Parametric Linkage Analysis Identifies Five Novel Genome-wide Significant Loci for Familial Lung Cancer

Anthony M. Musolf[1], Claire L. Simpson[1,2], Mariza de Andrade[3], Diptasri Mandal[4], Colette Gaba[5], Ping Yang[3], Yafang Li[6], Ming You[7], Elena Y. Kupert[7], Marshall W. Anderson[7], Ann G. Schwartz[8], Susan M. Pinney[9], Christopher I. Amos[6], and Joan E. Bailey-Wilson[1*]

[1] National Human Genome Research Institute, National Institutes of Health, Baltimore, MD

[2]Department of Genetics, Genomics and Informatics, University of Tennessee Health Science Center, Memphis, TN

[3]Mayo Clinic, Rochester, MN

[4] Department of Genetics, Louisiana State University Health Sciences Center, New Orleans, LA

[5]Department of Medicine, University of Toledo Dana Cancer Center, Toledo, OH

[6]Geisel School of Medicine, Dartmouth College, Lebanon, NH

[7]Cancer Center, Medical College of Wisconsin, Milwaukee, WI

[8]Karmanos Cancer Institute, Wayne State University, Detroit, MI

[9]Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH

*Correspondence: Joan E. Bailey-Wilson, 333 Cassell Dr, Baltimore, MD 21224, USA, email: jebw@mail.nih.gov, tel: 1-443-740-2921

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Abstract

Objective: One of four American cancer patients dies of lung cancer. Environmental factors such as tobacco smoking are known to affect lung cancer risk. However, there is a genetic factor to lung cancer risk as well. Here, we perform parametric linkage analysis on family-based genotype data in an effort to find genetic loci linked to the disease. **Methods:** 197 individuals from families with a high risk history of lung cancer were recruited and genotyped using an Illumina array. Parametric linkage analyses were performed using an affected-only phenotype model with an autosomal dominant inheritance using a disease allele frequency of 0.01. Three types of analyses were performed: single variant two-point, collapsed haplotype pattern variant two-point, and multipoint analysis. **Results:** Five novel genome-wide significant loci were identified at 18p11.23, 2p22.2, 14q13.1, 16p13, and 20q13.11. The families most informative for linkage were also determined. **Conclusions:** The five novel signals are good candidate regions, containing genes that have been implicated as having somatic changes in lung cancer or other cancers (though not in germ line cells). Targeted sequencing on the significant loci is planned to determine the causal variants at these loci.

Introduction

Lung cancer is the most lethal cancer in the United States. While mortality for the disease has decreased as we have learned more about the relationship between tobacco smoke and lung cancer, an estimated 158,080 Americans will die of lung cancer in 2016 - approximately 25% of all cancer-related deaths in the country [1].

Environmental exposure to chemical agents found in tobacco smoke [2-5], occupational hazards from mining, asbestos exposure, shipbuilding, and petroleum refining [6] are known to increase the risk of lung cancer. Tobacco smoking is by far the most deleterious; it is directly responsible for approximately 85-90% of lung cancer risk [7-9]. The incidence of lung cancer due to smoking is higher in men (90%) than women (70%) [10].

Though it is evident that the vast majority of lung cancer cases are due to the smoking of tobacco products, this does not account for every case. Approximately 10-15% of nonsmokers develop lung cancer. While a percentage could be due to secondhand smoking, studies have shown that it is responsible for only 16-24% of lung cancer in nonsmokers. Further, the number of lung cancer cases in nonsmokers may actually be increasing, in spite of stricter laws against public tobacco smoking [11].

Lung cancer has been found to have a strong genetic component in addition to its well-publicized environmental components. Familial aggregation of the disease was first identified in 1963 by Tokuhata and Lilienfeld [12,13], who observed that nonsmoking relatives of smoking lung cancer cases had a higher risk of susceptibility than nonsmoking relatives of smoking controls. Further studies in Louisiana [14], Utah [15,16], Texas [17] and Michigan [18] confirmed a higher risk of lung cancer in for individuals with an affected family member after adjusting for smoking histories.

Recently, much work on lung cancer genetics has focused on genome-wide association studies (GWAS). The majority of GWAS are population-based and focus on the identification of common, low penetrance variants with a moderate to small effect on disease risk. Several recent GWAS have provided highly significant and reproducible results for lung cancer. Three studies identified the 15q25 region (which contains the neuronal acetylcholine receptor gene cluster subunits *CHRNA3, CHRNA5*, and *CHRNB4*) was associated with increased risk [19-21]. Other GWAS in European populations have found significant associations to 6p21 and 5p15 [19,21-23] while studies in Asian populations have replicated these findings and found new associations at 3q28 [24-26].

Linkage analyses, which use family-based data to find rare, highly penetrant loci, have not been as prevalent as GWAS in the literature. This is likely due to the expensive and time consuming nature of collecting family-based samples instead of population-based samples. The first evidence for genome-wide significant linkage of a lung cancer susceptibility locus was to 6q23-25 [27]. The subjects present in this study had been collected from across the United States by the Genetic Epidemiology of Lung Cancer Consortium (GELCC). The GELCC continues to collect samples from high risk lung cancer families. An update was published 2010 that found further evidence for linkage on 6q and suggestive linkage to chromosomes 1q, 5q, 8q, 9p, 12q, 14q, and 16q [28]. Here, we present linkage analysis on 25 new families that have been recruited by the GELCC from 2008-2014.

