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No Evidence for Parent of Origin Influencing Premature Ovarian Failure in Fragile X Premutation Carriers

To the Editor:
We were interested to read, in the February edition of the *Journal,* an article by Hundscheid et al. (2000) that reported an imprinting effect on the fragile X premutation, such that paternally inherited premutations are more likely to give rise to premature ovarian failure (POF). We were impressed by the rigorous design of the study, which ensured that all women were interviewed personally and that adherence to strict criteria for the definition of POF was maintained. However, we were very surprised by the results of the study, in light of the results of our own study of a similar cohort of women from Wessex, United Kingdom.

We interviewed 352 women from families with the fragile X premutation about their reproductive and menstrual histories; of these women, 116 carried premutation-sized (51–200-repeat) alleles and were from 62 families (Murray et al., in press). These families have been investigated extensively during the past 15 years, and, therefore, we have almost complete ascertainment of "at-risk" individuals. All premutation and full-mutation carriers and their unaffected first- and second-degree relatives were invited to participate in the study and were interviewed either in person or via the telephone. Women who were using the contraceptive pill were excluded from the analysis, and those women who were still menstruating or who had undergone a hysterectomy were taken as censored values. We used Kaplan-Meier survival analysis to demonstrate that the premutation group had a mean age at menopause of 47.87 years, compared with 52.96 years for the full-mutation and normal groups combined (Murray et al., in press). It was apparent, from inspection of our data, that there were no parent-of-origin differences. In a separate study of women ascertained through the presence of POF, six pedigrees were illustrated (Murray et al. 1998); we could determine the origin of the premutation in only four women with POF (who were from two families), and the origin was maternal in each case.

However, the study by Hundscheid et al. (2000) prompted us to reevaluate our data and to analyze them in a similar fashion. Our population of 116 premutation carriers was comprised of 40 carriers with maternal transmissions, 51 carriers with paternal transmissions, and 25 carriers for whom we were not able to determine the origin of transmission. The results of survival analysis comparing maternal and paternal premutations demonstrated no significant shift in age at menopause between the two groups ($\chi^2_1 = 0.0143$; $P = .91$). POF was defined as spontaneous cessation of menses for >1 year, before age 40 years—a definition that is essentially the same as that in the study by Hundscheid et al. Similar to table 1 in the study by Hundscheid et al., table 1 in our study shows that we have only considered females that were of age ⩾40 years at the time of the interview; POF is not significantly more common in either group (two-tailed Fisher's exact test; $P = .669$).

The results of analyses of 116 female premutation carriers from families with fragile X do not provide any evidence with which to support the suggestion that there is imprinting of the *FMR1* gene. We can provide no explanation for the discrepancy between our data and the material presented in the report by Hundscheid et al. (2000). In both studies, survival analysis was used to estimate the distribution of age at menopause in an uncensored cohort, since any method that excludes premenopausal subjects underestimates the mean. Survival analysis extracts full and unbiased information from all relationships to probands, which have different frequencies of paternally inherited fragile X premutations (PIP) and maternally

Table 1

Origin of Premutation in Women of Age ⩾40 Years at the Time of the Interview

Classification	No. of Premutations of Origin		Total
	PIP	MIP	
Age at Menopause:			
<40 years	2	5	7
⩾40 years	6	10	16
Proved ovarian function[a]	4	3	7
Total	12	18	30

[a] Not menopausal, with follicular-phase FSH level <40 U/liter.

inherited fragile X premutations (MIP). Daughters of normal transmitting males all have PIP, sisters of probands never have PIP, mothers of probands have a ratio of nearly 3:1, and so forth (Morton and Macpherson 1992). Neither the present study nor the study by Hundscheid et al. categorizes relationship, which presumably accounts for the observed difference in frequencies; however, this is irrelevant if survival analysis is used correctly. In both studies, all subjects were interviewed and hearsay evidence was rejected. In an unspecified proportion of cases, Hundscheid et al. obtained age at menopause from medical records, whereas we accepted the subject's recall. Our definitions of POF, spontaneous menopause, unnatural menopause, menstrual history, and medication are indistinguishable from those of Hundscheid et al. We based our classification of MIP and PIP on several microsatellites in the FRAXA region, classifying 25 cases as being of unknown origin, according to conservative criteria. Hundscheid et al. did not specify whether markers were tested, how their classification was made, or how many subjects were unclassifiable. Regardless of whether this is consequential, the fact remains that we observed a significant difference between women with MIP and control individuals (log-rank $\chi_1^2 = 8.52$; $P = .0035$), whereas Hundscheid et al. did not. We are unable to explain this difference.

It would be very interesting to know whether other investigators find parent-of-origin differences in the frequency of POF in premutation carriers. As in all recent studies, the protocol should include interviews of all available female relatives, with rigorous definition of menopausal variables and mode of origin and with correct use of survival analysis. Only then will studies by different groups pass from debate to discovery.

