and Kruglyak [1995] threshold for suggestive linkage). We were also unable to replicate any of the linkage results on chromosomes 1, 3, or 6 that were observed in the original genome scan in whites.

Our LOD score of 4.66 for the confirmation of linkage to chromosome 3p exceeds the statistically significant threshold of 3.7 implied by Morton (1998) for 2-df tests of linkage (ours was a 2-df test, because we estimated both additive and dominance effects at the QTL). In the original, smaller sample, the LOD score of 2.50 was merely suggestive of linkage. As with any complex genetic trait, it is encouraging to observe replication of a linkage finding. Replication by an independent team—with a different population, sampling method, or analysis technique—would further strengthen the evidence that a locus on chromosome 3p contributes to variation in CRCL.

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Mode of Inheritance and Susceptibility Locus for Restless Legs Syndrome, on Chromosome 12q

To the Editor:

We read with interest the report by Desautels et al. (2001), who have described a susceptibility locus for restless legs syndrome (RLS), on chromosome 12q, in a family with putative autosomal recessive inheritance of RLS. RLS is a movement disorder characterized by a desire to move the extremities, often associated with motor restlessness, paresthesias/dysesthesias, worsening of symptoms at rest with at least temporary relief by activity, and worsening of symptoms in the evening or night (Walters 1995). A positive family history can be found in >40% of the idiopathic cases. Most reports of familial cases, as well as twin studies, suggest autosomal dominant transmission (Winkelman et al. 2001) with high penetrance (Trenkwalder et al. 1996; Lazzarini et al. 1999; Ondo et al. 2000).

To evaluate the role of the described chromosome 12q locus for RLS, we ascertained two large South Tyrolean families (E and LA) with clinically definite RLS. Inheritance followed a classic pattern of autosomal dominant transmission. Pedigrees of the families are shown in figure 1. The diagnosis was established according to the criteria of the International Restless Legs Syndrome Study Group (Walters et al. 1995). Genomic DNA was isolated from 51 family members (family E includes 9 [7 female and 2 male] affected individuals, with mean age at onset 31 ± 7 years; family LA includes 10 [7 female and 3 male] affected individuals, with mean age at onset 37 ± 9 years). Genotyping of the following DNA markers that span the candidate region containing the recently described locus on chromosome 12q was performed on an automated-sequencing machine (Li-Cor): D12S1064 (95.03 cM), D12S1044 (96.54 cM), D12S393 (104.12 cM), and D12S78 (111.87 cM). The marker-map positions are based on the sex-averaged maps from the Center for Medical Genetics, Marshfield Medical Research Foundation. Linkage analysis was conducted using the FASTLINK (Schäffer et al. 1994) and VITESSE programs (O'Connell and Weeks 1995). For

