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Segregation of Genetic Hemochromatosis Indexed by Latent Capacity of Transferrin

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Summary

A genetic analysis of the segregation of hereditary hemochromatosis, indexed by the measurement of latent capacity of transferrin (LCAP), was undertaken in an ascertained sample of 147 pedigrees from Brittany, France. There were no mean differences by sex in the distribution of LCAP in the control sample, although in the family data there was a higher representation of males with low values than of females with low values, consistent with the higher proportion of affected males. The results of bivariate segregation analysis revealed no systematic evidence for heterozygous expression either in the biochemical domain of LCAP abnormalities or in increased liability to overt symptomatic disease. Joint consideration of the quantitative variable with hemochromatosis affection status allowed clear resolution of a recessive single-gene inheritance pattern in these families.

Introduction

Hemochromatosis can be characterized as an iron-metabolism disorder, in which chronic augmented intestinal absorption from dietary sources leads to increases in total body iron stores, hepatic deposition of iron storage aggregates, and clinical sequalae, notably including cirrhosis, skin hyperpigmentation, and diabetes mellitus. It has been possible to attribute this disease to a single recessive gene locus by showing tight linkage to the HLA A locus on chromosome six (Simon et al. 1977, 1980; Cartwright et al. 1979; Kravitz et al. 1979; Dadone et al. 1982; Lalouel et al. 1985); males tend to be affected more frequently and earlier than females, possibly because of the protective effects of menstruation and pregnancy and other, as yet unidentified factors (Edwards et al. 1988). Although the precise nature of the genetic defect has not been characterized, individuals with the hemochromatosis genotype can be identified via linkage studies while they are still asymptomatic. The consensus from studies of this kind is that homozygotes with the disease show pathological values in biochemical measures of iron metabolism much earlier than they show the onset of actual clinical symptoms, which occurs in males usually between the ages of 30 and 50 years. Furthermore, while heterozygotes may show moderate abnormalities in biochemical measures with advancing age, they rarely accumulate sufficiently large iron stores to cause the appearance of the typical clinical symptoms (Beaumont et al. 1979; Cartwright et al. 1979; Borwein et al. 1983; Bassett et al. 1984). Therefore, heterozygous expression of the hemochromatosis gene appears to be limited to biochemical abnormalities.

There is an ongoing study of the genetics and expression of hemochromatosis in Brittany, France (Le Mignon 1982). A bivariate segregation analysis of the affection status in conjunction with serum iron concentration as an indicator of underlying liability in 147 pedigrees ascertained through probands revealed no evidence to support heterozygote expression either for overt disease or for serum iron abnormalities (Lalouel et al. 1985). For the purposes of investigating the mode of inheritance of the hemochromatosis locus, serum iron concentration proved to be a valuable indicator of liability, adding considerable information to the analysis. In the present investigation, we consider an alternative

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biological indicator, latent capacity of transferrin, in an analysis of the same set of families, in order to address the issue of heterozygote expression both in clinical and in biochemical terms.

Material and Methods

The French study includes both family data and an independent control sample, which have been described elsewhere (Le Mignon 1982; Lalouel et al. 1985). In brief, 147 families were ascertained from a pool of 274 identified cases. The family structure varies according to which relatives were available for examination, and, in all, there were 907 valid measurements of latent capacity (LCAP), which was measured by the methods of Ramsay (1957*a*, 1957*b*) as being the amount of iron required to achieve saturation of all transferrin binding sites.

The control group, comprising hospitalized patients free of liver disease, hospital staff members, and non–blood relatives of probands from the family data, was used for initial adjustments of the data. The procedures are described in detail by Borecki et al. (submitted). Essentially, there was no relationship of LCAP with age in the control sample, so the family data were simply standardized by sex-specific means and SDs, estimated in the control sample. These data then were submitted to a distribution analysis. On the basis of likelihood methods, three components with equal variance and no residual skewness described the male distribution best, while two components with equal variance and with residual skewness was the most parsimonious model for the female distribution. The fit of a three-component model to the female data did not provide a significantly better likelihood. However, under the common three-component model with no additional power transformation, comparable results were found for males and females. The estimated component means on the standardized scale were −3.93, −1.82, and −0.06, with a common variance of 0.286, in males, and they were −3.63, −1.24, and 0.27, with a common variance of 0.398, in females. Thus, no additional power transformation was applied to the data to ensure comparable scales for males and females.