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References

Hundscheid RDL, Sistermans EA, Thomas CMG, Braat DDM, Straatman H, Kiemeney LALM, Oostra BA, et al (2000) Imprinting effect in premature ovarian failure confined to paternally inherited fragile X premutations. Am J Hum Genet 66:413–418

Morton NE, Macpherson JN (1992) Population genetics of the fragile-X syndrome: multiallelic model for the FMR1 locus. Proc Natl Acad Sci USA 89:4215–4217

Murray A, Ennis S, MacSwiney F, Webb J, Morton N. Reproductive and menstrual history of females with fragile X expansions. Eur J Hum Genet (in press)

Murray A, Webb J, Grimley S, Conway G, Jacobs P (1998) Studies of FRAXA and FRAXE in women with premature ovarian failure. J Med Genet 35:637–640

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Premature Ovarian Failure Is Associated with Maternally and Paternally Inherited Premutation in Brazilian Families with Fragile X

To the Editor:

Strong evidence has been produced that indicates FMR1 premutation as a risk factor for premature ovarian failure (POF) (Cronister et al. 1991; Schwartz et al. 1994; Vianna-Morgante et al. 1996, 1999; Murray et al. 1998; Uzzielli et al. 1999). The most extensive survey was a collaborative study engaging nine centers in different countries that showed that 16% of women with premutation suffered POF compared with 0.4% of their noncarrier relatives (Allingham-Hawkins et al. 1999). In a recent study of Dutch families with fragile X, Hundscheid et al. (2000) disclosed a parent-of-origin effect of the premutation such that POF occurred with a significant frequency only in women who inherited the premutation from their fathers.

We investigated parental origin of the premutation and occurrence of POF in 113 female carriers in families with fragile X, ascertained through mentally retarded patients. In these families, women aged ⩾25 years who had been tested for the fragile X mutation were interviewed personally by one of us (A.M.V.-M.) about their menstrual, gynecological, and reproductive histories, after appropriate informed consent. Those who had undergone hysterectomy or oophorectomy were not included in the study. POF was defined as spontaneous cessation of menstruation at age <40 years, for at least 1 year. Part of the present sample was included in our previous study of the frequency of POF in fragile X carriers (Vianna-Morgante et al. 1999). Parental origin of the premutation could be determined in 59/113 women: 27 of the premutations were maternally inherited (MIP) and 32 were paternally inherited (PIP). The 27 women with a MIP belonged to 21 sibships (average 1.29 daughters, range 1–3 daughters), and the 32 women with a PIP belonged to 19 sibships (average 1.68

Table 1

Characteristics of Women Carrying an FMR1 PIP or MIP and Their Noncarrier Relatives

Characteristic (Age Group)	Data for Premutation Carriers		Data for Noncarriers
	PIP (n = 32)	MIP (n = 27)	(n = 50)
Mean age, years	39.18 ± 7.03	39.84 ± 12.89	39.20 ± 12.35
Mean age at menopause, years	36.50 ± 9.85 (n = 13)	34.67 ± 11.96 (n = 9)	50.67 ± 5.07 (n = 9)
POF (⩾40 years)	5/15	2/10	0/50
POF (<40 years)	4/17	3/17	0/50
POF (all ages)	9/32	5/27	0/50

daughters, range 1–5 daughters). Age at examination did not differ between the two groups (medians: MIP, 36.83; PIP, 38.875; P = .5328 [Mann-Whitney test]). Among women with a MIP, five had experienced POF, and it occurred in nine women with a PIP, a difference that was not statistically significant (P = .5411 [Fisher's exact test]). Age at menopause in the two groups did not differ either (medians: MIP, 38 [n = 9]; PIP, 35 [n = 13]; P = 1.0 [Mann-Whitney test]) but were significantly lower than age at menopause among 50 of their relatives who carried normal alleles (median age: 51 years [n = 9]; P = .0014 [Kruskal Wallis test]). These results are summarized in table 1.

In conclusion, our data do not support the hypothesis of a parent-of-origin effect of the FMR1 premutation on ovarian function such that only the paternally inherited premutation is significantly associated with POF. The association of POF with PIP and MIP in one pedigree as shown by Vianna-Morgante et al. (1996) further denies an effect confined to paternally inherited premutation. The finding of a possible genomic imprinting effect, reported by Hundscheid et al. (2000), may be peculiar to the Dutch population. Otherwise the difference between theirs and the present survey may be the result of an undiagnosed ascertainment bias. Data on other populations are urgently needed, if only considering their implications for genetic counseling.

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References

Allingham-Hawkins DJ, Babul-Hirj R, Chitayat D, Holden JJ, Yang KT, Lee C, Hudson R, et al (1999) Fragile X premutation is a significant risk factor for premature ovarian failure: the International Collaborative POF in Fragile X Study—preliminary data. Am J Med Genet 83: 322–325

Cronister A, Schreiner R, Wittenberger M, Amiri K, Harris K, Hagerman RJ (1991) Heterozygous fragile X female: historical, physical, cognitive, and cytogenetic figures. Am J Med Genet 38:269–274

Hundscheid RDL, Sistermans EA, Thomas CMG, Braat DDM, Sraatman H, Kiemeney LALM, Oostra BA, et al (2000) Imprinting effect in premature ovarian failure confined to paternally inherited fragile X premutations. Am J Hum Genet 66:413–418

Murray A, Webb J, Grimley J, Conway G, Jacobs P (1998) Studies of FRAXA and FRAXE in women with premature ovarian failure. J Med Genet 35:637–640

Schwartz CE, Dean J, Howard-Peebles PN, Bugge M, Mikkelsen M, Tommerup N, Hull C, et al (1994) Obstetrical and gynecological complications in fragile X carriers: a multicenter study. Am J Med Genet 51:400–402

Uzielli ML, Guarducci S, Lapi E, Cecconi A, Ricci U, Ricotti G, Biondi C, et al (1999) Premature ovarian failure (POF) and fragile X premutated females: from POF to fragile X carrier identification, from fragile X carrier diagnosis to POF association data. Am J Med Genet 84:300–303

Vianna-Morgante AM, Costa SS, Pares AS, Verreschi IN (1996) FRAXA premutation associated with premature ovarian failure. Am J Med Genet 64:373–375

Vianna-Morgante AM, Costa SS, Pavanello RCM, Mingroni-Netto RC (1999) Premature ovarian failure (POF) in Brazilian fragile X carriers. Genet Mol Biol 22:471–474

