Linkage Analysis of DRD2, a Marker Linked to the Ataxia-Telangiectasia Gene, in 64 Families with Premenopausal Bilateral Breast Cancer


Abstract

Recent reports suggest that subjects who are heterozygous for the ataxia-telangiectasia gene are at increased risk of breast cancer. We conducted linkage analyses of 64 families with premenopausal bilateral breast cancer using DRD2, a marker linked to the ataxia-telangiectasia locus at 11q22-23. We assumed a model with dominant transmission of breast cancer. Lod scores summed over all families provided strong evidence against tight linkage (e.g., a lod score of -6.08 at θ = 0.0001), although a single family provides suggestive evidence of tight linkage to DRD2. Evidence against linkage to 11q was strongest among families that may involve the BRCA1 breast cancer susceptibility gene on 17q21. However, we did not observe evidence of linkage to 11q among the remaining subgroup with neither a family history of ovarian cancer nor the appearance of linkage to 17q21.

Introduction

Recently, Swift et al. (1) reported evidence that subjects who are heterozygous for the A-T2 gene have an elevated risk of breast cancer. The ratio of observed to expected cases was 5.1 (P < 0.01). This increased risk is consistent with their previous study (2), as well as studies of smaller populations from the United Kingdom (3) and Norway (4). The combined relative risk estimate for mothers in the two European studies was 14.9 (95% confidence interval of 4.05-38.1; Ref. 5). Based on the observed effect of A-T on breast cancer risk and the estimate that between 0.68 and 7.7% of the Caucasian population are A-T heterozygotes, Swift et al. (2) estimated that 8.8% of breast cancer cases may be A-T heterozygotes. Given the high familial risk among such families and consequent high probability that inherited breast cancer susceptibility genes are segregating among them, a much higher proportion of cases among families with premenopausal bilateral occurrence of breast cancer may involve this gene. We report here results of a linkage analysis of 64 families with premenopausal bilateral breast cancer using DRD2, a marker on chromosome 11q reported to be between 4.2 and 9.4 sex-averaged cM distal to the A-T locus (6).

Materials and Methods

Family Data. Families for this analysis were identified as part of a large, ongoing genetic-epidemiological study of bilateral breast cancer. Cases of bilateral breast cancer with a diagnosis prior to 50 years of age were identified from the Los Angeles County Tumor Registry (1970-1989), the Connecticut Tumor Registry (1935-1989), and the major hospitals in Montreal and Quebec City, which identify 95% of all cases in these cities and the southern Quebec Province (1975-1989). Index cases were sent questionnaires regarding family history of breast cancer. We received 434 completed questionnaires: 200, 156, and 78, respectively, from the Los Angeles, Connecticut, and Montreal study sites. From the questionnaires, we identified multiple-case families potentially informative for genetic linkage. Blood samples were obtained from as many living members as possible. DNA was extracted and stored for future analysis. We have typed 68 multiple-case families and collected DNA from 428 members. Two families were not studied because of unresolved genetic incompatibilities, and two were not studied because DNA has not been obtained from critical family members. The families range in size from 4 members with 2 cases (both living) to 84 members (44 dead, 40 living) with 10 cases of breast cancer (5 dead, 5 living). Sixty-four of the families were studied for this analysis.

Determination of DRD2 Alleles. PCR reactions were performed with one radioactively labeled and one unlabeled primer, and products were visualized on modified acrylamide gels. Primers used were 509 (5'-CAGGAGCAC-GTTTCTCTACAC-3') and 419 (5'-GGAGGGCGGTGCGTTCAT-3') (7). One primer was end labeled with [α-32P]ATP (specific activity, 6000 Ci/mmol; Amersham) using T4 polynucleotide kinase (New England Biolabs) according to standard protocols (8). The primer concentrations were 0.5 pmol of each of the two primers. The reaction concentrations in the reaction mixture were optimal at 0.8 mM MgCl2 and 50 mM KCl. The reactions were overlaid with ~100 μl of light mineral oil.

