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Chromosomes 4 and 8 Implicated in a Genome Wide SNP Linkage Scan of 762 Prostate Cancer Families Collected by the ICPCG

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Abstract

Background—In spite of intensive efforts, understanding of the genetic aspects of familial prostate cancer remains largely incomplete. In a previous microsatellite-based linkage scan of 1233 prostate cancer (PC) families, we identified suggestive evidence for linkage (i.e. LOD≥1.86) at 5q12, 15q11, 17q21, 22q12, and two loci on 8p, with additional regions implicated in subsets of families defined by age at diagnosis, disease aggressiveness, or number of affected members.

Methods—In an attempt to replicate these findings and increase linkage resolution, we used the Illumina 6000 SNP linkage panel to perform a genome-wide linkage scan of an independent set of 762 multiplex PC families, collected by 11 ICPCG groups.

Results—Of the regions identified previously, modest evidence of replication was observed only on the short arm of chromosome 8, where HLOD scores of 1.63 and 3.60 were observed in the complete set of families and families with young average age at diagnosis, respectively. The most significant linkage signals found in the complete set of families were observed across a broad, 37 cM interval on 4q13-25, with LOD scores ranging from 2.02 to 2.62, increasing to 4.50 in families with older average age at diagnosis. In families with multiple cases presenting with more aggressive disease, LOD scores over 3.0 were observed at 8q24 in the vicinity of previously identified common PC risk variants, as well as *MYC*, an important gene in PC biology.

Conclusions—These results will be useful in prioritizing future susceptibility gene discovery efforts in this common cancer.

Introduction

The recent discoveries of multiple SNPs across the genome as common, reproducible genetic risk factors for prostate cancer (PC) have been impressive. Over 30 common sequence variants have now been confirmed to be associated with PC risk, emphasizing the polygenic nature of inherited susceptibility for this disease (1). In spite of the substantial progress in this area, current estimates suggest that the identified loci do not explain the majority of the excess risk associated with PC family history (1), one of the most reproducible risk factors for PC (2,3).

Attempts to map PC susceptibility genes by linkage analysis of individual family collections have yielded few reproducible leads despite numerous genome-wide scans, most likely due to genetic and disease heterogeneity (4–6). In an effort to address this question more effectively, we previously carried out a large linkage study that included 1233 PC families collected by members of the International Consortium for Prostate Cancer Genetics (ICPCG) (7). This study provided strong evidence that one or a few major genes cannot account for the majority of disease in PC families. At the same time, a number of loci demonstrated suggestive linkage signals, consistent with a complex genetic etiology for this disease. To extend these studies using a higher resolution marker set, and to assess which of

these linkage signals might warrant additional investigation, in this report we describe a second combined linkage analysis with 6000 SNPs to interrogate an independent set of 762 families collected by the ICPCG.

RESULTS

Study Population - 762 Prostate Cancer Families

Table 1 summarizes the characteristics of the 762 PC families from the 11 ICPCG groups participating in this analysis. Fifty-three percent of families had a mean age at diagnosis of <65 years, and 21% had four or more affected family members. Most of the families (65%) were collected in Europe or Australia, with the remainder collected in the U.S. The current analysis was restricted to Caucasian families; analysis of linkage results from African American pedigrees collected by members of the ICPCG will be described in a separate report.

SNP Scan Linkage Results

Shown in Figure 1 and Table 2 are the linkage results for the entire set of 762 families using dominant (dom), recessive (rec), and nonparametric (KCLOD or asm) linkage models. The strongest evidence of linkage in the complete set of families is located in a broad region with multiple peaks on the proximal and mid-q arm of chromosome 4. A maximum HLOD=2.62 was observed under a recessive model at 4q22 at 97 cM, along with several other peaks over 1.86 between 74 cM and 115 cM, 4q13-25. An examination of LOD scores by individual family collection indicates that six of the seven largest family collections had scores over 0.9 in the 12 cM interval between 83 cM and 102 cM on this chromosome, using either a recessive or asm model (Table 3).

One other region, 18q11, reached the threshold for suggestive evidence of linkage: rec HLOD=1.93 at 43cM. Including results from all three linkage models, LOD scores over 1.5 were observed at 8p11 (59 cM), 8q24 (142 cM), 11p15 (7 cM), 12q23 (114 cM), 16q21 (81 cM), and multiple positions on both arms of chromosome 2 (2p16, 77 cM; 2p11, 108 cM; 2q14, 131 cM; and 2q35, 216 cM) (Table 2).

Linkage Signals in Subsets of Families

To explore variables that might impact the linkage results, we analyzed subsets of families characterized by young or old average age at diagnosis (≤65, vs 65 or older), five or more affected individuals, or multiple members affected with more aggressive disease. For the 405 families with young age of diagnosis, the most significant evidence for linkage was observed on 8p11 at 59 cM (dom HLOD=3.60 vs 1.63 at this same position in all families). Two other regions showing suggestive linkage in this family subset were observed, at 3p24 (asm LOD=2.05 at 35 cM), and 1q44 (asm LOD =1.95 at 269 cM) (Figure 2, Table 4).

In families with an average age at diagnosis of 65 or greater (n=357), LOD scores over 1.86 are seen in a broad region spanning the centromere of chromosome 4 (51cM-122cM), which overlaps with the strongest region of linkage observed in the complete set of families. The peak for this subset analysis was at 95cM, 4q22, with a rec HLOD =4.50. Other positions with LOD scores over 1.86 in this subset of families were observed on chromosomes 12, 13 and 18 (dom HLOD = 3.14, rec HLOD = 2.23 and 1.91 at 113 cM, 15 cM and 43 cM, respectively) (Figure 3, Table 4).

The strongest linkage signals in families with more aggressive disease were seen on chromosome 8 where LOD scores reached over 3.0 across a 14 cM interval at 8q24 (126–140 cM, high score 3.17 at 132 cM, dom model). Four other regions were of interest in this

group: 1q43 (dom HLOD = 2.11 at 255 cM), 2q35 (asm LOD = 2.24 at 218 cM), and 4q32 (dom HLOD = 1.94 at 149 cM), 12q24 (dom HLOD = 2.23 at 146 cM) (Figure 4, Table 4).

In families with 5 or more affected individuals, suggestive evidence of linkage was observed at three regions: 13q34 (asm LOD = 2.46, 128 cM), 15q14 (40 cM, dom HLOD = 2.0), and 16p12.1 (rec HLOD = 2.0, 48 cM) (Figure 5, Table 4). On chromosome 21, an HLOD of 1.78 was observed at 45cM, in the vicinity of the *ERG* and *TMPRSS2* genes, in this family subset (Figure 5c).

Comparison of Two Linkage Scans in ICPCG Families

To search for reproducible linkage signals, we compared the results of this SNP linkage scan (designated here SNP scan) with our previous scan of 1,233 families using microsatellite markers (MS scan) (7). Of the six regions of suggestive linkage found in the MS scan, none were supported by LOD scores reaching the threshold for suggestive linkage, i.e. ≥1.86, in the SNP scan. However, more modest evidence of replication was observed on the proximal short arm of chromosome 8. In the SNP scan, an HLOD of 1.63 was observed at 8p11 (59 cM) under a dominant model. In the MS scan, two signals were observed on 8p, one at 60 cM (1.94) and one at 46 cM (1.97), under recessive and dominant models respectively. For the remaining four regions of suggestive linkage found in our first scan, at 5q12, 15q11, 17q21 and 22q12, little or no evidence for linkage was seen in the SNP scan (LOD scores <0.4).

Similarly, for all regions reaching LOD scores of 1.5 or greater in the complete set of families analyzed in the SNP scan, including the multiple loci on chromosomes 2 and 4, 8p11, 11p15, 12q23, 16q21, and 18q11, the highest score observed in the MS scan was 0.53 at 133 cM on chromosome 2.

Comparison of Two Linkage Scans in Subsets of Families

In families with an young age at diagnosis, dom HLOD scores ≥ 1.86 were observed on 3p in both scans, although the peak locations differed by over 20cM (35 cM in SNP scan and 57 cM in MS scan). When comparing regions of linkage in the scans of families with five or more affected members, peaks over 1.86 were observed within 15cM of each other on 16p12 (at 34cM in MS scan and 49cM in SNP scan) (asm LOD 2.04 and 2.14 respectively).