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Reply to the Letters from Murray et al. and Vianna-Morgante and Costa

To the Editor:
It was with great interest that we read the letter to the editor by Murray et al. (2000 [in this issue]), and we thank the authors for their comments. We agree that it would be very interesting to know whether other investigators observe a parent-of-origin effect in the development of premature ovarian failure (POF) in premutation carriers, as we have stated in our paper (Hundscheid et al. 2000). Murray et al. reevaluated their results and did not observe such an effect. There may be several explanations for this. The etiology of POF is extensive and comprises genetic, nongenetic, and multifactorial components. Therefore, it is not unlikely that there are differences between families with fragile X who are from the United Kingdom and those that are from The Netherlands. More importantly, differences in study design (especially for multicenter studies) will inevitably lead to other results and, subsequently, will lead to other conclusions.

We were very surprised by the mean age at menopause mentioned by Murray et al. In the premutation group, the mean age at menopause was 47.87 years, compared with 52.96 years in the full-mutation and normal groups combined. In our ongoing study, the mean age at menopause in premutation carriers who had experienced spontaneous menopause was 42.0 years (unpublished data); this finding is in line with observations made elsewhere (see Partington et al. 1996). When we performed Kaplan-Meier survival analysis on the entire group of premutation carriers, the mean age at menopause was 45 years. This finding strongly suggests that the study population of Murray et al. differs from ours. To verify our observation of imprinting, the mean age at menopause has to be comparable. A significantly lower mean age may point to a different population, a difference in the occurrence of POF, or the number of premutation carriers that are postmenopausal. If this is the case, then it is obvious that one cannot compare the two studies.

Murray et al. have shown that, of 116 women who were premutation carriers, 51 had a paternally inherited fragile X premutation (PIP), 40 had a maternally inherited fragile X mutation (MIP), and 25 had an unknown parental origin of premutation. Of the 91 women in whom the parental origin of the premutation could be established, only 30 (33%) were of age ⩾40 years. We have described 148 premutation carriers in whom the parental origin of the premutation could be determined: 106 women with a PIP and 42 women with a MIP. Of the 106 women with a PIP, 82 were of age ⩾40 years, and, of the 42 women with a MIP, 27 were of age ⩾40 years. Thus, in our study, 109 (74%) of 148 women were of age ⩾40 years and did not experience nonspontaneous cessation of menstruation at age <40 years. Therefore, we have to conclude that the proportion of women in whom the occurrence of POF can be established (only in women of age ⩾40 years) is significantly higher in our study, compared with the study by Murray et al. This may be the result of other methodology, which may also account for the low numbers of observed women with a PIP. This makes it rather impossible to compare the data.

University Hospital Nijmegen has been extensively studying families with fragile X, tracing possible carriers in several generations. We have estimated that the overall frequency of carriers of a PIP is approximately three times higher than that of carriers of a MIP. On the basis of this finding, we cannot reason why Murray et al. identified 51 women with a PIP and 40 women with a MIP. This other PIP:MIP ratio may be an indication that Murray et al. did not study the families to the same extent that we did. Murray et al. might possibly have included a large proportion of first-degree relatives in the younger generation. This will result in a different population with other observations that cannot be compared.

Since the etiology of POF is extensive, we think that it is of paramount importance to check medical histories with attending physicians, to avoid misclassification. Checking the dates with attending physicians may also help to avoid a patient's recall bias; postmenopausal women have a tendency to round off their age at menopause to the nearest age that ends in the numeral 0 or 5 (Partington et al. 1996).

We have to conclude that, in a comparison of our study with that of Murray et al., there are differences in methodology, mean age at menopause, and number of women in whom the occurrence of POF can be established. This probably reflects a different population, and we therefore doubt whether the results can be compared. We agree that it is remarkable that Murray et al. did not observe the same parent-of-origin effect that we observed. Therefore, we would like to invite groups with a population and methodology comparable to ours to verify our observation and to report their findings.

The reply we addressed to the letter to the editor submitted by Murray et al. applies to that submitted by Vianna-Morgante and Costa (2000 [in this issue]) as well. The population in the study by Vianna-Morgante and Costa is very young, compared with that in our study. The population's median age at examination, for women with a MIP (n = 27), was 36.83 years, and, for women with a PIP (n = 32), the median age was 38.875 years. In our study, for women with a MIP (n = 42), the median age at examination was 51.5 years, and, for

those with a PIP ($n = 106$), the median age was 50.0 years. Besides the fact that the PIP:MIP ratio (32:27) mentioned in the letter by Vianna-Morgante and Costa differs substantially from ours (106:42), we have a much older population. Moreover, this major difference in the study population is again emphasized by the fact that, in the study by Vianna-Morgante and Costa, 15 women with a PIP and 10 women with a MIP were of age ⩾40 years. This number is very low compared with our finding (82 women with a PIP and 27 with a MIP, all of age ⩾40 years). Again, this points out that their population is different than ours—a fact that obviously will lead to other results.

Vianna-Morgante and Costa have compared the occurrence of POF in women with a PIP with that in women with a MIP, and they have concluded that there is no difference between the two groups. However, their analysis incorporated data on women of age <40 years. Since POF is defined as a condition occurring at age <40 years, it can only be established reliably in women of age ⩾40 years. Not only will establishment of the occurrence of POF in women who have not reached the age of 40 years result in a higher risk for misclassification toward POF, but, in the majority of cases, occurrence of the condition cannot even be established. Hence, we think it is remarkable that the authors also included women of age <40 years in their study. Moreover, the mean age of the participants in their study is below the cutoff level for age. Therefore, the numbers they presented probably will not represent the final (as established only in women of age ⩾40 years) occurrence and distribution of POF. In the letter, 14 (24%) of 59 women with either a PIP or a MIP had POF—a finding that is an underestimation of the real (probably even higher) occurrence of POF. Since 16% of women with premutations experience POF (Allingham-Hawkins et al. 1999), we wonder whether the population in the study of Vianna-Morgante and Costa (in which ⩾24% of the women had POF) is randomly selected. In our ongoing study of families with fragile X, we have randomly selected women on the basis of mutation and not on the basis of indication of POF.