Methods

Patient Recruitment and Family Data Description

Participants with a strong familial history of lung cancer were recruited by the GELCC at eight sites across the United States. We defined "strong family history of lung cancer" as having three or more first degree relatives diagnosed with lung cancer. This resulted in the collection of 197 individuals from 25 high risk families. There were 4 two-generation families, 14 three-generation families, 6 four-generation families, and 1 five-generation family. There was an average of approximately 11.04 people per pedigree.

Blood, saliva, and archival tissue were collected for all participants. For the majority of affected participants, cancer status was substantiated through medical records, pathology reports, and death certificates. For the individuals where such documentation did not exist, diagnoses were verified by the reporting of multiple family members. Further information such as birthdays, age at onset, vital statistics, and smoking exposure statistics were also collected.

Genotyping and Quality Control

Genotyping was performed at the Center for Inherited Disease Research (CIDR) at Johns Hopkins University using an Illumina HumanCore-12v1-0 array. 192 of 197 samples were successfully genotyped. 298,830 SNPs were genotyped for each individual. Data cleaning was performed by PLINK [29]; 4,186 SNPs and 0 individuals were removed for having a missingness of 1% or greater. 149 ungenotyped individuals were included in the genotyped pedigrees to create proper familial relationships; these individuals were used to connect pedigrees that would have otherwise been disjointed. Examples would be a child where just one parent was genotyped, or two siblings with neither parent genotyped (likely because the parents were deceased). This also allowed for the calculation of identity-by-descent (IBD) values and to observe any Mendelian inconsistencies in the data. Linkage analysis methods use the genotype information on genotyped family members to calculate the probabilities of specific genotypes/haplotypes of the ungenotyped ancestors in the pedigrees.

IBD values were calculated by PLINK and PRESTPLUS [30] to confirm correct familial relationships; one individual was dropped due to an incorrect relationship (the genotypes for this individual were found to be a duplication of another individual in a different pedigree and thus were most likely due to a pipetting error. This individual was an unaffected child with no offspring in the third generation of a pedigree; thus the loss of genotype information from this person resulted in a small power loss in that family). Sib-pair [31] was used to check all pedigrees for Mendelian errors. SNPs containing Mendelian errors in a single family were removed from the offending family but kept for analyses in the other families. SNPs containing Mendelian errors in two or more families were removed from all families. When there is Mendelian error in only a single family, it is likely due to a single genotype error at that marker in that family. It is not a systemic problem in genotyping the marker but a random, single event error that causes the Mendelian inconsistency. If there is a Mendelian error across multiple families, this is more likely to be a systemic problem in genotyping the marker in any individual. Thus, the genotyping for all individuals is less reliable and thus the SNP is dropped for all families. At this stage, the Mendelian inconsistencies were not caused by familial relationships errors, as we had already checked the IBD values for all individuals and found them to be accurate for the given relationships except for the one person whose genotypes were dropped due to Mendelian inconsistencies (see above). When analyzing SNP array genotype data, it is expected that each family will exhibit a small number of Mendelian inconsistencies due to genotyping errors. True family inconsistencies due to misspecifications of the relationships OR pipetting errors during sample preparation result in very large numbers of Mendelian inconsistencies across many SNPs and changes in the overall IBD sharing values between the relative pairs in question. There were 887 total Mendelian inconsistencies, but only 133 that appeared in multiple families. 48,192 markers that were monomorphic throughout the entire population were also removed. After data cleaning, 246,319 SNPs remained for analysis.

Allele frequencies for the entire data set were then calculated by Sib-pair. Seventeen married-in spouses with genotype information but no offspring were used in the allele frequency calculations but dropped from the linkage analyses. Genetic positions for all SNPs were obtained from the Rutgers Map version 3 [32] using physical positions from GRCh37. Full diagnostics of the samples analyzed, including average age and percent smokers, can be found in Table 1.

Parametric Linkage Analyses

All linkages analyses were affected-only analyses; affected individuals were coded as affected; unaffected or unknown individuals were coded as having missing phenotypes. This allowed for the high degree of uncertainty between smoking and lung cancer risk as well as jointly allowing for smoking status (80% of affected individuals in the pedigrees smoked). The genetic model assumed a disease allele frequency (DAF) of 1% under an autosomal dominant model.

Historically we have used a low penetrance model of 10% for carriers and a 1% phenocopy rate in linkage analyses of other families because segregation analyses suggest that the lung cancer variant is most likely not highly penetrant in the absence of personal smoking. Given that the linkage analysis methods used do not allow the inclusion of smoking as a covariate in any simple manner, this low penetrance model was used previously to attempt to deal with lack of smoking exposure among many at risk relatives. However, because the families being analyzed in this study consisted of a vast majority of smokers and we coded all unaffected individuals as unknown phenotype, a higher penetrance model made more sense. Thus we performed analyses using our low penetrance model (as done in prior studies) and two higher penetrance models that we believe are more appropriate for this particular data set. As expected, the higher penetrance models produced stronger evidence in favor of linkage in five regions compared to the low penetrance model. However, we found no change in the significant signals between the 40% and 80% penetrance models and the difference between the LOD scores was not statistically significant (though the LOD scores for 80% were slightly higher in magnitude). Given the uncertainty of the correct model to use, we decided to present the results of the more conservative intermediate penetrance model.

