Genetic Epidemiology 2:255–262 (1985)

Studies on Vitiligo.
II. Familial Aggregation and Genetics

S.K. Das, Partha P. Majumder, T.K. Majumdar, and B. Haldar

Anthropometry and Human Genetics Unit (S.K.D.), and Applied Statistics, Surveys and Computing Division (P.P.M.), Indian Statistical Institute, and National Medical College (T.K.M., B.H.), Calcutta, India

Data on 298 pedigrees, each collected through an affected proband, have been analyzed to study familial aggregation and genetics of vitiligo. The extent of familial aggregation is statistically significant at the 5% level. The disease does not appear to be inherited in a simple dominant or recessive fashion. The heritability of liability to the disease is 46% ± 4.82%. Neither common family environment nor a major locus with additional sources (environmental and/or polygenic) can be excluded as a cause of familial aggregation. Association of the disease with six polymorphic genetic marker loci have been studied. Significant associations with ACP1 and RH loci have been found. This and earlier studies indicate that the disease is associated with genetic loci on different chromosomes, which points to a polygenic nature of the disease.

Key words: vitiligo, familial aggregation, heritability, genetic markers

INTRODUCTION

In an effort to understand the etiology of vitiligo (an idiopathic dermatological disorder that is characterized by pale, milk-white macules that tend to become progressive over time [Mosher et al, 1979]) a survey was undertaken in Calcutta, India. In this survey, retrospective epidemiological data were gathered from 15,685 individuals drawn from the general population, and pedigree data were collected through vitiligo patients. Most sampled individuals were Bengali speaking and belonged to the Hindu caste. Details of the methods of data collection are given in Das et al [1985]. Analyses of these data revealed the following facts, which are relevant here [Das et al, 1985]: 1) the overall prevalence of vitiligo is 0.459%; 2) there are no statistically significant sex or age differences in prevalence rates; and 3) there is about

Received for publication February 12, 1985; revision received April 12, 1985.

Address correspondence to Dr. Partha P. Majumder, Applied Statistics, Surveys and Computing Division, Indian Statistical Institute, 203 Barrackpore Trunk Road, Calcutta 700035, India.

a 4.5-fold increase in prevalence among close biological relatives (parents, sibs, offspring, grandparents, uncles/aunts, nephews/nieces, and first cousins) of affected individuals.

The purpose of this paper is to present results on familial aggregation and genetics of the disease, and its association with genetic markers.

FAMILIAL AGGREGATION

As mentioned in Das et al [1985], pedigree data were collected through affected individuals who came for medical treatment/consultation to the Dermatology Department of the Calcutta National Medical College Hospital and the Calcutta Skin Institute. The detailed family histories of the probands were gathered by examining all surviving members by repeated household visits. Clinical examinations were performed by a professional dermatologist (T.K.M.). In all, 298 pedigrees, each ascertained through exactly one affected proband, were collected. However, since the structures of the pedigrees were not fixed a priori, there is a good deal of "missing" information in this data base. In the present analysis, we have, therefore, used a relatively complete subset of each pedigree. This subset comprises the following relatives of each proband: spouse, children, sibs, and parents. Of the 298 pedigrees, 37 pedigrees had at least one affected relative of the proband, and the remaining 261 probands had no affected relatives.

Testing the hypothesis that there is familial aggregation in a pedigree comprising N relatives of the proband is equivalent to testing whether among these N relatives there is a greater number of affected individuals than is expected among N randomly drawn individuals from the population. The probability that there are R affected individuals in a set of N randomly drawn individuals is