The bivariate segregation analysis model utilized in this investigation is an extension of the POINTER logic (Lalouel and Morton 1981; Lalouel et al. 1983), attributable to Lalouel et al. (1985), and is implemented in the computer program YPOINT (see fig. 1). Let x denote the standardized LCAP and let y denote the liability to overt disease such that an individual becomes

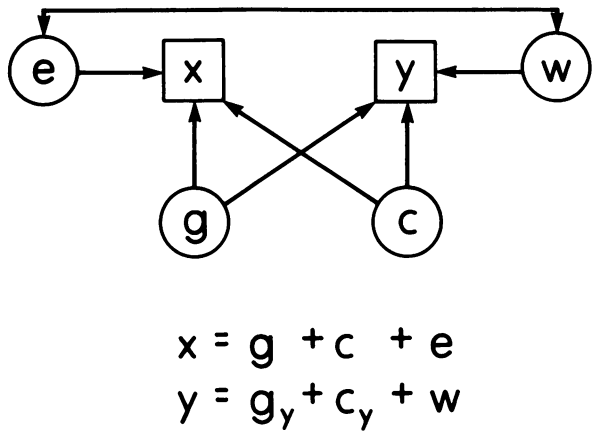

Figure 1 Representation of YPOINT model. The phenotypes are x (quantitative) and y (liability to affection). A single major gene (g) and a set of heritable factors (c) can have different effects on each of the two phenotypes. Correlation between the two traits can result from these pleiotropic determinants or from a nonfamilial residual correlation.

affected whenever y exceeds some threshold. This model postulates that the same three factors—namely, a major locus (g), a multifactorial transmissible component (c), and a nontransmitted environmental component (e)—exert specific effects on x and y. Thus, different displacements, dominance effects, and heritabilities are considered for x and y via common genetic factors: $x = g + c + e$ and $y = g_y + \alpha c + \beta e + w$, so that the variances of x and y are $V = G + C + E$ and $V_y = G_y + \alpha^2 C + \beta^2 E + W$. The component w, with variance W, represents a residual specific to the liability. With respect to the x trait (LCAP), d (dominance; $d = 0$ is recessive, and $d = 1$ is dominant), t (displacement), and q (gene frequency) are defined vis-à-vis a major gene effect, and $H = C/V$ is the multifactorial heritability. Correspondingly for y (liability to hemochromatosis), four additional parameters are defined: d_y, t_y, $H_y = \alpha^2 C/V_y$, and environmental covariance, $E_y = \beta E$. The overall mean and variance of y are arbitrary and are taken here as 0 and 1, respectively. Thus, W is not an independent parameter ($W = 1\text{-}G_y\text{-}\alpha^2 C\text{-}\beta^2 E$). The residual variance, E, with respect to LCAP is estimated in this case, along with the overall mean, u. It should be noted that the formulation of the model requires the quantity E_y to be positive. LCAP and liability to disease are inversely correlated; that is, low values of LCAP are associated with higher risk of disease. Therefore, for these analyses, the sign of each LCAP value was reversed in order to satisfy the program requirements.

Liability classes were assigned on the basis of sex and age groups to reflect both the higher proportion of affected males and the variable age at onset of clinically recognizable symptoms. Four age groups—20–39 years, 40–59 years, 60–79, and 80 years and older—by sex gave rise to eight liability classes, with frequencies derived from the 1975 French national census. With the Le Mignon (1982) data on the age at first diagnosis for a sample of 220 male and 43 female probands, and with an estimated prevalence in the general population of .0011 (Alexandre 1975), the following cumulative risks were estimated: .00098, .00357, .00541, and .00559 in males and .00019, 00064, 00104, and .00109 in females (Lalouel et al. 1985). The ascertainment probability under incomplete selection calculated by Lalouel et al. (1985) was .118, and this estimate was used in the present analysis.

