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Pseudoautosomal Linkage of Hodgkin Disease

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Summary

Heritable factors appear to account for much of the risk for Hodgkin disease (HD). There is evidence for an HLA-linked gene, but other predisposing loci remain unaccounted for. The observation of a family coinheriting both HD and Leri-Weill dyschondrosteosis (LWD) suggests that a gene conferring risk for HD resides adjacent to the LWD locus. The gene responsible for LWD, SHOX, localizes to the short-arm pseudoautosomal region (PAR) of the X and Y chromosomes. A unique segregation pattern for PAR-linked genes has been predicted—that affected sibs will tend to be same sex. An excess of sex-concordant affected sib pairs with HD has been noted but has been attributed to an environmental etiology. These two observations—sex concordance in sib pairs with HD and cosegregation of HD and LWD—impelled a test of the hypothesis that there is a PAR-localized gene for HD. By first scoring recombinations dissociating sex from phenotype in individuals from pedigrees with LWD, we determined a male maximum recombination frequency (θ_{max}) of .405. This places SHOX near the short-arm telomeres of the sex chromosome and supports the prediction that PAR recombination is obligatory for spermatogenesis. By inferring recombinations between HD and sexual phenotype in sib pairs, we predict, for the postulated HD gene, a male θ_{max} as high as .254, which places it in proximity to SHOX. Morton's nonparametric affected-sib-pair "β" model was used in the evaluation of linkage between HD and phenotypic sex and gave a LOD score of 2.41. Using this approach, we reevaluated evidence for HLA linkage in HD in haplotyped sib pairs and found a LOD score of 2.00. The resulting β values indicate that the putative PAR- and HLA-linked loci account for 29% and 40%, respectively, of the heritability of HD in an American population.

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Introduction

Hodgkin disease (HD [MIM 236000]), a common lymph-node cancer of uncertain cellular origin, differs from other tumors in that the bulk of the cells are not malignant but, rather, are infiltrates of normal inflammatory cells. On the basis of proband studies, it has been estimated that as much as one-third (Hafez et al. 1985) of the liability for HD is hereditary. Additional evidence supporting an inherited predisposition to HD comes from twin studies, racial-incidence patterns, and occurrences of multiplex families (reviewed by Horwitz, in press). Observations in a small number of affected relative pairs suggest the presence of an HLA-linked locus (Chakravarti et al. 1986), but this can account for only a minority of the familial risk observed in proband studies (Shugart and Collins 1998). Other loci that may contribute to genetic risk for HD are unknown. A prohibitive factor is the extreme rarity of familial HD; most accounts are historical, the individuals unsampled (although a few have been HLA typed), and the prospects for definitive molecular-genetic linkage analysis dim.

However, both the recent molecular cloning of a disease, Leri-Weill dyschondrosteosis (LWD), which has anecdotally been noted in association with HD, and a reported quirk—sex concordance among affected sib pairs—in the epidemiology of HD, might be taken as clues supporting a pseudoautosomal localization for a gene predisposing to HD. In this report, we take advantage of the disparity in the frequency of sex-concordant affected sib pairs with HD, to test for pseudoautosomal linkage.

LWD is a skeletal dysplasia characterized by short stature, shortened forelimbs, and distal radio-ulnar (Madelung) deformity. Gokhale et al. (1995) have reported a unique pair of sisters with both HD and LWD. There was a multigenerational, maternal history of LWD and, in maternal relatives, a further history of HD without known LWD. Those authors raised the possibility that either (*a*) LWD and HD resulted from a mutation deleting adjacent genes responsible for both illnesses or (*b*) genes for the two diseases were linked. At the time of the report, LWD was attributed to autosomal dominant inheritance.

However, LWD recently has been determined to result

from mutations—typically, large deletions—of the SHOX homeobox gene that maps to the pseudoautosomal regions on the short arm of the X and Y chromosomes (Belin et al. 1998; Shears et al. 1998). This finding now suggests that a pseudoautosomal location should be considered the locus hypothesized to confirm HD risk in the family reported by Gokhale et al.

Although, across their length, the X and Y chromosomes are divergent in sequence, the pseudoautosomal regions (PARs) represent two segments of homology that comprise 2.5 Mb and 320 kb on the terminal short and long arms, respectively (Freije et al. 1992). The PARs are the only sites of recombination between the X and Y chromosomes in male meioses. In fact, a single recombination in the p-arm PAR is thought to be an obligatory event in male spermatogenesis (Rouyer et al. 1986; Page et al. 1987). The recombination frequency (θ) for the male PAR is estimated to be 10-fold greater than that for the female PAR. A meiotic θ of just .02 is observed in the q-arm PAR (Freije et al. 1992; Li and Hamer 1995), which, although high for such a small region, is still much lower than that exhibited across the entire length of an arm of an autosome.