Performing an affecteds-only analysis with these penetrance models has the effect that non-smoking unaffected individuals do not contribute information about “not-sharing” genotypes with “affected” individuals in the linkage calculations, thus mitigating the fact that we do not have good age/smoking penetrance distributions to use in our analyses. Furthermore, since most affecteds are smokers and the few non-smokers who are affected are considered to be at very high genetic risk, the moderate phenocopy rate used in the penetrance models allows for the fact that some heavy-smoking affected individuals in these families might not be carrying the same risk variant carried by the other affected members of their family.

Three distinct types of parametric linkage analyses were performed. The first was the standard single variant two-point linkage analysis that observes linkage between a single SNP and the disease trait using an Elston-Stewart algorithm implemented by TwoPointLods [33]. Multipoint linkage analysis was performed by SimWalk2 [34-36]. SNPs were pruned prior to the multipoint linkage analyses in order to remove intermarker linkage disequilibrium that could lead to increased type I error rates. Markers were grouped into 1 cM bins and the SNP with the highest minor allele frequency (thus the highest information content), was chosen to represent the bin. This resulted in approximately 3,000 SNPs for the multipoint analysis. Once linkage analysis was complete, all variants were annotated by ANNOVAR [37,38].

To compensate for some of this loss of information in the multipoint analysis, we used the collapsed haplotype pattern method (CHP) implemented through SEQLinkage [39]. CHP combines SNPs into multiallelic pseudo-markers. These pseudo-markers correspond to annotated genes in RefSeq. The pruning for intermarker LD that is necessary to run programs like SimWalk2 is not needed under this scenario, so more information is retained. This approach has shown to be powerful and maintain proper type I error rates when SNPs with rare minor alleles in the analysis. We restricted CHP analysis to markers with a minor allele frequency of 10% and under (approximately 35,000 SNPs). The regional markers are sometimes further divided into smaller subunits based on observed recombination events within a gene. After the regional pseudo-markers were created, standard two-point linkage analysis was performed on the new markers using MERLIN [40]. This method will henceforth be referred to as CHP two-point linkage.

Results

CHP two-point linkage analysis identified five significant linkage signals located on five chromosomes (Figure 1, Table 2). Here, we use the Lander and Kruglyak values of HLOD >= 3.3 and HLOD >=1.9 as the respective thresholds for genome-wide significance and suggestion [41]. A LOD score of 3.3 corresponds to a p-value of 4.9 x 10^{-5} and a LOD score of 1.9 corresponds to 1.7 x 10^{-3}. The highest HLOD was 4.11 located on 18p11.23 and centered on the *PTPRM* gene. The other significant signals were located at *LRP1B* (HLOD = 3.90) at 2p22.2, *NPAS3* (HLOD = 3.73) at 14q13.1, *RBFOX1* (HLOD = 3.36) at 16p13, and *PTPRT* (HLOD = 3.34) at 20q13.11. A further 74 suggestive signals were found throughout the genome (Supplemental Table 1).

Multipoint analysis yielded no significant linkage signals and three suggestive linkage signals (Figure 2, Table 3). All three suggestive signals were located on 17q21.33. Further the top 9 SNPs were all located in the 17q21.32 – q22 region (Figure 3). The highest HLOD (1.97) was located an intron of *CA10*; the two other suggestive HLOD scores (1.96 and 1.92) were located in an intron of *UTP18* and the intergenic region of *CA10* and *C17orf112*. The highest exonic SNP (HLOD = 1.87) was also located in 17q21.33, in the *AMAP1* gene. The 17q21.32-q22 signal was primarily driven by three families – family 138 (HLOD range 0.44 – 0.80), family 147 (HLOD range 0.51 – 0.55), and family 148 (HLOD range 0.34 – 0.45).

Two-point analysis did not reveal any significant or suggestive markers (Supplemental Figure 2). The highest overall HLOD (1.80) was located on 17p12 in an intergenic region between *ELAC2* and *HS3T3A1*.

Since these families had not been previously analyzed, this set of linkage analyses allowed us to determine which families were informative for linkage at all. Five families were not informative for linkage at all (meaning they had no nonzero LOD scores for any of the three types of analyses) (Supplemental Table 2). The other twenty families showed varying degrees of information. From these twenty, there were eight families that had LOD scores above or approximately equal to 0.5 for all three types of analyses. We considered these families highly informative for linkage and will be the most useful for future sequencing studies.

Discussion

CHP two-point analysis located five novel significant linkage signals for familial lung cancer in this genotype data. While these signals had not previously been identified for linkage, all of these signals had been previously implicated in somatic changes in lung cancer in cell lines or in vivo. The protein tyrosine phosphatase gene *PTPRM*, located on 18p11.23, was the highest linkage peak. Protein tyrosine phosphatases regulate cellular growth and the mitotic cycle and are known oncogenes. *PTPRM* in particular has been implicated as an oncogene for lung cancer [42]. It has also been found to affect methylation patterns in lung cancer tumor cells compared to non-tumor cells [43] and has been shown to be activated in *KRAS* mutant lung adenocarcinomas [44].