Of the three regions reaching suggestive linkage (6p22, 11q14, and 20q11) in our previous MS scan of families with aggressive disease, one region, 11q14 provided some evidence of an overlapping signal in the SNP scan with a rec HLOD score of 0.8 at 100 cM. Also in this group of families, coinciding linkage signals occurred at 8q24, where dom HLOD scores of 1.17 and 3.05 were observed in the same positions (137 cM) in the MS and SNP scans, respectively.

Discussion

In this report, we describe a genome-wide linkage study of 762 families collected by members of the ICPCG. This is the second largest collection of PC families analyzed to date to assess linkage across the genome. A primary rationale behind this study was to determine whether linkage signals observed in an earlier microsatellite linkage scan of 1,233 families could be replicated, as a means to identify loci warranting further study.

Of the six regions of suggestive linkage observed in the previous MS scan of 1,233 PC families (7), one region, 8p11, attained a LOD score over 1.5 in the present SNP scan. In addition, overlapping linkage signals in the two scans provided some evidence of replication in defined subsets of families analyzed. Both families with young age at diagnosis and

families with five or more affected individuals had moderate linkage signals at 3p24 and 16p12, respectively, in both scans. In addition, the subset of families with clinically aggressive disease showed linkage to 8q24 in both scans. Thus, while overall replication of previous linkage peaks was quite limited, several loci, particularly on chromosome 8, showed consistent linkage signals in two large, jndependent collections of prostate cancer families.

Chromosome 8 has long been suggested to harbor both prostate tumor suppressor gene(s) and oncogene(s) due to the frequent copy number alterations (deletions of 8p and gains of 8q, respectively) occurring somatically in specimens of prostate tumor tissue (8–10, reviewed in 11, 12). At the germline level, linkage at 8p has been observed in PC family collections from Japan, Sweden, Germany and the US (13–17), although in the majority of these studies the signals observed were more telomeric than the one observed here. In addition, a large case-control study conducted by PRACTICAL found two SNPs at 8p21, near *NKX3.1*, to be associated with PC risk (18).

The 8q24 locus been extensively analyzed by GWAS, with five or more regions reproducibly shown to be associated with PC risk (19–21). Historically, the region was first identified through a fine-mapping study of a linkage peak observed in a genome-wide scan of Icelandic PC families (22). Linkage to this region was also reported by Camp et al (23) in extended PC families from Utah. It will be of interest to determine whether any of the susceptibility loci identified in the original study and the association studies since, contribute to the linkage signals observed here. A gene of particular interest for PC, *MYC*, lies in the region of linkage observed in this study in families with more aggressive disease. Previous studies have demonstrated the common up-regulation of this gene early in human prostate carcinogenesis (24, 25), amplification of the gene in advanced PC (26, 27), and the ability of prostate specific expression of this gene to induce PC in animal models (28, 29). Such studies, together with recent work demonstrating interactions between risk loci and *MYC* regulatory elements have led to the hypothesis that the 8q24 risk alleles that have been identified to date modify PCa risk mechanistically by altering *MYC* regulation and expression (30–34).

In the complete family collection, the strongest linkage signals in this study were observed on the proximal and mid q arm of chromosome 4. One important aspect of this signal is the contributions provided by the multiple different family collections. Interestingly, six of the seven largest family collections (ranging from 35 to 174 families) had LOD scores over 0.9 in the 12 cM interval between 93 cM and 105 cM on this chromosome, whereas the four smaller collections ($n < 25$ families) contributed little evidence to this signal. Curiously, the six positive family collections include all five groups originating from Europe/Australia, suggesting a possible geographical association to the chromosome 4 linkage, although limited sample size of the US family sets, or chance occurrence are also possible explanations for this observation. It is of interest that recent GWAS findings have led to the identification and confirmation of several SNPs on 4q22 and 4q24, in introns of *PDLIM5*, and upstream of *TET2*, respectively, as being associated with PC risk (18). Whether or not common risk alleles at these or nearby loci play a role in the linkage signal observed in the larger family collections studied here is a question for further investigation.

Stanford et al (35) recently reported a SNP based linkage scan in which several linkage signals were reported that coincide with results from this study. Specifically, coincident peaks were observed at 15q13-14 and 2q14-21 in this study and the one reported here, although the signals were observed in different subsets of PC families. Evidence of linkage to the long arm of chromosome 8 (8q22) was observed in the complete set of 289 Caucasian families.

One of the aims of this study was to replicate findings from our earlier MS scan (7); however few loci were observed in both studies. While this is disappointing, it is not surprising given the known genetic heterogeneity of PC. Indeed, a limitation of our study is the potentially heterogeneous genetic and environmental influences arising from a collection of families from multiple locations across the U.S., Europe, and Australia. Over half of the families (65%) studied in this scan were collected in Europe or Australia, while the majority of families (79%) studied in our previous MS scan were collected in the U.S. Differences in intensity of PC screening in Europe versus the U.S. may lead to substantial differences in the distribution of disease stage at diagnosis (e.g., lower stage due to widespread PSA testing in the U.S.). While we have attempted to address some of these differences by examining specific subsets of PC families stratified by age at diagnosis and clinical and pathologic variables of the disease, this may not be sufficient to account for the heterogeneity that may be introduced by the differences in clinical practices between the continents.

It should be noted that with respect to comparability with our previous MS scan, while in general the family characteristics were quite similar, the families in this scan had on average fewer members affected with PC (~2.3 per family compared to~3.5 in the MS scan). This fact could have implications with respect to the linkage evidence on 8q24. Smaller numbers of affected individuals within families could reflect a greater presence of sporadic disease. While little is known about the role of 8q24 susceptibility variants in familial PC, there is unequivocal evidence that these risk alleles are associated with sporadic PC even though the relative risks associated with these risk alleles are small to modest. It is interesting to note that the Icelandic families in which the 8q24 locus was originally identified through linkage analysis were of similar average size to the families in this study (22).

The strengths of this study are its large size and increased genetic information and resolution due to the use of dense SNP panels for genotyping. While the wide area of family ascertainment may generate heterogeneity, the large number of families afforded by this approach increases power and possibly results in the identification of more robust genetic signals. Finally, the large number of families increases our ability to examine potentially more homogeneous subsets of families while still maintaining reasonable levels of power.

In summary, in an examination of results from a high resolution SNP scan of 762 families and a previous MS scan of 1233 independent PC families, no locus emerges as an unequivocally strong candidate. However, our results suggest that a broad region on proximal 4q, and multiple regions on chromosome 8 are possible candidate regions harboring PC susceptibility loci. In light of evidence from this and previous studies, further analysis of these regions appears warranted.

Methods

Ascertainment of Families

The ICPCG study populations have been previously described in detail (36). Each group within the ICPCG recruited PC families and eleven ICPCG groups contributed to this combined genome-wide screen: ACTANE (Anglo/Canadian/Texan/Australian/Norwegian/ European Union Biomed), Centre de Recherche sur les Pathologies Prostatiques, (CeRePP) in France, Johns Hopkins University (JHU), Mayo Clinic, University of Michigan, Northwestern University, *PROGRESS* (Prostate Cancer Genetic Research Study, Fred Hutchinson Cancer Research Center), University of Tampere in Finland, University of Ulm in Germany, Karolinska Insititute in Sweden, and University of Utah. There were 762 PC pedigrees in this combined analysis. The research protocols and informed consent procedures were approved by each group's institutional review board.

Definition of Affection Status and Classification of Pedigrees

Affected individuals were defined as men diagnosed with PC that had been confirmed by either medical records or death certificates. Self- or relative-reported affected men without either medical records or death certificate confirmation were considered as having unknown affection status. All men without a diagnosis of PC were coded as having unknown affection status, regardless of whether they had undergone screening for PC. Hence, all analyses were based on the sharing of marker genotypes among affected individuals, with no consideration of the phenotype for the remaining subjects. Family members not considered affected nonetheless contributed genotype information, when available, to increase the linkage information content among the affected men. Although such an approach may result in some loss of power, it provided a uniform approach across all participating groups, particularly important because screening of unaffected men varied across groups.

