Letters to the Editor

Am. J. Hum. Genet. 73:1465–1466, 2003

Further Evidence of *IBD5/CARD15 (NOD2)* Epistasis in the Susceptibility to Ulcerative Colitis

To the Editor:

We read with interest the two recent articles describing analyses of the *IBD5* (MIM 606348) risk haplotype and inflammatory bowel disease (IBD) in European cohorts (Giallourakis et al. 2003; Mirza et al. 2003). In both European cohorts, the association with the *IBD5* risk haplotype and Crohn disease (CD [MIM 266600]) was replicated. Mirza et al. (2003) additionally provided evidence for interaction between *IBD5* and *CARD15 (NOD2)* (MIM 605956) in CD, a finding not seen in the subsequent paper by Giallourakis et al. (2003) or in our own transmission/disequilibrium testing (TDT) an alysis (Negoro et al. 2003). Subsequent genotype-phenotype analysis by Giallourakis et al. (2003) found no association between *IBD5* and clinical subgroups of patients with CD. In contrast, our group has recently published a case-control study of U.K. whites demonstrating that the association between *IBD5* and CD was confined to those individuals with perianal disease (Armuzzi et al. 2003). It would be interesting to know whether association with this particular phenotype was evaluated in the Giallourakis cohort.

In 187 trios with ulcerative colitis (UC [MIM 191390]), Giallourakis et al. (2003) also reported a novel association between the *IBD5* risk haplotype and UC. This association was most pronounced in those individuals possessing one of the three common CD-associated *CARD15* variant alleles, suggesting an epistatic relationship between these replicated CD loci in patients with UC. In our British patients, we observed a similar linkage disequilibrium (LD) pattern across this locus. However, using both TDT (105 transmissions to 124 nontransmissions, $P = .24$) and case-control studies, we were unable to demonstrate association between the *IBD5* risk haplotype and UC (Armuzzi et al. 2003; Negoro et al. 2003). However, stratification of the *IBD5* results in UC by *CARD15* status was not performed.

Following the report by Giallourakis et al. (2003), we therefore stratified our trios to assess the transmissions of IGR2060a_1 (an *IBD5* risk haplotype–tagging SNP) from heterozygous parents to affected offspring who also carried at least one *CARD15* risk allele, revealing 14 transmissions to 12 nontransmissions ($P = .78$). The lack of association may reflect a true relationship, or it may be a type I error due to the relatively weak power of this analysis. We therefore genotyped for the three common *CARD15* variants and *IBD5* haplotype–tagging SNP to assess any epistatic association in a more powerful case-control study of 278 patients with UC (largely independent of the UC trios) and 232 healthy controls (HC). We found a novel association between the *CARD15* 702Trp variant and UC (table 1). This association was not significant in the *IBD5* wild-type homozygotes but became significant in the *IBD5* heterozygotes and even more significant in the *IBD5* "risk" homozygotes, supporting the theory of an epistatic relation between the *IBD5* locus and *CARD15* in the susceptibility to UC (table 1). However, there was no

Table 1

Case-Control Analysis of *CARD15* 702Trp Allele Association with UC, Stratified by *IBD5* Status

Patient Group	702Trp Allele Frequency (%)	*P* Value (compared to HC)[a]	Relative Risk (95% CIs)[a]	PAR[b]
Healthy controls (232)	2.80			
Ulcerative colitis, overall (278)	5.94	.016	2.14 (1.11–4.12)	.03
IBD5 "nonrisk" homozygotes (75)	3.33	.737	NA	NA
IBD5 heterozygotes (152)	6.25	.019	2.28 (1.11–4.70)	.04
IBD5 "risk" homozygotes (51)	8.82	.004	3.40 (1.41–8.18)	.06

[a] *P* value and RR were calculated using the Fisher's Exact Test.
[b] PAR = population attributable risk.

Table 2

Transmissions versus Nontransmissions in Common CD-Associated *CARD15* Variants from the TDT of 244 UC Trios

Variant	Transmissions	Nontransmissions	*P* Value
702Trp	24	16	.26
908Arg	3	12	.066
Leu1007fsinsC	8	15	.22

such relationship between UC and the 908Arg or Leu1007fsinsC *CARD15* alleles (908Arg: HC 0.65%, UC 0.73%, $P = .73$; Leu1007fsinsC: HC 2.14%, UC 0.74%, $P = .057$), despite *IBD5* stratification (data not shown). We found no particular UC phenotype (need for surgery, age at onset, disease distribution) associated with the *CARD15* 702Trp allele, with or without *IBD5* stratification.

We believe that these data confirm the presence of an *IBD5/CARD15* epistatic relationship in the susceptibility to UC (although the overall population effect is relatively small [table 1]), in contrast to the nonepistatic relationship between *IBD5/CARD15* and CD seen in both our populations and that of Giallourakis et al. (2003). Our data, however, suggest that this epistatic effect is seen exclusively with the 702Trp variant, supporting other data that imply that the 702Trp polymorphism may possess unique properties not shared with the other *CARD15* CD-associated variants (Bonen et al. 2003; Rahman et al. 2003; Sugimura et al. 2003). Indeed, our data suggest a trend toward undertransmission/reduced allele frequency of 908Arg/Leu1007fsinsC in UC (table 2 and the paragraph above). Further work is needed to determine whether the epistatic *IBD5/CARD15* interaction in UC is a "global" *CARD15* phenomenon or, as we have suggested, is restricted to the 702Trp allele. These data support the theory that UC and CD are related polygenic conditions that share some, but not all, susceptibility genes.

