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Linkage Disequilibrium in the Human Insulin/Insulin-Like Growth Factor II Region of Human Chromosome 11

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Summary

Caucasian (N = 128) and Chinese (N = 84) subjects were typed for RFLPs in the insulin (*INS*)/insulin-like growth factor II (*IGF2*) region of chromosome 11. Both the analysis of extended haplotypes and the pairwise measures of linkage disequilibrium among the RFLPs indicate that there is extensive linkage disequilibrium in the *INS/IGF2* region. The disequilibrium extends across the hypervariable region (HVR) located just 5′ to the *INS* gene and encompasses a region of at least 40 kbp. Previous studies had suggested that linkage disequilibrium in the *INS* region was negligible and that this region may therefore contain a "recombinational hotspot" (Chakravarti et al. 1986). However, results of this and another recent study (Thompson et al. 1988) highlight the importance of the frequencies of associated alleles in the ability to detect linkage disequilibrium. Thus, the previous failure to detect disequilibrium in the *INS* region may have reflected a lack of power, rather than a true absence of disequilibrium in this region.

Introduction

The insulin (*INS*)/insulin-like growth factor II (*IGF2*) gene region in the distal (p15.5) region of the short arm of chromosome 11 (Human Gene Mapping Workshop 9, Paris) has been implicated in a number of diseases through association studies (insulin-dependent diabetes mellitus [Bell et al. 1984], atherosclerosis (Mandrup-Poulsen et al. 1984), and diabetic hypertriglyceridemia [Jowett et al. 1984]) as well as linkage studies (manic depressive disease in an Old Order Amish pedigree [Egeland et al. 1987]). Chakravarti et al. (1986) presented data from relatively small samples of U.S. blacks, Caucasians, and Pima Indians suggesting that linkage disequilibrium in the insulin gene region is negligible. On the basis of these observations, they concluded that recombination within the insulin gene region may occur more frequently than expected if crossing—over were uniform throughout the human genome. However, as noted by Brown (1975) and more recently by Thompson et al. (1988), the failure to find evidence for linkage disequilibrium does not necessarily denote its absence. This is because certain combinations of allelic association (e.g., low-frequency allele in disequilibrium with high-frequency allele) will not generate significant evidence for linkage disequilibrium except with very large sample sizes, even when disequilibrium is very near its theoretical maximum.

As part of a continuing study of candidate genes that might contribute to the development of diabetes mellitus in Caucasians and Chinese-Americans, we have examined a number of RFLPs in the *INS/IGF2* region including the insulin gene hypervariable region (HVR), which is a variable numbers of tandem repeats (VNTR)-type RFLP, as well as site-type RFLPs on either side of the HVR. These RFLPs span 40–45 kbp. Our data indicate that there is significant linkage disequilibrium in the region of the *INS/IGF2* genes in both Caucasian and Chinese populations, suggesting that it may be premature to invoke, on the basis of previously reported low levels of linkage disequilibrium, a region of increased recombination—or a recombinational hot spot—near the insulin gene.

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Material and Methods

Subjects

Caucasian (N = 128) and Chinese (N = 84) subjects who have no history of diabetes mellitus were recruited from the San Francisco area as controls for studies of genetic risk factors in diabetes mellitus. Chinese subjects had no non-Chinese ancestry.

DNA Analysis

DNA prepared from peripheral blood lymphocytes was digested with the appropriate restriction endonuclease, electrophoresed, and blotted; nitrocellulose filters were hybridized with ^{32}P-labeled nick-translated probes as described elsewhere (Bell et al. 1981). RFLPs at five sites in the *INS/IGF2* region were typed for the present study (fig. 1, table 1). The HVR RFLPs were designated as described elsewhere (Bell et al. 1984) with 1 being the smaller and 3 the larger allele. For the other RFLPs examined in the present study, the larger allele is designated as allele 1.

Data Analysis

Haplotype frequencies for pairs of RFLPs and for extended haplotypes were estimated using the "myriad haplotype" algorithm of MacLean and Morton (1985) (implemented in a FORTRAN computer program for up to eight loci and available on request). Individuals typed for all of the particular RFLPs under study were included in estimating haplotypes; for example, 47 Caucasian subjects were included in the analyses of the *Taq*I and HVR RFLPs while 38 Caucasian subjects were included in the analyses of the *Rsa*I and HVR RFLPs.