Another member of the protein tyrosine phosphatase family, *PTPRT* was also found to be significant for linkage. *PTPRT*, located on 20q13, has been shown to be mutated in lung cancer cells and may be involved in cellular adhesion and tumor migration [45]. Whole exome sequencing of matched pairs of lung carcinomas and normal tissue found an increase of somatic mutation of this gene [46] and mutational analysis of *PTPRT* suggested a potential role as a tumor suppressor in colorectal cancer [47].

The low density lipoprotein receptor *LRP1B*, located at 2p22.2, had the second highest HLOD score and is well documented as being deleted in tumor cells. It is a likely tumor suppressor gene in multiple cancers, including lung cancer [48]. The gene is inactivated in nearly 50% of non-small cell lung cancer cell lines [49]; its normal function when active includes inhibiting cellular migration [50]. All previous reports of *LRP1B* inactivation are somatic mutations or deletions; this is the first report of an *LRP1B* mutation in the germ line affecting familial lung cancer risk.

The significant signal at 16p13.3 centered on the RNA binding protein *RBFOX1* (HLOD = 3.48). This gene has been found to be deleted in malignant mesothelioma cell lines [51]. Furthermore, *RBFOX1* has been linked to disease recurrence in colon cancer in array-CGH [52] and significantly associated with increased survival of chemotherapy treated breast cancer patients in a Finnish GWAS study [53]. Our study is the first to report a familial linkage to the region.

The transcription factor *NPAS3* at 14q13.1 has not previously been found to have any links to lung cancer. It has been shown to be critical for lung development [54]. In addition, knockdown of *NPAS3* has been shown to induce the growth of malignant astrocytomas in cell lines and overexpressed *NPAS3* suppressed transformation in malignant glioma cell lines, leading to speculation that *NPAS3* functions as a tumor suppressor [55].

While the multipoint analysis found no significant signals, one suggestive region was found at 17q22.33. This region contains *AMAP1*, which is overexpressed in breast cancer tumors [56]and has been found to play a role in both metastasis [57] and the epithelial-mesenchymal transition [58]. The membrane trafficking protein *TOM1L1* is also located near this region and had been implicated in both breast cancer [59] and colorectal cancer [60].

The single variant two-point analyses found no significant or suggestive variants. This is likely due to the lack of information within these pedigrees for single variant two-point analysis. We had no more than two genotyped affected individuals per family. Therefore, the linkage analysis algorithms use the information from the genotyped affected and unaffected individuals to calculate the probability of a given genotype for additional ancestors in the family (particularly affected family members). This is a standard property of linkage analysis in general. However, imputation of genotypes for the ungenotyped affected individuals is less informative at single SNP than when multiple SNPs are combined into haplotypes. The calculation of genotype probabilities for ungenotyped affecteds is less accurate when using single SNP loci as opposed to multiple SNPs combined into more informative multiallelic haplotypes, as was done in the CHP analysis. This resulted in the higher information content and thus higher power in the CHP two-point analyses.

Another interesting observation is the amount of overlap between the three linkage methods. There was some overlap between the CHP two-point results and the multipoint results, as both analyses localized a signal to the 18q21-23 region, though the magnitude of the signal was much higher in the CHP two-point analysis. The lower magnitude was most likely due to the heavy pruning of the data required to perform the multipoint analysis. Further, large degrees of overlap are unlikely between the CHP two-point and multipoint analyses because the data sets necessitate different types of filtering; multipoint analysis required the binning of SNPs and selection based on the highest MAF, while the CHP two-point analysis required SNPs with a MAF <= 0.1. CHP two-point analysis also used multiallelic pseudo-markers instead of the bilallelic markers used in the multipoint and single variant two-point analyses, resulting in greater information content and consequently higher power for the CHP two-point analysis.

The linkage analyses also allowed us to determine which families were informative for linkage; again critical because no family had more than two genotyped affecteds. Twenty of the twenty-five families were informative for at least one of the linkage analyses. Eight were highly informative. The information content of these families gives them priority for future sequencing studies. We will likely perform targeted sequencing on the five loci of interest identified from this study; the sequencing will focus on these eight families. Similarly, the GELCC is performing whole exome sequencing (WES) on families from throughout its entire data set (not just the new families used here). The information gained from the LOD score metrics from these analyses identified that the top four families (i.e. the 4 most informative of the eight highly informative families identified in this data set) will be included in this WES effort.

We note that we did not see any replication of the previously published linkage signal identified on 6q in these families. Lung cancer (like all cancers) is a highly heterogeneous phenotype and it is not unlikely that the majority of the families here might have different causal loci. In fact, in Bailey-Wilson et al. [27] of the 6q linkage, only a small proportion of the families were strongly linked to this region.

One interesting additional note regarding this data set. We have data for age, age at onset, and smoking status for these families. However, there is currently no reliable way to add covariates to most linkage analysis programs, particularly for multipoint linkage analysis. Development of linkage analysis software stagnated after the explosion of GWAS studies in the early 2000s. Our approach in this study was to control for smoking status by performing affected-only linkage analysis, using the genotypes from unaffected individuals solely to impute genotypes of ungenotyped affecteds and to help compute the probability of identity by descent sharing of alleles by the affected relative pairs using linkage analysis algorithms. This approach was helped by the fact that approximately 80% of the affected individuals were known to smoke. As family-based studies have begun to come back into vogue in recent years, this will hopefully result in the development of additional linkage software that can include covariates.