For subset analyses, pedigrees were stratified according to the following criteria: 1) average age at diagnosis within families, contrasting <65 years to 65+ years; 2) families with aggressive disease based on criteria previously described (37). Briefly, families meeting these criteria had three or more affected individuals with PC with at least one of the following clinicopathologic characteristics: Gleason score 7 or higher, TNM stage of T3 or T4, pretreatment serum PSA ≥20 ng/mL, or death from PC before age 65. In these families, other cases not meeting any of the criteria for aggressive disease were classified as having unknown disease status; 3) families having 5 or more affected individuals..

Genotyping

Genome-wide SNP linkage scan genotyping was performed at the Center for Inherited Disease Research using Illumina's HumanLinkage-12 Genotyping BeadChip (http://www.cidr.jhmi.edu/human_snp.html). These chips assay 6,090 SNP markers, with an average intermarker distance of 0.58 cM across the genome and an average marker heterozygosity of 0.43 in Caucasians.

Statistical Analysis: Linkage-Analysis Methods

The computer programs Pedcheck (http://watson.hgen.pitt.edu/register/docs/pedcheck.html) and PREST (http://galton.uchicago.edu/~mcpeek/software/prest/) were used for checking whether the genotypes of individuals within a pedigree are consistent with their specified relationships. Based on these analyses, 58 individuals were removed from further analysis.

Both parametric and non-parametric linkage analyses were performed using Merlin software (38). The parametric LOD scores were computed using either a dominant or a recessive model, as described in elsewhere (5). LOD scores allowing for linkage heterogeneity among families (HLOD) were estimated using HOMOG (39). Non-parametric LOD scores were calculated using the Kong and Cox exponential allele sharing model score (herein referred to as asm) (40). Marker allele frequencies for each SNP were estimated by counting alleles across all genotyped subjects, ignoring genetic relationships. Multipoint linkage statistics were calculated at 0.5 cM intervals across the genome.

We used the r-square option (≥ 0.1) of Merlin to remove SNPs that were in linkage disequilibrium (LD). This is necessary to reduce the positive bias of strong marker LD among flanking SNPs on linkage results, and to reduce the memory and time requirements for large pedigrees. To further fit pedigree data into the memory limits of Merlin software, trimming of family members was conducted. Un-genotyped subjects or subjects with missing phenotypes were trimmed. Trimming was performed on each pedigree to obtain a maximum bit size of 24.

To facilitate comparison of the results of this SNP scan with our previous scan using microsatellite (MS) markers, we aligned the results of these two linkage scans based on physical map positions (Build 35) of both the microsatellite markers and SNP markers.

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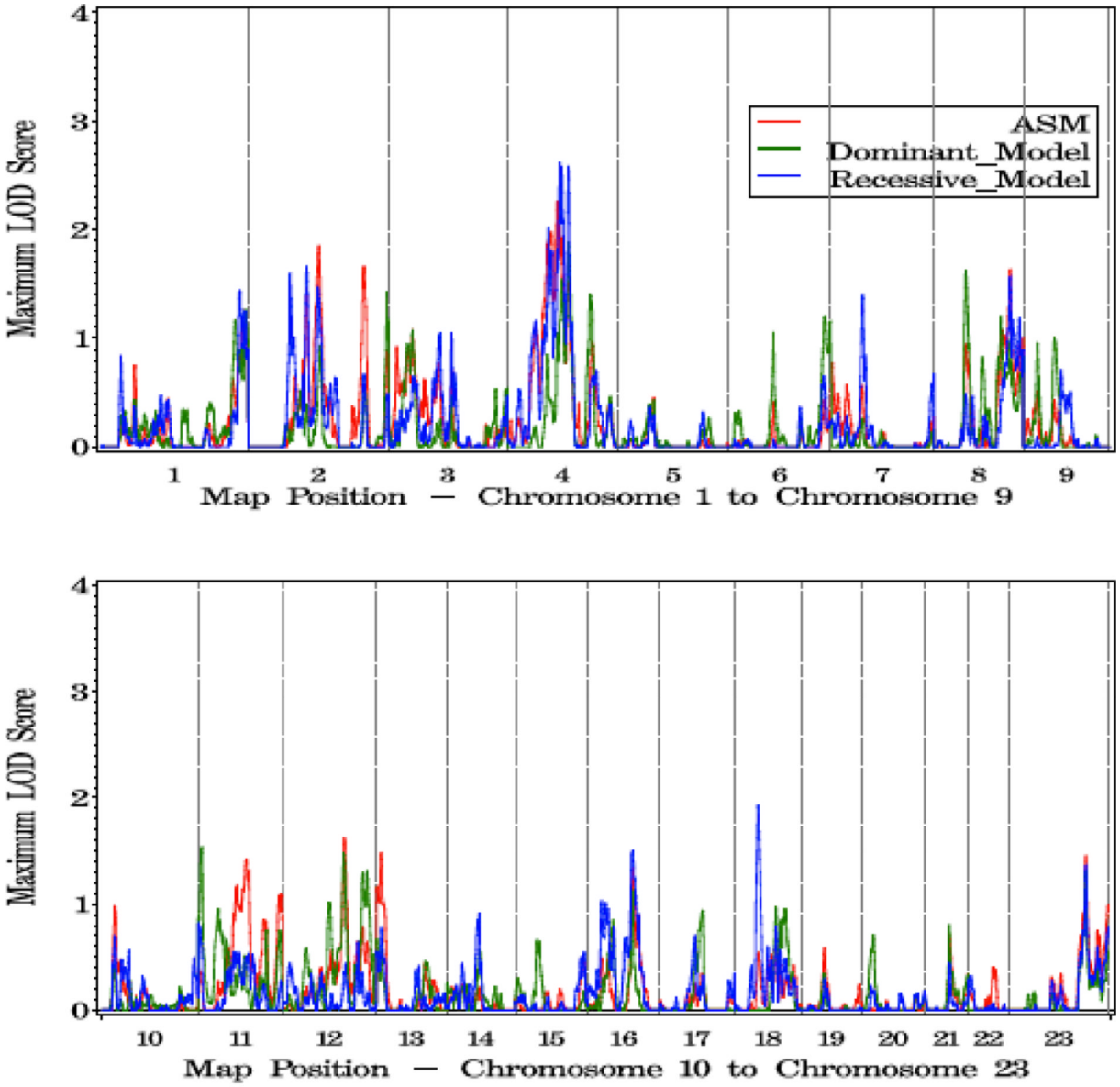
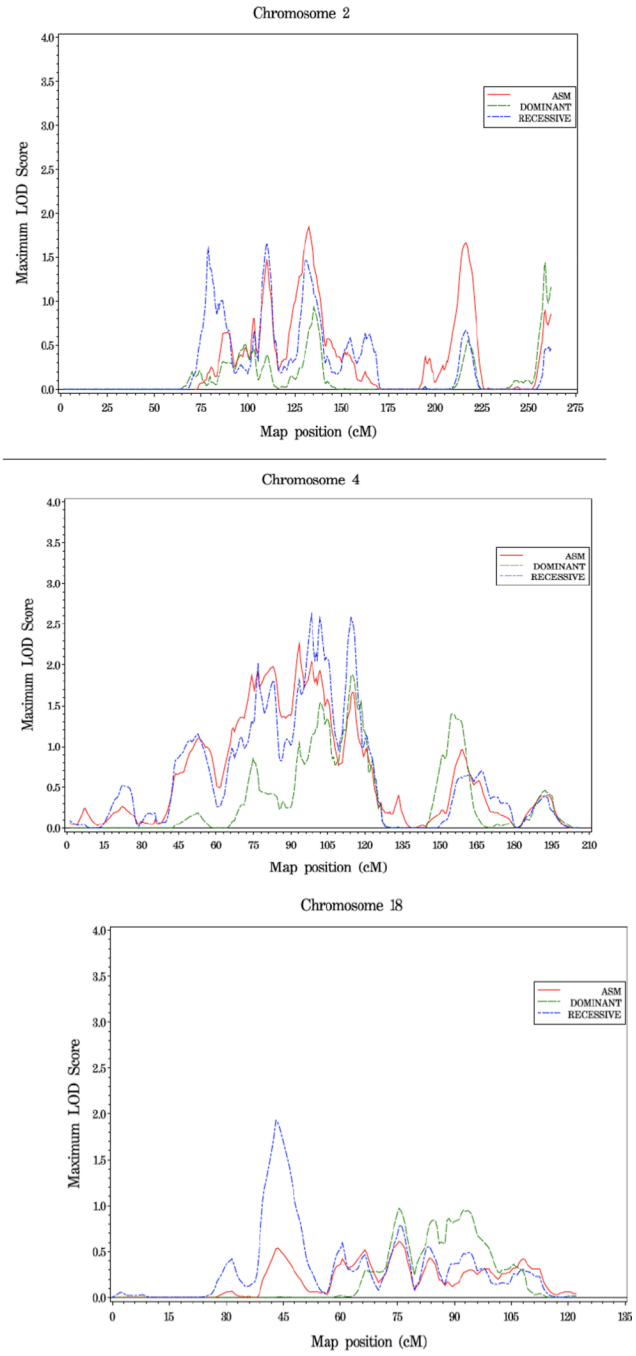
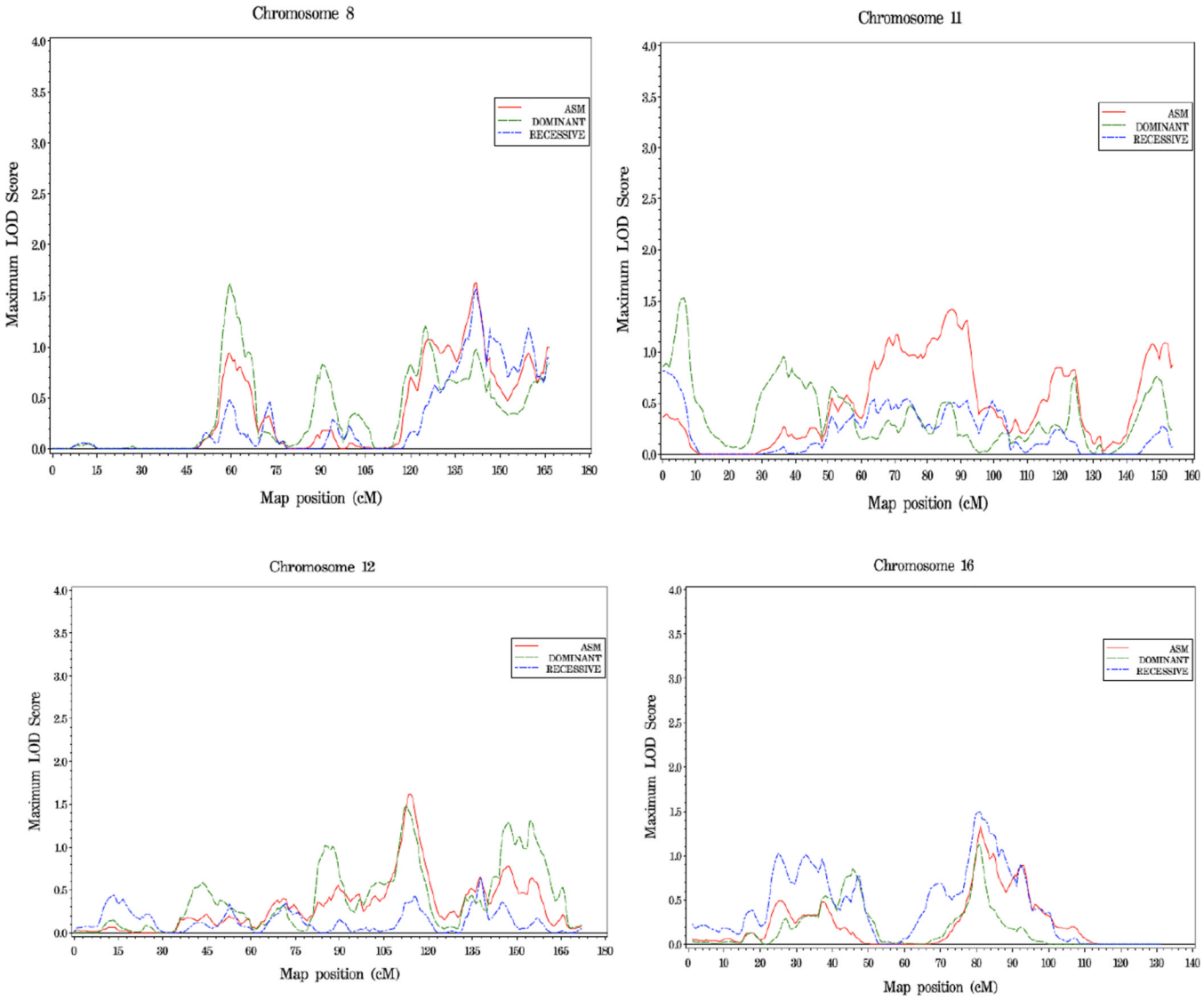


