scores range from 0.20 to 1.28. Third, we assessed the sensitivity of the LOD scores to the disease model when the parameters are adjusted to make the predicted prevalence consistent with the observed prevalence. Model F ($q = 0.026$), which differs from model E ($q = 0.001$) only in the value of disease-allele frequency q, has slightly lower LOD scores at small values of θ than model E. Model G, which differs from model E only in the phenocopy rate (model G $f_0 = 0.05$), also has smaller LOD scores at small values of θ than model E. Thus, increasing the disease-allele frequency or the phenocopy rate to bring the population prevalence K up to ~5% lowered the LOD score slightly.

Chen et al. (2004) claim to have identified a "significant" RLS susceptibility locus on chromosome 9p24-22. Their results might not actually be significant, as a result of the many methodological errors we have described above. The use of equal marker-allele frequencies, overinterpretation of the significance of the NPL scores, and "validation" analysis in only 2 of the 15 families are some of the potential problems that lead us to believe that there is no convincing evidence for an RLS locus on chromosome 9p. Chen et al. (2004) should model linkage heterogeneity and should use the proper marker-allele frequencies, penetrances, and disease-allele frequency when they reanalyze their data.

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Electronic-Database Information

The URLs for data presented herein are as follows:

GDB Human Genome Database, http://gdbwww.gdb.org/ (for *D9S274* alleles [accession number GDB:245741])
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for RLS)

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Reply to Ray and Weeks: Linkage for Restless Legs Syndrome on Chromosome 9p Is Significant

To the Editor:

Ray and Weeks (2005 [in this issue]) discussed some concerns about the statistical methodology of our genomewide linkage-scan study that identified a novel susceptibility locus for restless legs syndrome (RLS) on chromosome 9p24-22 (Chen et al. 2004). Before we systematically address their concerns, it is important to note that the 9p24-22 RLS locus has been replicated in a large family from Germany, with a significant LOD score found by an independent research group (J. Winkelmann, personal communication). This independent replication in a different study population has strongly validated our initial linkage finding.

The Genetic Component of RLS: We are aware of the issue of a bias in estimation of familial correlations and heritability in a population enrolled for genetic stud-

ies. Therefore, we clearly stated this in our article (e.g., in the "Results" section, we noted, "The derived heritability…was 0.60, which indicates that RLS is a highly heritable trait in this ascertained cohort," and, in the "Discussion" section, we noted, "It is important to note that familial correlations and heritability were estimated for an ascertained cohort, and the results may not be generalizable to the RLS population at large" [Chen et al. 2004, pp. 881 and 882]). Since the sampling schemes for the recruited RLS pedigrees are complicated and cannot be described by a single form of ascertainment, a formulated correction for ascertainment bias was not undertaken because of the lack of an appropriate method and software package. Nevertheless, our conclusion about the genetic nature of the disease was based on the analysis of diverse types of relative pairs. A large number of relative pairs of more remote types (i.e., second-, third-, and fourth-degree relations) were used for the inference, and these data might be less influenced by the ascertainment. We agree that familial risk-ratio estimation by use of the ascertained cohort could be biased, and a high population prevalence of 12% was thus used to obtain a conservative estimate. The determination of the genetic component of a complex human disease will perhaps continue to be a critical issue, since human pedigrees are often ascertained from clinical settings, and the sampling schemes may not be defined by a single ascertainment form. Nevertheless, genetic analysis of the collected samples for evidence of genetic factors, as a requisite step toward identification of disease genes, cannot be ignored. Without familial aggregation in which genetic factors play an important role, the expense and effort involved in further gene mapping and cloning would be wasted (Farrer and Cupples 1998).

Model-Free Linkage Analysis: Ray and Weeks (2005) were concerned about our reporting of statistical significance. However, their arguments, which are based on the criteria of Lander and Kruglyak (1995) and rough transformation between different statistics through only the link of a *P* value (see table 1 in Ray and Weeks 2005), may not be the most appropriate. First, Lander and Kruglyak's criteria for genomewide significance were based on a dense map and on complete information extraction, which do not apply to our experiment, which had a less dense marker map (10-cM density, on average) and a fraction of missing genotypes. Using extensive simulations, Wiltshire et al. (2002) demonstrated that the criteria proposed by Lander and Kruglyak (1995) were too stringent for an initial 10-cM genome scan. Second, direct translation of a *P* value for different statistics can be criticized because of the high reliance of *P* values on sample sizes (Witte et al. 1996), especially for a small-to-medium sample size like that of our study. Third, a nominal *P* value derived from the asymptotic distribution of a linkage statistic can be different from the estimate obtained from permutation. In other words, the asymptotic distribution of a statistic can deviate substantially from the observed value, especially for small sample sizes. Fourth, the nonparametric linkage (NPL) statistic used in our genomewide scan is deemed to be quite conservative, meaning that *P* values of .05 occur