Results

The parameter estimates and likelihood values are shown in table 1. The model in which no familial resemblance is postulated, with the only estimated parameters being E, u, and the covariance E_y (line 7), yields −.434 as the maximum likelihood estimate of the correlation between LCAP and the liability. The most parsimonious model is shown in the first line, which also serves as a general model against which null hypotheses can be tested. It consists of a significant contribution of a major gene, with frequency .061, to the liability to disease and to LCAP. The test of the recessive hypothesis for hemochromatosis ($d_y = 0$, line 2 vs. line 1) was significant ($\chi_1^2 = 6.24$), suggesting possible heterozygote expression. The effect of the gene on LCAP was clearly recessive for low LCAP values, d consistently being estimated to the boundary value of zero. There was also a residual multifactorial family resemblance (line 3 vs. line 1, $\chi_1^2 = 15.69$) accounting for 26% of the total variability. This result suggests that LCAP is not among those biological parameters of iron metabolism that show abnormalities in heterozygotes. Additional explorations of the data revealed no evidence of either a residual covariance ($E_y = 0$) or a multifactorial covariance ($H_y = 0$) between the two traits. Therefore, all the covariance between LCAP values and liability to disease can be explained by the segregation of the hemochromatosis gene. It is possible that, in this particular analysis, the pleiotropic gene effect may overpredict the correlation between LCAP and liability, thus requiring E_y to have a compensatory negative effect. As a negative value for E_y is not permitted in this model, one would expect E_y to be estimated at the

Table 1

YPOINT Model Parameter Estimates and Likelihood Function Values for Joint Analysis of Latent Capacity with Hemochromatosis Affection Status

A. Total Sample									
E	u	d	t	q	H	d_y	t_y	E_y	$-2\ln L + c$
.661	.398	.000	3.291	.061	.260	.305	8.088	.000	.00
.684	.393	.000	3.286	.061	.233	[0]	13.260	.000	6.24
.886	.380	[0]	3.300	.061	[0]	.146	10.630	[0]	15.69
1.339	.917	[0]	[0]	.006	.322	.411	4.280	.943	246.77
2.448	.954	[0]	[0]	.130	[0]	[0]	4.818	.418	406.39
1.096	1.567	.000	3.194	.289	.370	[0]	[0]	.752	626.80
1.610	.613	[0]	[0]	[0]	[0]	[0]	[0]	.551	663.07
3.237	1.408	[0]	[0]	[0]	[0]	[0]	[0]	[0]	1331.78
B. When Seven Subjects with a Diagnosis of "Probably Affected" Are Deleted[a]									
E	u	d	t	q	H	d_y	t_y	E_y	$-2\ln L + c$
.653	.402	[0]	3.307	.061	.257	.214	9.679	[0]	.00
.657	.399	[0]	3.304	.062	.257[b]	[0]	12.620	[0]	3.10
±.470	±.039		±.053	±.001	. . .		±1.882		

NOTE.—Parameters in square brackets are fixed at the values shown.

[a] Reported likelihoods scaled with a different constant than were those above.

[b] Derived parameter. The heritable variance is actually estimated: $\hat{C} = .241 \pm .049$.

smallest permissible value, i.e., zero. However, it is unclear whether this led to $E_y = 0$ in these analyses or whether there is a real lack of residual correlation. In no instance was there evidence of sporadic (nongenetic) cases among these data.