Despite advances in treatment and prevention, lung cancer still remains the leading cancer killer in the United States. Our linkage analyses identified genome-wide specific signals on 18p11.23, 2p22.2, 14q13.1, 16p13, and 20q13.11. While several of the signals centered on genes with a previous implication to lung cancer (though not in the germ line), we want to further elucidate the causal variant(s) underlying each signal, so targeted sequencing of these regions is planned. The denser map will allow us a greater ability to pinpoint the exact variant(s) that is causing each signal. Once a preliminary causal variant has been identified, laboratory based work will be performed to confirm the finding.

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Author Contributions: AMM and CLS performed statistical analyses of the data. MA, DM, CG, PY, YL, MY, MWA, AGS, SMP, CIA, JEBW designed the study, obtained funding, obtained the genotype data, and were involved in enrollment of study participants. EYK performed laboratory work on the study. AMM wrote the manuscript. All other authors reviewed and edited the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

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Table 1: Characteristics of Individuals used in Linkage Analyses

	Affected	**Unaffected/Unknown**	**Total**
Genotyped	35	130	165
Ungenotyped	37	112	149
Average Age	70	63.8	66.5
Avg. Age at Onset	63.7	N/A	N/A
Number Smokers	55	74	122
Percentage Smoker	0.76	0.31	0.71

Diagnostic information on the individuals from the 25 extended families used in the linkage analyses after quality control and removal of married-in spouses. Average age, average age at onset, and smoking statistics were calculated using individuals with available data.

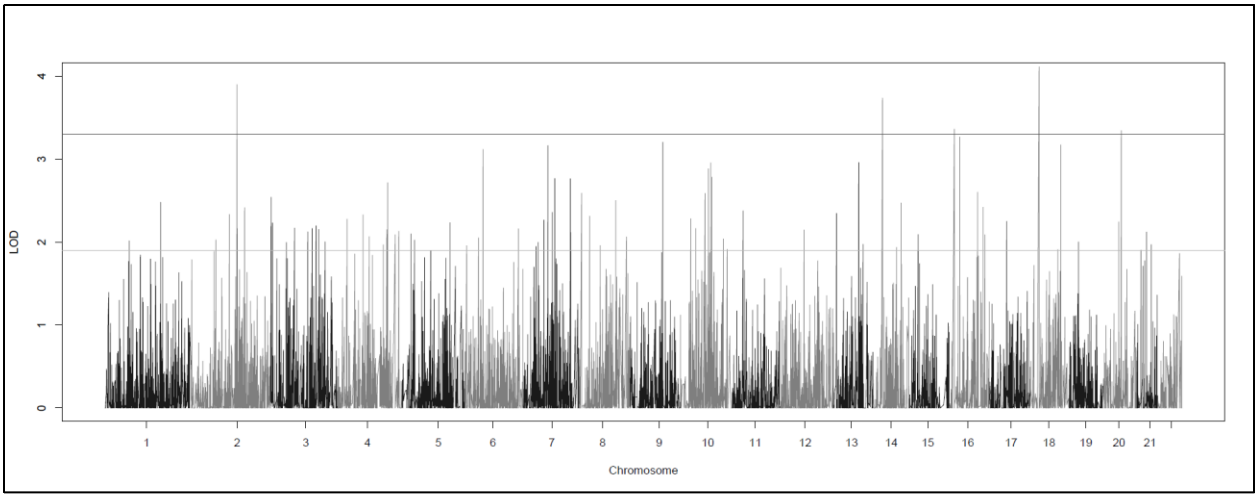


Figure 1: Genome-wide HLOD Plot of CHP Variant Two-point Linkage Analysis: The heterogeneity LOD (HLOD) scores calculated across all 25 families for the CHP variant two-point linkage analysis performed by SEQLinkage and MERLIN. The lines at 3.3 and 1.9 represent the thesholds for the respective significant and suggestive LOD scores as recommended by Lander and Kruglylak.

Table 2: Genome-wide Significant HLOD Scores in CHP Variant Two-point Linkage Analysis

CHR	POS	GENE	LOD	ALPHA	HLOD
18p11.23	29.36403	*PTPRM*[1]	4.1099	1	4.1099
2p22.2	152.1074	*LRP1B*[1]	3.8964	1	3.8964
14q13.1	32.13378	*NPAS3*[1]	3.7337	1	3.7337
16p13	18.02736	*RBFOX1*[1]	3.3597	1	3.3597
20q13.11	62.31841	*PTPRT*[1]	3.3425	1	3.3425

The genome-wide significant (>=3.3) heterogeneity LOD (HLOD) scores from the CHP variant two-point linkage analysis performed by SEQLinkage and MERLIN. CHR stands for chromosome, POS is the start position in cM of the regional marker, GENE is the name of the gene within which the positional marker is located, LOD is the cumulative LOD score across all families, alpha is a measure of the percentage of families linked to that regional marker and is calculated jointly with HLOD, the heterogeneity LOD score. The brackets next to the gene name indicate the gene has been broken into pieces and the number in the bracket represents the particular piece.

