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While breast cancer in many families is due to the known breast cancer genes, there is still a sizable proportion of families that do not show linkage to these known genes. Thus, it is important that other genetic loci responsible for these cases be investigated.

The past year was mostly spent on developing theory for estimating linkage disequilibrium between a generalized mendelian locus and a nearby marker locus (Task 4). Work is progressing satisfactorily and results will be implemented in a computer program. A manuscript will be written in the next funding period.

Also, in collaboration with other researchers at Columbia University, we investigated four families with Cowden's syndrome (CS), which is inherited in an autosomal manner and is associated, among other things, with breast tumors. A gene for CS has previously been localized on chromosome 10, in the vicinity of a gene known as MMAC1. Genetic linkage of our families was investigated and showed that two of them are likely to segregate a disease gene near the MMAC1 gene. Cases and controls collected by our colleagues will be investigated with the new methods for disequilibrium when available.

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INTRODUCTION

The work reported below addresses Task 4, *To improve the currently available methods for assessing linkage through allelic association (linkage disequilibrium).* This work is statistical in nature, that is, it addresses method development and implementation of the resulting methodology in computer programs. Specifically, it involves estimating linkage disequilibrium between a mendelian disease gene and a nearby marker locus.

While this development work is ongoing and is expected to be completed in the next research period, collaboration with other researchers at Columbia University was established in order to obtain family data on which the new methods can be tried once fully developed and implemented. The disease gene investigated was that responsible for Cowden's Syndrome.

BODY

When linkage between a disease and marker locus is very tight, one often also observes linkage disequilibrium (LD) (allelic association), that is, differences in marker allele frequencies between cases and controls. Well-known examples of disease loci in disequilibrium with nearby markers are cystic fibrosis and Duchenne muscular dystrophy. It has been recommended that LD analysis is more efficient for finding disease loci of small effects (Risch and Merikangas 1996), and that LD is suitable for fine-mapping of disease loci (Lander and Kruglyak 1995).

Depending on the mode of inheritance of the disease, a more efficient analysis than simply comparing allele frequencies in cases and controls is possible. For a dominant and fully penetrant disease without phenocopies, Chakravarti et al. (1984) have shown how LD may be estimated by maximum likelihood. The procedure currently under development will be applicable to dominant or recessive diseases with incomplete penetrance and presence of phenocopies. Details of the approach are described in the original grant proposal (task 4). Theoretical methods development is completed and work has begun on implementing the approach in a computer program. A manuscript will be written in the next funding period.

Cowden's Syndrome (CS) is an autosomal dominant disease associated, among other things, with breast cancer. In a majority of families with CS, the disease appears to be due to a gene on chromosome 10 in close vicinity of a gene known as MMAC1. Mutations in the MMCA1 gene have been shown to occur in cases with CS. In collaboration with other researchers at Columbia University, we investigated four families in which CS occurs. Genetic linkage with three marker loci near the MMCA1 gene was investigated. Two of the families were consistent with linkage, while in two other families linkage was excluded. Cases and controls collected for this work will be analyzed by the newly developed LD methods.

CONCLUSIONS

Linkage analysis in small families with Cowden's Syndrome (autosomal dominant) suggested linkage to chromosome 10 markers in two families and absence of linkage in another

two families. Methods development is in progress for investigating linkage disequilibrium between mendelian traits such as Cowden's Syndrome and genetic markers. These methods will be applied to cases and controls collected by researcher colleagues at Columbia University.

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Tsou HC, Teng D, Ping XL, Brancolini V, Davis T, Hu R, Xie XX, Gruener AC, Schrager CA, Christiano AM, Eng C, Steck P, Ott J, Tavitigian SV, Peacocke M (1997) Role of MMAC1 mutations in early onset breast cancer: Causative in association with Cowden's syndrome and excluded in BRCA1-negative cases (in press)

APPENDIX

Copies of the manuscript by Tsou et al. (1997) are included in the appendix.