Last but not least, for both groups of women, the authors calculated the median and mean age at menopause (see also table 1 in the study by Vianna-Morgante and Costa). The numbers on which these calculations are based are very small (13 women with a PIP and 9 women with a MIP), and, to us, it is not clear which data the authors have included in their calculations. If the authors included women with POF when they calculated mean age at menopause, then their calculation would not result in a reliable mean age at menopause. For instance, for women with a PIP, the mean age at menopause was based on 13 women who have experienced spontaneous menopause. If the authors also used the data on the women with POF, then it can be inferred that only four women who did not experience POF were used in this calculation. The mean age at menopause that is presented is not representative of that in all women with a PIP. Thus, on the basis of these numbers, we do not subscribe to the authors' conclusion that there is no difference between the two groups, as far as age at menopause is concerned.

In conclusion, neither Murray et al. nor Vianna-Morgante and Costa can confirm our observation of a parent-of-origin effect. Both groups have younger populations, other PIP:MIP ratios, and a sample size that is much smaller than ours. We therefore do not follow Vianna-Morgante and Costa's suggestion that a "possible genomic imprinting effect may be peculiar to the Dutch population," since no sufficient convincing evidence of this is provided. Nevertheless, if the parent-of-origin effect that we have observed cannot be demonstrated by other authors, then we have to conclude either that the parent-of-origin effect is unique to the Dutch population with fragile X or that we all are overlooking some other factors (bias or nonbias). Whatever is causing this discrepancy, it will be of major importance with regard to future research (and which methodology is to be used) in this particular field. However, we think that it is too premature to draw final conclusions with regard to the parent-of-origin effect. Our population and methodology differ too much from those described by Murray et al. and by Vianna-Morgante and Costa. Further research is warranted to verify our observation.

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References

Allingham-Hawkins DJ, Babul-Hirji R, Chitayat D, Holden JJ, Yang KT, Lee C, Hudson R, et al (1999) Fragile X premutation is a significant risk factor for premature ovarian failure: the International Collaborative POF in Fragile X study—preliminary data. Am J Med Genet 83:322–325

Hundscheid RDL, Sistermans EA, Thomas CMG, Braat DDM, Straatman H, Kiemeney LALM, Oostra BA, et al (2000) Imprinting effect in premature ovarian failure confined to paternally inherited fragile X premutations. Am J Hum Genet 66:413–418

Partington MW, Moore DY, Turner GM (1996) Confirmation of early menopause in fragile X carriers. Am J Med Genet 64:370–372

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Inflated False-Positive Rates in Hardy-Weinberg and Linkage-Equilibrium Tests Are Due to Sampling on the Basis of Rare Familial Phenotypes in Finite Populations

To the Editor:

If it is assumed that genotypes of some locus ($\mathbf{G}_\mathrm{D}$) are in Hardy-Weinberg equilibrium (HWE) in a population and that these genotypes are correlated with some phenotype (**Ph**), then, among "cases" in the tail of the distribution of **Ph** (equivalently, affected with rare disease), the $\mathbf{G}_\mathrm{D}$ will show Hardy-Weinberg disequilibrium (HWD) (Nielsen et al. 1999; Deng et al. 2000; Göring and Terwilliger 2000). However, this does not imply that "generally, in individuals at either end of the quantitative-trait distribution, HWD exists if and only if there exists a whole-population LD [i.e., "linkage disequilibrium"]" (Deng et al. 2000, p. 1030). The "only if" part of this sentence is not correct. Even Deng et al. (2000, p. 1044) point out that "an absence of HWD does not imply that a marker locus and a QTL are not in LD" and that, for completely random marker loci, there will be inflated false-positive rates in tests for HWD (and LD as well), because "cases" of familial disease tend to be more related than "controls," for the following reasons.

Assume that **Ph** is correlated in families, without specifying whether this is due to genetic or shared environmental factors. Let the prevalence, $\phi = P$(individual B is a case), and the familial relative risk $\lambda = P$(individual B is a case | relative A is a case)/ϕ (Weiss et al. 1982; Risch 1990). Then, P(A *and* B are affected | A and B are relatives) = $\lambda\phi^2$, and P(A *and* B are affected) = ϕ^2 if they are randomly ascertained. This implies that P(A and B are relatives | A *and* B are affected) = $\lambda\phi^2 P$(A and B are relatives)/ϕ^2 = λP(A and B are relatives). If $\lambda > 1$, then ascertainment of "cases" ascertains relatives with greater probability than does random ascertainment of "controls," leading to increased false-positive evidence of HWD and LD throughout the genome. This effect will be largest when λ is large, ϕ is small, and the population is small and/or structured (such that P[A and B are relatives] is nontrivial). In a sense, this is related to the problem of population stratification when the phenotype being studied correlates with a familial stratum, regardless of whether the trait is "genetic" (see Chase 1977).