Acknowledgments

This work was supported by the British Medical Research Council (LINK Grant, D.Mc.G. and D.v.H.), the Digestive Disorders Foundation/Belmont Trust (D.Mc.G.), the International Organization for the Study of Inflammatory Bowel Disease (D.Mc.G.), the National Association for Colitis and Crohn's Disease (T.A.), the Broad Foundation (T.A.), the Hamanako Symposium (K.N.), and Oxagen Ltd.

DERMOT P. B. MCGOVERN,[1,2] DAVID A. VAN HEEL,[1] KENICHI NEGORO,[1] TARIQ AHMAD,[2] AND DEREK P. JEWELL[2]

[1]*The Wellcome Trust Centre for Human Genetics and* [2]*Gastroenterology Department, University of Oxford, Oxford*

Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for Crohn disease, ulcerative colitis, *IBD5*, and *CARD15 [NOD2]*)

References

Armuzzi A, Ahmad T, Ling KL, De Silva A, Cullen S, Van Heel D, Orchard TR, Welsh KI, Marshall SE, Jewell DP (2003) Genotype-phenotype analysis of the Crohn's disease susceptibility haplotype on chromosome 5q31. Gut 52: 1133–1139

Bonen DK, Ogura Y, Nicolae DL, Inohara N, Saab L, Tanabe T, Chen FF, Foster SJ, Duerr RH, Brant SR, Cho JH, Nunez G (2003) Crohn's disease-associated *NOD2* variants share a signaling defect in response to lipopolysaccharide and peptidoglycan. Gastroenterology 124:140–146

Giallourakis C, Stoll M, Miller K, Hampe J, Lander ES, Daly MJ, Schreiber S, Rioux JD (2003) *IBD5* is a general risk factor for inflammatory bowel disease: replication of association with Crohn disease and identification of a novel association with ulcerative colitis. Am J Hum Genet 73:205–211

Mirza MM, Fisher SA, King K, Cuthbert AP, Hampe J, Sanderson J, Mansfield J, Donaldson P, Macpherson AJ, Forbes A, Schreiber S, Lewis CM, Mathew CG (2003) Genetic evidence for interaction of the 5q31 cytokine locus and the *CARD15* gene in Crohn disease. Am J Hum Genet 72:1018–1022

Negoro K, McGovern DP, Kinouchi Y, Takahashi S, Lench NJ, Shimosegawa T, Carey A, Cardon LR, Jewell DP, van Heel DA (2003) Analysis of the *IBD5* locus and potential gene-gene interactions in Crohn's disease. Gut 52:541–546

Rahman P, Bartlett S, Siannis F, Pellett FJ, Farewell VT, Peddle L, Schentag CT, Alderdice CA, Hamilton S, Khraishi M, Tobin Y, Hefferton D, Gladman DD (2003) *CARD15*: a pleiotropic autoimmune gene that confers susceptibility to psoriatic arthritis. Am J Hum Genet 73:677–681

Sugimura K, Taylor KD, Lin YC, Hang T, Wang D, Tang YM, Fischel-Ghodsian N, Targan SR, Rotter JI, Yang H (2003) A novel *NOD2/CARD15* haplotype conferring risk for Crohn disease in Ashkenazi Jews. Am J Hum Genet 72:509–518

Address for correspondence and reprints: Dr. Dermot P. B. McGovern, Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Headington, Oxford, OX3 7BN, United Kingdom. E-mail: dermot@well.ox.ac.uk

© 2003 by The American Society of Human Genetics. All rights reserved.
0002-9297/2003/7306-0024$15.00

Am. J. Hum. Genet. 73:1467–1468, 2003

Reports of the Death of the Epistasis Model Are Greatly Exaggerated

To the Editor:

I was surprised by the conclusions drawn by Vieland and Huang (2003) that linkage studies of affected sibling pairs (ASPs) cannot, in general, as a matter of mathematical principle, be used to distinguish heterogeneity from epistatic models. A glance at the citation list suggests that the authors have overlooked a critical body of scholarly work that is directly relevant to this issue and that flatly contradicts their conclusions.

Epistasis (interaction) between genes influencing inherited traits has been recognized since 1865, when the results of Gregor Mendel's hybridization experiments were published. Fisher (1918) was the first to partition genetic variance into a series of additive components corresponding to the "main effects" (additive and dominance components) attributable to individual genotypes and "interactions" (epistatic components) determined by combinations of genotypes. Cockerham (1954) used orthogonal contrasts to decompose the epistatic variance into several components; for a two-locus example, under the assumption of linkage equilibrium, the total genetic variance can be written as $(V_G) = V_{A1} + V_{A2} + V_{D1} + V_{D2} + V_{A1A2} + V_{A1D2} + V_{D1A2} + V_{D2D2}$, where V_{A1} and V_{A2} are additive components for the first and second loci; V_{D1} and V_{D2} are dominance components; and V_{A1A2}, V_{A1D2}, V_{D1A2}, and V_{D2D2} are additive-additive, additive-dominance, dominance-additive, and dominance-dominance components, respectively, for the two loci. Epistasis is present in the model when one or more of the V_{A1A2}, V_{A1D2}, V_{D1A2}, and V_{D2D2} components are >0. In experimental intercrosses, analysis-of-variance (ANOVA) techniques are traditionally used to assess the significance of each component, and the classic two-locus statistical test for epistasis compares the fit of the general epistasis model (eight components) to a nested (hierarchical) model with four main effects (i.e., $V_{A1A2} = V_{A1D2} = V_{D1A2} = V_{D2D2} = 0$). More elaborate methods using models based on the variance-components framework have been developed (e.g., Kao and Zeng 2002). It is commonplace to colloquially refer to the main-effects model as the "additive" model. The real-world meaning of the additive model is crystal clear: the effects of each locus on the phenotype are independent of each other—the very same definition of "genetic heterogeneity" used by Vieland and Huang (2003). Or, to put it another way, it doesn't matter on what genetic background you choose to estimate the effects of a locus, you will measure the same effect.