1a

1b





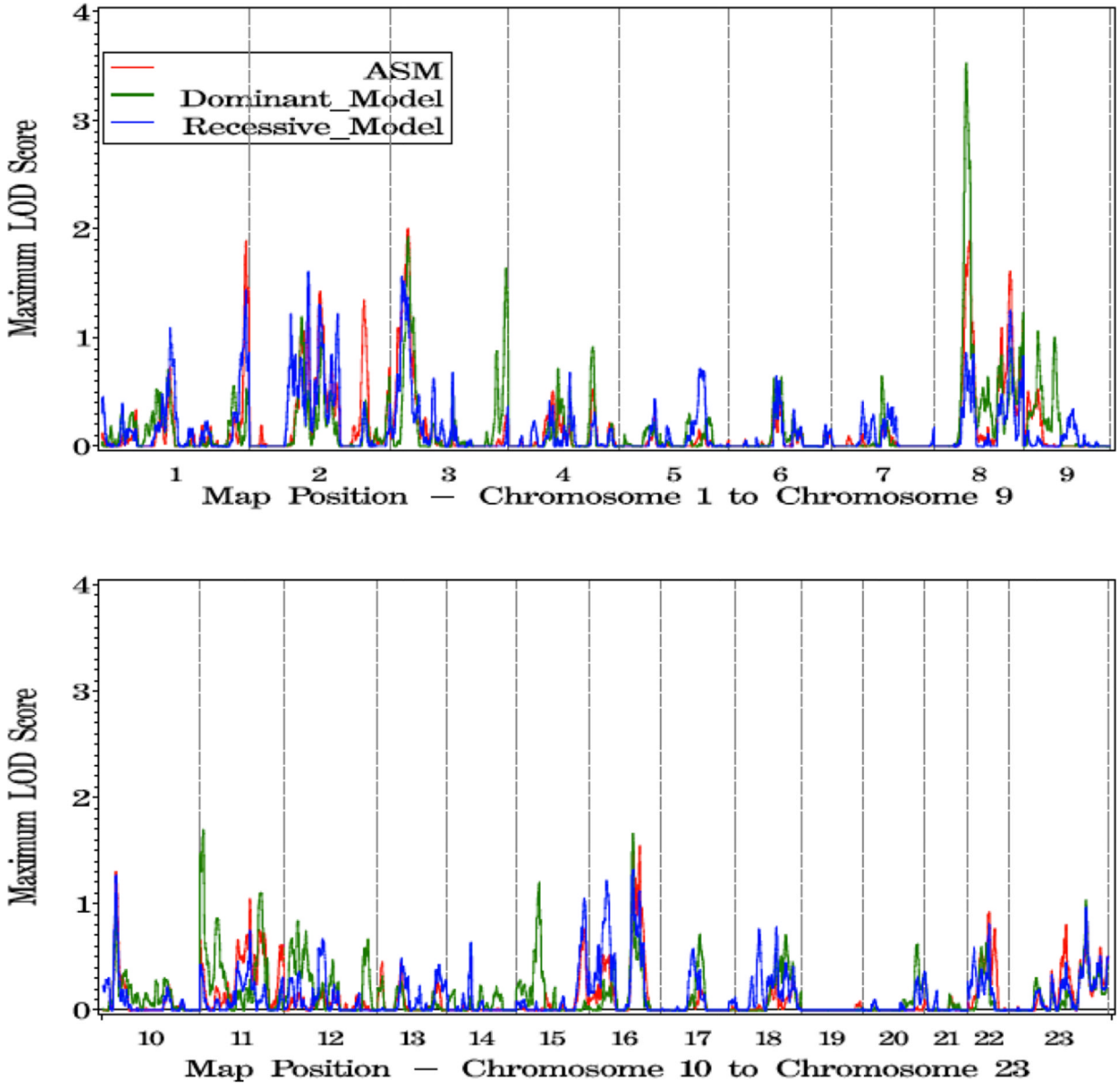
1c

Figure 1.

a. Plot of LOD scores for all families by chromosome.

b. Chromosomes with LOD scores > 1.86 in all families.

c. Chromosomes with LOD Scores > 1.5 in all families.