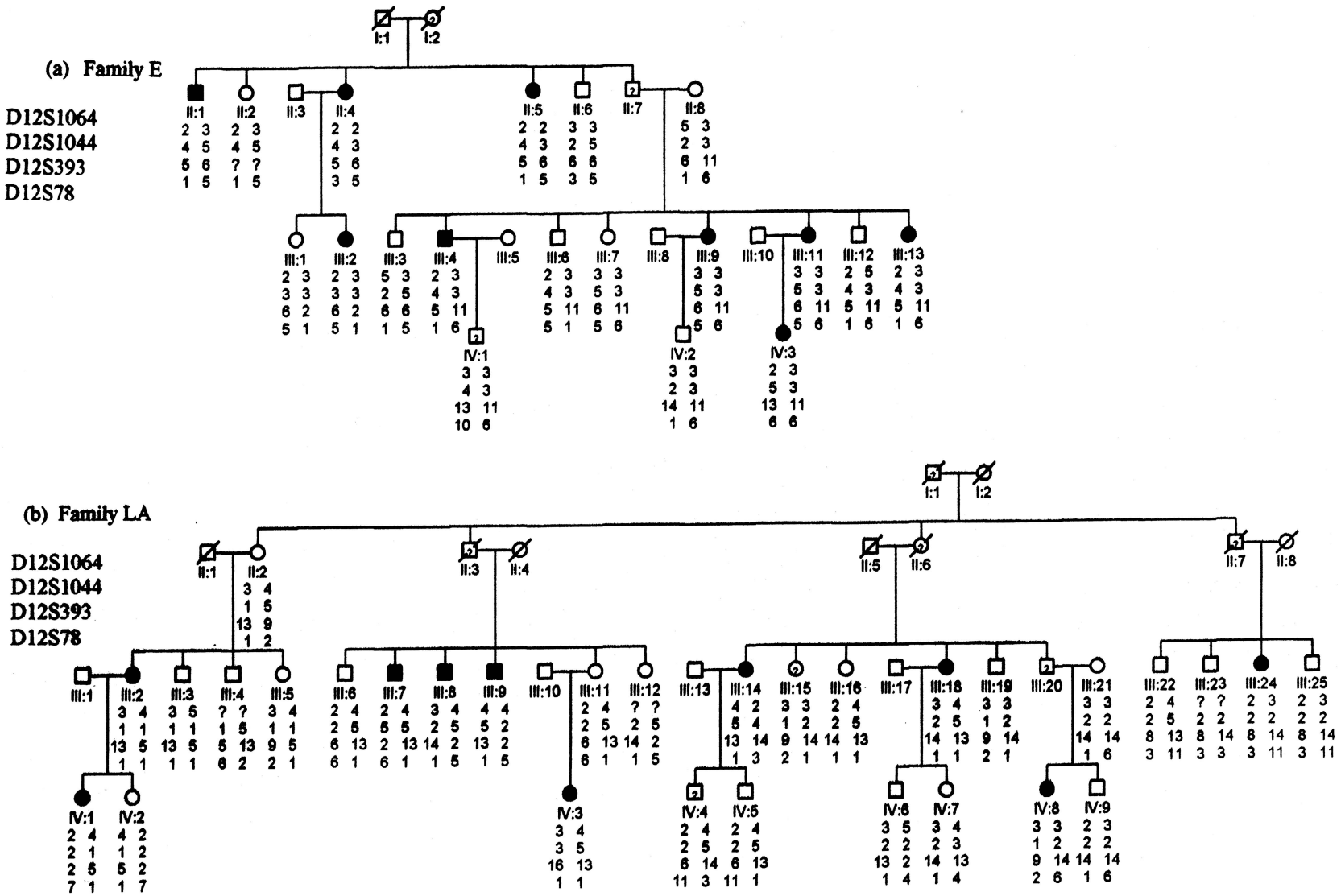

Figure 1 Pedigrees of families E and LA, with haplotypes for the four markers that span ~13 cM on chromosome 12p. The order of markers is indicated to the left of each generation. Individuals affected with RLS are denoted by solid symbols, those with possible RLS are denoted with a question mark (?) (and are considered unknown with respect to disease status in the linkage analysis), and those with clear symbols and haplotype data were unaffected at the time of clinical assessment. Generation numbers are given above the haplotype data.

each family, we considered both a dominant model and a recessive model for RLS. For the former, we used a conservative, affecteds-only model and a disease-allele frequency of 0.001. For the latter, we adopted the model parameters used by Desautels et al. They also used an affecteds-only model, but they incorporated a disease-allele frequency of 0.25 and a high phenocopy rate of 0.80.

In both of the families that we studied, linkage was unambiguously excluded using a dominant model (multipoint LOD scores across the region ranged from −2.46 to −6.67 in family LA and from −1.61 to −5.14 in family E). In the larger family (LA), linkage was also excluded using the recessive model (multipoint LOD scores across the region ranged from −2.16 to −4.23). In family E, we obtained no positive evidence for linkage by use of the recessive model (multipoint LOD scores across the region ranged from −0.51 to 0.47). With nine affected individuals, the maximum potential LOD score for this family is at least 2.5.