Table 1

Revised Pairwise LOD Scores between RLS and Chromosome 9p24-22 Markers Obtained by Model-Based Linkage Analysis in Two Extended Kindreds with RLS

Marker and Kindred	LOD Score at θ =					
	.00	.05	.10	.20	.30	.40
D9S1779:						
40004	.00	.00	.00	.00	.00	.00
40015	−1.32	.11	.24	.20	.05	−.03
Total	−1.32	.11	.24	.20	.05	−.03
D9S1871:						
40004	.30	.28	.25	.20	.14	.08
40015	1.90	1.75	1.59	1.25	.88	.47
Total	2.20	2.03	1.84	1.45	1.02	.55
D9S2169:						
40004	.60	.56	.51	.40	.29	.16
40015	1.87	1.70	1.53	1.17	.78	.38
Total	2.47	2.26	2.04	1.57	1.07	.54
D9S286:						
40004	1.49	1.36	1.22	.92	.58	.25
40015	2.04	1.86	1.68	1.31	.91	.48
Total	3.53	3.22	2.90	2.23	1.49	.73
D9S168:						
40004	.00	.00	.00	.00	.00	.00
40015	1.87	1.70	1.53	1.16	.78	.37
Total	1.87	1.70	1.53	1.16	.78	.37
D9S268:						
40004	.18	.10	.02	−.11	−.15	−.10
40015	1.54	1.41	1.27	.99	.69	.35
Total	1.72	1.51	1.29	.88	.54	.25
D9S274:						
40004	1.19	1.07	.93	.66	.38	.13
40015	1.91	1.75	1.59	1.25	.88	.48
Total	3.10	2.82	2.52	1.91	1.25	.61
D9S1839:						
40004	.00	.00	.00	.00	.00	.00
40015	.80	.70	.60	.40	.21	.06
Total	.80	.70	.60	.40	.21	.06
D9S162:						
40004	−1.41	−.18	.02	.12	.07	.00
40015	2.04	1.88	1.70	1.31	.92	.47
Total	.63	1.70	1.72	1.43	.99	.47
D9S1121:						
40004	−1.95	−1.24	−.80	−.40	−.23	−.13
40015	.15	.13	.10	.06	.03	.01
Total	−1.80	−1.11	−.70	−.34	−.20	−.12

Note.—LOD scores were computed with the assumption of a disease-gene frequency of 0.001, a penetrance rate of 95%, a phenocopy rate of 0.001 (as used in the original analysis), and marker-allele frequencies estimated by maximum likelihood. The combined LOD score for *D9S286* at $\theta = 0$ is 3.49 when the disease-gene frequency is increased to 0.003 and the phenocopy rate is increased to 0.005 and is 3.24 with a disease-gene frequency of 0.003, a phenocopy rate of 0.05, and a penetrance rate of 0.95.