Role of *MMAC1* Mutations in Early Onset Breast Cancer: Causative in Association with Cowden's Syndrome and Excluded in *BRCA1*-Negative Cases

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Summary

Cowden's syndrome (CS) is an autosomal dominant disorder associated with the development of hamartomas and benign tumors in a variety of tissues, including the skin, thyroid, breast, endometrium, and brain. It has been suggested that women with CS are at increased risk for breast cancer. A locus for CS was recently defined on chromosome 10 in 12 families, resulting in the identification of the Cowden critical interval between the markers *D10S215* and *D10S541*. More recently, affected individuals in four families with CS have been shown to have germline mutations in a gene known as *PTEN* or *MMAC1*, which is located in the Cowden critical interval on chromosome 10. In this study, we report three novel *MMAC1* mutations in CS, and demonstrate that *MMAC1* mutations are associated with CS and breast cancer. Further, we also show that certain families and individuals with CS do not have mutations in the coding sequence of *MMAC1*. Finally, we did not detect *MMAC1* mutations in a subpopulation of individuals with early onset breast cancer suggesting that germline mutations in this gene do not appear to be common in this group.

Introduction

Cowden's syndrome (CS) (Lloyd and Dennis 1963), or multiple hamartoma syndrome (Weary et al. 1972), is an autosomal dominant disorder associated with the development of hamartomas and benign tumours in a variety of tissues, including the skin, the thyroid, the breast, the colon and the brain. It has been suggested that women with CS are at increased risk for breast cancer (Brownstein et al. 1978) and, as in other susceptibility syndromes, they appear to develop breast cancer at an early age (Schrager et al. 1997a). CS is also associated with a specific skin lesion, the trichilemmoma (tumor of the follicular infundibulum), and thus this breast cancer susceptibility syndrome can be recognized by the presence of a cutaneous biomarker (Brownstein et al. 1977, 1978). We have studied in detail the clinical and pathological findings (Schrager et al. 1997a, 1997b) in this syndrome and have demonstrated that the mean age of presentation with malignant breast disease in CS is 46 years, with the age range of presentation with breast cancer in affected women from 33 to 74 years (Schrager et al. 1997a). Moreover, very few of the women with CS that we studied had a family history of breast cancer (Schrager et al. 1997a). Of interest, men with CS appear not to be at increased risk for the development of breast cancer (Brownstein et al. 1978; Schrager et al. 1997a). We have also shown that women with CS develop exuberant benign breast disease and frequently report a history of multiple breast biopsies prior to the development of breast cancer (Schrager et al. 1997b). The history of skin disease and benign breast disease can therefore allow identification of affected individuals prior to the development of breast cancer in this high risk population.

It has been previously demonstrated that a locus for CS exists on chromosome 10 (Nelen et al. 1996). In that study, a total of 12 families were

examined resulting in the identification of the Cowden critical interval between markers *D10S215* and *D10S564*. Certain affected individuals in these families had CS and Lhermette-Duclos disease (LDD) (Nelen et al. 1996; Liaw et al. 1997), a rare brain disorder characterised by a dysplastic gangliocytoma of the cerebellum (Albrecht et al. 1992). Fine mapping of this area refined this initial result (Liaw et al. 1997), supporting a location for the CS gene between markers *D10S215 and D10S541*. More recently, affected individuals in four families with CS have been shown to have germline mutations (Liaw et al. 1997) in a gene known as *PTEN* (Li et al. 1997), *MMAC1* (Steck et al. 1997) or *TEP1* (Li, D.-M., et al. 1997) which is located in the Cowden critical interval on chromosome 10. Of interest, the predicted *MMAC1* protein contains sequence motifs with significant homology to the catalytic domain of protein phosphatases, and to the cytoskeletal proteins, tensin and auxillin (Li et al. 1997; Steck et al. 1997). Moreover, coding region mutations in *MMAC1* were observed in human tumors or tumor cell lines of the breast, brain, prostate and kidney (Li et al 1997; Steck et al. 1997). While the function of this gene is unknown, it is likely that *MMAC1* plays a role in the control of cell proliferation and its loss of function is important in the development of human tumors.

Methods

Clinical Materials: Approval for this study was obtained by the Investigation Review Board of Columbia Presbyterian Medical Center. Blood samples were obtained after informed consent from individuals with Cowden's syndrome. An aliquot was used for DNA extraction, while peripheral blood mononuclear cells were purfied from a second sample and used to generate an EBV-transformed lymphoblastoid cell line. The diagnosis of CS was made using the International Cowden's Consortium CD diagnostic criteria (Nelen et al. 1996). For individuals with early onset breast cancer, the sample consists of 63 women who developed