Risch (1990) introduced an elegant mathematical approach to the generalized study of complex human diseases. Identity-by-descent (IBD) vectors in ASPs could be modeled "on the back of an envelope" using mathematically simple models of gene interaction. His "additive," two-locus model carefully defines the joint penetrance (the probability that an individual with a particular multilocus genotype is affected) as a sum of "penetrance summands," one for each locus. The critical issue here is that the "penetrance summands" are deliberate abstractions—they are distinct from marginal, locus-specific penetrances. This is because the sole purpose of the "penetrance summands" is to specify the joint penetrances and thus specify the joint IBD probability vector (an analogous trick was used by Risch et al. [1993] and extended by Bonyadi et al. [1997] to analyze affected animals in backcrosses and intercrosses). The marginal IBD probability vector (IBD observed at each constituent locus) can then be easily solved but *not* some marginal penetrance vector. If I understand them correctly, it is these marginal penetrance vectors (one for each locus) that Vieland and Huang (2003) seek to estimate. The reason why this search is pointless in the context of ASP linkage studies can be understood by reference to the work of James (1971) and Suarez et al. (1978). First, the expected probabilities of the three IBD configurations in an ASP can be calculated from a set of allele frequencies and single-locus penetrances (for any number of alleles; a minimum of four parameters for a two-allele model). However, there is no inverse solution, since the penetrances and allele frequencies cannot be identified starting from a set of IBD probabilities. This was confirmed for ASPs by Whittemore et al. (1991), who pointed out that the inverse solution *can* be solved in larger families. It is this unique statistical property of ASPs that validates the term "non-parametric" to test statistics based on IBDs and ASPs. For aficionados of the ASP paradigm, this is valued as a strength (Farrall 1997*b*); for detractors, however, it is apparently perceived to be a weakness (Greenberg et al. 1996; Spence et al. 2003). The point here is that ASPs are good for detecting linkage (via IBD distortion), but they are hopeless for measuring allele-specific or genotype-specific parameters. This latter objective is of great interest to both "earlier generations" and the "next generation" of gene mappers, but I suspect that more insights will be gained through genotype/haplotype association techniques than by pure linkage tests.

Anyway, Cordell and colleagues (1995) built on the findings of Risch (1990) to expand and generalize the variance-components model for two-locus disease models; in effect, they implemented the ASP equivalent of Cockerham's variance-components model. This was informative, since it led to the conclusion that the main-effects model (see above) was equivalent to Risch's additive model; Risch had chosen this moniker well. Consequently, for ASPs, the classic linkage test for epis-

tasis was immediately obvious: use likelihood-ratio tests to compare the general epistasis model with the additive model (or GEN-ADD in Cordell et al. 1995). This test has been successfully applied (Cordell et al. 1995) and theoretically extended to the case of linked susceptibility genes (Farrall 1997*a*). The existence and mathematical justification of this linkage test directly contradicts the main conclusion of Vieland and Huang (2003).

Vieland and Huang (2003) comment on their surprise on reaching their conclusions. They had counted the number of degrees of freedom in a two-locus IBD matrix (eight) and were suspicious that there might be eight underlying parameters to describe a saturated model. Of course, the eight degrees of freedom are mirrored by the eight variance components in the general epistasis model of Risch (1990) and Cordell (1995). It seems that it will be impossible to reconcile the variance-components epistasis ASP model with the conclusions of Vieland and Huang (2003). I look forward to Vieland and Huang's critique of the variance-components epistasis model and its application to ASP data and also to their re-evaluation of their findings.

MARTIN FARRALL
Department of Cardiovascular Medicine
University of Oxford

References

Bonyadi M, Rushholme SAB, Cousins F, Farrall M, Akhurst RJ (1997) Mapping of a major modifier of embryonic lethality in TGFβ1 knockout mice. Nat Genet 15:207–212

Cockerham CC (1954) An extension of the concept of partitioning hereditary variance for analysis of covariances among relatives when epistasis is present. Genetics 39:859–882

Cordell HJ, Todd JA, Bennett ST, Kawaguchi Y, Farrall M. (1995) Two-locus maximum lod score analysis of a multifactorial trait: joint consideration of IDDM2 and IDDM4 with IDDM1 in type 1 diabetes. Am J Hum Genet 57:920–34

Farrall M (1997*a*) Affected sibpair linkage tests for multiple linked susceptibility genes. Genet Epidemiol 14:103–115

——— (1997*b*) LOD wars: The affected-sib-pair paradigm strikes back! Am J Hum Genet 60:735–738