A unique pattern of inheritance should result from a mutation in a gene in the PAR (Crow et al. 1994). If the locus were situated on the centromeric portion of the p-arm PAR, proximal to the invariant recombination, then the mutant allele would consistently segregate, during male meiosis, with sexual phenotype. A man could possess the mutant allele on either his X or Y chromosome. If it were on the X chromosome, then only his daughters would inherit the allele, whereas if it resided on the Y chromosome, then only his sons would inherit the allele. A similar prediction pertains to loci situated in the q-arm PAR, where recombination between the X and Y chromosomes is rare. The result is that multiple affected sibs would be sex concordant (i.e., "MM" or "FF," where "M" denotes "male" and "F" denotes "female"). In the case of paternal inheritance of a gene situated at the telomeric boundary of the short-arm PAR, where recombination probability is .5, there would be no difference with a random sex distribution, and sex-discordant (i.e., "MF") and sex-concordant sib pairs would be equally probable. Since the mutant allele can reside on either of a woman's X chromosomes, maternal inheritance would result in random segregation with sex. Overall, there should be a deficiency of sex-discordant, compared with sex-concordant, affected sib pairs, with the magnitude of the difference being twice as great for a recessive gene as for a dominant gene and lessening with closing proximity to the telomere. Interestingly, an excess of sex-concordant sib pairs with HD has been noted (Grufferman et al. 1977).

Unequal frequencies of sex-discordant and sex-concordant affected sib pairs may be used as the basis of a test for linkage to the pseudoautosomal region. We therefore identified all known sibships with multiple occurrences of HD and evaluated linkage with sexual phenotype, using a nonparametric allele-sharing method. We also attempted to genetically localize a putative gene for HD, with respect to SHOX, in the PAR and so first applied parametric linkage analysis to enumerate recombination events in families with both HD and LWD.

Subjects and Methods

Pedigrees with LWD

To identify all pedigrees with LWD, the literature was systematically searched. We began with Online Mendelian Inheritance in Man (OMIM)–cited references for LWD (MIM 127300). Each article cited in the bibliography of each of these articles was searched, and so on, until a finite set of interreferencing papers was constructed. Acceptable criteria for LWD included either the authors' designation of such or, in the absence of this, pedigrees that met all of the following criteria: (1) presence of Madelung deformity, (2) mesomelic disproportionate short stature, and (3) multiple generations of individuals affected with findings 1 and 2. Pedigrees with LWD that have been described elsewhere (Rullier et al. 1968; Felman and Kirkpatrick 1969; Lisker et al. 1972; Espiritu et al. 1975; Belin et al. 1998; Shears et al. 1998) were identified and were used in the linkage analysis. An overlapping but separate set that also includes at least an affected sib pair but not necessarily multiple affected generations is listed in table 1.

Pedigrees with HD

Pedigrees with HD that were from the Montefiore Medical Center in the Bronx were ascertained by completion of a questionnaire when all probands with hematopoietic malignancy presented for diagnosis or treatment, beginning in 1988. The family history was corroborated by a review of medical records.

For affected sib pairs prior to 1977, we relied on the previous reports and reviews by Grufferman et al. (1977). For published reports after this date and for second-degree and more-distant ($\geq$2°) relative pairs, we identified families through a Medline search followed, as above, by a review of the bibliographies of all cited articles, until no additional referenced pedigrees could be found.

We required families with HD to have at least one pair of affected sibs. There were no cases of such families with HD and an affected parent, and parental affection status is not considered in the BETA program's allele-sharing method. Some families with HD had $\geq$2° relatives who were affected, but these individuals were not included in the linkage analysis, since they do not con-

Table 1

Affected-Sib-Pair Sex Concordance in LWD

Reference and Pedigree	No. of Affected Sib Pairs		
	MM	MF	FF
Espiritu et al. (1975)	0	2	1
Felman and Kirkpatrick (1969)	0	0	1
Lichtenstein et al. (1980)	0	1[a]	0
Carter and Currey (1974)	0	1[a]	0
Fryns and Van Den Berghe (1979)	0	0	2
Herdman et al. (1966)	0	1	0
Lisker et al. (1972)	0	1	0
Rullier et al. (1968)	1	0	0
Anton et al. (1938):			
45 and 46	1	0	0
134 and 135	0	0	1
Shears et al. (1998):			
1	3[a]	5[a]	1
2	0	1	0
3	0	2	1
4	0	0	1
6	1[a]	0	0
Gokhale et al. (1995)	0	0	1
Belin et al. (1998):			
4	0	0	1[a]
5	0	1	0
6	0	0	1[a]
8	1	0	0
Total	7	15	11

[a] Includes sibships with more than two affected members but that were truncated after the first two cases.

tribute to the BETA allele-sharing method. Stratification by either age at onset or histological subtype is not possible, since, for many of the cases, these data were not available.