breast cancer before age 35 (average age at diagnosis is 27.7 yrs), did not have a clinical diagnosis of CS, and who had previously been shown not to carry clearly deleterious mutations in BRCA1 (5 women in the sample carried missense polymorphisms of unknown significance)(Shattuck-Eidens et al. 1997). These women are a subset of a sample of 798 unrelated individuals from 20 collaborating institutions, chosen from families which were generally at an elevated risk of carrying *BRCA1* mutations. Most families were chosen because of multiple cases of breast cancer, early age of breast cancer diagnosis, and incidence of ovarian cancer, as these conditions have been previously shown to be associated with germline mutations of *BRCA1*. Some of the families extended to second degree relatives. All samples from institutions in the United States were collected from individuals participating in research studies on the genetics of breast cancer. Each individual read and signed informed consent documents approved by the local institutional review board. All samples from institutions outside of the United States were collected according to the appropriate guidelines concerning research involving human subjects imposed by the institution's equivalent authorities. Only one representative from each family was included in the sample, and no families known to be linked by genetic markers to *BRCA1* were included. This is a heterogeneous sample which represents the diversity amongst patients who present at high-risk clinics as opposed to the more controlled sampling done for family or population studies. This has directed our analyses towards methods which do not require that samplefrequencies of subgroups reflect frequencies in the general population. Therefore we can assess, for example, the probability that a woman with breast cancer diagnosed at age 30 carries a deleterious *BRCA1* or *MMAC1* mutation, but we cannot estimate the frequency of such women in the general population. All the samples used in the *MMAC1* study were stripped of identifiers.

DNA extraction: After informed consent was obtained, patients genomic DNA was extracted from whole blood or lymphoblastoid cell lines using QIAamp blood Maxi Kit. Concentration was measured by OD_{260} and purity was checked by the ratio of OD_{260}/OD_{280}.

Genotyping: Primer pairs for the chromosome 10 locus were obtained from Research Genetics. The forward strand primer was end-labeled in the presence of ^{33}P-γATP and polynucleotide kinase. PCR reactions were performed in a total reaction volume of 30 microliters. The reactions consisted of 10 mM of each primer, 200 mM of deoxynucleotides, 1.5 units of Taq DNA polymerase and 50 ng of genomic DNA. PCR was performed for 35 cycles with 45 seconds denaturation at 94°C, 45 seconds anealing at 55°C and 1 minute elongation at 72°C. A final 10 minutes elongation was used. PCR reactions were stopped by addition of 20 microliters of stop solution (95% formamide, 1 mM EDTA, 0.25% bromophenol blue, 0.25% xylene cyanol). Then reactions were denatured for 5 minutes at 94°C and the products were separated on a 8% denaturing polyacrylamide gel. Allele sizes were determined by comparing to the SequaMark (Research Genetics) which was included as a size standard on the gels.

Linkage analysis: Two-point linkage analysis was performed using MLINK. Individuals below 20 years were considered as unknown. Disease gene frequency was set equal to 0.000001 and marker allele frequencies were estimated using ILINK. Both MLINK and ILINK are from the LINKAGE package Version 5.2 (Lathrop et al. 1984). Reconstruction of the most probable haplotypes in family D was obtained using GENEHUNTER (Kruglyak et al. 1996). Pedigrees were drawn using Cyrillic Version 2.02.

Mutation detection: We performed nested PCR amplifications on genomic DNAs and screened the resulting amplicons for sequence variants as previously described, with several modifications (Steck et al. 1997). First, exon 6 was screened as a single secondary amplicon amplified using the exon 6 FB-RR primer pair. Second, exon 8 was screened as two secondary amplicons using the following FB-RQ and FC-RR primers:

CA6.HB $^{5'}$-GTTTTCCCAGTCACGACGAGGTGACAGATTTTCTTTTTTA-$^{3'}$

CA6.HQ $^{5'}$-AGGAAACAGCTATGACCATTCGGTTGGCTTTGTCTTTA-$^{3'}$

CA6.HC $^{5'}$-GTTTTCCCAGTCACGACGCATTTGCAGTATAGAGCGT-$^{3'}$

CA6.HR $^{5'}$-AGGAAACAGCTATGACCATAGCTGTACTCCTAGAATTA-$^{3'}$

Third, since mononucleotide runs in certain introns caused poor dye-primer sequencing, we obtained dye-terminator sequence data on secondary amplicons exon8 FB-RQ and exon 9 FB-RR using the nested primers

$^{5'}$-TTTTTTTTTAGGACAAAATGTTTC-$^{3'}$

$^{5'}$-AAT TCA GAC TTT TGT AAT TTG TG-$^{3'}$

We obtained more than 95% double stranded coverage of the *MMAC1* coding sequence for all genomic DNAs screened; all mutations were confirmed by sequencing a newly amplified product.