$$P(R \mid N) = \binom{N}{R} p^R (1-p)^{N-R} ,$$

where p denotes the probability of affection of a random member of the population (which, as in the present case, is independent of age and sex). If for a pedigree, $P(R \mid N) \leqslant 0.05$, than one would conclude that there is a significant familial aggregation at the 5% level. Straightforward calculations show that for $P = 0.00459$, the prevalence rate of vitiligo, $P(R \geqslant 1 \mid N) < 0.05$ for all $N = 2,3,\ldots,11$, while $P(R=1 \mid N = 12) > 0.05$ and $P(R \geqslant 2 \mid N=12) < 0.05$. In the present set of 37 pedigrees each with at least one affected relative of the proband, there are: 1) 30 pedigrees each with $R=1$ and $N \leqslant 11$; 2) one pedigree with $R=1$ and $N=12$; and 3) six pedigrees with $R \geqslant 2$ and $N \leqslant 12$. From the calculations presented above, it is clear that except for the pedigree with $R=1$ and $N=12$, for the remaining 36 pedigrees $P(R \mid N) \leqslant 0.05$. Of the 298 pedigrees the expected number of pedigrees yielding $P(R \mid N) \leqslant 0.05$ by chance is, at the 5% level, only 15 ($\approx 298 \times 0.05$). This shows that there is a statistically significant evidence of familial aggregation.

ESTIMATION OF HERITABILITY

As mentioned earlier, of the 298 pedigrees in only 37 pedigrees there is at least one affected individual other than the proband; except for the probands, the remaining

261 pedigrees are devoid of any vitiligo cases. As is often the case with many human diseases [Falconer, 1965, 1981; Bulmer, 1980], a close examination of the pedigrees indicates that the disease is not inherited in any exclusive and strict Mendelian fashion. In none of the pedigrees is there any consanguineous mating contrary to what would be expected if the disease were due to a single recessive gene. Furthermore, the recurrence risks are much lower than the 25% or 50% figures expected, respectively, under simple completely penetrant recessive or dominant models. Thus, a fully penetrant single gene alone cannot explain the familial aggregation. Involvement of an incompletely penetrant major gene, perhaps with other sources (common family environment and/or polygenes), cannot, however, be ruled out. Since the power to discriminate among the alternative models based on this limited data set is very low, we tentatively entertain, on grounds of parsimony, a polygenic liability threshold model [Falconer, 1965], and estimate the heritability of liability to the disease. However, the possible involvement of an additional major locus cannot be discounted. In the set of 298 pedigrees, out of 1731 first-degree relatives (with coefficient of relationship = 0.5) of the proband, 46 were affected, which yields a prevalence rate of 2.657% among relatives of probands. As mentioned earlier, the prevalence rate in the general population is 0.459% (72 affected individuals out of a total of 15,685 individuals). Using these figures, the heritability [Falconer, 1965] is estimated to be 46.06% $\pm$ 4.82%. It, therefore, seems that although genes may play a role, environmental factors also play a large role in the familial aggregation of the disease.

ASSOCIATION WITH GENETIC MARKERS

The examination of the association of a disease with other genetic marker loci is of obvious importance in genetic epidemiology. Evidence for such associations can provide helpful clues regarding the genetics of the disease. Earlier studies have indicated that among vitiligo patients there is a significant excess of M blood group individuals [Wasfi et al, 1980] and of G-6-PD deficient males [Saha et al, 1982]. Significant association with some HLA antigens have also been noted [Metzker et al, 1980; Foley et al, 1983].

In the present study, we made efforts to type the blood of each proband at the following 13 genetic marker loci: ABO, RH, ACP1, PGM1, ESD, AK1, HP, HB, TF, CP, LDH, MDH, and ALB. However, some probands refused to give blood and the lack of a constant supply of laboratory reagents resulted in variable sample sizes at the various marker loci. The phenotypic distributions at seven marker loci are presented in Table I; the remaining six loci are either completely or largely monomorphic (HB: AA = 150, AE = 3; TF: CC = 100; CP: BB = 102; LDH: normal = 102; MDH: normal = 102; ALB: normal = 102). It must be mentioned that although the phenotypic distributions are presented separately for the various types of vitiligo, because of the small sample sizes within each type, only the pooled data were used in the analysis. In order to examine association of the disease with marker loci, we selected reports of a few studies for which the sample sizes were large, the sampled individuals were of an ethnic composition similar to the vitiligo probands, and the places of sampling were Calcutta and neighborhood. (One of us, S.K.D., has been associated with all these studies, and the blood-typing work for all the these studies was performed at the Human Genetics Laboratory of the Indian Statistical Institute, Calcutta.) Since comparable data at the ESD locus are not available, the phenotypic