Linkage Analysis

To test for pseudoautosomal linkage, the pedigrees were coded with two loci. The first locus addressed affection status. For sibships for which parental information was unavailable, parents were considered to be of unknown status. Unaffected sibs were included only when the phenotype was known or reported. The second locus consisted of numbered alleles corresponding to the X and Y chromosomes. Males were coded 1-2 and females were coded 1-1. The frequency of allele 1 was entered as .75, allele 2 as .25; but, because sex of the parents is always known, the results are not a function of allele frequency.

To test for HLA linkage, in cases in which parental haplotypes either were not reported or could not be deduced, parents were assumed to be heterozygotes with no shared haplotypes. No intra-HLA recombination events were observed in the pedigrees with HD for which HLA-haplotype information was available. A single allele with frequency arbitrarily set equal to .25 was used to simulate a nonrecombinant haplotype. (Actual allele frequencies are irrelevant, since all matings were constructed with informative parental haplotypes.)

Two-point parametric LOD scores and maximum θ (θ_{max}) values were determined by the MLINK and ILINK programs, respectively, of FASTLINK (Lathrop and Lalouel 1984; Cottingham et al. 1993). For the parametric approach, HD-gene frequency was set at .00004 (Gruffferman et al. 1977), and LWD-gene frequency was arbitrarily assigned to be .01, in the absence of data on its prevalence. Penetrance for HD, either for heterozygotes in the pseudoautosomal dominant model or for homozygotes in the pseudoautosomal recessive model, was initially determined, on the basis of MZ-twin-concordance frequency (Mack et al. 1995), to be .056, but it was allowed to be iteratively estimated by ILINK. Penetrance for LWD was arbitrarily set as .9 in heterozygotes, since there is evidence that it is nearly, but not quite, complete (Goepp et al. 1978; Lichtenstein et al. 1980), and it was allowed to be iteratively estimated by ILINK. A similar penetrance value was assigned for homozygotes, who are thought to phenotypically manifest the Langer syndrome of mesomelic dwarfism. The few instances of these individuals in the pedigrees with LWD were coded accordingly. The female (F)-to-male (M) recombination ratio for pseudoautosomal segregation was set as 0.1 (Rouyer et al. 1986), but iterative estimation by ILINK also was allowed. (Since female meioses were generally not informative in testing for linkage with sexual phenotype, this had little bearing on the results.)

Nonparametric allele sharing in sibs was conducted by means of the BETA program using the β method of Morton (Collins et al. 1996; Morton 1996; Lio and Morton 1997).

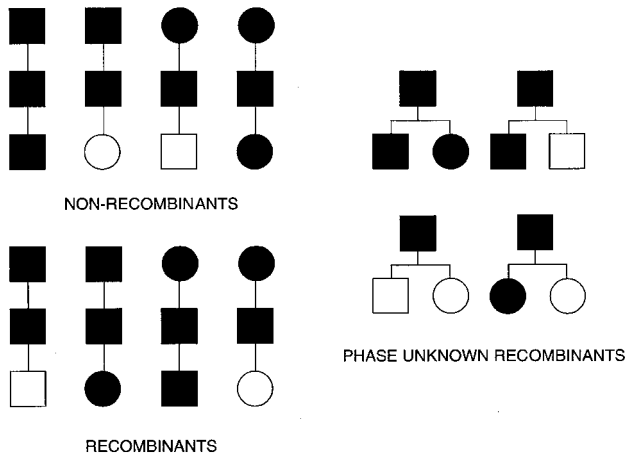

Figure 1 Hypothetical two- and three-generation pedigrees illustrating all possible combinations of scorable recombination events for a gene localizing to the PAR.

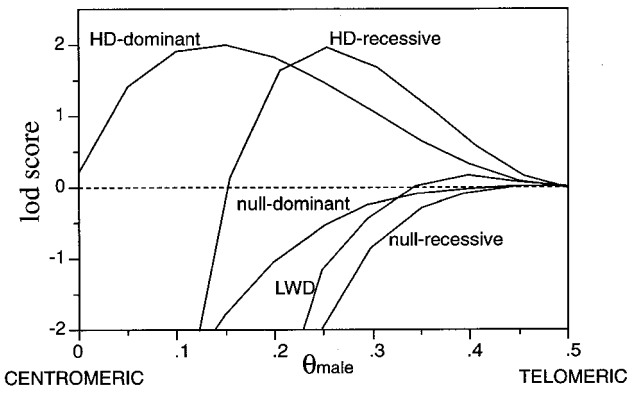

Figure 2 Parametric LOD score determined as a function of male PAR θ. The result for LWD is shown in comparison with the result for HD, under both the dominant and the recessive models of inheritance. Also shown is the result with the synthetic set of families with HD that corresponds to sex-dependent penetrance without sex linkage, under both the dominant and the recessive models of inheritance.