2a

2b

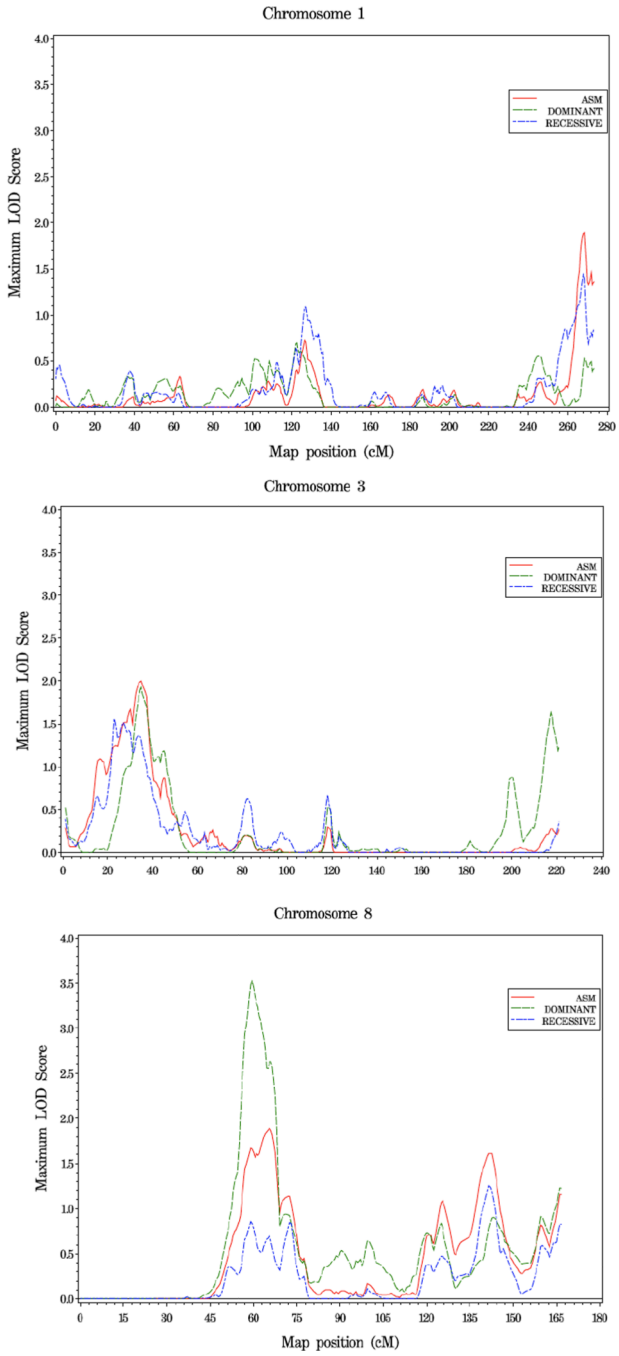
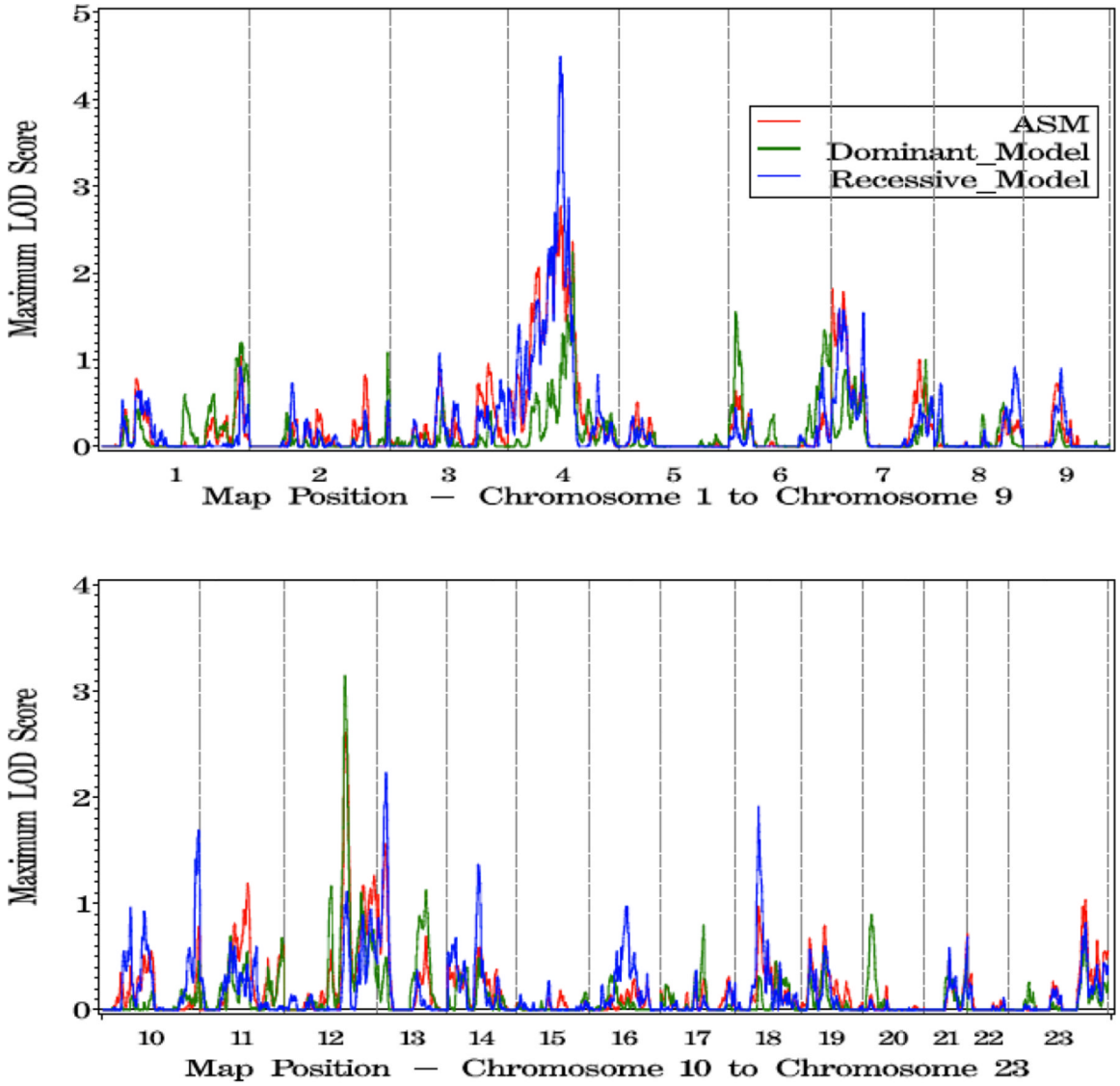