TABLE I. Phenotypic Frequencies at Genetic Marker Loci Among Vitiligo Patients and in the General Population*

Genetic marker loci	Among vitiligo patients					In general Bengali Population	
	Vitiligo types						
	Localized	Generalized			Total	Frequency	Source
		Vulgaris	Acrofacial	Universal			
ABO							
O	8 (26.67)	30 (43.48)	13 (52.00)	7 (31.82)	58 (39.73)	186 (31.37)	Chaudhuri et al [1969]
A	10 (33.33)	12 (17.39)	5 (20.00)	5 (22.73)	32 (21.92)	147 (24.79)	
B	9 (30.00)	23 (33.33)	6 (24.00)	9 (40.91)	47 (32.19)	212 (35.75)	
AB	3 (10.00)	4 (5.80)	1 (10.00)	1 (4.55)	9 (6.16)	48 (8.09)	
Total	30 (20.55)	69 (47.26)	25 (17.12)	22 (15.07)	146	593	
RH							
CCDEE	1 (3.70)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.93)	0 (0.00)	Chaudhuri et al [1969]
CCDEe	1 (3.70)	0 (0.00)	1 (6.25)	0 (0.00)	2 (1.87)	6 (1.55)	
CCDee	12 (44.44)	22 (44.00)	5 (31.25)	4 (28.57)	43 (40.19)	169 (43.78)	
CCddee	0 (0.00)	1 (2.00)	0 (0.00)	0 (0.00)	1 (0.93)	0 (0.00)	
CcDEE	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	6 (1.55)	
CcDEe	1 (3.70)	3 (6.00)	3 (18.75)	2 (14.19)	9 (8.41)	56 (14.51)	
CcDee	5 (18.52)	9 (18.00)	4 (25.00)	5 (35.71)	23 (21.50)	106 (27.46)	
Ccddee	0 (0.00)	1 (2.00)	1 (6.25)	1 (7.14)	3 (2.80)	1 (0.26)	
ccDEE	0 (0.00)	1 (2.00)	0 (0.00)	0 (0.00)	1 (0.93)	4 (1.04)	
ccDEe	1 (3.70)	5 (10.00)	0 (0.00)	1 (7.14)	7 (6.54)	16 (4.15)	
ccDee	5 (18.52)	8 (16.00)	1 (6.25)	0 (0.00)	14 (13.08)	18 (4.66)	
ccddee	1 (3.70)	0 (0.00)	1 (6.25)	1 (7.14)	3 (2.80)	4 (1.04)	
Total	27 (25.23)	52 (46.73)	16 (14.95)	14 (13.08)	107	386	

ACP1							
AA	0 (0.00)	3 (3.90)	2 (7.14)	0 (0.00)	5 (3.07)	24 (9.41)	Das et al
AB	9 (27.27)	26 (33.77)	11 (39.29)	14 (56.00)	60 (36.81)	89 (34.90)	[1970]
BB	24 (72.73)	48 (62.34)	15 (53.57)	11 (44.00)	98 (60.12)	142 (55.69)	
Total	33 (20.25)	77 (47.24)	28 (17.18)	25 (15.34)	163	255	
PGM1							
1-1	12 (36.36)	41 (53.95)	14 (45.16)	10 (40.00)	77 (46.67)	140 (52.24)	Das et al
2-1	13 (39.39)	31 (40.76)	14 (45.16)	10 (40.00)	68 (41.21)	95 (35.45)	[1970]
2-2	8 (24.24)	4 (5.26)	3 (9.68)	5 (20.00)	20 (12.12)	33 (12.31)	
Total	33 (20.00)	76 (40.06)	31 (18.79)	25 (15.15)	165	268	
ESD							
1-1	20 (62.50)	40 (52.63)	17 (60.71)	11 (64.71)	88 (57.52)		
2-1	11 (34.38)	32 (42.11)	10 (35.71)	5(29.41)	58 (37.91)		
2-2	1 (3.13)	4 (5.26)	1 (3.57)	1 (5.88)	7 (4.58)		
Total	32 (20.92)	76 (49.67)	28 (18.30)	17 (11.11)	153		
AK1							
1-1	23 (74.19)	64 (84.21)	27 (96.43)	14 (93.33)	128 (85.33)	226 (83.39)	Das et al
2-1	8 (25.81)	11 (14.47)	1 (3.57)	1 (6.67)	21 (14.00)	43 (15.87)	[1970]
2-2	0 (0.00)	1 (1.32)	0 (0.00)	0 (0.00)	1 (0.67)	2 (0.74)	
Total	31 (20.67)	76 (50.67)	28 (18.67)	15 (10.00)	150	271	
HP							
1-1	0 (0.00)	3 (6.38)	0 (0.00)	0 (0.00)	3 (2.97)	8 (2.60)	Mukherjee and
2-1	4 (14.81)	13 (27.66)	4 (25.00)	2 (18.18)	23 (22.77)	79 (25.25)	Das [1970]
2-2	23 (85.19)	31 (65.96)	12 (75.00)	9 (81.82)	75 (75.26)	221 (71.75)	
Total	27 (26.73)	47 (46.53)	16 (15.84)	11 (10.89)	101	308	