Results

Linkage Analysis and Mutation Screening In CS Kindreds

In order to extend the observations indicating a CS locus on chromosome 10, we performed a two point linkage analysis using five markers located in the Cowden critical interval, on four families with clinical evidence of CS (Nelen et al. 1996). All families were examined in detail and the diagnosis of this syndrome was made using the International Cowden's Consortium CD diagnostic criteria

(Nelen et al 1996). Two small families displayed positive LOD scores that could not exclude linkage to three loci on chromosome 10 (see family A and B, Table I). Two other families with clinical findings identical to those described above, showed significant negative lod-scores for some of the markers in this region (families C and D, Table I). A heterogeneity test was also performed which gave non-significant results (data not shown). These findings were confirmed by the haplotypes construction (Figure 1). In particular, in family C, individual 2 transmits to both her affected children the haplotype inherited from her unaffected father. Finally, in family D, individuals 2 and 20 have inherited a haplotype different from one of their affected relatives.

Using a PCR and sequencing based approach, we examined the 9 exons and associated splice junctions of *MMAC1*, using the described primers (Steck et al. 1997), in 16 affected individuals from these 4 families. Of interest, 4 of these 16 individuals had breast cancer, and 2 of the 4 had breast cancer prior to the age of 40. We failed to detect mutations in the coding sequence in these 16 individuals from these 4 families with the classic symptoms and signs of CS.

Mutational Analysis in Individuals with CS

We then screened a set of 31 affected individuals from 23 families with CS whose kindreds had not been used in our linkage studies. Of the 31 individuals, 13 were related individuals from 5 families. Thus, a total of 23 unrelated probands were screened. A single affected female (Walton et al. 1986) demonstrated a frameshift mutation in exon 7 of the coding sequence (see Figure 2). Specifically, we demonstrated an AT insertion after nucleotide 791 (791insAT), thus resulting in a frameshift and downstream premature termination codon. Of interest, this woman developed mammogram negative breast cancer at the age of

36, which was discovered at the time of prophylactic mastectomy (Walton et al. 1986). The proband had an unaffected brother, as well as an affected daughter. Direct sequencing of exon 7 in these individuals demonstrated the presence of the identical mutation in the affected daughter (Figure 2) and the absence of the mutation in the unaffected brother. In studying a second individual with CS and early onset breast cancer (age 33), we demonstrated a three base insertion in exon 2 (137ins3), resulting in the insertion of a single amino acid (Asn). Finally, in another woman with bilateral breast cancer (Schrager et al. 1997a) and endometrial cancer, we identified a 13 base pair frame shift deletion in exon 8 (915del12). These data demonstrate 3 more mutant alleles of *MMAC1* that are associated with CS (Liaw et al. 1997), and in particular, with CS and breast cancer (Brownstein et al. 1978; Schrager et al. 1997a). However, in 27 individuals from 20 families, we did not detect mutations in the coding sequences of *MMAC1*. In this population, 7 of these individuals had breast cancer, although all of these women developed breast cancer after the age of 40. One of these 7 individuals had bilateral breast cancer. In total, therefore, combining the family data, as well as these individuals, we detected coding sequence mutations in 4 individuals from 3 CS families, but did not detect coding sequence alteratons (ie:missense or silent variants) in 43 other individuals from 24 families with CS.

Mutational Analysis in Women with Early Onset Breast Cancer

A strong case has been made for the existence of a genetic mechanism regulating breast tumor formation in early onset breast cancer (the development of breast cancer before the age of 40) (Claus et al. 1990). As CS is inherited in an autosomal dominant fashion, the genetic mechanisms regulating the development of breast cancer in this population may also play a role in the development of early onset breast cancer. Since we detected germline *MMAC1* mutations in CS