The penetrance of each genotype in each liability class was computed on the basis of two models, one in which the hemochromatosis gene was completely recessive and one with heterozygous disease expression (table 2). In either case, the models predict some disease expression in older males without the homozygous recessive disease genotype. The expression is limited to male heterozygotes in the case where d_y is nonzero, with no penetrance in normal homozygotes (see bottom half of table 2). Among these family data there were seven cases diagnosed as "probably affected" and, therefore, treated as "affected" in this set of analyses. On the supposition that these cases may be responsible for the evidence of heterozygous expression, the two salient pleiotropic models ($d_y = 0$ and d_y estimated) were refit, deleting these individuals. The two hypotheses are presented in the bottom of table 1. Although both models yield parameter estimates similar to those of the corresponding models using the data including the probably affected subjects, the test of $d_y = 0$ was no longer significant ($\chi^2_1 = 3.10$). Further investigation into these individuals revealed that, for the most part, there were minimal clinical abnormalities. Two individuals had mild degrees of hepatomegaly, one was diabetic, and another had associated cutaneous manifestations. The diagnosis of probable hemochromatosis was made predominantly on the basis of biochemical abnormalities, such as high levels of transferrin saturation (76%–100% — and, therefore, low latent capacities) or elevated serum ferritin values (230–575 μM/L). The position of these individuals within their respective family structures always probabilistically favored heterozygosity as the most likely genotype, although, except for one subject, all these individuals shared both HLA haplotypes with the proband. Since it thus has been confirmed that their most likely genotype is homozygosity for the hemochromatosis allele, these individuals may be considered preclinical, and this analysis does not support disease expression of heterozygotes other than for the previously reported biochemical abnormalities.

The model parameter estimates can be used to predict the mean LCAP values for each distinct genotype. These are −0.386 for the normal and heterozygous genotypes and −3.677 for the hemochromatosis recessive genotype. On the original scale (units in μM/L), this translates to 42.6 and 7.1 in males and to 42.1 and 6.4 in females. The actual distribution of LCAP scores in the family data is shown in figure 2, where it is overlaid with the predicted population distribution from YPOINT parameter estimates (under the parsimonious model $d = d_y = 0$). Those values of −3 and lower represent probands and other affected relatives from the family data, which, of course, would not be represented with such high frequency in the general population.

Table 2

Penetrance Estimates under Two Models: (1) Complete Recessivity of Hemochromatosis Allele ($d_y = 0$) and (2) Incomplete Dominance of Normal Allele ($d_y = .305$)

A. $d_y = 0$			
Age (years)	Genotype[a]		
	OO	HO	HH
Males:			
≤39	.00000	.00000	.26054
40–59	.00000	.00000	.94912
60–79	.00165	.00165	1.00000
80+	.00184	.00184	1.00000
Females:			
≤39	.00000	.00000	.05051
40–59	.00000	.00000	.17015
60–79	.00000	.00000	.27650
80+	.00000	.00000	.28979
B. $d_y = .305$			
Age (years)	Genotype[a]		
	OO	HO	HH
Males:			
≤39	.00000	.00000	.26148
40–59	.00000	.00000	.95254
60–79	.00000	.01446	1.00000
80+	.00000	.01603	1.00000
Females:			
≤39	.00000	.00000	.05070
40–59	.00000	.00000	.17076
60–79	.00000	.00000	.27749
80+	.00000	.00000	.29083

[a] O = normal allele; H = hemochromatosis allele.

Discussion

In this analysis, the latent capacity of transferrin was used as a biological indicator in the investigation of the segregation and expression of the hemochromatosis gene. In the first analysis of these data, Lalouel et

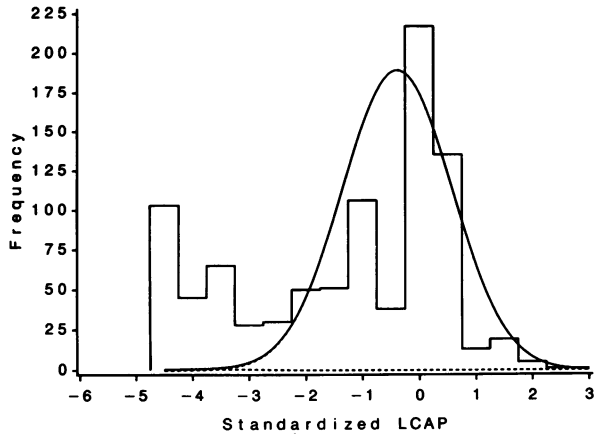

Figure 2 Distribution of standardized LCAP scores in family data. The expected distribution in the general population is superimposed. The excess of individuals with low values reflects the increased proportion of affected individuals in this ascertained data set.