Results

Genetic Localization of SHOX

We wished to determine a genetic-map position in the PAR for the putative HD gene. Since our hypothesis for PAR localization of a gene for HD was based, in part, on the observation of a family coinheriting HD and LWD, we first determined a genetic location for SHOX, on the basis of observed recombination events dissociating sex from disease phenotype. For a PAR-linked gene, recombinations between the X and Y chromosomes may be counted by tabulation of the sex and affection status of grandchildren born to the affected son of an affected grandparent of known sex (fig. 1). When the affected grandparent is male, then it can be inferred that the SHOX mutation resides on the Y chromosome. Consequently, the grandfather's affected son's affected daughters and unaffected sons are recombinants, whereas the affected grandson and unaffected granddaughter are nonrecombinants. Conversely, when the affected grandparent is female, then it can be inferred that the mutation resides on the X chromosome. Consequently, the grandmother's affected son's affected sons and unaffected daughters are recombinants, whereas the affected granddaughters and unaffected grandsons are nonrecombinants. Eleven families with LWD were collectively informative for 52 meioses of such structure. Twenty of the meioses represented recombinations, corresponding to a male θ_{max} of .385. In some cases, however, the affected grandparent is undetermined, but a phase-unknown recombination event can be inferred, since an affected male fathered affected children of both sexes, unaffected children of both sexes, and/or one affected and one unaffected child of the same sex. To maximally extract information from the families with LWD, we also calculated θ by using a pedigree likelihood approach. We thereby performed parametric linkage analysis between sexual phenotype and LWD-affection phenotype, using the LOD-score method and a pseudoautosomal dominant model of inheritance, on a set of 294 individuals from 21 published pedigrees with LWD. The male θ_{max} was found to be .405, with a corresponding maximum LOD score (Z_{max}) score of .161 (fig. 2), which is in good agreement with the value derived from the subset consisting of only phase-determinable meioses. (The weak Z_{max} reflects the genetic distance of the "marker"—here, phenotypic sex—from the phenotype; however, exclusionary LOD scores at lower values of θ help to tentatively localize SHOX to the distal PAR.) This result places SHOX close to the telomeric boundary of the PAR.

Expected Skewing in Sex Concordance, for Sib Pairs with LWD

We next quantified the skewing in sib-pair sex ratios that would result from a θ of .405 for a pseudoautosomal dominant gene. In figure 3, we derive a function relating the male p-arm PAR θ to the frequency, f, of sex discordant affected sib pairs: $f = \theta - (\theta^2 + \frac{1}{4})$. Therefore, sex discordance should be observed in 49.1% of sib pairs with LWD, a result nearly indistinguishable from a random outcome. Among the published pedigrees with LWD (table 1), we counted a total of 33 affected sib pairs, 15 (45.5%) of which were sex discordant. There

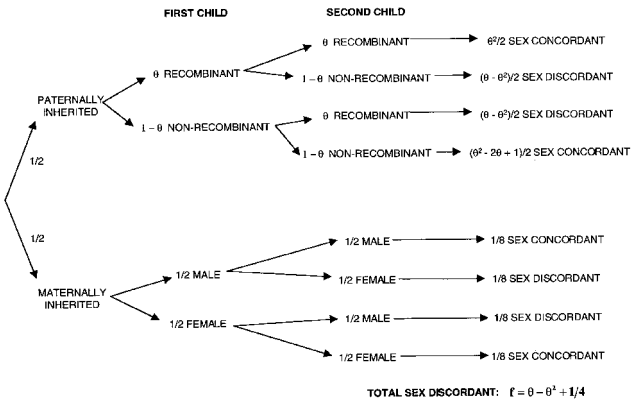

Figure 3 Derivation of a function relating the male PAR θ to the expected frequency, f, of sex-discordant affected sib pairs. The branching diagram illustrates the outcome of all possible consecutive meiotic recombinations for a dominant gene residing in the PAR. In this case, f is equal to the sum of the four outcomes leading to sex-discordant affected sib pairs.