If the "case" phenotype is a good predictor of $\mathbf{G}_\mathrm{D}$ (a prerequisite for mapping to be powerful), then a large portion of the "case" sample will share some risk allele IBD from some common ancestor. The coalescent path connecting these chromosomes historically defines the most distant possible relationship among the "cases" carrying this allele, defining an upper bound on how "unrelated" they could possibly be. Again, this implies that ascertainment of affected individuals increases the probability of ascertainment of relatives. And the less frequent the shared risk allele is, the more closely related the "case" individuals will be (see Terwilliger, in press), leading to potential deviations from HWE and LE in unrelated parts of the genome as well.

The more closely related two people are, the larger the proportion of their genomes that they will share, as measured by their kinship coefficient (also see Terwilliger et al. 1997). If cases are "more related" than controls, then they will, with higher probability than will be seen in controls, share alleles IBD at random places in the genome, leading to increased false-positive rates in HWD and LD tests. This anticonservative behavior may be minor in studies of a single marker locus, but, when one considers the effects of testing hundreds of thousands of markers jointly in a genome scan, often making inferences based on the most significant values of the test statistic over the genome, the inflation of the type I error can have significant import. Furthermore, because the effect of small deviations, from HWE and/or LE, that are induced by such sampling is to shift the distribution slightly upward, the anticonservative bias will increase as we look farther out into the tail of the pointwise distribution (data not shown—but similar in shape to what appears in fig. 4 of Göring and Terwilliger 2000), leading to potentially gross inflation of genome-wide false-positive rates. To test for such problems, one can do a Monte Carlo randomization, as was done, in a case-control study of a small genetically homogeneous population isolate, by Hovatta et al. (1999), who kept the genotypes (for the whole genome scan) of all individuals constant and randomized their phenotypes ("case" and "control"). The simulation showed that their sample had approximately twice as many positives as would be expected from the randomization test, consistent with what is expected for reasons described in this note. When the fundamental assumption that "cases" and "controls" are independent and identically distributed with respect to random marker-locus genotype frequencies throughout the genome appears to have been rejected, it is essential to maintain skepticism in the interpretation of the results of such an analysis.

Unfortunately, the conditions in which "cases" are most likely to be relatives (e.g., small populations, rare

diseases, large familial correlations) are the same cases in which LD and HWD tests are likely to be useful (see Zöllner and von Haeseler 2000; Terwilliger, in press). In a study of more-common phenotypes and larger, more diverse populations, it is highly unlikely that marginal effects of single-risk alleles of a given locus are going to be etiologically important—in which case, LD and HWD tests will have little or no power (see Terwilliger and Weiss 1998; Terwilliger and Göring 2000; Weiss and Terwilliger, in press). And small populations with unusual histories are also more likely to have some population-level deviation from HWE in general, and, if one does not ascertain population controls, then there is no way to validate this critical assumption of the model. Although the paranoia about population stratification that leads people to mistrust case-control samples may be exaggerated, the absence of a sample of controls poses even greater danger.

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References

Chase GA (1977) Genetic linkage, gene-locus assignment, and the association of alleles with diseases. Transplant Proc 9: 167–171

Deng HW, Chen WM, Recker RR (2000) QTL fine mapping by measuring and testing for Hardy-Weinberg and linkage disequilibrium at a series of linked marker loci in extreme samples of populations. Am J Hum Genet 66:1027–1045

Göring HHH, Terwilliger JD (2000) Linkage analysis in the presence of errors. IV. Joint pseudomarker analysis of linkage and/or linkage disequilibrium on a mixture of pedigrees and singletons when the mode of inheritance cannot be accurately specified. Am J Hum Genet 66:1310–1327

Hovatta I, Varilo T, Suvisaari J, Terwilliger JD, Ollikainen V, Arajärvi R, Juvonen H, et al (1999) A genomewide screen for schizophrenia genes in an isolated Finnish subpopulation, suggesting multiple susceptibility loci. Am J Hum Genet 65:1114–1124

Nielsen DM, Ehm MG, Weir BS (1999) Detecting marker-disease association by testing for Hardy-Weinberg disequilibrium at a marker locus. Am J Hum Genet 63:1531–1540

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

Terwilliger JD. On the resolution and feasibility of genome scanning approaches to unraveling the genetic components of multifactorial phenotypes. In: Rao DC, Province MA (eds) Genetic dissection of complex phenotypes: challenges for the new millennium. Academic Press, New York (in press)

Terwilliger JD, Göring HHH (2000) Gene mapping in the 20th and 21st centuries: statistical methods, data analysis, and experimental design. Hum Biol 72:63–132

Terwilliger JD, Shannon WD, Lathrop GM, Nolan JP, Goldin LR, Chase GA, Weeks DE (1997) True and false positive peaks in genomewide scans: applications of length-biased sampling to linkage mapping. Am J Hum Genet 61:430–438

Terwilliger JD, Weiss KM (1998) Linkage disequilibrium mapping of complex disease: fantasy or reality? Curr Opin Biotechnol 9:578–594

Weiss KM, Chakraborty R, Majumder PP, Smouse PE (1982) Problems in the assessment of relative risk of chronic disease among biological relatives of affected individuals. J Chronic Dis 35:539–551

Weiss KM, Terwilliger JD. How many diseases do you have to study to map one gene with SNPs? Nat Genet (in press)

Zöllner S, von Haeseler A (2000) A coalescent approach to study linkage disequilibrium between single-nucleotide polymorphisms. Am J Hum Genet 66:615–628

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QTL Fine Mapping, in Extreme Samples of Finite Populations, for Complex Traits with Familial Correlation Due to Polygenes

To the Editor:

Recently, Deng et al. (2000) developed a QTL fine-mapping approach on the basis of Hardy-Weinberg (HW) or linkage-disequilibrium (LD) patterns in extreme samples of large and random-mating populations in which HW equilibrium holds. This approach is based on robust linkage results that have already localized a quantitative-trait locus (QTL) to a large genomic region (e.g., ~30 cM). The purpose is to fine map the QTL to a small region of ~1 cM, through examination of the patterns of deviation from HW and linkage equilibrium at a series of closely linked marker loci in extreme samples of populations. The deviation can be measured by a number of indices (including some test statistics—e.g., the test statistics for HW equilibrium) (Deng et al. 2000). Our approach is an extension of those of Feder et al. (1996) and Nielsen et al. (1998) for fine mapping of disease-

susceptibility loci. Feder et al. have successfully used this approach by examining HW disequilibrium patterns in affected cases, at a series of closely linked marker loci, to fine map a susceptibility locus for hereditary hemochromatosis, to a region of ~600 kb.