Ten ng of genomic DNA was amplified in a 5-μl PCR reaction using AmpliTaq DNA polymerase (Perkin Elmer Cetus) according to the specifications of the manufacturer. Reactions were amplified on an MJ research programmable thermal controller PTC-100, using the following thermocycling protocol: initial denaturation at 94°C for 5 min, followed by 25 cycles of 94°C for 30 s, 58°C annealing for 30 s, and 72°C elongation for 10 s. PCR products were combined with 10-fold loading buffer consisting of xylene cyanol and bromophenol blue dyes in 15% Ficoll and electrophoresed on 8% denaturing Long Ranger gels (AT Biochem) for 2 h at 1200 V of constant voltage. Gels were wrapped in Saran Wrap and vacuum dried at 80°C for 30 min with a gel dryer (model FB.GD.45; Fisher Biotech) and then exposed directly to Kodak XAR film for 2-16 h at room temperature without intensifying screens.

 Autoradiograms were independently scored twice. Fragment sizes were measured relative to size standards consisting of 5'-end-labeledMspI fragments of pBR322 (sizes provided, 76 and 90 base pairs) and HaellI fragments of pBR322 (sizes provided, 68 and 89 base pairs). Scoring was also confirmed by gels containing DRD2 allele ladders derived from previously typed individuals. Individuals from two previously typed CEPH pedigrees were used to confirm allele designations.

Analytical Methods. The 1992 version of the computer program MENDEL (9) was run on an IBM/9000 model 900 under Multiple Viral System to calculate lod scores for a single recombination parameter. The program was modified to incorporate the age-at-onset step function model reported by Claus et al. (10). The model gives cumulative incidence as genotype-dependent step functions with steps at every 10 years from age 30-80 years. For gene carriers, the cumulative incidence begins at 0.0167 for those younger than 30 years and increases to 1.000 for those 80 years or older. For noncarriers, the cumulative incidence begins at 0.0002 and increases to 0.1254 at 80 years. We estimated the density at each step by dividing the increase in step height by the step length. The penetrance function is given by the density for affected individuals or by 1 - cumulative incidence for unaffected persons.

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The abbreviations used are: A-T, ataxia-telangiectasia; PCR, polymerase chain reaction; bp, base pair fragment; MLE, maximum likelihood estimate.

The frequency of the high-risk allele was set to 0.0006, and probabilities for males were set equal to those for 20- to 29-year-old females.

Lod scores were calculated for 21 values of θ: 0.000001, 0.02, 0.50, 0.575, . . . , 0.450, 0.475, 0.500. Vectors of lod scores were summed for all families and for subgroups defined by ages at onset (families with all cases younger than 45 years at diagnosis versus families with one or more cases 45 years or older at diagnosis), family history of ovarian cancer, synchronicity (proband had both primaries diagnosed within 1 year versus >1 year), span of ages at diagnosis (families with all breast cancer diagnoses within 10 years of age versus families with a difference in ages at diagnosis >10 years for at least two breast cancer cases), evidence suggestive of involvement of the BRCA1 locus on 17q, and histopathological diagnosis. Histopathological diagnosis was based on histopathological findings (11) described in the original pathology report or rapid report form of the Los Angeles County Tumor Registry. Families were included in subgroups based on histopathological diagnosis only if confirmed diagnosis was available for tumors of both breasts of the proband and for a tumor of at least one additional case in the family. Four groups of families were defined: families in which all cases with confirmed diagnoses had tumors classified as ductal carcinoma in situ, invasive ductal carcinoma, or spatial variants thereof (ductal); families in which all cases with confirmed diagnoses had tumors displaying only lobular elements (lobular); families in which all cases with confirmed diagnoses had at least one tumor displaying both lobular and ductal elements (mixed); and families in which cases with confirmed diagnoses were discordant with respect to histopathological diagnosis (discordant), i.e., at least one case had a histopathological diagnosis differing from that of the proband. We conducted two separate sets of analyses, one using frequencies of the DRD2 alleles estimated among original members of A-T families without inbreeding and the other using allele frequencies estimated among original members of our breast cancer families. The two sets of frequencies were similar. Frequencies estimated among the breast cancer families were 0.3% for the 76 bpf, 15.5% for the 78 bpf, 8.8% for the 80 bpf, 51.6% for the 82 bpf, 22.6% for the 84 bpf, and 1.2% for the 86 bpf.