Table 2

Affected-Relative-Pair Sex Concordance in HD

	Sibs			≥2° Relatives[a]		
Reference and Pedigree	MM	MF	FF	MM	MF	FF
Hors and Dausset (1983)	1	4	1	2	2	4
Hors et al. (1985)	1	5	5	2	1	1
Hafez et al. (1985)	1	2	0	0	0	0
Haim et al. (1982)	0	1	1	0	0	0
Berberich et al. (1983)	0	1	0	1	4	2
Durosinmi et al. (1989)	1	0	0	0	0	0
Cimino et al. (1988)	1	0	0	0	0	0
Weintraub et al. (1996)	1	1	0	6	2	0
Gokhale et al. (1995)	0	0	1	1	0	0
Nagel et al. (1978)	0	0	0	1	8	0
Bowers et al. (1977)	0	0	1	0	0	0
Greene et al. (1979)	2	4	1	2	4	2
Montefiore Medical Center collection	2	4	1	2	1	0
Grufferman et al. (1977):						
Original series	7	1	5			
Literature[b]	24	16	6			
DeVore and Doan (1957)[b]				9	9	4
Creagan and Fraumeni (1972)				0	1	1
Rigby et al. (1966):						
0063				0	1	0
0161				1	0	0
Vianna et al. (1974)				2	4	1
Total	41	39	22	29	37	15

Note.—Although some cases are cited by more than one reference, they are included in this table only once.

[a] Each of all possible combinations counted; thus, one individual may be counted more than once. Blank entries indicate that no data were available.

[b] The study by Grufferman et al. (1977) was used to review the literature—for sibs but not that for ≥2° relatives—prior to 1977.

is partial overlap of the data sets, but the results obtained by these two differing approaches are not significantly different. It should therefore be expected that, for a SHOX contiguous-gene –deletion syndrome, skewing in the frequencies of sex-concordant sib pairs will not be large and will occur only occur with deletions extending in a centromeric direction.

Families with HD

The observation of sex concordance among affected sib pairs with HD was first noted by Grufferman et al. (1977), both in a proprietary series and in a review of prior literature reports in which HD was suggested to result from an environmental factor more likely to be shared by same-sex sibs. A significant sex distortion was not confirmed in a subsequent, smaller review of cases published later (Chakravarti et al. 1986). We surveyed all available pedigrees with HD, including those listed by Grufferman et al. and Chakravarti et al., new reports since then, and a proprietary collection, from the Montefiore Medical Center, ascertained by recording the family history when the symptomatic proband presented for treatment of any hematological malignancy. As listed in table 2, we identified 102 affected sib pairs. As shown in table 3, we also noted seven affected sib triplets, two affected sib quadruplets, and one affected sib quintuplet. For all of the Montefiore Medical Center collection, as well as for some of the published reports, additional family information, including number and sex of unaffected sibs, is known. The relative prevalence of HD is somewhat greater for males than for females, and the frequency of males in the sib-pair data set is .59. There is an excess of sex-concordant pairs, but it does not achieve significance ($\chi^2 = 4.40$, 2 df, $P < .2$) when compared with the random distribution (MM = .3481, MF = .4838, and FF = .1681) anticipated for the observed proportion of male and female cases. We therefore examined a control group consisting of all ≥2° relative pairs (regardless of affection status of the intervening relative[s]) drawn from these families (table 2). If the hypothesis of PAR linkage were true, then sex concordance should be diminished in pairs that are more distantly related, since, whenever there is an intervening female meiotic transmission, recombination events between two X chromosomes will be noninformative. The

Table 3

Sex Concordance in Large Sibships with HD

No. Affected in Sibship and Sex Distribution	Reference
3:	
MMF	Greene et al. (1979)
MMF	Nunez-Roldan et al. (1979)
FFF	McBride and Fennelly (1977)
MMF	Hors and Dausset (1983)
MMM	Donhuijsen-Ant et al. (1988)
MFF	Razis et al. (1959)
MMF	Montefiore Medical Center collection
4:	
MMMF	Robertson et al. (1987)
FFFF	Nagel et al. (1978)
5:	
MMMMM	Kajii et al. (1968)

proportion of males (.59) in this control group of 81 relative pairs is the same as that in the test group of sib pairs, but there is less skewing in the distribution of sex-concordant pairs (54.3% are sex concordant in the $\geq 2°$ relative pairs, compared with 61.8% in the sib pairs). The sex distribution of affected pairs in the collection of families with HD could be a function of genetic relationship between the individuals. Since these χ^2 tests, however, do not fully extract genetic information from the available pedigrees, genetic-linkage analysis is a more appropriate means to further test this hypothesis.