Fisher RA (1918) The correlation between relatives on the supposition of Mendelian inheritance. Proc R Soc Edinb 52: 399–433

Greenberg DA, Hodge SE, Vieland VJ, Spence MA (1996) Affecteds-only methods are not a panacea. Am J Hum Genet 58:892–895

James JW (1971) Frequency in relatives for an all-or-none trait. Ann Hum Genet 35:47–49

Kao CH, Zeng ZB (2002) Modeling epistasis of quantitative trait loci using Cockerham's model. Genetics 160:1243–1261

Risch N (1990) Linkage strategies for genetically complex traits. I. Multilocus models. Am J Hum Genet 46:222–228

Risch N, Ghosh S, Todd JA (1993) Statistical evaluation of multiple-locus linkage data in experimental species and its relevance to human studies: application to nonobese diabetic (NOD) mouse and human insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 53:702–714

Spence MA, Greenberg DA, Hodge SE, Vieland VJ (2003) The emperor's new methods. Am J Hum Genet 72:1084–1087

Suarez BK, Rice J, Reich T (1978) The generalized sib pair IBD distribution: its use in the detection of linkage. Ann Hum Genet 42:87–94

Vieland VJ, Huang J (2003) Two-locus heterogeneity cannot be distinguished from two-locus epistasis on the basis of affected-sib-pair data. Am J Hum Genet 73:223–232

Whittemore AS, Keller JB, Ward MJ (1991) Family data determine all parameters in Mendelian incomplete penetrance models. Ann Hum Genet 55:175–177

Address for correspondence and reprints: Dr. Martin Farrall, Department of Cardiovascular Medicine, University of Oxford, The Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford OX3 7BN, United Kingdom. E-mail: Martin.Farrall@well.ox.ac.uk

© 2003 by The American Society of Human Genetics. All rights reserved.
0002-9297/2003/7306-0025$15.00

Am. J. Hum. Genet. 73:1468–1471, 2003

Affected-Sib-Pair Data Can Be Used to Distinguish Two-Locus Heterogeneity from Two-Locus Epistasis

To the Editor:

I was surprised by the conclusions of Vieland and Huang (2003), who maintain that two-locus heterogeneity cannot be distinguished from two-locus epistasis on the basis of affected-sib-pair (ASP) data. Since a number of previous studies (not cited or discussed by Vieland and Huang [2003]) have, in fact, used ASP data to distinguish between two-locus heterogeneity and two-locus epistasis (see, for example, Cordell et al. 1995, 2000; Farrall 1997), there appears to be some contradiction between the conclusions drawn by Vieland and Huang (2003) and previous work.

An obvious explanation for the contradiction would be that the definitions of heterogeneity and epistasis used by Vieland and Huang (2003) differ from those used in previous studies. There is still some debate in the literature over the precise mathematical definition of epistasis, and, indeed, the term is often used without definition, so that it is difficult to know which definition is being assumed in any given situation (Cordell 2002).

Most models are defined in terms of an underlying penetrance matrix for the effects of two diallelic loci,

$$\begin{pmatrix} w_{11} & w_{12} & w_{13} \\ w_{21} & w_{22} & w_{23} \\ w_{31} & w_{32} & w_{33} \end{pmatrix},$$

where w_{ij} is the penetrance for genotype i at locus 1 and genotype j at locus 2 (i.e., the probability of disease, given that an individual has $i - 1$ copies of the risk allele at locus 1 and $j - 1$ copies of the risk allele at locus 2). Vieland and Huang appear to only consider the situation in which the underlying penetrance matrix takes one of the following forms,

$$\begin{pmatrix} f_P & f_P & f_A \\ f_P & f_P & f_A \\ f_B & f_B & f_{AB} \end{pmatrix}, \begin{pmatrix} f_P & f_A & f_A \\ f_P & f_A & f_A \\ f_B & f_{AB} & f_{AB} \end{pmatrix}, \text{ or } \begin{pmatrix} f_P & f_A & f_A \\ f_B & f_{AB} & f_{AB} \\ f_B & f_{AB} & f_{AB} \end{pmatrix},$$

which they refer to as RR (recessive-recessive), RD (recessive-dominant), and DD (dominant-dominant), respectively. Given this parameterization, they choose to define two-locus heterogeneity as the parameter restriction

$$f_{AB} = f_A + f_B - f_A f_B$$

and two-locus epistasis as any penetrance model not satisfying this restriction.

As pointed out by Vieland and Huang (2003), this definition does not coincide with the definition of a heterogeneity model used by Risch (1990), nor does it coincide with his definitions of an additive or a multiplicative model, all of which have, in various situations, been considered to represent a lack of epistasis (Cordell 2002). Thus, we have one immediate explanation for the apparent contradiction between the conclusions of Vieland and Huang (2003) and the results of Cordell et al. (1995, 2000) and Farrall (1997), who used the Risch (1990) definitions of heterogeneity, additivity, and multiplicativity: it is possible that ASP data *can* be used to distinguish two-locus heterogeneity from two-locus epistasis when these concepts are defined in terms of the Risch (1990) models of heterogeneity, additivity, and multiplicativity, but not when they are defined using the definition proposed by Vieland and Huang (2003).