The recessive model suggested by Desautels et al. requires a disease-allele frequency on the order of a common polymorphism. Furthermore, they specify a genotype-specific penetrance value of 0.80 for f_0, which represents the probability that homozygous normal individuals (i.e., non–disease-allele carriers) are affected—or, more simply, the phenocopy rate. For relatively common diseases, the population prevalence of the disorder is often used as an estimate of f_0. A phenocopy rate of 80% far exceeds the population prevalence (2%–10%) of idiopathic and secondary RLS combined. We reproduced the results from Desautels et al., by analysis of the same family that they studied and by use of their marker data and model parameters. We then specified lower (but still generous) rates of 10% and 20% and obtained lower LOD scores. Specifically, the highest two-point LOD score was 0.35 ($s = 0.0$), at D12S1300, by use of the 20% phenocopy model. Although RLS is not a rare disease, its prevalence in the general population does not reach the proportions suggested by the model advocated for this family.

The family studied by Desautels et al. is French Canadian and derives from a population that, at least historically, has been a genetic isolate. Families from such populations may be particularly useful for identifying disease and susceptibility genes for common disorders. Of interest in this family is the fact that two of the four married-in spouses were diagnosed with "probable" RLS and therefore were potentially homozygous disease-allele carriers (although they were considered as having "unknown" disease status in the linkage analysis). This "trilineality" may be the result of increased relatedness in this kindred, given their population history, and could strengthen Desautels et al.'s results if further explored and documented. Desautels et al., however, have not discussed this matter in their article.

In conclusion, we did not confirm the susceptibility locus for RLS, on chromosome 12q, in either of the families that we studied. We also question certain parameters used in the recessive model by Desautels et al., and we suggest that additional information on family structure may be useful in the search for RLS genes in this French Canadian family.

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Electronic-Database Information

The URL for data presented herein is as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/ (for sex-averaged maps)

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Reply to Kock et al.

To the Editor:

In their study, Kock et al. (2002 [in this issue]) investigated two families from South Tyrol, to test for linkage with a restless legs syndrome (RLS)–susceptibility locus, on chromosome 12q, that was recently identified by our group (Desautels et al. 2001). They genotyped a total of 51 subjects by use of four markers spanning a 17-cM interval on the candidate region. Assuming both a dominant and a recessive mode of inheritance, Kock et al. failed to replicate our reported linkage on chromosome 12q in the families that they studied. In addition, aiming to reproduce our original results, they subsequently analyzed the same family that we studied by use of our marker data and the published recessive model, assuming a phenocopy rate of 80%. Being unable to replicate our findings by use of our own material, Kock et al. have raised some concerns regarding the recessive model described in our article.

Unfortunately, the published version of our original article reporting linkage between RLS and a locus on chromosome 12q (Desautels et al. 2001) contained an error. Indeed, the information presented for the penetrance values and phenocopy rates in the published table (p. 1267) does not correspond to the values used in our analyses. The genetic models used by our group were based on realistic assumptions, which are listed below, in the rectified version of the table (table 1; for the recessive model, the disease-allele frequency was set at 0.25 with a reduced penetrance of 0.8 and an estimated phenocopy rate of 0.005). The error involves only the presentation of the table, not the parametric analyses per se. Therefore, this will clarify the issue pertaining to our model raised by Kock et al., since their criticisms were based on misinterpretations induced by this unfortunate error.

Table 1

Genetic Models Used in the Parametric Analysis

Model of Inheritance	Allele Frequency		Penetrance		
	p	q	f_1	f_2	f_0
Dominant 1	.99	.01	.9	.9	0
Dominant 2	.95	.05	.5	.5	.005
Recessive	.75	.25	.8	.005	.005

NOTE.—θ Between males and females was considered to be equal.

The nonreplication of our positive linkage in two South Tyrolean families' results raises the hypothesis that genetic heterogeneity is present. Accordingly, since the publication of the original study, additional families have been recruited and investigated for the candidate interval. Although linkage to chromosome 12q has been confirmed in some kindreds, our analyses indicate that a number of families are definitely not linked to this region, further supporting the heterogeneity hypothesis (A. Desautels, G. Turecki, J. Montplaisir, A. S. Walters, B. L. Ehrenberg, K. Brisebois, A. K. Desautels, W. G. Johnson, E. Lugaresi, G. Coccagna, D. L. Picchietti, A. Lazzarini, Y. Gingras, and G. A. Rouleau, unpublished data). Taken together, these data suggest that other putative loci, besides the locus on chromosome 12q that has recently been identified by our group, could be involved in the etiology of this common sleep disorder.

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