Table 1

Allele Frequencies in the *INS/IGF2* Region

Enzyme/Probe and Subjects (N)	Allele Size (kbp)			Allele Frequency		
	1	2	3	1	2	3
*Rsa*I/phins500:						
Chinese (72)	1.4	1.2		.09	.91	
Caucasian (36)				.44	.56	
*Taq*I/phins500:						
Chinese (73)	4.8	2.8, 2.0		.12	.88	
Caucasian (47)				.13	.87	
*Rsa*I/phins96:						
Chinese (73)	2.1		3.7	.95		.05
Caucasian (128)				.71		.29
*Bam*HI/phins311:						
Chinese (73)	2.2	1.2		.79	.21	
Caucasian (56)				.59	.41	
*Apa*I/phig2-11:						
Chinese (84)	3.5	2.4		.46	.54	

We calculated a measure of linkage disequilibrium, D', for all pairs of RFLPs. This measure was first suggested by Lewontin (1964) and provides information on linkage disequilibrium relative to the maximum (or minimum) possible for a pair of loci. Thus, the D' measure of linkage disequilibrium is frequency independent; it has the same range of values regardless of the frequencies of the alleles that are associated. As noted recently by Hedrick (1987), the property of frequency independence does not hold for many other measures of linkage disequilibrium that have been proposed, including the Δ value used by Chakravarti et al. (1986) to assess

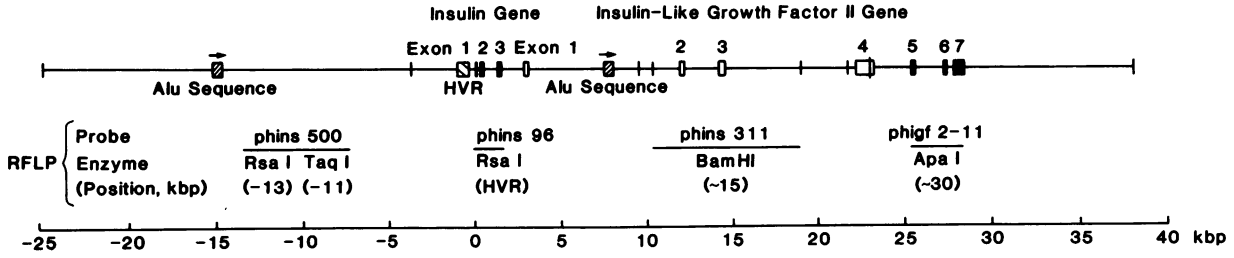

Figure 1 Map of the human *INS/IGF2* region. The zero coordinate corresponds to the transcriptional start site of the *INS* gene. The vertical lines denote *Eco*RI sites. The exons of the *INS* and *IGF2* genes are indicated by filled and open boxes. The open boxes in the *IGF2* gene denote exons encoding various segments of the 5′-untranslated region of the mRNA. Two types of IGF-II transcripts have been identified, one encoded by exons 1–3 and 5–7 and the other by exons 4–7 (Bell et al. 1985; dePagter-Holthuizen et al. 1987). The positions of the hypervariable region (HVR) flanking the insulin gene and of two *Alu*-type dispersed middle repetitive sequences are also indicated. RFLPs are noted together with their approximate locations. The *Taq*I/phins500 and *Rsa*I/phins500 RFLPs are described by Chakravarti et al. (1986), the HVR *Rsa*I/phins96 RFLP by Bell et al. (1984), the *Bam*HI/phins311 RFLP by Xiang et al. (1987), and the *Apa*I/phigf2–11 RFLP by Xiang et al. (1988).

linkage disequilibrium. The D′ value is calculated as $D' = D/D_{max}$, where $D = h_{11} - pq$ (h_{11} is the observed frequency of haplotypes with allele 1 at locus 1 and allele 1 at locus 2 and p and q are the frequencies of allele 1 at loci 1 and 2, respectively) and where $D_{max} = \min[pq, (1-p)(1-q)]$ for $D<0$ or $\min[p(1-q), (1-p)q]$ for $D>0$. Following Thompson et al. (1988), we report D' as positive when the rarer alleles at each of the two loci is associated and as negative when the rarer allele at the locus is associated with the more common allele at the second locus. Although we report the D' value because its frequency independence makes it most useful for comparisons, the test for whether the observed disequilibrium differs significantly from 0 is a test on D, using the value $[D\sqrt{N/p(1-p)q(1-q)}]^2$, which is asymptotically distributed as a χ^2 with 1 df under the null hypothesis of $D = 0$.