Details of the methodology for distinguishing between the Risch (1990) two-locus models of heterogeneity, additivity, and multiplicativity using ASP data are described in Cordell et al. (1995, 2000) and Farrall (1997). Briefly, these authors show that the 3 × 3 matrix of (2, 1, 0) identity-by-descent (IBD)–sharing probabilities for ASPs can be written in terms of the prior IBD-sharing probabilities and eight variance-component–ratio parameters: V_{A_1}/K^2, V_{D_1}/K^2, V_{A_2}/K^2, V_{D_2}/K^2, $V_{A_1A_2}/K^2$, $V_{A_1D_2}/K^2$, $V_{D_1A_2}/K^2$, and $V_{D_1D_2}/K^2$. Here, K corresponds to the population prevalence of disease; V_{A_i} and V_{D_i} correspond to the additive and dominance variances due to locus i; and $V_{A_1A_2}$, $V_{A_1D_2}$, $V_{A_2D_1}$, and $V_{D_1D_2}$ to the additive × additive, additive × dominance, dominance × additive, and dominance × dominance variances due to locus 1 and locus 2, respectively (Kempthorne 1957). Although these parameters, together with the underlying penetrances and allele frequencies from which they are derived, are not individually identifiable from the 3 × 3 matrix of IBD sharing, the eight variance-component–ratio parameters are identifiable. The fit of different penetrance models is compared by performing likelihood ratio tests, with the likelihood defined in terms of these eight variance-component–ratio parameters. The general epistatic (saturated) model corresponds to a situation in which the eight parameters are allowed to vary freely; the additive model (which can be shown to be virtually indistinguishable from the heterogeneity model with regard to IBD sharing among ASPs) corresponds to the restriction that $V_{A_1A_2}/K^2 = V_{A_1D_2}/K^2 = V_{D_1A_2}/K^2 = V_{D_1D_2}/K^2 = 0$; and the multiplicative model corresponds to the combined restrictions $V_{A_1A_2}/K^2 = V_{A_1}/K^2 \times V_{A_2}/K^2$, $V_{A_1D_2}/K^2 = V_{A_1}/K^2 \times V_{D_2}/K^2$, $V_{D_1A_2}/K^2 = V_{D_1}/K^2 \times V_{A_2}/K^2$, and $V_{D_1D_2}/K^2 = V_{D_1}/K^2 \times V_{D_2}/K^2$.

Although the definition of heterogeneity proposed by Vieland and Huang (2003) does not precisely correspond to that used by Risch (1990), these definitions can, in fact, be shown to be equivalent in the special case of a model with no phenocopies ($f_P = 0$). The rationale for the model proposed by Vieland and Huang (2003) appears to come from the desire to express the population prevalence, K, in the form

$$K = K_A + K_B - K_A K_B ,$$

which is a natural expression for the probability of the union of two independent events. In the Risch heterogeneity model, the penetrances w_{ij} may be written as $x_i + y_j - x_i y_j$, and Risch (1990) showed that, with this parameterization, the population prevalence can also be written as

$$K = K_1 + K_2 - K_1 K_2 ,$$

where K_1 and K_2 correspond to contributions of locus 1 and 2, respectively, so that the Risch model also leads to the desired population prevalence structure. Note that the actual definitions of K_1 and K_2 in the Risch formulation differ from the definitions of K_A and K_B in the Vieland and Huang formulation, except when $f_P = 0$. It is not clear whether the Vieland and Huang definition of heterogeneity,

$$f_{AB} = f_A + f_B - f_A f_B ,$$

in fact leads to the desired prevalence structure if $f_P \neq 0$, since their calculation of the prevalence, K, as

$$K = q_A^2(1 - q_B^2)f_A + (1 - q_A^2)q_B^2 f_B + q_A^2 q_B^2 f_{AB}$$

(which does lead to the desired structure when K_A and K_B are defined as $q_A^2 f_A$ and $q_B^2 f_B$, respectively) in fact only holds when $f_P = 0$. In the RR model of Vieland and Huang (2003), the Risch heterogeneity model can be shown to correspond to the restriction

$$f_{AB} = \frac{f_A + f_B - f_A f_B - f_P}{1 - f_P},$$

which might be considered to be a more general form of heterogeneity than that proposed by Vieland and Huang (2003).

Nevertheless, Vieland and Huang (2003) are correct in stating that, given a set of penetrances satisfying either the Risch (1990) or the Vieland and Huang (2003) definition of heterogeneity, it is possible to find another set of penetrances, equally compatible with the observed IBD sharing, that does not satisfy the respective definition of heterogeneity. This is because for any set of penetrances, w_{ij}, it can be shown that multiplying each penetrance by a constant, C, leads to an identical set of variance-component ratios and thus to an identical set of IBD-sharing probabilities. For the additive and multiplicative models of Risch (1990), this has no effect on the underlying penetrance structure, since we may write the new penetrance as $W_{ij} = Cw_{ij} = Cx_i + Cy_j = X_i + Y_j$, for the additive model, and $W_{ij} = Cw_{ij} = \sqrt{C}x_i \times \sqrt{C}y_j = X_i \times Y_j$, for the multiplicative model. For the heterogeneity model, however, we have $W_{ij} = Cw_{ij} = Cx_i + Cy_j - Cx_iy_j$, which cannot in general be written as $X_i + Y_j - X_iY_j$. Similarly, one can show that, on the prevalence scale, the additive and multiplicative structures ($K = K_1 + K_2$ and $K = K_1K_2$, respectively) are unaltered by multiplying the penetrance matrix by a constant, but the heterogeneity structure becomes $K = CK_1 + CK_2 - CK_1K_2$ or, equivalently, $K/C = K_1 + K_2 - K_1K_2$. Thus, the models fitted by Cordell et al. (1995, 2000) and Farrall (1997) can be thought of as implicitly using this as their definition of heterogeneity on the prevalence scale, for any constant value of C. Although perhaps less satisfactory than the original structure, $K = K_1 + K_2 - K_1K_2$, it can nevertheless be seen to correspond to a situation in which the effects of the two loci act in the required form with regard to the scaled prevalence, K/C, rather than with regard to the prevalence itself. Alternatively, because of the close correspondence between the Risch heterogeneity and additive models with regard to IBD sharing among ASPs (Cordell et al. 1995), one can simply consider "heterogeneity" to be defined as corresponding to an additive model for the penetrance and prevalence structures.