As pointed out by Terwilliger (2000 [in this issue]), in the development of our fine-mapping approach for genes underlying common complex traits, familial correlations of complex traits are ignored, and large randomly mating populations are assumed. In response to the concerns of Terwilliger, we performed simulations to investigate the performance of our approach in finite populations, in the presence of familial correlations for the trait under study.

The simulation procedures are roughly the same as those described by Deng et al. (2000), except that families with familial correlations for the trait are simulated for finite populations. In brief, an evolving population of size N is simulated for 50 generations. In each generation, random pairs of individuals are mated to generate the next generation. The number of children per family is generated from a Poisson distribution with a mean of 2. To maintain a constant population size of N from generation to generation, if the number of children generated is $>N$, random children are discarded, so that the total number of children is N for the next generation. If the number of children simulated is $<N$, random parental pairs are included, to generate more children (according to the Poisson distribution for each pair), until there are N children generated for the next generation. Without loss of generality, the family correlation is simulated via 10 unlinked biallelic background polygenic QTLs. The effect of the background polygenic QTLs is indexed by the heritability (h^2_{pg}) attributable to them. Each polygenic QTL has the same recessive effect, so that its heritability is $h^2_{pg}/10$; the frequency of the allele causing lower trait values is .2. The correlations among family members can be easily computed from h^2_{pg} and from the heritability (h^2) of the QTL being tested. In simulations, the frequency of the allele causing lower trait values at the QTL is p = .1, the marker-allele frequency p_M = .2, and h^2 = .20. If the marker is linked to the QTL, $D^0_{A_1M}$ (the amount of LD simulated at the 0 generation) = .08. The initial LD may be caused by various evolutionary scenarios, such as admixture of populations differentiated at the QTL and marker frequencies. At the 50th simulated generation, extreme samples are taken from the simulated populations. For a series of closely linked marker loci that are simulated around the QTL being tested, we performed QTL fine mapping by the five-point moving-average technique (Deng et al. 2000). The first stage of fine mapping (Deng et al. 2000) is more robust than the second stage and is little affected by finite population sizes and familial correlations. Therefore, we present, in figure 1, only the results for the second stage of fine mapping, when highly dense markers (~0.2 cM apart from one another) are typed around the QTL position. In figure 1, the Y-axis is the probability that the true QTL position is within a certain distance (X-axis) from the peak of the LD measure q_{excess} (Deng et al. 2000) at a series of closely linked markers. Measurement of disequilibrium by q_{excess} uses individuals from the bottom and the top 10 percentiles (100 each) in study populations. The results for other LD measures (including those for HW disequilibrium—the χ^2_2 and χ^2_4 [Deng et al. 2000]) are essentially the same.

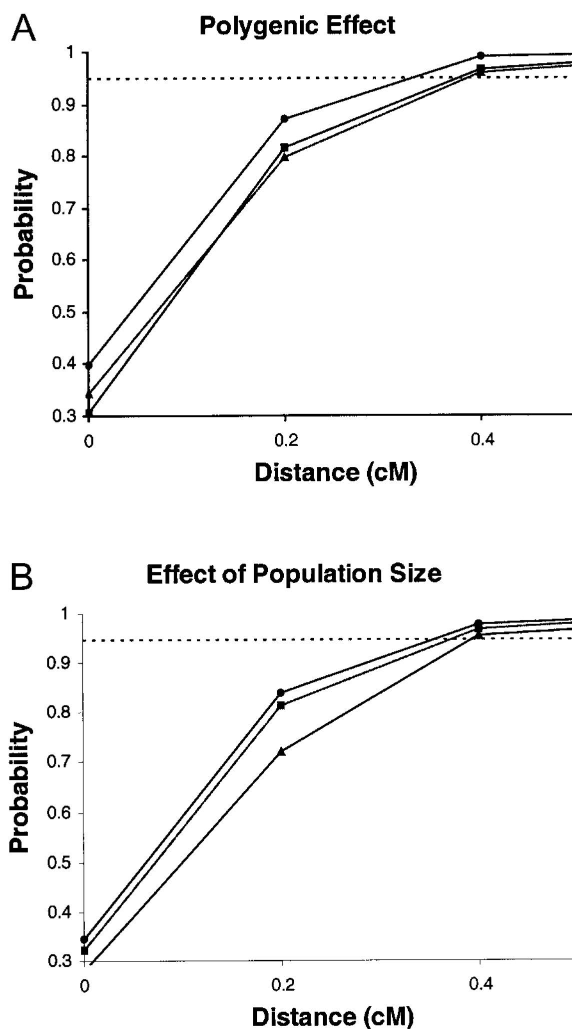

Figure 1 Effects of polygenic QTLs and population sizes on the QTL fine-mapping approach of Deng et al. (2000). For plot *A*, the population site is 5,000; circles, triangles, and squares represent the data for situations in which h^2_{pg} = .0, .3, and .6, respectively. For plot *B*, h^2_{pg} = .5; circles, squares, and triangles represent the data for situations in which the population sizes are 15,000, 5,000, and 2,000, respectively.