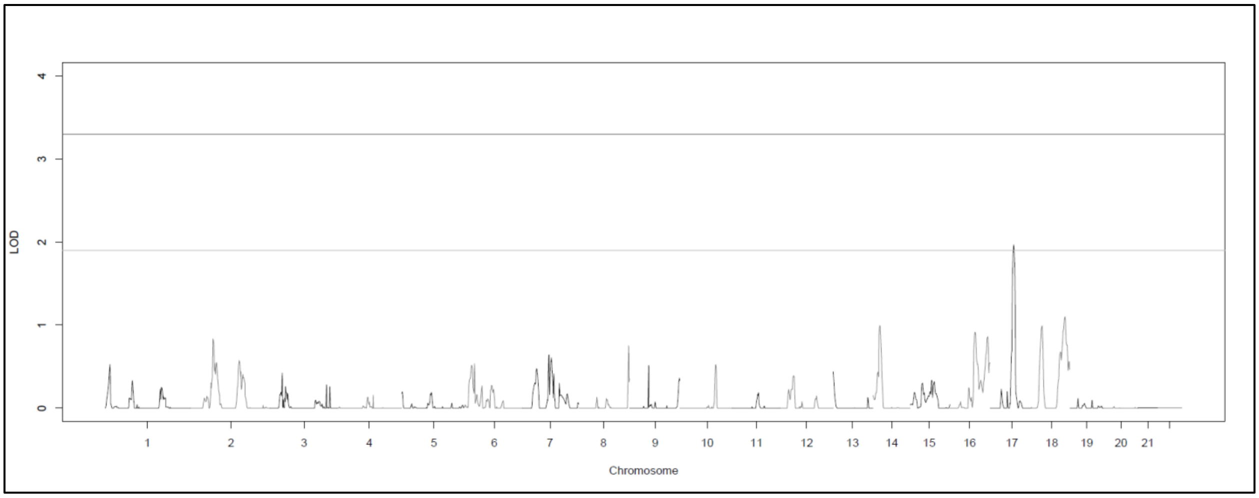


Figure 2: Genome-wide HLOD Plot of Multipoint Linkage Analysis: The heterogeneity LOD (HLOD) scores calculated across all 25 families for the multipoint linkage analysis performed by SimWalk2. SNP pruning was necessary before running SimWalk2, which accounts for the less dense map than the two-point analysis. The lines at 3.3 and 1.9 represent the thesholds for the respective significant and suggestive LOD scores as recommended by Lander and Kruglylak.

Table 3: Top Nine HLOD Scores in Multipoint Linkage Analysis

CHR	rsID	POS	LOD	ALPHA	HLOD	FUNCTION	GENE
17q21.33	rs1263965	77.8514	1.966	1	1.966	intronic	*CA10*
17q21.33	rs6504702	77.3418	1.957	1	1.957	intronic	*UTP18*
17q21.33	rs7218763	78.9483	1.921	1	1.921	intergenic	*CA10,C17orf112*
17q21.33	rs9890721	76.1046	1.874	1	1.874	exonic	*AMAP1*
17q21.33	rs1881140	75.5546	1.853	1	1.853	intergenic	*LOC101927230,TMEM92*
17q22	12165058	80.8282	1.715	1	1.715	intronic	*TOM1L1*
17q22	rs888207	81.3318	1.666	1	1.666	intergenic	*HLF,MMD*
17q21.32	rs4794031	73.5562	1.584	1	1.584	intergenic	*FLJ40194,MIR6129*
17q22	rs9896667	82.8156	1.2045	0.95	1.209	intergenic	*PCTP,ANKFN1*
17q21.33	11870935	72.4112	1.0765	0.825	1.163	intronic	*KPNB1*

The top nine HLOD scores from the multipoint analysis performed by SimWalk2. All were located between 17q21.32-q22. The top three SNPs are genome-wide suggestive (>= HLOD 1.9) as recommended by Lander and Kruglyak. CHR stands for chromosome, rsID is the SNP name, POS is the start position in cM of the SNP, LOD is the cumulative LOD score across all families, alpha is a measure of the percentage of families linked to the marker and is calculated jointly with HLOD, the heterogeneity LOD score, FUNCTION is the location of the SNP, and GENE is the gene or nearby genes. Annotations for all SNPs were performed by ANNOVAR.