We compared the haplotype frequencies estimated for the entire set of RFLPs with those expected on the basis of no disequilibrium. In addition, the observed distribution of haplotypes that could be unambiguously determined (individuals heterozygous at less than two RFLPs) was compared with that expected on the basis of no disequilibrium. We also used raw data from Chakravarti et al. (1986) on the *Rsa*I, *Taq*I, and HVR RFLPs in Caucasian subjects to calculate D' values to compare with those calculated from our Caucasian subjects and to determine whether D differed significantly from 0 in their data for these loci taken pairwise.

Results

The data on the *INS/IGF2* genotypes in our Chinese and Caucasian samples are provided in the Appendix. The haplotype frequencies estimated from all Chinese subjects typed for the five *INS/IGF2* RFLPs and the haplotypes observed in individuals who could be unambiguously haplotyped were both significantly different from those expected on the basis of no linkage disequilibrium (table 2). Similarly, the Caucasian sample demonstrates significant disequilibrium when extended haplotypes in the *INS/IGF2* region are considered (table 3). Pairwise measures of linkage disequilibrium for these RFLPs in Chinese (table 4) and Caucasian (table 5) subjects indicate that there is extensive linkage disequilibrium between the various sites, even between the *Taq*I and *Apa*I sites, which are separated by 40 kbp. It is also apparent from tables 4 and 5 that the ability to detect deviations from no disequilibrium is quite sensitive to the frequencies of the alleles that are associated. For example, the D' value for the *Rsa*I and *Taq*I RFLPs

Table 2

Insulin Gene Region Haplotype Frequencies Estimated from all Chinese Subjects, Haplotype Frequencies Expected for No Linkage Disequilibrium, and Haplotype Numbers Observed in Individuals Heterozygous for Less than Two RFLPs.

Haplotype					Estimated Frequency	Expected Frequency	No. Observed
*Rsa*I	*Taq*I	HVR	*Bam*HI	*Apa*I			
1	1	1	1	2	.01	.00	
1	2	1	1	1	.02	.03	1
1	2	1	1	2	.02	.03	
1	2	1	2	1	.00	.01	
1	2	1	2	2	.00	.01	
1	2	3	1	2	.05	.00	
2	1	1	1	1	.00	.04	
2	1	1	1	2	.02	.04	2
2	1	1	2	1	.00	.01	
2	1	1	2	2	.10	.01	2
2	2	1	1	1	.44	.28	39
2	2	1	1	2	.24	.32	28
2	2	1	2	1	.01	.08	
2	2	1	2	2	.09	.08	4
2	2	3	1	1	.00	.01	
2	2	3	1	2	.01	.02	

NOTE.—Haplotype frequencies were estimated for 72 individuals. Statistics: estimated compared with expected haplotype frequencies, $\chi^2 = 28.9$, 6 df, $P < .001$; observed (unambiguous) compared with expected haplotype frequencies, $\chi^2 = 31.7$, 4 df, $P < .001$. In χ^2 calculations, all cells with expected numbers less than five were pooled.

Table 3

Insulin Gene Region Haplotype Frequencies Estimated from All Caucasian Subjects, Haplotype Frequencies Expected for No Linkage Disequilibrium, and Haplotype Numbers Observed in Individuals Heterozygous for Less than Two RFLPs

Haplotypes						
*Rsa*I	*Taq*I	HVR	*Bam*HI	Estimated Frequency	Expected Frequency	No. Observed
1	1	1	1	.00	.02	
1	1	1	2	.00	.02	
1	1	3	1	.00	.01	
1	1	3	2	.00	.01	
1	2	1	1	.02	.16	1
1	2	1	2	.19	.11	4
1	2	3	1	.18	.07	7
1	2	3	2	.03	.05	1
2	1	1	1	.02	.03	1
2	1	1	2	.13	.02	4
2	1	3	1	.00	.01	
2	1	3	2	.00	.01	
2	2	1	1	.30	.20	11
2	2	1	2	.07	.14	3
2	2	3	1	.07	.08	2
2	2	3	2	.00	.06	

Note.—Haplotype frequencies were estimated for 31 individuals. Statistics: estimated compared with expected haplotypes, $\chi^2 = 17.3$, 5df, $P < .001$; observed (unambiguous) compared with expected, $\chi^2 = 6.6$, 2 df, $P < .05$. In χ^2 calculations, cells with expected numbers less than five were pooled.