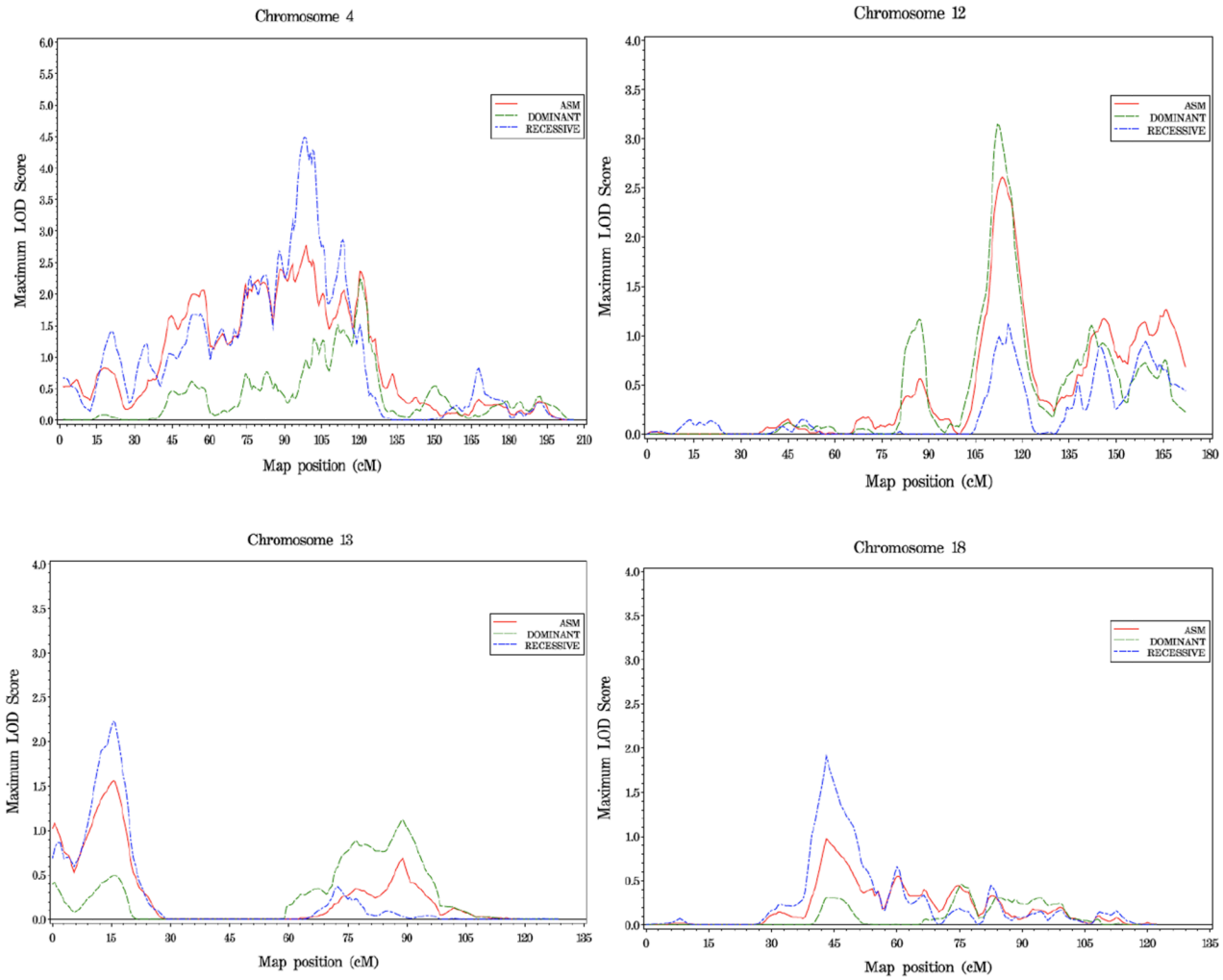
Figure 2.

a. Plot of LOD Scores for families with average age at diagnosis under 65.

b. Chromosomes with LOD scores ≥ 1.86 in families with young age at diagnosis (<65).