The Risch definition of heterogeneity is much more general than the Vieland and Huang formulation, as it does not assume dominance or recessiveness at either locus. It has the advantage of extending to multiallelic systems and does not, as suggested by Vieland and Huang (2003), preclude models with no phenocopies (which can be modeled, for example, by allowing $x_1 = y_1 = 0$). Moreover, we have seen that a generalization of this formulation leads to models for IBD-sharing probabilities that can be tested using ASP data. For all these reasons, the Risch (1990) definition would seem to be preferable to that proposed by Vieland and Huang (2003). A final question of interest is whether the penetrance models implied by either of the prevalence structures, $K = K_1 + K_2 - K_1K_2$ or $K/C = K_1 + K_2 - K_1K_2$, do in fact correspond to some biological mechanism of interest. There is still considerable debate within the literature concerning the biological interpretation of mathematical models of epistasis (Cordell 2002). Some would argue that biological models of interest at the micro scale (at the level of biochemical reactions, for example) are indistinguishable when measured at the macro scale of epidemiological studies, since many different underlying models can lead to essentially the same disease risks (Thompson 1991). As mentioned, several authors have considered departure from a multiplicative model as an indication of epistasis, which can be tested on the basis of a positive correlation between IBD-sharing probabilities at the relevant loci (Holmans 2002). This definition leads to natural tests of interaction on the log-odds scale in the standard epidemiological framework, but it is unclear whether there is any advantage to this definition with regard to elucidation of the underlying biological mechanisms. Others have used tests based on different aspects of the correlational structure of genotype data across loci (e.g., Cox et al. 1999). The relationship between these tests and tests based on mathematical models for the penetrance matrix remains to be elucidated.

HEATHER J. CORDELL
Department of Medical Genetics
University of Cambridge

References

Cordell HJ, Todd JA, Bennett ST, Kawaguchi Y, Farrall M (1995) Two-locus maximum lod score analysis of a multifactorial trait: joint consideration of *IDDM2* and *IDDM4* with *IDDM1* in type 1 diabetes. Am J Hum Genet 57:920–934

Cordell HJ, Wedig GC, Jacobs KB, Elston RC (2000) Multilocus linkage tests based on affected relative pairs. Am J Hum Genet 66:1273–1286

Cordell HJ (2002) Epistasis: what it means, what it doesn't mean, and statistical methods to detect it in humans. Hum Mol Genet 11:2463–2468

Cox NJ, Frigge M, Nicolae DL, Concannon P, Hanis CL, Bell GI, Kong A (1999) Loci on chromosomes 2 (*NIDDM1*) and 15 interact to increase susceptibility to diabetes in Mexican Americans. Nat Genet 21:213–215

Farrall M (1997) Affected sibpair linkage tests for multiple linked susceptibility genes. Genet Epidemiol 14: 103–115

Holmans (2002) Detecting gene-gene interactions using affected sib pair analysis with covariates. Hum Hered 53:92–102

Kempthorne, O (1957) An introduction to genetic statistics. John Wiley & Sons, New York

Risch N (1990) Linkage strategies for genetically complex traits. I. Multilocus models. Am J Hum Genet 46:222–228

Thompson WD (1991) Effect modification and the limits of biological inference from epidemiologic data. J Clin Epidemiol 44:221–232

Vieland VJ, Huang J (2003) Two-locus heterogeneity cannot be distinguished from two-locus epistasis on the basis of affected-sib-pair data. Am J Hum Genet 73:223–232

Address for correspondence and reprints: Dr. Heather J. Cordell, Department of Medical Genetics, Wellcome Trust Centre/MRC Building, Cambridge Institute for Medical Research, Addenbrookes Hospital, Hills Road, Cambridge, CB2 2XY, United Kingdom. E-mail: heather.cordell@cimr.cam.ac.uk

© 2003 by The American Society of Human Genetics. All rights reserved.
0002-9297/2003/7306-0026$15.00

Am. J. Hum. Genet. 73:1471–1473, 2003

Reply to Cordell and Farrall

To the Editor:

"…Vieland and Huang (2003) are correct in stating that, given a set of penetrances satisfying either the Risch (1990) or the Vieland and Huang (2003) definition of heterogeneity, it is possible to find another set of penetrances, equally compatible with the observed IBD [identity-by-descent] sharing, that does not satisfy the respective definition of heterogeneity." Thus concludes Cordell (2003 [in this issue]), and interested readers may wish to consult the section of her text immediately following that statement for a recapitulation of our proof. This means that affected sibling pairs (ASPs) cannot be used to distinguish two-locus heterogeneity (2L HET) from two-locus epistasis (2L EPI), as we defined these terms, which is exactly what we claimed to have proved in our paper (Vieland and Huang 2003). (More precisely, this completes the proof for HET models; see Vieland and Huang [2003] for the extension to EPI models.)