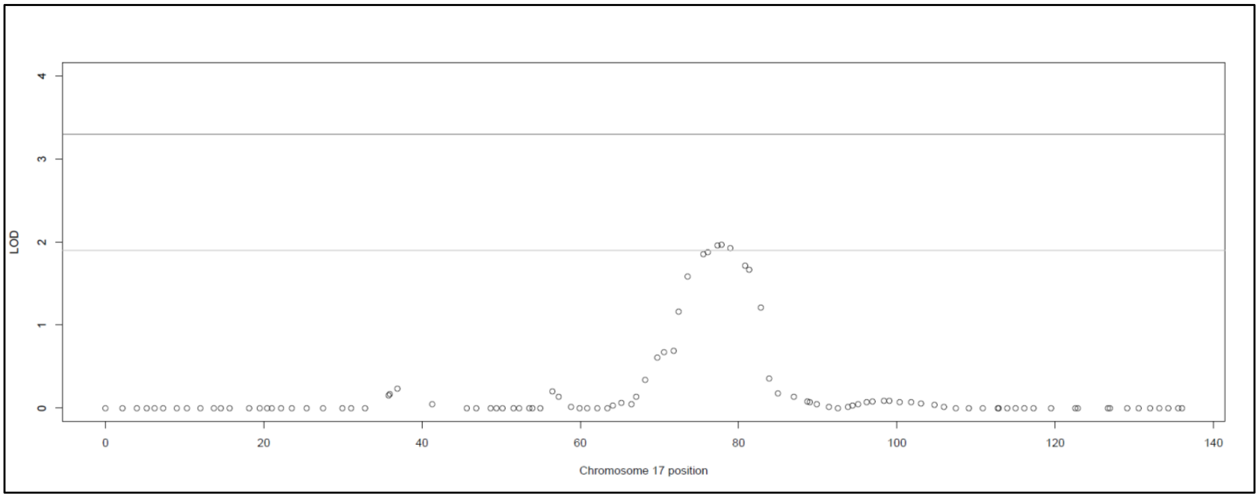


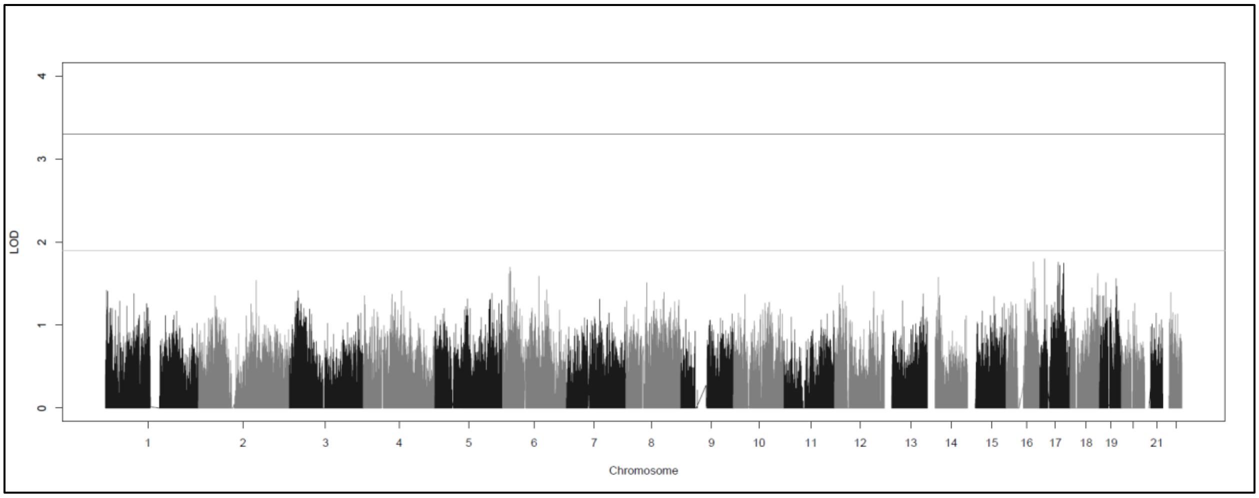
Figure 3: Multipoint HLOD Plot of Chromosome 17: The heterogeneity LOD (HLOD) scores calculated across all 25 families at chromosome 17 for the multipoint linkage analysis performed by SimWalk2. The lines at 3.3 and 1.9 represent the thesholds for the respective significant and suggestive LOD scores as recommended by Lander and Kruglylak.

Supplemental Table 1: Genome-wide Suggestive HLOD Scores in CHP Variant Two-point Linkage Analysis

CHR	POS	GENE	LOD	ALPHA	HLOD
16	36.10678	ABCC1[1]	3.2645	1	3.2645
9	109.3256	ABCA1[1]	3.2015	1	3.2015
18	102.3015	CCDC102B[1]	3.1704	1	3.1704
7	82.7502	WBSCR17[1]	3.1612	1	3.1612
6	60.88568	DNAH8[1]	3.1154	1	3.1154
13	84.28189	GPC5[1]	2.9583	1	2.9583
10	102.3388	NRG3[1]	2.9532	1	2.9532
10	94.27962	C10orf11[1]	2.8822	1	2.8822
10	105.5822	GRID1[1]	2.7809	1	2.7809
7	105.5729	PPP1R9A[1]	2.7656	1	2.7656
7	157.2949	CNTNAP2[2]	2.7621	1	2.7621
4	166.6779	MARCH1[1]	2.7143	1	2.7143
16	95.38696	ADAMTS18[1]	2.5993	1	2.5993
8	7.747743	CSMD1[1]	2.5869	1	2.5869
10	82.80332	CTNNA3[1]	2.5852	1	2.5852
3	2.332563	CNTN6[1]	2.5391	1	2.5391
8	121.7699	SLC30A8[1]	2.4993	1	2.4993
1	185.1424	TNR[1]	2.4784	1	2.4784
14	94.3429	SLC24A4[1]	2.4698	1	2.4698
16	113.593	CDH13[1]	2.4198	1	2.4198
2	177.0431	MYO3B[1]	2.4139	1	2.4139
11	35.68421	NAV2[1]	2.3752	1	2.3752
7	97.2799	SEMA3A[1]	2.3575	1	2.3575
13	10.40546	SPATA13[1]	2.3454	1	2.3454
2	126.3212	DPP10[1]	2.3323	1	2.3323
4	84.84841	SLC4A4[1]	2.3279	1	2.3279
8	34.61141	PSD3[1]	2.311	1	2.311
10	35.83745	FAM107B[1]	2.2802	1	2.2802
4	31.32766	LDB2[1]	2.2748	1	2.2748
7	69.55921	ABCA13[1]	2.2649	1	2.2649
17	58.52668	ASIC2[1]	2.2483	1	2.2483
20	53.84893	C20orf112[1]	2.2419	1	2.2419
5	160.1693	TNIP1[1]	2.2325	1	2.2325
3	6.61202	CNTN4[2]	2.2296	1	2.2296
3	152.147	SLC9A9[1]	2.1962	1	2.1962
3	79.88634	FHIT[1]	2.1678	1	2.1678
3	138.7392	CPNE4[1]	2.1624	1	2.1624
10	52.00449	MPP7[1]	2.162	1	2.162
2	153.422	KYNU[1]	2.1612	1	2.1612