Results

Table 1 presents for each family the mean age at diagnosis, MLE of θ, and lod scores for θ = 0.000001 and θ = 0.025. Fig. 1 presents the summed lod scores for values of θ for all families and illustrates the strong evidence against tight linkage of breast cancer to the DRD2 locus in the majority of the families. The summary lod score is -6.018 for θ = 0.000001 and -3.362 for θ = 0.025. One family (BCS069) had a lod score of 0.935 at θ = 0.000001; no characteristic that we analyzed distinguished this family from the others. A lod score of -2.0 occurred for a value of θ between 0.05 and 0.075. Using linear interpolation between these points, we estimated that, for the lod scores summed over all families, lod(θ) is <-2.0 for θ in the interval (0, 0.063). These results were obtained by specifying DRD2 allele frequencies estimated from the breast cancer families. Very similar results (e.g., summary lod score of -5.3974 at θ = 0.000001) were obtained using allele frequencies estimated from the A-T families.

Since etiologic heterogeneity of breast cancer is likely, we used both predivided sample and admixture tests to investigate heterogeneity of linkage between breast cancer and DRD2 in our data. To implement the predivided sample analysis, we stratified the families on variables that may define sources of heterogeneity and summed vectors of lod scores separately for each stratum. The program MTEST was used to conduct likelihood ratio tests for heterogeneity between the strata of each variable. We observed no significant evidence of heterogeneity when we divided our families by the following variables: ages at diagnosis (all cases younger than 45 years at diagnosis, one or more cases 45 years or older at diagnosis) (P = 0.683); pathology (ductal, lobular, mixed, discordant) (P = 0.919); family history of ovarian cancer (yes, no) (P = 0.585); synchronicity of diagnoses of primary tumors in proband (within 1 year, >1 year apart) (P = 0.510); age span between diagnoses of cases within a family (span ≤10 years, span >10 years) (P = 0.776).

Separate analyses have suggested linkage between breast cancer and markers in the region of the BRCA1 gene on chromosome 17q in a subset of our families. We used results of our linkage analyses between breast cancer and mfd188, a marker locus on 17q, in 67 pedigrees with bilateral breast cancer, with a focus on heterogeneity, submitted.
define subgroups of families appearing to be linked (lod score > 0.20 at \( \Theta = 0.000001 \)) and unlinked (lod score \( \leq 0.2 \) at \( \Theta = 0.000001 \)) to the \( BRCA1 \) region. By this criterion, breast cancer appeared to be linked to 17q in nine families. Results suggest that the strongest evidence against linkage comes from families that may be linked to 17q (Fig. 2). However, even within the remaining families, we did not observe evidence of linkage (e.g., at \( \Theta = 0.000001 \), the summed lod score was –0.422). We obtained similar results when we increased or decreased the lod score criterion. Since the \( BRCA1 \) locus appears to be involved in familial breast and ovarian cancer, we defined a second composite variable consisting of either linkage to mfd188 by the above criteria or positive family history of ovarian cancer. Nine of our families had at least one case of ovarian cancer. Of these, four were found to be linked to mfd188 by the above criteria, and by definition, all nine were included in the composite \( BRCA1 \) group. Using the predivided sample test, we found no significant difference between linked and unlinked groups (P = 0.648) or levels of the composite variable (P = 0.695).

We used the program HOMOG to conduct admixture tests comparing three hypotheses: no linkage to \( DRD2 \), linkage to \( DRD2 \) with homogeneity, and linkage to \( DRD2 \) with heterogeneity. We were unable to reject the hypothesis of no linkage (\( \Theta = 0.5 \)) against the alternative of linkage with homogeneity (\( \Theta < 0.5 \); one-sided P = 0.189). For admixture testing the parameter \( \alpha \) is defined as the proportion of linked families. We were also unable to reject the hypothesis of no linkage (\( \Theta = 0.5 \), \( \alpha = 0.0 \)) against linkage with heterogeneity (\( \Theta < 0.5 \), \( \alpha < 1.0 \); one-sided P = 0.339). Finally, we were unable to reject linkage with homogeneity (\( \Theta < 0.5 \), \( \alpha = 1.0 \)) against the alternative of linkage with heterogeneity (\( \Theta < 0.5 \), \( \alpha < 1.0 \); one-sided P = 0.500).