Even with small population sizes (as small as 2,000)

and large familial correlations (as reflected by h^2_{pg}, which is as large as .6), our QTL fine-mapping approach not only remains powerful but also is valid and robust (fig. 1). Under the parameters simulated, the correlation between full sibs is .36, and that between a parent and a child is .27, when $h^2_{pg} = .5$ (plot *B* in fig. 1). Finite population sizes and familial correlations may lower the power of our QTL fine-mapping approach, especially when the marker is extremely close (<0.2 cM) to the true QTL position. However, the effect is very small. In particular, when the distance of the peak from the true QTL position is >0.5 cM, our power of QTL fine mapping is little affected. Recall that the purpose of our QTL fine-mapping approach is to narrow a large genomic region found in regular linkage analyses to a small region of ∼1 cM, for further physical mapping to clone the QTL. With finite population sizes and familial correlations, our approach can have >95% probability to correctly position the QTL to a region <0.8 cM (fig. 1).

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References

Deng HW, Chen WM, Recker RR (2000) QTL fine mapping by measuring and testing for Hardy-Weinberg and linkage disequilibrium at a series of linked marker loci in extreme samples of populations. Am J Hum Genet 66:1027–1045

Feder JN, Gnirke A, Thomas W, Tsuchihasi Z (1996) A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 13:399–408

Nielsen DM, Ehm MG, Weir BS (1998) Detecting marker-disease association by testing for Hardy-Weinberg disequilibrium at a marker locus. Am J Hum Genet 63:1531–1540

Terwilliger JD (2000) Inflated false-positive rates in Hardy-Weinberg and linkage-equilibrium tests are due to sampling on the basis of rare familial phenotypes in finite populations. Am J Hum Genet 67:258–259 (in this issue)

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Reply to Deng and Chen

To the Editor:
The simulations done by Deng and Chen (2000), in response to my letter (Terwilliger 2000), are completely consistent with one of the points that I was trying to make. The pointwise mean and variance of the distribution of the test statistics are slightly inflated in extreme samples from small populations, so that, when such analyses are performed over much larger genomic regions, as in a genome scan, these seemingly minor pointwise effects can be dramatic. This is the real danger in such studies, which could lead to a potential sea of false positives in the literature, swamping the likely dearth of true-positive findings (see Weiss and Terwilliger, in press). The effects of "extreme sampling" are going to be much greater when the frequency of the phenotype is <10% (which is very common for a disease phenotype) and/or the effective population size is smaller (e.g., because of rapid population expansion and/or more-extreme isolation), as seen in the schizophrenia study by Hovatta et al. (1999). But, even under this "best-case scenario," Deng and Chen showed that there is an inflation of mean and variance of their statistics under H_0, even for $P \leqslant .05$, and, when one gets closer to the critical values needed in a genomewide sense (which must be more, not less, strict than those used in linkage analysis—$P < .0001$), the inflation must be larger still (also see Terwilliger and Göring 2000 and Terwilliger, in press). Furthermore, under the model that I described, the familial correlations in phenotype could have absolutely nothing to do with genetic factors at all (like "ability to speak Finnish" in a sample of Americans); yet the same problems would result, because familial phenotypes correlate with familial substrata of the population, leading to potentially increased rates of false evidence of both Hardy-Weinberg and linkage disequilibrium, compared with what is seen in random samples from the whole population.

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References

Deng HW, Chen WM (2000) QTL fine mapping in extreme samples of finite populations for complex traits with familial correlation due to polygenes. Am J Hum Genet 67:259–261 (in this issue)

Hovatta I, Varilo T, Suvisaari J, Terwilliger JD, Ollikainen V,

Arajärvi R, Juvonen H, et al. (1999) A genomewide screen for schizophrenia genes in an isolated Finnish subpopulation, suggesting multiple susceptibility loci. Am J Hum Genet 65:1114–1124

Terwilliger JD (2000) Inflated false-positive rates in Hardy-Weinberg and linkage-equilibrium tests are due to sampling on the basis of rare familial phenotypes in finite populations. Am J Hum Genet 67:258–259 (in this issue)

———. On the resolution and feasibility of genome scanning approaches to unraveling the genetic components of multifactorial phenotypes. In: Rao DC, Province MA (eds) Genetic dissection of complex phenotypes: challenges for the new millennium. Academic Press, San Diego (in press)

Terwilliger JD, Göring HHH (2000) Gene mapping in the 20th and 21st centuries: statistical methods, data analysis, and experimental design. Hum Biol 72:63–132

Weiss KM, Terwilliger J. How many diseases do you have to study to map one gene with SNPs? Nat Genet (in press)

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Interpretation of Linkage Data for a Huntington-Like Disorder Mapping to 4p15.3

To The Editor:

Kambouris et al. (2000) report on the mapping of a neurodegenerative disorder on the basis of a sibship of 10 individuals whose parents are first cousins. Using a model of autosomal recessive inheritance, linkage analysis detects a maximum two-point LOD score (Z_{max}) of 3.03 at recombination fraction (θ) 0.

The authors of the report postulate the genetic interval as a 7-cM region bounded by *D4S2366* and *D4S2983,* because all affected individuals are homozygous for the two markers (*D4S431* and *D4S394*) in between. Figure 1 in their article demonstrates a haplotype analysis in which the parents (III:2 and III:3), although first cousins, share very few alleles in the putative linked region.

