notwithstanding, all these arguments were mere speculations up to the point when Dr. Mary Furlan Feitosa, from the Department of Genetics of Fundação Oswaldo Cruz, Rio de Janeiro, and co-workers decided to reopen the investigation on the familial distribution of the Mitsuda reaction by complex segregation analysis, using the unified model of Lalouel *et al.* (1983). The family data comprised 544 nuclear families of leprosy patients who have received medical care from the late Dr. Reynaldo Quagliato in the Campinas Dispensary of the ancient Department of Leprosy Prophylaxis of the State of São Paulo. All the lepromin tests were performed by Dr. Reynaldo Quagliato and the reactions were classified as recommended by the Sixth International Congress of Leprosy (Madrid, 1953). However, taking into account the studies of Bechelli *et al.* (1959) and Azulay *et al.* (1960) on the histologic correspondence of the macroscopic Mitsuda reactions in leprosy contacts, the +, ++ and +++ reactions have been taken as positive responses, while the - and reactios have been classified as negative Mitsuda reactions.

The model of segregation analysis used by Feitosa *et al.* (1996) assumes an underlying liability scale to which a major locus, a multifactorial component, and the random environment contribute independently. In the single major locus it is supposed two alleles, *A, a,* with frequencies respectively *p* and *q,* being *p+q =1,* and with the resulting genotypes *AA, Aa* and *aa* distributed in Hardy-Weinberg proportions, that is to say, as *(p+q)".* The distance between the two homozygous genotype class means *(AA* and *aa)* is called *displacement* and represented by *t.* The position of the heterozygous genotype mean relative to the means of the two homozygous genotypes is referred as the *degree of dominance* and represented by *d.* If a phenotype is completely dominant *(AA =Aa), d* will be 1, being *d* = 0.5 when codominance is observed, and 0.5<d<1 when dominance is partial.

Variation around each major genotype mean is assumed to be normally distributed, with common variance C+E, being C the variance due to multifactorial transmissible effects and E the residual environmental variance component that is not transmitted within families. The total phenotypic variance is denoted V, and the ratio C/V = H is the heritability, which reflect the proportion of the total phenotypic variance due to multifactorial effects. Additional parameters can be estimated to test deviations from Mendelian parent-offspring transmission of the major gene. These parameters symbolized by *AA, Aa* and *aa* denote the probabilities of transmitting the allele

Table 2. Segregation analysis of the Mitsuda reaction (Feitosa *et. al.,* 1996).

MODEL	d	t	q	H	τ_{AA}	τ_{Aa}	τ_{aa}	-2lnL+c	Estimated parameters
Mendelian mixed	0.811	1.983	0.474	0.0*	1	1/2	0	0.07	4
No major gene	0	0	0	0.658	-	-	-	27.87	1
With no multifactorial component	0.811	1.983	0.474	0	1	1/2	0	0.07	3
Sporadic	0	0	0	0	-	-	-	238.57	0
Recessive mendelian	0	1.861	0.882	0	1	1/2	0	35.52	2
Additive mendelian	1/2	2.438	0.468	0	1	1/2	0	13.56	2
Dominant mendelian	1	1.653	0.452	0	1	1/2	0	9.15	2
D, t, q, H, τ_{AA} , τ_{Aa} , τ_{aa}	0.805	1.987	0.473	0.0*	1.0*	0.492	0.0*	0.00	7
D, t, q, H, $\tau_{AA} = \tau_{Aa} = \tau_{aa}$	0.351	1.596	0.173	0.0*	1.0*	1.0*	1.0*	198.28	5

* Reached its bound.

A for genotypes *AA, Aa* and *aa.*

Different hypotheses were tested by estimating or fixing parameters of the complete model. Standard tests of each hypothesis were performed, using the likelihood ratio criterion. The ascertainment probability was taken as 1 (complete selection) since it is believed that almost all cases of leprosy in the area of Campinas were ascertained by Dr. Reynaldo Quagliato. Only one population liability class was used with a prevalence of 0.6 (Beiguelman, 1962[6]).

The results of the segregation analysis summarized in Table 2 show that, in comparison with the mendelian mixed model, both the hypotheses of no family resemblance (x^2= 238.57 - 0.07 = 238.50; 4 d.f..; P < 0,0001) and no major gene (x^2= 27.87 - 0.07 = 27.80; 3 d.f.; P < 0.002) are rejected, and no multifactorial component (x^2 = 0.07 - 0.07 = 0; 1 d.f.; P = 1) accepted. Moreover, while the mendelian transmission is easily accepted (x^2 = 0.07 - 0.0 = 0.07; 3 d.f.; P > 0.99), equal transmission is rejected (x^2= 198 28 - 0.00 = 198.28; 2 d.f.; P < 0.0001). On the other hand the strictly recessive, additive and dominant models were rejected in favor of a partial dominant effect *(d*= 0.811).

As it seen, one may conclude that the most promising results from genetic studies in leprosy have been obtained by complex segregation analysis of the familial distribution of Mitsuda reaction, since it suggest that, by means of molecular genetics methods, it might be possible to identify a locus responsible for this reaction. Since this would also mean that a possibility exist to recognize a gene responsible for the susceptibility to lepromatous leprosy, one may ask why segregation analysis of families of lepromatous patients failed? The best explanation seems to be that the level of complexity necessary to the manifestation of the Mitsuda reaction is obviously lower than that required for the manifestation of lepromatous leprosy.

Since it is known that in the distal area of the lower arm of human chromosome number 2 (2q31-2q37) there is a homologous region to the mouse's *Bcg* locus, which is responsible for the inborn resistance or susceptibility to several pathogens including mycobacteria (Schurr *et al.,* 1990), it seems attractive to start linkage studies on the Mitsuda reaction with genetic markers of this chromosomal region. Lets hope that this trial can be more successful than the investigations which had as a goal linkage studies between leprosy and genetic markers located in the distal area of the lower arm of chromosome 2 (Shaw *et al.,* 1993; Levee *et al.,* 1994).

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