3a

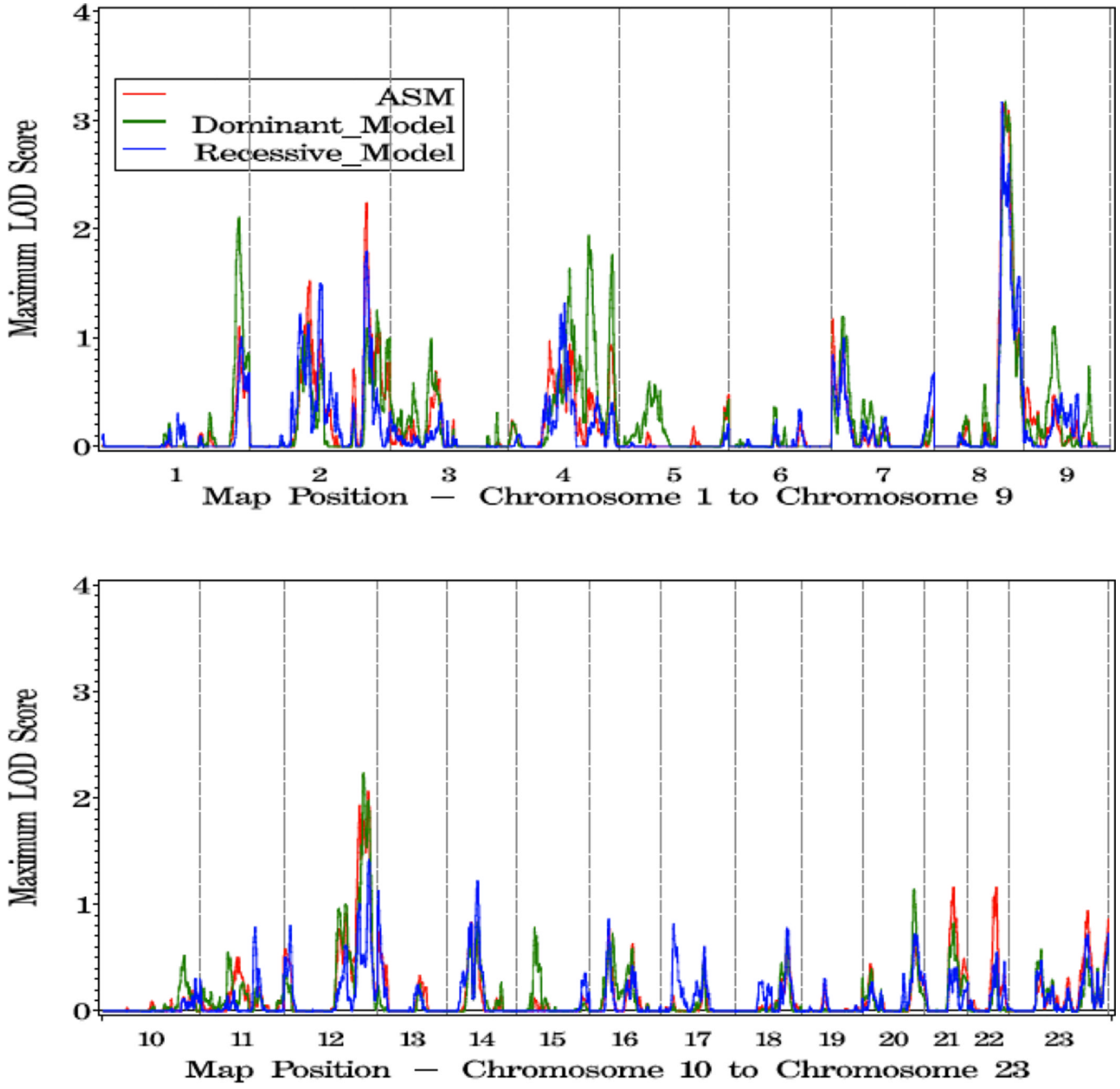


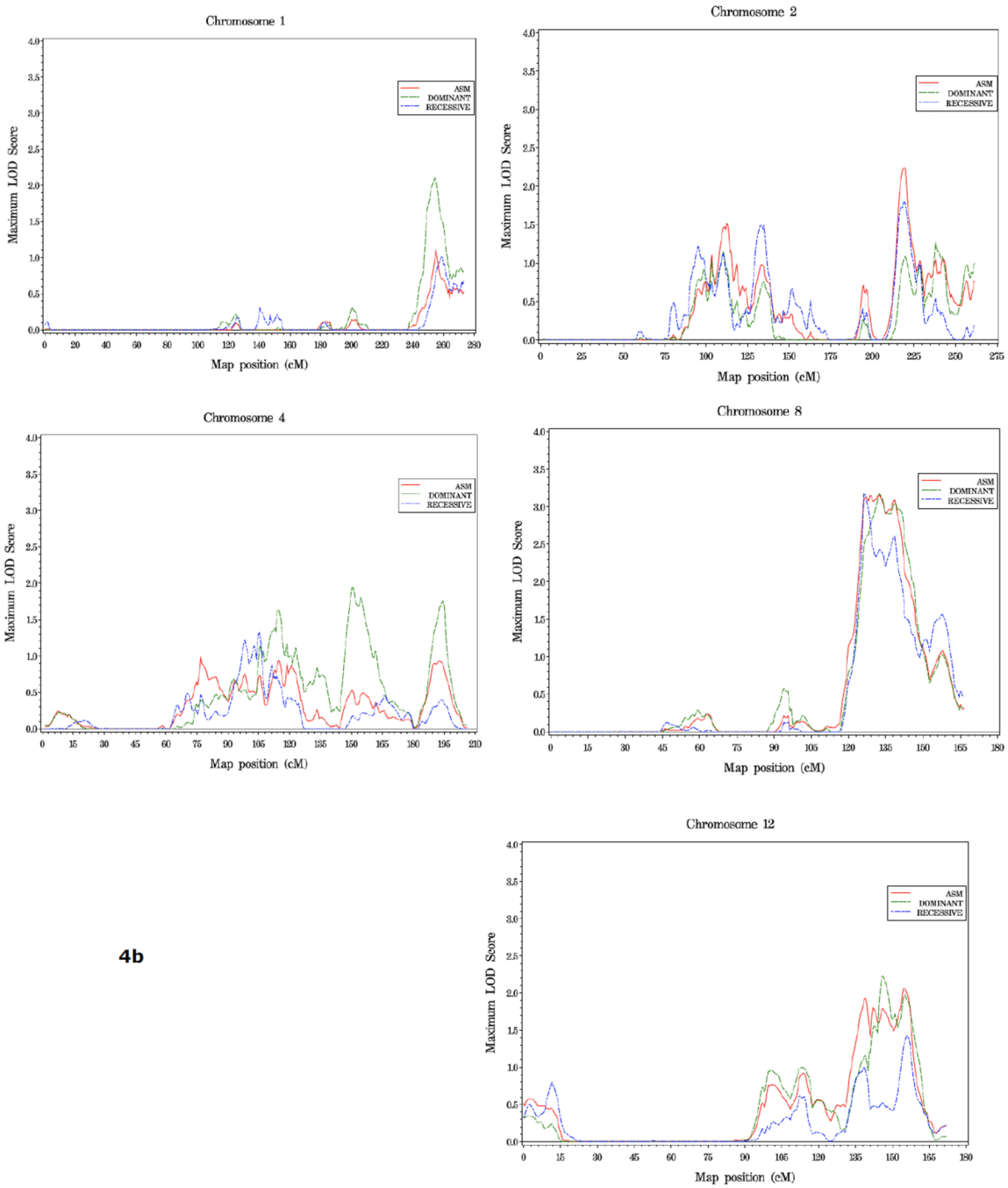
3b

Figure 3.

a. Plot of LOD Scores for families with average age at diagnosis ≥ 65.

b. Chromosomes with LOD scores ≥ 1.86 in families with average age at diagnosis ≥65.



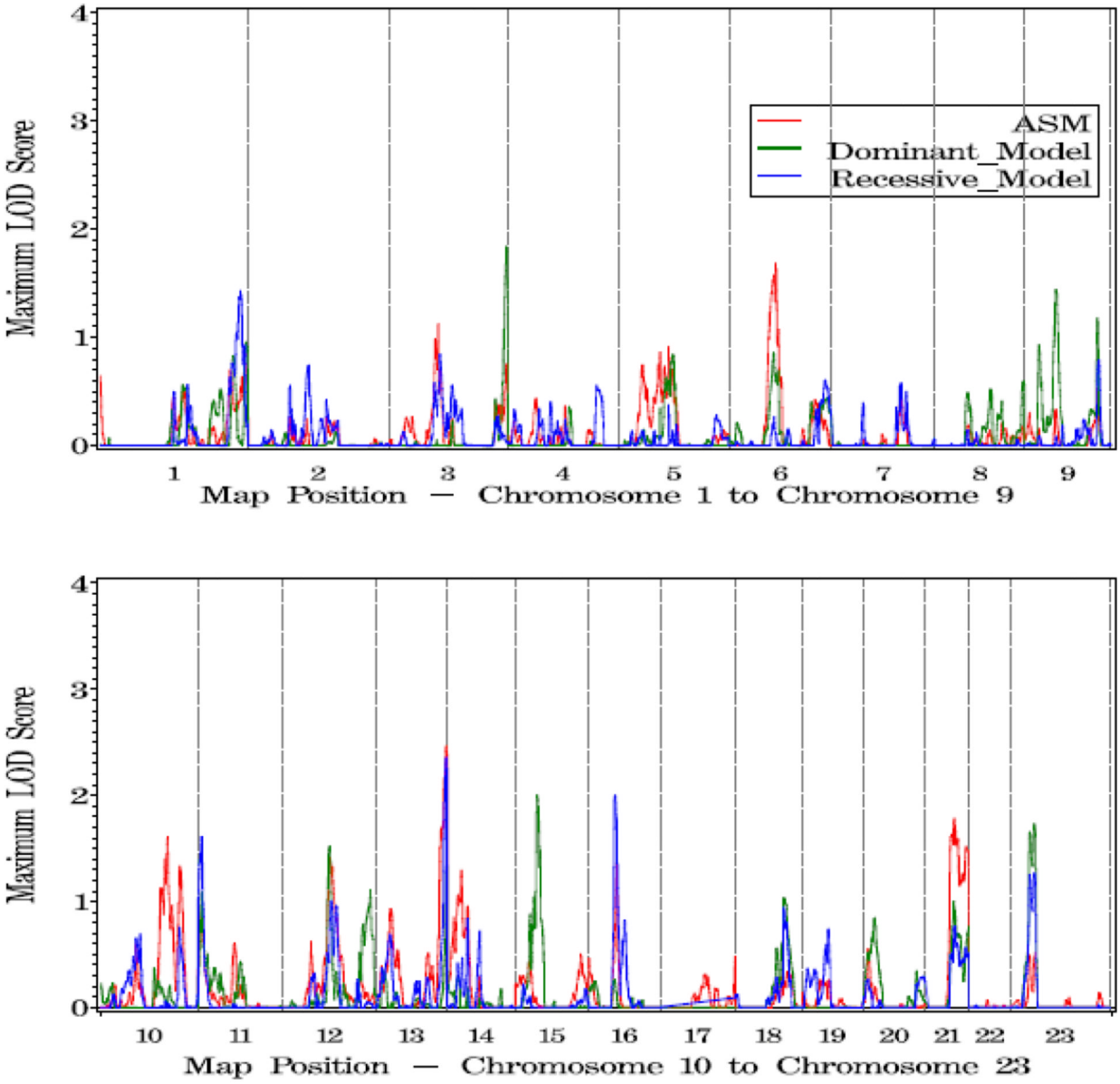


4b

Figure 4.

a. Plot of LOD Scores for families with more aggressive disease.

b. Chromosomes with LOD scores ≥1.86 in families with more aggressive disease.