Cordell argues, however, that we would be able to differentiate 2L HET from 2L EPI in ASP data, *if we were to change what we meant by these terms*. This is certainly true, and the literature is replete with alternative, often conflicting, mathematical representations of HET and EPI. (See Cordell [2002] and Vieland and Huang [2003] for further discussion.) So how do we decide on our definitions in the first place?

In selecting the definition of 2L HET to be used in Vieland and Huang (2003), we took as our primary objective the derivation of a mathematical expression that would capture a class of 2L models, such that any geneticist would agree they represented locus HET in its classical form. We therefore focused our discussion on models with simple dominance structures—that is, where the (marginal) mode of inheritance was either dominant or recessive at each locus—although relaxing this assumption, as in Risch's (1990) definition, does not affect our proofs. (Risch's definition also differs from ours in the way "phenocopies" are handled, although it does allow for $f_P = 0$, in Vieland and Huang's [2003] notation, as Cordell notes.) The resulting definition of HET (Vieland and Huang 2003; equation 2) seems to us impeccable, in the sense that any penetrance table that is consistent with it is readily seen to represent the classical concept of locus HET in terms of independent gene action, as it applies to the known heterogeneous Mendelian disorders. We then defined 2L EPI as any model that did not qualify as HET, on the grounds that either the genes act independently or they do not.

We stand by our mathematical definitions as genetically well justified and appropriate to the subject matter of our paper. As far as we can tell, Cordell is also fundamentally in agreement with our definition of HET from a *genetic* point of view, at least if the definition is given in the generalized form of Risch (1990).

Cordell nevertheless proposes to adopt a different definition for the purposes of reconciling the findings of Vieland and Huang (2003) with earlier work, in which she and her colleagues developed and applied a test for distinguishing 2L HET (as defined by Risch) from 2L EPI in ASPs (Cordell et al. 1995). In particular, she proposes to replace the definition based on a particular structure in the prevalence, K (as in the work of Risch [1990] and Vieland and Huang [2003]), with a definition based instead on K/C, where C is a constant (see Cordell's letter in the current issue for details), saying that the models fitted in the 1995 paper "can be thought of as implicitly using this…definition of heterogeneity on the prevalence scale."

The significance of this shift to a definition of 2L HET "on the prevalence scale" is obscure in the extreme, until one recognizes that the new definition is in essence a simple restatement of our main result. Letting $f_A^* = f_A/C$, $f_B^* = f_B/C$, and $f_{AB}^* = f_{AB}/C$, Cordell's new definition of HET can be written as $f_{AB}^* = f_A^* + f_B^* - f_A^* f_B^*$. This produces the requisite structure "on the prevalence scale," which is seen, for example, by substituting these expressions back into the equations on p. 225 of Vieland and Huang (2003). [We note a typographical error in

the second line of the second equation on p. 225 of Vieland and Huang (2003), which should read as follows: $q_A^2 f_A + q_B^2 f_B - q_A^2 q_B^2 (f_A + f_B - f_{AB})$.] But in terms of the original penetrances, a little algebra shows that this translates back to a definition of 2L HET as $f_{AB} = f_A + f_B - (1/C)(f_A \times f_B)$. When $C = 1$, therefore, Cordell's definition and ours coincide; for any other value of C, models conforming to her definition of HET will satisfy neither our definition nor that of Risch. But they will produce identical IBD probabilities, because the original penetrance ratios—f_A/f_{AB}, etc.—and the rescaled penetrance ratios—f_A^*/f_{AB}^*, etc.—are identical (see Vieland and Huang [2003], p. 227–228, for details).

Cordell's new definition of HET "works" by simply *reclassifying* as HET the infinitely many corresponding EPI models, which, as Vieland and Huang (2003) proved, cannot be distinguished from HET by their IBD probability structure. We persist in calling these models "EPI" because (1) they fail to qualify as HET under our genetically based definition (or that of Risch) and (2) because their structure precludes expression in terms of probabilistic independence across the two loci, which we take as the sine qua non of any reasonable definition of HET.

The new definition thus vindicates the Cordell et al. (1995) procedure as a statistical test. We can continue to refer to this as a test of 2L HET versus 2L EPI if we like, but only insofar as we are willing to consider epistasis between loci as a form of HET. This is surely putting the cart before the horse. If we wish to use statistical modeling to learn something about real diseases, we need to start with the genetic definitions of our terms and then seek mathematical representations appropriate to statistical modeling—not the other way around. This is the only procedure for ensuring that our statistical conclusions have genetic relevance.

The language that Cordell and Farrall use to describe variance-components (VC) models for dichotomous traits additionally complicates the issue of definitions. The fully saturated 2L VC model contains locus-specific, or "main-effects," terms, plus terms involving both loci, or "interaction" terms. The saturated model is referred to, with solid historical precedent, as Farrall (2003) notes, as "the general epistatic…model" (Cordell 2003 [in this issue]); a test of the fit of the main-effects–only model against the saturated model is called a test of "whether epistatic components of variance are required in the model" (Cordell et al. 1995).