al. (1985) argued convincingly for the value of serum iron as an indicator of underlying liability, largely because it is generally elevated in individuals absorbing excess iron, is uncorrelated with the progress of the disease, and also appears to be identically distributed in males and females. As a biological indicator of hemochromatosis in a segregatin analysis of this type, the LCAP measure provides information slightly different from that provided by serum iron. Transferrin protein serves to move the absorbed iron to target tissues, and, with excess iron, more of the binding sites on transferrin molecules will be saturated, with a correspondingly decreased latent capacity. For susceptible hemochromatosis-homozygous individuals, decreased latent capacity will be correlated with the progress of the disease, up to the point where all binding sites are saturated, leaving no latent capacity to bind more iron. In this sense, latent capacity may provide more information than serum iron to identify correctly preclinical hemochromatosis homozygotes.

There are some noteworthy features regarding the distribution of LCAP values. There is a pole at zero, also evident on the standardized scale, as seen in figure 2. An assumption of the model is that the genotype-specific distributions are normal, with equal variance. Violation of this assumption could affect the estimates of LCAP component means and residual variance. Latent capacity of transferrin also appears to have a similar distribution in control males and females, although, in the ascertained family data, there is a higher representation of males with low values than of females with low values, consistent with the higher proportion of affected males. However, there is further evidence of heterogeneity by sex in the distribution of LCAP values. Our previous admixture analysis of these family data supported both the parsimonious model of three components in males, and the parsimonious model of two components in females (Borecki et al., in press). Although the present segregation model allows for differential disease expression in males and females via sex-specific liabilities, it does not allow for sex-specific differences in the distribution of the quantitative index. In fact, when the dominance parameter is not constrained to the inclusive interval between 0 and 1 (i.e., recessivity to complete dominance), the maximum likelihood estimate of d is negative. This result could be a reflection of the constraints imposed by such simplifying assumptions, as it is not biologically plausible.

Most other studies (e.g., see Cartwright et al. 1979; Kravitz et al. 1979; Dadone et al. 1982) have investigated transferrin saturation, as opposed to the latent capacity, and have reported partial expression in heterozygotes. Although there is an inverse correlation between these measures, transferrin saturation is standardized to the total iron-binding capacity of each individual and thus may not be strictly comparable. Our own study of the distribution of LCAP by genotype, assigned on the basis of HLA haplotype sharing with the respective proband, suggested that, while heterozygotes have lower mean values on the original scale than do homozygous normal individuals (Borecki et al., in press), this difference was not detected as heterozygote expression of LCAP abnormalities under this analysis model.

The information added to the analysis by the inclusion of LCAP as a quantitative indicator permits the conclusion that heterozygotes do not evidence any greater liability to overt disease than do homozygous normal individuals. This is consistent with the findings from previous family studies (Beaumont et al. 1979; Cartwright et al. 1979; Bassett et al. 1981; Borwein et al. 1983). The gene-frequency estimate obtained from this analysis was .061 ± .001, comparable to .056 (Cartwright et al. 1979) and .069 (Dadone et al. 1982) for a Utah population, .055 (Borwein et al. 1983) in a Canadian population, and .069 (Olsson et al. 1983) in a Swedish population.

The inferred segregation pattern of the hemochromatosis gene from the present analysis is qualitatively the same as that from the previous analysis of the same data, in which serum iron was used as the biological indicator of liability (Lalouel et al. 1985). Whereas

Lalouel et al. argued for recessivity of the hemochromatosis allele more on the basis of biological considerations than on the basis of statistical support, the information contained in the measure of latent capacity enables the conclusion of a recessive mode of inheritance.

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