associated with early onset breast cancer, and mutations in this gene occur at relatively high frequency in breast tumors and breast tumor cell lines (Steck et al. 1997, Li et al. 1997), we wanted to further investigate the role of germline *MMAC1* mutations in early onset breast cancer. In an effort to bias ourselves towards a sample set potentially enriched in germline *MMAC1* mutations, we sequenced the gene in 63 women who developed breast cancer before age 35 (average age at diagnosis 27.7 years), did not appear to have a clinical diagnosis of CS, and who had previously been shown not to carry clearly deleterious mutations in *BRCA1* (5 women in the sample carried missense polymorphisms of unknown significance). No coding sequence alterations were detected in the 9 exons of *MMAC1* in this sample set. In contrast, using the exact same mutation detection and analysis criteria on a similarly ascertained set of non-Ashkenazi breast cancer affecteds (without exclusion of *BRCA1* carriers), we would expect to detect 7 deleterious mutations and 5 missense polymorphisms of unknown significance in *BRCA1* (Shattuck-Eidens et al. 1997). Furthermore, outside of the 4 CS patients carrying germline mutations in *MMAC1* described above, we have detected no sequence polymorphisms in the coding sequence of this gene in more than 200 germline chromosomes, and in fact find only one sequence difference (silent) between the human and chimpanzee sequences. If the frequency of coding and proximal splice junction sequence variants in *MMAC1* were 5% in the population from which this sample was drawn, then we would have had a 95% chance of detecting one or more such variant.

Discussion

Cowden syndrome is distinct among autosomal dominant genetic syndromes that predispose to the development of breast cancer as it has a unique cutaneous biomarker, the trichilemmoma (Brownstein et al. 1997, 1978). Furthermore,

women with CS frequently give a history of multiple breast biopsies for benign breast disease prior to the development of breast cancer (Schrager et al. 1997a, 1997b). Most of these women did not have a family history of breast cancer. To date, the most well described association of CS with organ specific cancer susceptibility is the female breast (Brownstein et al. 1977, Schrager et al. 1997a, 1997b). Other organ systems that appear to develop cancer with increased frequency in these individuals such as the thyroid. In contrast to other autosomal breast cancer susceptibility syndromes, such as the one associated with mutations in *BRCA1* (Ford et al. 1995), the development of ovarian cancer in this syndrome is quite rare. However, CS shares with these syndromes an earlier age of onset of breast cancer, as well as an increased likelihood of bilateral breast cancer (Schrager et al. 1997a, 1997b).

Previous observations demonstrated linkage of CS to chromosome 10q22-23 (Nelen et al. 1996). Furthermore, it is also now evident that mutations in a gene (Liaw et al 1997) known as *PTEN* (Li et al. 1997), *MMAC1* (Steck et al. 1997) or *TEP1* (Li and Sun 1997) found in the Cowden's critical interval on chromosome 10, are associated with CS individuals (Liaw et al 1997). In the observations reported here, we identify 3 new germline mutations in the coding sequence of *MMAC1* associated with CS, and specifically in individuals with CS and breast cancer. In two related individuals with CS, we described a frameshift mutation in exon 7, resulting in a premature termination codon, that is identical in an affected mother and her affected daughter. This *MMAC1* mutation appears to be associated with early onset breast cancer, as one of the two affected individuals developed breast cancer at age 36. In a third affected individual, we identified a 13 base pair deletion in exon 8. While this individual did not develop breast cancer at an early age, she had a history of bilateral breast cancer. Of interest, she also developed endometrial cancer while on tamoxifen. Given that endometrial cancer

has been associated with CS (Starink et al. 1986) and with tamoxifen use (Fornander et al. 1989), the contribution of both risk factors to the development of disease in this one women is unknown. However, this raises the possibility that the subpopulation of women who develop endometrial cancer while on tamoxifen may have CS and/or mutations in *MMAC1*. Finally, we identified a 3 base insertion in exon 2 in a another woman who developed breast cancer at the age of 33.

In the set of CS individuals that we studied, we detected germline *MMAC1* mutations 4 individuals from 3 families, but did not observe any coding sequence alterations in the remaining 43 individuals from 24 unrelated families. These data supported our limited linkage information, suggesting that all CS families may not link to the locus identified on chromosome 10. While the experiments we performed do not rule out mutations in the 5' regulatory regions or in the 3' untranslated region of *MMAC1*, or other mechanisms that alter its expression level, such as methylation silencing, as being associated with CS, both the linkage data and the DNA sequencing results support the idea that the CS may be genetically heterogeneous. Tuberous sclerosis, another autosomal dominant disorder associated with the formation of hamartomas in the skin and other organs, has been shown to be genetically heterogeneous with distinct loci located at chromosome 9q34 (Haines et al. 1991) and chromosome 16p13.3 (Kandt et al. 1992). Our results suggest that this may also be true for CS. Why this was not demonstrated in the initial observations is not clear, but could be due to the ethnic backgrounds of the initial families examined (Nelen et al. 1996; Liaw et al. 1997). Moreover, certain of these individuals presented with CS and Lhermette-Duclos disease, which we have never seen in a CS proband or in a CS family (Nelen et al. 1996; Liaw et al. 1997). Alternatively, we may have ascertained cases in an inappropriate manner.