6	178.6219	PACRG[1]	2.1584	1	2.1584
3	160.781	LINC01214[1]	2.1508	1	2.1508
4	163.6827	FSTL5[1]	2.1489	1	2.1489
12	78.19477	FAM19A2[1]	2.1455	1	2.1455
4	203.5172	SORBS2[1]	2.1295	0.9938	2.1297
3	124.0329	LSAMP[1]	2.123	1	2.123
21	32.30334	GRIK1[1]	2.1207	0.9917	2.121
7	158.5582	MIR548F3[1]	2.1136	1	2.1136
5	30.71129	CTNND2[2]	2.0978	1	2.0978
15	27.82131	RYR3[1]	2.0905	1	2.0905
4	192.2219	TENM3[1]	2.088	1	2.088
16	119.3982	COTL1[1]	2.0871	1	2.0871
4	104.9059	CCSER1[1]	2.0645	1	2.0645
8	156.986	FAM135B[1]	2.0615	1	2.0615
6	45.88286	CASC15[1]	2.05	1	2.05
8	7.747743	CSMD1[2]	2.0458	1	2.0458
10	143.7745	MIR5694[1]	2.0371	1	2.0371
2	81.27133	LINC01122[1]	2.025	1	2.025
5	41.81669	CDH18[1]	2.0225	1	2.0225
1	80.14709	SLC1A7[1]	2.0135	1	2.0135
3	181.8731	NAALADL2[1]	2.003	1	2.003
19	31.13983	DNM2[1]	2.0009	1	2.0009
7	50.29992	PDE1C[1]	1.9958	1	1.9958
3	52.79558	RBMS3[1]	1.9946	1	1.9946
13	98.90918	NALCN[1]	1.9716	1	1.9716
21	47.89004	ERG[1]	1.9682	1	1.9682
4	151.989	LRBA[1]	1.9664	1	1.9664
8	69.97262	XKR4	1.9567	1	1.9567
6	5.959985	GMDS[1]	1.9556	1	1.9556
7	42.64313	JAZF1[1]	1.9462	1	1.9462
14	79.11249	CEP128[1]	1.9351	1	1.9351
10	156.932	DOCK1[1]	1.9109	1	1.9109
18	92.15627	PHLPP1[1]	1.9092	1	1.9092
7	158.7946	MIR548T[1]	1.9024	1	1.9024
21	13.0434	CHODL[1]	1.9017	1	1.9017

The genome-wide suggestive (>=1.9) heterogeneity LOD (HLOD) scores from the CHP variant two-point linkage analysis performed by SEQLinkage and MERLIN. CHR stands for chromosome, POS is the start position in cM of the regional marker, GENE is the name of the gene within which the positional marker is located, LOD is the cumulative LOD score across all families, alpha is a measure of the percentage of families linked to that regional marker and is calculated jointly with HLOD, the heterogeneity LOD score. The brackets next to the gene name indicate the gene has been broken into pieces and the number in the bracket represents the particular piece.



Supplemental Figure 1: Genome-wide HLOD Plot of Single Variant Two-point Linkage Analysis: The heterogeneity LOD (HLOD) scores calculated across all 25 families for the multipoint linkage analysis performed by TwoPointLods. The lines at 3.3 and 1.9 represent the thesholds for the respective significant and suggestive LOD scores as recommended by Lander and Kruglylak.

Supplemental Table 2: Highest LOD Score for each Families

FID	SV TP LOD	MP LOD	CHP TP LOD
137	0.2886	0.356	0.3362
138	0.8152	0.817	0.8176
139	0.171	0.208	0.2004
140	0.4849	0.542	0.8027
141	0.2635	0.281	0.2741
143	0.4779	0.505	0.4188
144	0	0	0
145	0.5962	0.774	0.7452
147	0.5502	0.55	0.5503
148	0.6733	0.807	0.7642
149	0.4692	0.545	0.5446
150	0.1497	0.197	0.264
151	0.2305	0.231	0.2306
153	0	0	0
154	0	0	0
155	0	0.057	0.5446
156	0	0	0
157	0	0.034	0.2277
159	0	0.03	0.2699
160	0.263	0.276	0.2766
161	0.2156	0.231	0.2236
162	0.2473	0.262	0.2476
163	0	0	0
164	0.4977	0.552	0.5086
165	0.265	0.27	0.2614

The overall highest LOD score for each of the 25 families. The three scores correspond to the three types of linkage analyses: single variant two-point (SV TP LOD), multipoint (MP LOD), and collapsed haplotype pattern variant two-point (CHP TP LOD). FID represents the family ID.