is the same in our Chinese, our Caucasian, and the Chakravarti et al. (1986) Caucasian population ($D' = -1.0$), but the D value is significantly different from 0 in only the latter two groups. This is because there is less power to detect disequilibrium when an allele with frequency of .09 is associated with an allele with frequency of .88 (the Chinese population) than when an allele with frequency of .46 is associated with an allele with frequency of .87 (the Caucasian populations). Similarly, comparing the disequilibrium observed between the *Rsa*I and HVR RFLPs with that observed between the *Taq*I and HVR RFLPs in the Chinese population indicates that only in the former is D significantly different from 0. Although the *Rsa*I and *Taq*I minor allele frequencies in Chinese are similar (.09 and .12, respectively), the rarer *Rsa*I allele is in disequilibrium with rarer HVR allele, while the rarer *Taq*I allele is associated with the more common HVR allele. The effects of the correspondence in frequency of the alleles in disequilibrium on the power to detect that disequilibrium are systematically examined in Thompson et al. (1988).

The only direct comparisons that can be made between our data and those of Chakravarti et al. (1986)

Table 4

D′ (Upper Right) and Kilobase Distance (kbp) between RFLPs (Lower Left) for *INS/IGF2* RFLPs in Chinese Subjects

	*Rsa*I	*Taq*I	HVR	*Bam*HI	*Apa*I
*Rsa*I		−1.0	.86***	−1.0	−.14
*Taq*I	2.0		−1.0	.76***	−1.0***
HVR	13.0	11.0		−1.0	−1.0**
*Bam*HI	28.0	26.0	15.0		−.88***
*Apa*I	43.0	41.0	30.0	15.0	

** D significantly different from 0 ($P < .01$).
*** D significantly different from 0 ($P < .001$).

Table 5

D′ Values for a San Francisco Caucasian Sample (Upper Right) and the Caucasian Sample (Lower Left), Examined by Chakravarti et al. (1986)

	*Rsa*I	*Taq*I	HVR	*Bam*HI
*Rsa*I		−1.0**	.63***	.07
*Taq*I	−1.0*		−1.0**	.81***
HVR	.38*	.07		−.68***
*Bam*HI	ND	ND	ND	

Note.—ND = not determined.
* D significantly different from 0 ($P < .05$).
** D significantly different from 0 ($P < .01$).
*** D significantly different from 0 ($P < .001$).

are with the *Rsa*I, *Taq*I, and HVR RFLPs in the Caucasian samples. As Chakravarti et al. (1986) originally reported, there is no disequilibrium evident in the overall distribution of *INS* haplotypes in their Caucasian sample. In contrast, there is disequilibrium in the overall distribution of *INS/IGF2* haplotypes in our Caucasian sample for all RFLPs typed (table 3), as well as for just the *Rsa*I, *Taq*I, and HVR RFLPs (data not shown). The numbers of individuals typed for the *Rsa*I, *Taq*I, and HVR RFLPs were similar in the two studies (31 in ours, 28 in Chakravarti et al. 1986). An examination of the pairwise measures of disequilibrium (table 5) suggests that the major differences appear to be in the observed disequilibrium between the *Taq*I and HVR sites. In our sample, the rarer *Taq*I allele is in significant disequilibrium with the common HVR allele ($D' = -1.0$; D significantly different from 0, $P < .01$), while in the Chakravarti et al. (1986) sample, the rarer *Taq*I allele is in disequilibrium with the rarer HVR allele ($D' = 0.07$; D not significantly different from 0). The reason for this discrepancy is not clear. Since neither sample is particularly large, chance may have played a role in either direction. However, the D' value for the *Rsa*I and *Taq*I RFLPs is -1.0 and D is significantly different from 0, for both studies. The D' value for the *Rsa*I and HVR RFLPs is larger in our Caucasian sample than in the Chakravarti et al. (1986) Caucasian sample (.63 and .38, respectively), but D is significantly different from 0 in both. Thus, both studies demonstrate evidence for significant disequilibrium in the immediate vicinity of the *Rsa*I and *Taq*I polymorphic sites, as well as in the region spanning the *Rsa*I and HVR RFLPs. These findings, and the evidence for extensive disequilibrium between the *Taq*I RFLP and RFLPs located 15 kbp or more 3′ to the HVR in both our Caucasian and Chinese samples, suggest that disequilibrium between the *Taq*I and HVR RFLPs in the Caucasian population is likely.

Discussion

Our data on haplotypes and pairwise measures of linkage disequilibrium indicate that there is extensive linkage disequilibrium in the *INS/IGF2* region in Chinese and Caucasian populations. This disequilibrium extends across the HVR and includes a region of over 40 kbp. Moreover, the disequilibrium between the HVR and the *Rsa*I or *Bam*HI RFLPs located 15 kbp on either side of it was similar, providing no support for a difference between these regions in the frequency of recombination.