But the main-effects model is identical to neither our definition of 2L HET nor that of Risch. That is to say, there are (dichotomous) 2L HET models that have these so-called epistatic components of variance in the VC equation. It may seem odd to say that HET models can involve interlocus interaction terms, but nevertheless, when the fitted VC model includes nonzero interaction terms, one might still be looking at a HET model—that is, a model in which the genes are acting independently on the phenotype (Vieland and Huang 2003; Risch 1990).

A rigorous, a priori definition of HET is necessary to systematically investigate which subclass of the saturated VC model actually represents locus HET in the usual genetic sense, and, indeed, this was the starting point of our own investigation. Although we gave our proof in terms of penetrance-based models rather than VC models, the VCs can be parameterized in terms of the more fundamental penetrance parameters, so that the Vieland and Huang (2003) proof applies to either framework, as Cordell (2003 [in this issue]) makes clear. Thus, shifting the discussion from penetrance-based models to VC models has nothing to do with the mathematics of our argument, and the language in which VC models are described should not distract us from the underlying issue.

Finally, we would like to address Farrall's (2003) comment that the method of Cordell et al. (1995) for distinguishing 2L HET from 2L EPI had already been "successfully applied" to an ASP data set of patients with insulin-dependent diabetes mellitus (IDDM). How could the method have been *successfully* applied, in view of the subsequent Vieland and Huang (2003) results?

The Cordell et al. (1995) paper actually included an important mathematical caveat, which should have raised a flag even at the time. Acknowledging that the VC parameters could not all be simultaneously (uniquely) estimated from ASP data, Cordell et al. constrained the maximization procedure by fixing the population prevalence, K, at a specific numerical value, and, for the multiplicative model, they fixed two prevalences, one for each locus. These ad hoc constraints solved the numerical problem but could have distorted the relative fit of different 2L models. (Indeed, there may be a connection between this procedure and Cordell's new definition of 2L HET on the prevalence scale.) Thus, they did not in fact *succeed* in completely fitting the models. The impact of their numerical procedures on comparative model fitting would need to be thoroughly investigated before we could interpret the results as telling us something interesting about IDDM.

Their analyses were also conducted under the assumption that IDDM is actually a 2L disease, an assumption that is almost certainly incorrect, as they pointed out (Cordell et al. 1995). But model fitting is based on parameter estimation, and the behavior of estimates based on the assumption of 2L inheritance has never been systematically investigated for models having more than two loci. Cordell et al. (2000) made this point explicitly, saying that for a complex disease, "we must beware of overinterpretation of the estimates of the variance components parameters, since…it is not clear to

what extent the parameter estimates generated under the assumption of a two-locus—or even a three-locus—disease model will resemble their true population quantities." This caution applies to comparative model-fitting results based on parameter estimation as well.

Thus, the results of the application of Cordell et al.'s (2000) methods to the IDDM data set needed all along to be interpreted with more than a modicum of caution. This is in no way meant to disparage the elegant mathematical work in that paper, and possibly the analyses do elucidate some interesting aspects of the data. However, the simple existence of a statistical procedure does not, in and of itself, ensure that its application to complex genetic data is appropriate or meaningful. To know what, if anything, the results of Cordell et al. (1995) could really have taught us about IDDM, we would need further evaluation of the method in application to multilocus data. Appropriate definitions of HET and EPI would need to be the starting point of any such evaluation, rather than the conclusion.

VERONICA J. VIELAND[1, 2] AND JIAN HUANG[1, 3]
[1]*Program in Public Health Genetics, College of Public Health,* [2]*Department of Psychiatry, Roy J. and Lucille A. Carver College of Medicine, and* [3]*Department of Statistics and Actuarial Science, College of Liberal Arts and Sciences, University of Iowa, Iowa City*

References

Cordell HJ (2003) Affected-sib-pair data can be used to distinguish two-locus heterogeneity from two-locus epistasis. Am J Hum Genet 1468–1471 (in this issue)

Cordell HJ (2002) Epistasis: what it means, what it doesn't mean, and statistical models to detect it in humans. Hum Mol Genet 11:2463–2468

Cordell HJ, Wedig GC, Jacobs KB, Elston RC (2000) Multilocus linkage tests based on affected relative pairs. Am J Hum Genet 66:1273–86

Cordell HJ, Todd JA, Bennett ST, Kawaguchi Y, Farrall M (1995) Two-locus maximum LOD score analysis of a multifactorial trait: joint consideration of IDDM2 and IDDM4 with IDDM1 in type 1 diabetes. Am J Hum Genet 57:920–934

Farrall M (2003) Reports of the death of the epistasis model are greatly exaggerated. Am J Hum Genet 1467–1468 (in this issue)

Risch N (1990) Linkage strategies for genetically complex traits. I. Multilocus models. Am J Hum Genet 46:222–228

Vieland VJ, Huang J (2003) Two-locus heterogeneity cannot be distinguished from two-locus epistasis on the basis of affected sib-pair data. Am J Hum Genet 73:223–232

Address for correspondence and reprints: Veronica Vieland, Center for Statistical Genetics Research, 2190 Westlawn, University of Iowa, Iowa City, IA 52242. E-mail: veronica-vieland@uiowa.edu

© 2003 by The American Society of Human Genetics. All rights reserved.
0002-9297/2003/7306-0027$15.00