Genetic Localization of the Putative HD Gene in the PAR, by Parametric Linkage Analysis

To localize the hypothesized HD gene in the PAR, we determined the male θ_{max} in a test of PAR linkage, using a pedigree likelihood parametric approach based on the LOD-score method (fig. 2). The pedigree data set consisted of 503 individuals from the 112 families with HD that are listed in tables 2 and 3 (with parents of unknown affection status being constructed as necessary). Two models were considered—pseudoautosomal dominant and pseudoautosomal recessive linkage of HD to sexual phenotype. Penetrance was initially estimated as .056, on the basis of the HD concordance frequency in MZ twins (Mack et al. 1995), but iteration was allowed. With dominant segregation $Z_{max} = 1.91$ and male $\theta_{max} = .152$, whereas for the recessive model, $Z_{max} = 1.91$ and male $\theta_{max} = .254$. On the basis of the LOD scores, the two models seem to be equally likely explanations, although both probably represent misspecifications, given the substantial evidence for complex inheritance of HD. In any event, the putative HD gene would localize reasonably close to SHOX, if a pseudoautosomal recessive mode of inheritance is assumed (fig. 2).

This approach tests only for linkage between sexual phenotype and disease phenotype. Sex may, of course, influence penetrance independently of localization of a gene on the sex chromosome (e.g., in the autosomal dominant syndromes of breast and ovarian cancer). Some amount of sex concordance in sib pairs is to be expected to result, just from the disparity in sex-dependent penetrance. It is therefore important to control for sex-dependent penetrance in HD; to do so, we synthesized a data set, on the basis of the expected distribution of affected sib pairs, triplets, quadruplets, and quintuplet, under the null hypothesis of sex-dependent penetrance without PAR linkage. Using the sex ratios observed in the families with HD (.59 male, .41 female) and rounding off to the closest integer, we determined that for 102 sib pairs the expected distribution is 36 MM, 49 MF, and 17 FF; that for 7 triplets it is 1 MMM, 3 MMF, 2 MFF, and 1 FFF; that for 2 quadruplets it is 2 MMFF; and that for 1 quintuplet it is 1 MMMFF. Families were constructed on the basis of these synthesized sibships consisting of affected sib pairs, triplets, quadruplets, and quintuplets (and with inclusion of all information from the real data set on unaffected sibs) and were parametrically analyzed for two-point linkage between phenotypic sex and affection, for an arbitrary phenotype, under both a dominant model and a recessive model (fig. 2). For both models, $Z_{max} = 0$ at $\theta_{max} = 0$, confirming that the evidence for sex linkage in HD is a specific consequence of the familial relationships between affected individuals; that is, sex and phenotype segregate independently in the family structures expected to randomly arise as a result of having the observed higher frequency of males—or, in other words, having a greater proportion of affected males than females does not, in itself, result in a false-positive, nonzero LOD score between the two, normally independently segregating traits here being tested for linkage (phenotypic sex and either autosomal dominant or autosomal recessive inheritance of an arbitrary phenotype).

Determination of Heritability of Risk for the Putative HD Gene, by Nonparametric Linkage Analysis

Although a nonparametric linkage test cannot determine θ_{max} in a two-point analysis between phenotypes, it is the most powerful linkage method for a complex trait such as HD (Lio and Morton 1997). We therefore tested for linkage between HD and phenotypic sex, in the collection of families listed in tables 2 and 3, using Morton's β method of allele sharing (Collins et al. 1996; Morton 1996; Lio and Morton 1997). Because this model is nonparametric, there is no requirement to estimate gene frequency or penetrance. In this model, a is defined as the negative of the natural log of the relative population risk $R_0 (R_0 = e^{-a})$, and β is defined as the natural log of the relative recurrence risk R_c of the disease

gene within a family (for sibs, $R_c \approx e^{-a+\beta}$). This model assumes that there is no allelic interaction on a logistic scale and that the probabilities of 0, 1, and 2 alleles being identical by descent in affected sib pairs is $1/(1 + e^{\beta})^2$, $2e^{\beta}/(1 + e^{\beta})^2$, and $e^{2\beta}/(1 + e^{\beta})^2$, respectively. On the basis of estimates that the sex-averaged prevalence of HD is ~.00004 (Grufferman et al. 1977), a is determined to be 10.1. Proband studies indicate a relative sib recurrence risk of HD of 210-fold (in an Egyptian population; Hafez et al. 1985) to 7-fold (in the Boston metropolitan area; Grufferman et al. 1977), giving a β value that ranges from 5.35 to 1.95, respectively. The total β reflects the sum of risk contributions from each component gene for a complex trait. The resulting LOD score determined on the basis of 146 affected sib pairs in these 112 families is 2.410, with component β of 0.562 (standard error 0.261). The hypothesized pseudoautosomal HD gene would thus be expected to account for 11% (95% confidence interval [CI] 1%–20%) to 29% (95% CI 2.3%–55%) of the total heritable risk for HD in Egyptian and Bostonian populations, respectively. As a control measure, we also tested the synthetic families derived under the null hypothesis of sex-dependent penetrance without PAR linkage. The β method determined a LOD score of 0.006 with component β of -0.0280 (standard error 0.251), a result that is close to 0 for both values and that suggests that the observed linkage to the PAR does not trivially result from the fact that HD is more common among males than among females.