These results appear to be quite different from those reported by Chakravarti et al. (1986). However, as noted in the Results section, the two studies differ in conclusions from the test on $D = 0$ with respect to only one pair of RFLPs in Caucasians, the only ethnic group common in both studies. Many of the conclusions of Chakravarti et al. (1986) were derived from studying a U.S. black population that showed no evidence of disequilibrium between the *Rsa*I or *Taq*I RFLPs and the HVR or RFLPs just 3′ to it. While it is true that there was little power to detect disequilibrium in some of these RFLP pairs, the general results for this U.S. black population appear to differ markedly from those for the Caucasian and Chinese populations. The linkage disequilibrium among markers in a region reflects the evolutionary history of those markers in the population being studied, as well as the recombination frequency in the region. Therefore, different ethnic groups would not necessarily be expected to have identical levels of linkage disequilibrium in the *INS/IGF2* region. Chakravarti et al. (1986) noted a considerable difference in the frequency of HVR alleles between U.S. black and African black populations, consistent with the recent admixture of Caucasian genes in the U.S. black population. Such admixture might lead to the apparent lack of disequilibrium in the *INS/IGF2* region in this U.S. black sample, even if both the original African black and the U.S. Caucasian populations had appreciable levels of disequilibrium. Alternatively, one could hypothesize that there are differences among these ethnic groups in the recombination frequency (at least in the *INS/IGF2* region), differences that account for the different levels of disequilibrium. The higher frequency of the larger HVR class 2 and class 3 alleles in black populations might be relevant to this hypothesis, given the previous speculation that it could be the HVR itself that promotes recombination; larger numbers of repeats may be more efficient at promoting recombination. There is no evidence in our data, however, for differences between the Chinese and Caucasian populations in levels of linkage disequilibrium in the *INS/IGF2* region, although Caucasians have a much higher frequency of the larger class 3 alleles (.29) than do Chinese (.05).

We have found evidence for appreciable linkage disequilibrium in the insulin gene region in Caucasians and Chinese. This suggests that the search for population associations between markers from this region and a disease need not be dismissed a priori simply on the basis of a lack of linkage disequilibrium. Moreover, the linkage disequilibrium observed in the *INS/IGF2* re-

gion in our data appears to be quite similar to that observed over comparable distances in other areas of the genome. For example, *Xba*I and *Bgl*II RFLPs at the human glucose transporter (*GLUT*) gene on chromosome 1 are separated by a distance of 30 kbp, with no known VNTR-type regions between them (Fukumoto et al. 1988). In the Chinese sample the D' value for these glucose transporter RFLPs is .73, which is similar to the D' value of .76 observed over the ~40 kbp between the *Taq*I and *Bam*HI RFLPs in the insulin gene region. In summary, we see no need to invoke a higher than average frequency of recombination for the insulin gene region, at least for Chinese and Caucasian populations.

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Appendix

INS/IGF2 RFLP Genotypes

No. of Individuals	Genotype				
	*Rsa*I	*Taq*I	HVR	*Bam*HI	*Apa*I
A. Chinese					
12	2 2	22	11	11	12
13	2 2	22	11	11	11
8	2 2	22	11	12	12
4	1 2	22	11	11	12
8	2 2	12	11	12	12
4	2 2	12	11	12	22
1	1 2	22	13	11	22
2	2 2	22	11	12	22
1	2 2	12	11	22	12
2	2 2	12	11	22	22
6	2 2	22	11	11	22
2	2 2	12	11	11	22
5	1 2	22	13	11	12
1	1 2	22	11	11	11
1	1 2	22	13	12	22
1	1 2	12	11	11	12
1	2 2	22	13	11	12
1	0	22	11	12	12
5	0	0	0	0	22
2	0	0	0	0	11
4	0	0	0	0	12

(*continued*)

Appendix (continued)