*Figures in parentheses denote percentages.

TABLE II. Comparison of Phenotypic Frequency Distributions of Vitiligo Patients and the General Population at Six Genetic Loci

Genetic marker	Chi-square	
	Value	d.f.
ABO	3.87	3
RH-C	12.13*	2
-D	7.14*	1
-E	0.85	2
ACP1	6.21*	2
PGM1	1.54	2
AK1	0.27	2
HP	0.36	2

*Significant at the 5% level.

distributions at the remaining six marker loci for the Bengali population are presented in Table I. To test the equality of phenotypic frequencies among vitiligo patients and the general Bengali population, contingency chi-square tests were performed for each polymorphic locus separately. In order to avoid vagaries of small cell frequencies, at the RH locus, phenotypes based on C, D and E antisera were treated separately. Results of this analysis are presented in Table II, which shows that the phenotypic distributions among vitiligo patients are significantly different from those of the general Bengali population at two loci—RH and ACP1. Among vitiligo patients, at the RH locus, there is an elevation of the frequencies of alleles c and d, and at the ACP1 locus of the allele B.

DISCUSSION

In this paper, we have examined the extent of familial aggregation of vitiligo, heritability of liability to the disease, and its association with several polymorphic marker loci. The number of families in which there is an elevation of incidence of the disease in comparison with that expected under the null hypothesis of no familial aggregation is found to be statistically significant at the 5% level. Lack of parental consanguinity and the fact that recurrence risks are much lower than those expected under simple dominant or recessive models suggest that a fully penetrant gene alone cannot explain the familial aggregation of the disease. We, therefore, tentatively hypothesized that the model underlying the manifestation of the disease is polygenic liability threshold model [Falconer, 1965]. Using this model, we estimated the heritability of liability to the disease to be 46.06% ± 4.82%. Despite a significant familial aggregation, the disease does not seem to be under strong genetic control, and the possibility of common family environment being a cause for familial aggregation cannot be ruled out. There is, however, the possibility that in addition to or instead of the polygenic liability threshold model, an incompletely penetrant gene may be involved in the familial transmission. This needs to be investigated more thoroughly using a considerably more informative data set and methods of complex segregation analysis, especially because autosomal dominant, autosomal recessive, and polygenic models have all been invoked as possible modes of inheritance [El-Mofty, 1968; Mehta et al, 1973]. Unfortunately, as is true for most rare diseases that are not

determined by a single gene, the number of affected individuals in each of the pedigrees is very small (in about 88% of the pedigrees the proband is the only affected individual). Therefore, it will require an extraordinary effort to collect data on large numbers of multiplex families needed for complex segregation analysis.