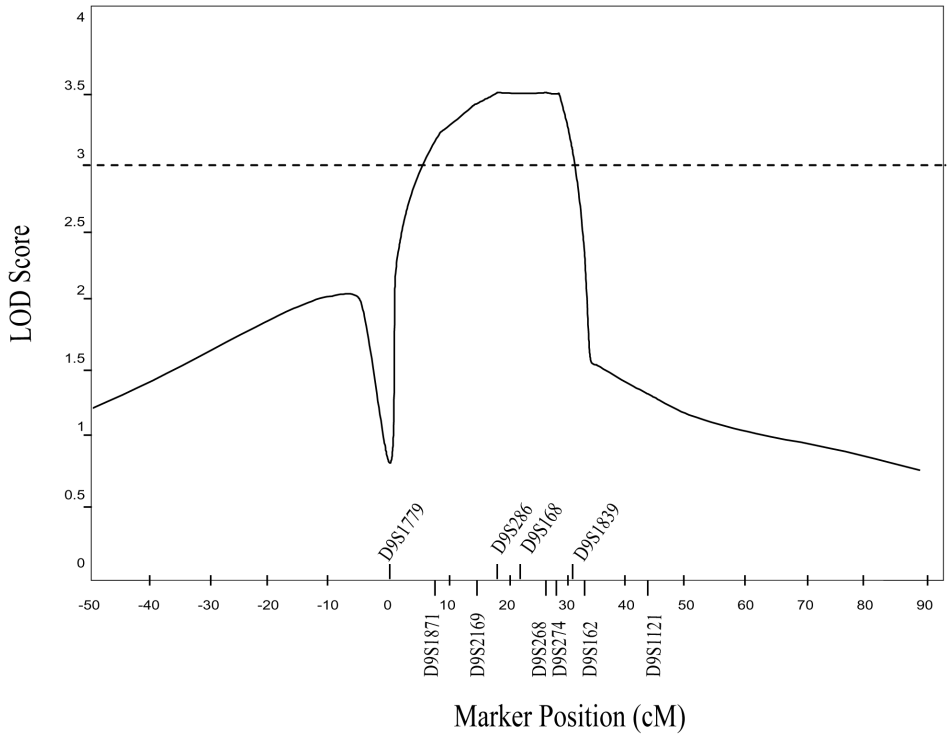

Figure 1 Multipoint LOD-score analysis for markers at the 9p24-22 RLS locus. Only one member of the twin pair was included in the analysis. The parameters used for the analysis include a disease-gene frequency of 0.001, marker-allele frequencies estimated by maximum likelihood, a penetrance rate of 0.95, and a phenocopy rate of 0.001.

less often than expected (Haines 1998). Thus, direct exchange between LOD and NPL measures is highly questionable. Since unified criteria for significant and suggestive linkages based on NPL scores have not been described in the literature, we defined NPL scores of ≥ 3.0 as significant evidence of linkage and scores of ≥ 2.0 as suggestive evidence of linkage on the basis of the nominal empirical *P* values. Similar criteria have been used by others (Kaminen et al. 2003; Laivuori et al. 2003; Friedrichsen et al. 2004; Middleton et al. 2004). Most importantly, our claim of significant linkage at the 9p RLS locus was based on multiple lines of evidence from NPL analysis, Haseman-Elston sib-pair regression (*P* value at the peak $<10^{-5}$; authors' unpublished data), and model-based linkage analysis.

Model-Based Linkage Analysis: In our study (Chen et al. 2004), we calculated the LOD scores using the program Linkage Package 5.2 (MLINK) directly connected to the pedigree program Cyrillic 2, which apparently treated twins as full siblings. This treatment leads to the upper-limit estimation of LOD scores. We have now recalculated the LOD scores by deleting the data from one member of the twin pair in the analysis. The linkage remains significant (table 1).

For model-based linkage analysis of one or a few pedigrees, the assumptions of the disease-gene frequency, marker-allele frequencies, penetrance, and phenocopy rates are largely based on educated guesses. These assumptions may vary among different research groups, since the genetic architecture of the disease is not clear in most cases. A disease-gene frequency of 0.026, as suggested by Ray and Weeks (2005), is too high, since RLS is genetically highly heterogeneous; many genes may be involved in development of RLS, and the frequency of one specific gene—for example, the chromosome 9p RLS gene—is expected to be much lower. The assumption made by Ray and Weeks (2005) is not realistic unless a single gene is the cause of the disorder in all patients with RLS (5%–12% of the population). To date, two other RLS loci have already been mapped to chromosomes 12 and 14, and many families do not link to the three known RLS loci (Desautels et al. 2001; Bonati et al. 2003; Chen et al. 2004).

To investigate whether the $1/n$ method (i.e., the assumption that there are equal allelic frequencies and that *n* equals the total number of alleles observed in the families studied) could lead to false evidence of linkage for these particular data, we calculated the LOD scores for our genotyping data by using the allele frequencies that were estimated with the maximum-likelihood meth-

od. The linkage for the 9p RLS linkage remains significant, with a combined two-point LOD score of 3.53 at *D9S286* (table 1) and a multipoint LOD score of 3.56 (fig. 1). (Note that these LOD scores were obtained when one member of the twin pair was removed from the analysis, and the overall LOD scores slightly increased when actual allele frequencies were used, as compared with the use of 1/*n* allele frequencies: 3.53 vs. 3.52 for *D9S286* and 3.10 vs. 2.95 for *D9S274*.) These results indicate that the LOD scores for the 9p RLS linkage do not change much if the maximum-likelihood estimates of the sample allele frequencies are used.

For model-based linkage analysis, genotyping is commonly performed for multiple mid- to large-size families, but linkage at a specific locus may be identified and reported in a subset of the families, since other families may be associated with different disease genes. We agree that model-based linkage analysis can also be performed for our complete genotyping data with the assumption of heterogeneity. To calculate heterogeneity LOD (HLOD) scores, we used the GeneHunter-Plus program, which maximizes the LOD scores over both recombination fraction θ and admixture parameter α. The HLOD score at the 9p RLS locus, with the assumption of autosomal dominant inheritance, reached 2.31. Similar results were obtained with the program HOMOG (HLOD = 2.46, with the assumption of autosomal dominant inheritance). These results are consistent with our original conclusion of the identification of an RLS locus on chromosome 9p24-22.

In summary, we feel that our evidence of genetic linkage for RLS on chromosome 9p is methodologically sound, and our finding is buttressed by the independent replication in a different population.

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