INS/IGF2 RFLP Genotypes

No. of Individuals	Genotype				
	*Rsa*I	*Taq*I	HVR	*Bam*HI	*Apa*I
B. Caucasian					
2	1 1	22	33	11	
1	2 2	22	13	11	
1	2 2	11	11	12	
1	1 2	22	33	11	
1	2 2	12	11	12	
3	1 2	22	13	12	
1	2 2	11	11	22	
4	1 2	22	11	12	
2	1 1	22	11	22	
4	2 2	22	11	11	
2	2 2	22	11	12	
2	1 2	12	11	22	
2	1 2	22	13	11	
1	1 1	22	33	12	
1	1 1	22	13	12	
1	2 2	12	13	12	
1	1 1	22	13	11	
1	2 2	12	11	22	
25	0	0	13	0	
2	0	22	13	11	
1	0	22	13	12	
1	0	22	11	11	
30	0	0	11	0	
1	0	12	13	12	
5	0	0	13	12	
2	0	22	11	12	
2	0	0	13	11	
6	0	0	11	12	
3	0	22	11	0	
1	0	0	11	11	
1	0	12	11	0	
3	0	22	33	0	
1	0	22	13	0	
1	0	12	13	0	
1	0	0	33	11	
2	0	0	33	0	
2	1 1	0	13	0	
1	0	0	33	12	
1	0	0	13	22	
1	2 2	0	11	0	
1	1 1	0	33	0	
1	0	0	11	22	
1	2 2	0	13	0	
1	0	0	12	0	

References

Bell, G. I., D. S. Gerhard, N. M. Fong, R. Sanchez-Pescador, and L. B. Rall. 1985. Isolation of the human insulin-like growth factor genes: insulin-like growth factor II and insulin genes are contiguous. Proc. Natl. Acad. Sci. USA 82:6450–6454.

Bell, G. I., S. Horita, and J. H. Karam. 1984. A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. Diabetes 33:176–183.

Bell, G. I., J. H. Karam and W. J. Rutter. 1981. Polymorphic DNA region adjacent to the 5′ end of the human insulin gene. Proc. Natl. Acad. Sci. USA 78:5759–5763.

Brown, A. H. D. 1975. Sample sizes required to detect linkage disequilibrium between two or three loci. Theor. Popul. Biol. 8:184–201.

Chakravarti, A., S. C. Elbein, and M. A. Permutt. 1986. Evidence for increased recombination near the human insulin gene: implication for disease association studies. Proc. Natl. Acad. Sci. USA 83:1045–1049,

dePagter-Holthuizen, P., M. Jansen, F.M.A. van Schiak, R. van der Kammem, C. Oosterwijk, J. L. Van den Brande, and J. S. Sussenbach. 1987. The human insulin-like growth factor II gene contains two development-specific promoters. FEBS Lett. 214:259–264.

Egeland, J. A., D. S. Gerhard, D. L. Pauls, J. N. Sussex, K. K. Kidd, C. R. Allen, A. M. Hostetter, and D. E. Housman. 1987. Bipolar affective disorders linked to DNA markers on chromosome 11. Nature 325:783–787.

Fukumoto, H., S. Seino, H. Imura, Y. Seino, and G. I. Bell. 1988. Characterization and expression of the human HepG2/erythrocyte glucose transporter gene. Diabetes 37:657–661.

Hedrick, P. W. 1987. Gametic disequilibrium measures: proceed with caution. Genetics 117:331–341.

Jowett, N. J., L. G. Williams, G. A. Hitman, and D. J. Galton. 1984. Diabetic hypertriglyceridemia and related 5′ flanking polymorphism of the human insulin gene. Br. Med. J. 228:96–99.

Lewontin, R. C. 1964. The interaction of selection and linkage. I. General considerations: heterotic models. Genetics 49:49–67.

MacLean, C. J., and N. E. Morton. 1985. Estimation of myriad haplotype frequencies. Genet. Epidemiol. 2: 263–272.

Mandrup-Poulsen, T., D. Owerbach, S. A. Mortensen, K. Johansen, H. Meinertz, H. Sorensen, and J. Nerup. 1984. DNA sequences flanking the insulin gene on chromosome 11 confer risk of atherosclerosis. Lancet 1:250–252.

Thompson, E. A., S. Deeb, D. Walker, and A. C. Motulsky. 1988. The detection of linkage disequilibrium between closely linked markers: RFLPs at the AI-CIII apolipoprotein genes. Am. J. Hum. Genet. 42:113–124.

Xiang, K., N. J. Cox, and G. I. Bell. 1988. *Apa*I and *Sat*I RFLPs at the insulin-like growth factor II (IGF2) locus on chromosome 11. Nucleic Acids Res. 16:3599.

Xiang, K., J. H. Karam, and G. I. Bell. 1987. *Bam*HI RFLP at the insulin-like growth factor II (IGF2) locus on chromosome 11. Nucleic Acids Res. 15:7655.