In order to get more clues regarding the genetics of the disease, we studied its association with several genetic marker loci and found significant association with ACP1 and RH loci. It is known [American Journal of Human Genetics, 1983] that the RH and ACP1 loci are on chromosomes 1 and 2, respectively. Earlier studies have reported associations with MN blood group locus on chromosome 4 [Wasfi et al, 1980], G-6-PD locus on the X chromosome [Saha et al, 1982], and some HLA antigens on chromosome 6 [Metzker et al, 1980]. Although such association studies cannot provide as strong evidences as linkage studies provide, nevertheless, the reported associations of the disease with genetic loci on several chromosomes, if true, would indicate that the disease may be polygenic. It may also be pointed out that it is possible that vitiligo is not a single homogenous disease, because in certain localized types there seems to be a neural involvement, while in some generalized types there seems to be an immunological involvement [Sawada and Ohashi, 1982]. Although our sample sizes do not yet permit us to rigorously analyze the data by various types of vitiligo, a cursory examination of the marker frequencies among the various vitiligo types presented in Table I indicate differences at some marker loci, perhaps supporting the hypothesis of genetic heterogeneity. We are now collecting further data, especially for purposes of linkage analysis, which we envisage will throw more light on the role of genes in the causation of vitiligo.

ACKNOWLEDGEMENTS

We are grateful to the Superintendent of Calcutta National Medical College, colleagues of the Anthropometry and Human Genetics Unit, and the Director, Indian Statistical Institute, Calcutta, for logistic and financial support. Thanks are also due to Professor B.P. Adhikari, Indian Statistical Institute, Calcutta, for valuable suggestions. Comments of an anonymous reviewer helped in improving the presentation substantially.

REFERENCES

American Journal of Human Genetics (1983): Human gene map. 35:134–156.

Bulmer MG (1980): "The Mathematical Theory of Quantitative Genetics." Oxford: Clarendon Press.

Chaudhuri S, Mukherjee BN, Ghosh J, Roychoudhury AK (1969): Study of blood groups, ABH secretors and hemoglobin variants in three upper castes of West Bengal, India. 30:129–132.

Das SK, Majumder PP, Chakraborty R, Majumdar TK, Haldar B (1985): Studies on vitiligo. I. Epidemiological profile in Calcutta, India. Genet Epidemiol 2:71–78.

Das SR, Mukherjee BN, Das SK, Blake NM, Kirk RL (1970): The distribution of some enzyme group systems among Bengalis. Indian J Med Res 58:866–875.

El-Mofty AM (1968): "Vitiligo and Psoralens." New York: Pergamon Press.

Falconer DS (1965): The inheritance of liability to certain diseases, estimated from the incidence among relatives. Ann Hum Gen 29:51–76.

Falconer DS (1981): "Introduction to Quantitative Genetics." 2nd Ed. New York: Longman.

Foley LM, Lowe NJ, Misheloff E, Tiwari JL (1983): Association of HLA-DR4 with vitiligo. J Am Acad Dermatol 8:39–40.

Mehta NR, Shah KC, Theodore C, Vyas VP, Patel AB (1973): Epidemiological study of vitiligo in Surat area, South Gujarat. Indian J Med Res 61:145–154.

Metzker A, Zamir R, Gajit E, David M, Feuerman EJ (1980): Vitiligo and the HLA system. Dermatologica 160:100–105.

Mosher DB, Fitzpatrick TB, Ortonne JP (1979): Abnormalities of pigmentation. In Fitzpatrick TB, Eisen AZ, Wolf K, Freedberg IK, Austen KF (eds): "Dermatology in General Medicine." New York: McGraw Hill, pp 582–590.

Mukherjee BN, Das SK (1979): The haptoglobin and transferrin types in West Bengal and a case of haptoglobin 'Johnson.' Hum Hered 20:209–214.

Saha N, Ahmed MA, Wasfi AI, El-Munshid HA (1982): Distribution of serum proteins, red cell enzymes and haemoglobins in vitiligo. Hum Hered 32:46–48.

Sawada Y, Obashi M (1982): Immunopathological studies of vitiligo vulgaris. In "XVI Congressus Internationalis Dermatologiae (Tokyo)." p 172 (abstract).

Wasfi AI, Saha N, El-Munshid HA, El-Sheik FS, Ahmed MA (1980): Genetic association in vitiligo. Clin Genet 17:415–417.

Edited by D.C. Rao