Test for Linkage between HD and HLA

Associations between particular HLA alleles and HD risk have been appreciated for some time. Furthermore, a segregation analysis by Chakravarti et al. (1986) implied the existence of an HLA-linked susceptibility gene. In that analysis, LOD scores from all HLA-haplotyped families were exclusionary. However, a transmission/disequilibrium test for linkage in the presence of genetic heterogeneity achieved significance under the hypothesis that HD in 60% of families resulted from a recessive HLA-linked gene. We therefore reevaluated linkage in Chakravarti et al.'s data set of 32 affected sib pairs (table V in Chakravarti et al. 1986) from 32 families, using Morton's β method. We determined a LOD score of 2.00 with $\beta = 0.78802 \pm 0.40926$, suggesting that the hypothesized HLA locus would contribute from 15% (95% CI 0%–29%) to 40% (95% CI 0%–82%) of the total genetic liability for HD in the Egyptian and Bostonian populations, respectively.

Discussion

We have investigated a predicted peculiarity of pseudoautosomal dominant segregation, on the basis of published reports of LWD. The observed θ_{max} for LWD, .405, places the SHOX gene close to the telomere of the short arms of the sex chromosomes, supporting the hypothesis that short-arm PAR recombination is an inevitable event in spermatogenesis. Sex in sib pairs with LWD would not be expected to differ from the random outcomes anticipated for an autosomally localized gene. By observing a separate but overlapping data set of sib pairs with LWD, we confirm that there is an approximately random distribution of sex concordance. The localization of SHOX near the telomeric boundary of the short-arm PAR is in good agreement with two prior results. First, molecular-genetic mapping of LWD (Shears et al. 1998) produced the greatest LOD score with DXYS233, the most telomerically positioned short-arm PAR marker. Recombination between DXYS233 and LWD was observed, however, indicating that SHOX is somewhat subtelomeric. Second, SHOX deletions also cause idiopathic short stature, and a location for SHOX can be inferred from a contiguous-gene deletion–syndrome map clinically observed for the terminal short arm of the sex chromosome (Ballabio et al. 1989). In that map, which correlated the extent of cytogenetic deletion with a range of observed phenotypes attributed to loss of that region of the X chromosome, short stature, presumably representing SHOX deletion, was localized to the most telomeric position, in relation to other phenotypes. At least two other genetic diseases—Kallman syndrome and chondrodysplasia punctata—in addition to LWD physically map to the short-arm PAR, but fitness for these diseases is reduced, particularly for males, on account of hypogenitalism and mental retardation, respectively, and a determination of male θ is precluded, since inheritance through a male parent is unreported. There are also equivocal linkage data supporting localization of a schizophrenia-predisposing gene to the short-arm PAR (Crow et al. 1994); however, at this time, LWD remains the only confirmed disease for which predicted pseudoautosomal segregation patterns may be tested. These findings raise the question of how pseudoautosomal localization of a disease comes to be suspected. Most current genomewide linkage "mapping panel" marker sets do not include pseudoautosomal coverage. Although the region is small, it represents one genomic area not routinely investigated for disease localization. Also, if the gene resides some distance from the centromeric boundary of the PAR, such as with SHOX, then segregation patterns are indistinguishable from autosomal dominant transmission, as has been proved here for LWD. It is only then that the rare patient with either a constitutional sex-chromosome translocation, such as

has been shown to be the case with LWD (Belin et al. 1998), or a potential contiguous gene–deletion syndrome, such as we conjecture could be the case with HD and LWD, brings the possibility of PAR linkage to attention. A corroborating reason to suspect PAR localization for HD is the previously reported excess of sex-concordant sib pairs (Grufferman et al. 1977). We find that the frequency of sex-concordant sib pairs for HD is skewed beyond random expectations, whereas there was no evidence of significant sex skewing in a somewhat smaller control set of affected relative pairs with HD who were more distantly related (i.e., $\geq 2°$); the excess sex concordance in sib pairs did not reach statistical significance, however. A significant proportion of the risk for HD appears to be attributable to genetic factors, but, at present, the lack of ample collections of appropriately sampled rare families with HD prohibits molecular-genetic approaches to screening for linkage. Here we have developed reasonable evidence for linkage of HD to sexual phenotype, but, unlike arbitrary molecular markers, the sex phenotype may markedly influence, first, the penetrance, second, the ascertainment, and third, the genetic relationships between affected individuals.