A strong case has been made for the existence of a genetic mechanism regulating breast tumor formation in early onset breast cancer (Claus et al. 1990). Indeed, early onset breast cancer has been associated with mutations in the *BRCA1* (Miki et al. 1994) and *BRCA2* (Wooster et al. 1995). CS is associated with early onset breast cancer, and the cancer is usually ductal carcinoma (Brownstein et al. 1977, Brownstein et al. 1978). Rachel Cowden, for whom the syndrome is named, apparently died of breast cancer at age 31 (Lloyd and Dennis 1963; Brownstein et al. 1978). As described herein, we have identified *MMAC1* mutations in 2 CS individuals with early onset breast cancer, as well as in 1 with bilateral breast cancer. However, when we searched for germline *MMAC1* mutations in a subgroup of women with early onset breast cancer, lacking the signs of CS and previously shown to have wildtype sequences of *BRCA1*, we failed to detect any sequence variants. These data suggest that germline mutations in *MMAC1* occur infrequently in at least this subpopulation of early onset breast cancer cases.

In summary, we extend the observation that *MMAC1* mutations are associated with CS (Liaw et al. 1997), and demonstrate that *MMAC1* mutations appear to be associated with CS and breast cancer. However, we also show that certain families and individuals with CS do not have mutations in the coding sequence of *MMAC1*. Finally, we failed to detect *MMAC1* mutations in a subpopulation of early onset breast cancer, suggesting that germline mutations in this gene do not appear to be common in at least a subpopulation of breast cancer cases who also do not demonstrate mutations in *BRCA1*.

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Table 1

Twopoint Analysis of CD Families with CA Repeat Markers

	0.0	0.01	0.05	0.1	0.2	0.3	0.4
FAMILY A							
D10S579	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D10S215	0.30	0.30	0.28	0.26	0.20	0.15	0.08
D10S541	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D10S1739	0.30	0.30	0.28	0.26	0.20	0.15	0.08
D10S564	0.30	0.30	0.28	0.26	0.20	0.15	0.08
FAMILY B							
D10S579	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D10S215	0.30	0.29	0.26	0.21	0.13	0.06	0.02
D10S541	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D10S1739	0.30	0.29	0.26	0.21	0.13	0.06	0.02
D10S564	0.30	0.29	0.26	0.21	0.13	0.06	0.02
FAMILY C							
D10S579	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D10S215	-infinity	-3.40	-2.00	-1.40	-0.80	-0.44	-0.19
D10S541	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D10S1739	-0.05	-0.06	-0.09	-0.13	-0.16	-0.15	-0.09
D10S564	-infinity	-3.40	-2.00	-1.40	-0.80	-0.44	-0.19
FAMILY D							
D10S579	-infinity	-1.52	-0.28	0.11	0.28	0.19	0.05
D10S215	-infinity	-1.58	-0.33	0.07	0.25	0.18	0.05
D10S541	-infinity	-1.44	-0.39	0.01	0.22	0.18	0.06
D10S1739	-2.20	-0.45	0.14	0.32	0.35	0.23	0.08
D10S564	-0.03	0.08	0.30	0.38	0.35	0.22	0.07

Table II

	Mutation	Exon/Intron	Predicted Effect
1.	791insAT	Exon 7	Frameshift
2.	915del13	Exon 8	Frameshift
3.	137ins3	Exon 2	One amino acid insertion (Asn)

Figure Legends

Figure 1 Haplotype construction with markers on chromosome 10 in four families with CS.

Figure 2 DNA Sequencing of *MMAC1* in a family with CS and early onset breast cancer. The affected mother (black circle) demonstrates a 2 base pair insertion (AT) in exon 5, which is not seen in her unaffected brother (open square). Her affected daughter has inherited the AT insertion.