Discussion

Our results suggest that the A-T gene is not responsible for the majority of breast cancer in these data, although it may be a cause of breast cancer in one or more of the families. Our findings must be interpreted in light of several factors upon which they depend: prior information about the genetic distance between \( DRD2 \) and the A-T locus, an assumed model for inheritance of breast cancer at the A-T locus, and the likely existence of etiological heterogeneity for breast cancer.

We interpolated summed lod scores to be \( \leq -2.0 \), the conventional critical value for exclusion of linkage, up to 6.3 cM from the \( DRD2 \) locus. Our interval extends into but does not cover the support interval for the A-T locus approximately 4.2–9.4 cM proximal to \( DRD2 \), identified by Foroud et al. (6); our interval nearly reaches the midpoint of the support interval, approximately 6.8 cM from \( DRD2 \). However, our interval can be interpreted as an exclusion region only under the assumption of genetic homogeneity, which is not reasonable for this disease. It is possible that an unidentified subset of our families involve the A-T gene and that the reported lod scores represent weighted sums from unknown proportions of linked and unlinked families.

It is possible that misspecification of the genetic model for breast cancer inheritance may have biased our lod scores and MLE of to some degree (14). The assumption of dominant inheritance of susceptibility is inherent in the hypothesis that A-T heterozygotes are at increased risk of breast cancer; therefore, it is unlikely that the degree of dominance was misspecified. However, the penetrances or the frequency of the high-risk allele could have been misspecified, but misspecification of these parameters is unlikely to have resulted in such strongly negative scores at \( \Theta = 0.000001 \) if breast cancer were tightly linked to \( DRD2 \) in most of our families.

Unresolved etiological heterogeneity could also explain our failure to detect linkage even if the A-T gene is a cause of breast cancer in one or more families. We addressed the possibility of genetic heterogeneity with admixture and predivided sample tests. Using the admixture test, we could not discriminate between the hypotheses of no linkage, linkage with homogeneity, and linkage with heterogeneity. However, the specific tests conducted were not well suited to provide insight into the heterogeneity question. It is unlikely that all breast cancer involves the A-T gene; therefore, we have strong prior belief that the hypothesis of linkage with homogeneity is false. Thus, the test of the null hypothesis of no linkage versus the alternate hypothesis of linkage with homogeneity is not of great interest. For the same reason, the test of linkage with heterogeneity against linkage with homogeneity is not of great interest. Furthermore, given that very large numbers are needed to detect linkage heterogeneity using a dominant model with incomplete penetrance for disease inheritance (15), we probably had extremely low power to detect heterogeneity using the admixture test. Given our prior knowledge, the final test of the null hypothesis of no linkage against the alternate hypothesis of linkage with heterogeneity is a meaningful comparison. Unfortunately, a recent report (16) indicates that this test does not have good asymptotic properties and, therefore, that the P value for this test cannot be interpreted as the probability of the observed or more extreme results given that the null hypothesis is true.

When we split our families into subgroups defined by a number of variables, including ages at onset, histopathological diagnosis, family history of ovarian cancer, synchronicity of diagnoses of primary tumors in proband, and span of ages at diagnosis of breast cancer within families, we obtained no significant evidence of heterogeneity using the predivided sample test. However, these variables may not discriminate well between those families in which the A-T gene is segregating and the others; therefore, mixtures of linked and unlinked
families might persist in the strata we defined. Much of the evidence against linkage came from the 14 families that may involve the BRCA1 locus on 17q, but we did not observe evidence of linkage among the remaining 54 families. However, the BRCA1 locus is probably not the only other locus conferring risk for breast cancer (17); consequently, among the 54 families there may be some among which the A-T gene is segregating and others among which it is not.

These results strongly suggest that the A-T gene is not a cause of breast cancer in most of the cases in these families, although it is possible that one or a few families within our series (particularly BCS069) may involve the A-T gene. It remains a possibility, of course, that the A-T gene is a cause of other forms of breast cancer not defined by multiple-case families with premenopausal, bilateral probands. Because power of standard linkage analyses is limited by reduced penetrance and the possibility of phenocopies, it is unlikely that a single family will produce compelling evidence for linkage to the A-T locus. Therefore, we believe that further elucidation of the role of the A-T gene in breast cancer and identification of individual breast cancer families or cases that involve this gene awaits cloning of the gene or at least a reliable, feasible method for detecting A-T heterozygotes.

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References

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