The fact that HD is somewhat more prevalent among males indicates that there should be an excess of MM affected sib pairs, even in the absence of genetic etiologies. If such an excess were sizable, then a positive LOD score might be expected, just as a result of this effect. We are able, however, to exclude sex-dependent penetrance issues as contributory to the finding of linkage, because simulated sibships corresponding to the sex distributions expected under the null hypothesis of no linkage yield Z_{max} values close to 0.

Sex may also influence the ascertainment of affected relative pairs (Penrose 1942). In fact, there is evidence for ascertainment bias in the pedigrees with LWD that were collected here. We find that the θ is greater for male probands than for female probands (data not shown). An interpretation of this result is that, among males, the sex in which expression of LWD is considered to be less severe (Goepp et al. 1978; Lichtenstein et al. 1980), a male is more likely to be found to be affected if his father is also affected. This may lead to an overestimation of the θ_{max} for LWD. Among the pedigrees with HD that were collected by Chakravarti et al. (1986) are both previously published families and families that they identified. In the group of published sib pairs, 16 shared two HLA haplotypes, 8 shared one HLA haplotype, and 5 shared zero HLA haplotypes. However, among the previously unpublished families included in Chakravarti et al.'s analysis, which presumably were ascertained independently of HLA haplotype, only one shared two HLA haplotypes, four shared one HLA haplotype, and one shared zero HLA haplotypes. (The sample size is small, but this agrees with the expected random distribution of 1.5:3:1.5.) A publication premium might therefore be affixed to families with HD that support the hypothesis of HLA linkage. It is perhaps more difficult to imagine sources for ascertainment bias that would influence the sex in collections of sibs affected with HD, since the disease is severe and almost always fatal if untreated and since, prior to this report, the hypothesis of pseudoautosomal segregation was not widely entertained. Nevertheless, most of the reports of sex-concordant sibs come from just a few published sources, the series by Grufferman et al. (1977), and the sibships with four and five affected individuals. One possible explanation is that, in the presence of a PAR-linked HD gene, there is actually ascertainment bias *against* sex-concordant sib pairs. The sibships with the greatest number of affected individuals are those that are most likely to have a genetic—as opposed to environmental or coincidental—etiology, and it is in those sibships that sex concordance is most pronounced. The sibships consisting of only affected pairs are more likely to include cases resulting from nongenetic or coincidental etiologies. And, among all the published sources on families with HD, only in the series by Grufferman et al. (1977) was a systematic attempt made to identify all potential cases in a limited geographic distribution. The high frequency of sex concordance may represent the fact that family structures are more likely to remain intact in younger sibships (since older sibs are more likely to have left the parental home and moved away from each other); the patients with earlier onset are more likely to have a genetic etiology for HD, at least according to suggestions from twin studies (Mack et al. 1995). We thus cannot exclude the possibility that we have actually underestimated the role of sex linkage in HD.

Finally, the most plausible genetic model for sex linkage is localization of a gene to the PAR. It may be of particular interest, then, that a cluster of cytokine-receptor genes, including components of the receptors for GM-CSF and IL-3, resides on the short arm PAR (Kremer et al. 1993) and that the receptor for IL9 maps to the long arm PAR (Kvaloy et al. 1994). There is some experimental evidence that these cytokines modulate the inflammatory reactions characteristic of HD (Merz et al. 1991), presumably in concert with HLA. Nevertheless, sex linkage may be legitimately measured in the absence of a genetic mechanism involving the sex chromosomes. Sib relationships are entangled with the family environment. The explanation that Grufferman et al. (1977) originally proposed to explain the excess sex concordance among affected sibs—that is, that a sister pair or brother pair are more likely to share a common environment than are a sister and brother pair—cannot be rejected here. On the other hand, Grufferman et al. found that differences in age between sib pairs were un-

related to risk for concordance of HD. Since it might be expected that sibs of more closely spaced ages would be more likely to share environments, the absence of such a cohort effect speaks against the likelihood of environmental contributions to HD; however, if, in the future, a sufficient number of sib pairs with HD become available for molecular genetic analysis, then it can be tested whether they are more likely to share a common maternal X chromosome—and discrimination between ascertainment biases and environmental and genetic factors responsible for the observed sex linkage of HD becomes possible.

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Electronic-Database Information

Accession numbers and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for HD [MIM 236000] and LWD [MIM 127300])

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