5a

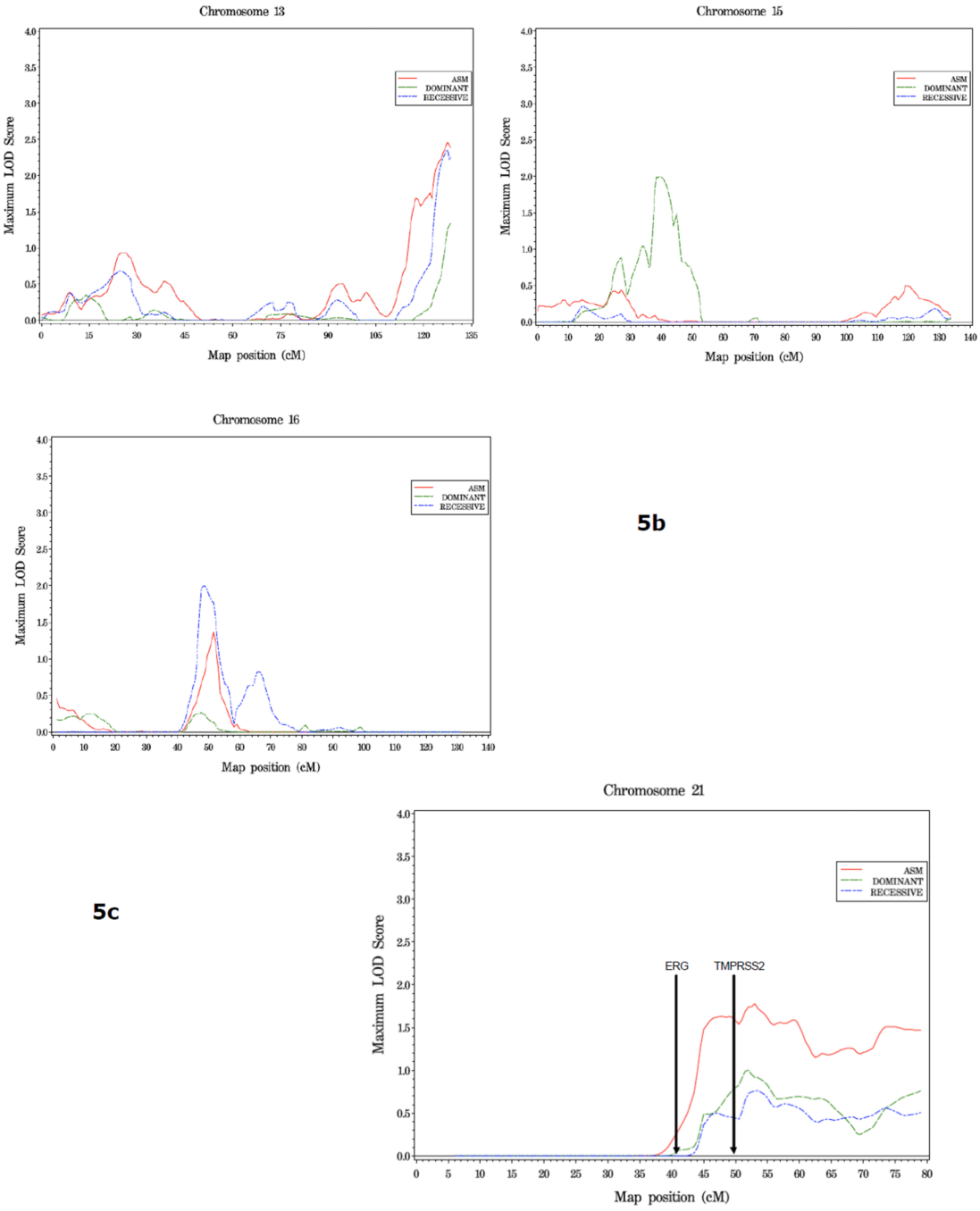


Figure 5.

a. Plot of LOD Scores for families with 5 or more affected members.

b. Chromosomes with LOD scores ≥ 1.86 in families with 5 or more affected members.

c. Chromosome 21 in families with 5 or more affected members. The position of ERG and TMPRSS2, two genes known to undergo common genomic rearrangement leading to gene fusion and activation of ERG (41), is noted.

Table 1

Characteristics of 762 ICPCG Families

	Mean Age at Diagnosis		Number of Affected Members				Families with Aggressive PCa	TOTAL		
ICPCG MEMBER	**<65**	**≥65**	**2**	**3**	**4**	**≥5**		**Families**	**Affected Individuals**	**Non-Affected Individuals**
ACTANE	108	71	89	72	13	5	150	179	380	83
Univ TAMPERE	24	33	16	32	6	3	42	57	144	159
CeRePP	87	87	110	49	11	4	63	174	385	73
FHCRC	3	10	12	1	0	0	3	13	27	0
JHU	19	5	2	10	5	7	10	24	78	24
MAYO Clinic	6	5	2	6	2	1	0	11	29	10
Univ MICHIGAN	88	43	55	58	12	6	0	131	344	0
Northwestern Univ	12	2	11	3	0	0	0	14	29	6
Univ UMEA	11	24	7	19	7	2	4	35	88	25
Univ ULM	34	16	17	28	5	0	17	50	114	21
Univ UTAH	13	61	0	0	55	19	59	74	143	268
TOTAL	405	357	321	278	116	47	348	762	1761	699

Table 2

LOD Score Summary - All Families (n = 762)[a]

Chr	pos (cM)	LOD[b]	model	region	SNP	pos (bp)
2	77	1.60	rec	2p16.1	rs1961245	54947346
2	108	1.66	rec	2p11.2	rs11395	86219368
2	131	1.85	asm	2q14.2	rs280192	121453918
2	216	1.66	asm	2q35	rs750365	218099958
4	77	2.02	rec	4q13.1	rs1489572	63718358
4	93	2.26	asm	4q21.23	rs1383972	86603603
4	98	2.62	rec	4q22.1	rs729685	90654547
4	102	2.58	rec	4q22.2	rs183993	95349298
4	114	2.58	rec	4q25	rs1879053	111616647
8	59	1.63	dom	8p11.21	rs868586	40824258
8	142	1.63	asm	8q24.22	rs1062064	133081648
11	7	1.53	dom	11p15.4	rs2231963	4581846
12	114	1.62	asm	12q23.2	rs1544921	100635554
16	81	1.50	rec	16q21	rs1027277	61242497
18	43	1.93	rec	18q11.2	rs948384	18260958

[a] All scores >1.5

[b] HLOD scores listed for dom and rec models

Table 3

LOD Scores on Chromosome 4

group	LOD[a]	pos (cM)	model	# families
ACTANE	**1.20**	**98**	**asm**	**179**
Univ Tampere	**1.36**	**83**	**rec**	**57**
CeRePP	**1.54**	**83**	**rec**	**174**
Univ Umea	**1.71**	**102**	**rec**	**35**
Univ Ulm	**0.94**	**102**	**rec**	**50**
Univ Utah	**1.00**	**93**	**rec**	**74**
MAYO Clinic	0.36	83	rec	11
FHCRC	0.00	83–102	rec	13
JHU	0.33	83	rec	24
Univ Michigan	0.00	83–102	rec	131
NW Univ	0.09	102	asm	14

[a]HLOD scores listed for rec model

Table 4

LOD Score Summary - Subsets of Familes

Chr	pos (cM)	LOD[a]	model	region	SNP	pos (bp)
Families with early age atdiagnosis (<65, n=410)						
1	269	1.95	asm	1q44	rs1148917	243595288
3	35	2.05	asm	3p24.3	rs826423	15316855
8	59	3.60	dom	8p11.21	rs868586	40824258
Families with older age at diagnosis (≥65, n=353)						
4	95	4.50	rec	4q22.1	rs729685	90654547
12	113	3.14	dom	12q23.2	rs1544921	100635554
13	15	2.23	rec	13q12.13	rs977655	25202569
18	43	1.91	rec	18q11.2	rs948384	18260958
Families with more aggressive disease (n=348)						
1	255	2.11	dom	1q43	rs528011	236344348
2	218	2.24	asm	2q35	rs746233	220209631
4	149	1.94	dom	4q32.1	rs716428	156864474
8	126	3.17	rec	8q24.13	rs2833	124055830
8	132	3.17	dom	8q24.21	rs7814955	127491272
8	139	3.09	asm	8q24.21	rs766811	130073850
12	146	2.23	dom	12q24.31	rs2197777	124356099
Families with five or more affected (n=47)						
13	128	2.46	asm	13q34	rs1885688	112942237
15	40	2.00	rec	15q14	rs276855	37318605
16	48	2.00	rec	16p12.1	rs991911	24198554

[a]HLOD scores listed for dom and rec models