First, the marker order presented in the report's figure 2*b* contradicts that presented in its haplotype analysis (fig. 1) and in the multipoint analysis (fig. 2*a*). The Marshfield sex-averaged linkage map places *D4S2366* between *D4S431* and *D4S394*. The haplotype and multipoint analyses place *D4S2366* centromeric to *D4S431* and *D4S394*. Since the parents share no alleles for *D4S2366,* interposing *D4S2366* between *D4S431* and *D4S394* would abolish this region of putative homozygosity by descent among the affected individuals. It appears more likely that it is by chance alone that the two parents share a "2" allele for *D4S431* and a "1" allele for *D4S394*. For example, the Foundation Jean Dausset CEPH genotype database reveals that the most common allele (205 bp) for *D4S394* has a frequency of 41%. Thus, if allele 1 for *D4S394* in the report's figure 1 is the 205-bp allele, the chances are 41% that parent III:2 inherited the 1 allele from the unrelated parent (II:1). Without genotype data for the parents and/or siblings of III:2 and III:3, identity by descent cannot be assumed.

Kambouris et al. make the assumption that the disorder is recessive, apparently because of the consanguinity in the family. Although they report $Z_{max} = 3.03$ at $\theta = 0$, under the assumption of 50% penetrance, the two-point LOD scores were likely calculated under a model of 100% penetrance. The two-point LOD scores would be expected to be lower under a model of 50% penetrance (two-point LOD score 2.7 at $\theta = 0$ for the four fully linked markers). The data could also support a model of autosomal dominance with reduced penetrance with the disorder segregating with the red haplotype, if the disease is not penetrant in parent III:2 and individual IV:8. The same argument could be made for parent III:3 and individual IV:10 and the purple haplotype. Testing a dominant model assuming 90% penetrance demonstrated a Z_{max} of 1.94 at $\theta = 0$, with marker *D4S412* (data not shown).

Even if it were assumed that the mode of inheritance is truly autosomal recessive, homozygous genotypes among the affected individuals are not absolutely required. If the linkage to this region is true, and if the red and purple haplotypes contain noncomplementing mutated alleles, the genetic interval would actually be defined by the telomeric recombination event in IV:2 and the centromeric recombination events in IV:4—that is, by *D4S3023* and *D4S1599,* defining a nonrecombinant region of 15 cM.

Finally, Kambouris et al. note that only chromosome 4 markers were genotyped. Testing markers at the already mapped locus on chromosome 20, for a similar Huntington-like disorder, would certainly seem pertinent. A two-point LOD score of 3.3 (not 3.0) is the generally accepted criterion for a 5% significance level (Lander and Schork 1994). A complete genome screen may well reveal another locus in which the parents are heterozygous for a common haplotype with a more convincing region of homozygosity.

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References

Kambouris M, Bohlega S, Al-Tahan A, Meyer BF (2000) Localization of the gene for a novel autosomal recessive neurodegenerative Huntington-like disorder to 4p15.3. Am J Hum Genet 66:445–452

Lander ES, Schork NJ (1994) Genetic dissection of complex traits. Science 265:2037–2048

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Reply to Lesperance and Burmeister

To the Editor:

Lesperance and Burmeister rightly draw attention to a discrepancy, in the placement of D4S2366, between that presented in haplotype/multipoint analysis and that presented in the Marshfield sex-averaged linkage map. The precise position of D4S2366 in relation to D4S431 and D4S394 is open to question, in the absence of their placement on current physical maps. Our placement of D4S2366 was based on its assignment 4–5 cM and 12–13 cM from the 4p telomere in the CHLC and Marshfield sex-averaged maps, respectively. Both a LOD score (3.03) and a homozygosity LOD score (4.71) were presented, because, as pointed out by Lesperance and Burmeister, although the parents were first cousins, relatively few alleles were shared in the linkage interval. Nonparametric linkage (NPL) analysis (data not shown) based on inherited-by-descent allele sharing among affected individuals was also performed, using multiple markers and genotyping data from all pedigree members. This analytical approach is least likely to be misled through inherited-by-state allele sharing, is least sensitive to specification of allele frequencies, and is model free (Kruglyak et al. 1996). Multipoint NPL analysis of markers D4S3023, D4S2366, D4S431, D4S394, D4S2983, and D4S1599 resulted in a Z score of 5.31 or level of significance $P < .00001$ (Kruglyak et al. 1996), indicating, with a high level of confidence, that affected individuals share by descent the 15-cM region between D4S3023 and D4S1599. On the basis of such data, it is our opinion that a whole-genome scan in search of more robust linkage is not warranted. We sought to consolidate evidence that the region encompassing D4S431 and D4S394 was homozygous by descent (HBD) in affected individuals, by genotyping them for markers D4S3007 and D4S2935, which are positioned between D4S431 and D4S394, in both the Généthon and Marshfield sex-averaged linkage maps. However, D4S3007 and D4S2935 were noninformative and partially informative, respectively, in the family studied. Given ambiguity in the placement of D4S2366, the 15-cM region defined by D4S3023 and D4S1599 should be regarded as the candidate interval, with initial focus on a putative region HBD between D4S2366 and D4S2983. Given the extremely rare nature of the disease studied and the extensive consanguinity in the pedigree, we strongly believe that this is an autosomal recessive disorder. However, in consideration of the fact that 50% of individuals within the sibship are affected, we did discuss the possibility of autosomal dominant inheritance with germline mosaicism explaining the absence of disease in either parent. A 90%-penetrant autosomal dominant disease, as suggested by Lesperance and Burmeister, cannot be excluded. Finally, the parametric LOD score of 3.03 is indeed calculated on the basis of 100% penetrance. The 50% figure that appeared in the original manuscript was a typographical error.

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References

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347–1363

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