

PARKINSON DISEASE LOCI IN THE MID-WESTERN AMISH

By

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Thesis

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LIST OF ABBREVIATIONS

AFF	Affecteds
CHR	Chromosome
DOM	Dominant model
HLOD	Heterogeneity logarithm of the odds of linkage
MAF	Minor allele frequency (adjusted for relationships using MQLS)
MB	Megabase
MQLS	Modified Quasi-Likelihood Score
OV	Overall
REC	Recessive model
SI	Support interval
SNP	Single nucleotide polymorphism
SUB-PED	Sub-pedigree
UNAFF	Unaffecteds
UNK	Unknown

CHAPTER I

INTRODUCTION

Parkinson disease (PD) is the second most common neurodegenerative disorder in adults and was originally defined as a motor disease based on the four hallmark characteristics: resting tremor, bradykinesia, rigidity, and postural instability.¹ However, it has since been realized that individuals with PD are affected by many other symptoms. Non-motor symptoms of PD include dementia, pain, mood disorders, sleep difficulties, and autonomic dysfunction. Not all symptoms are present for each individual diagnosed with PD, but the number and severity of symptoms tend to accumulate over time. The average age at diagnosis of PD is 70.5 years and most frequently occurs after the age of 60.²

The clinical expression and physiological systems involved in Parkinson disease are also complex, suggesting variability in the etiology. Development of PD in an individual has been attributed to environmental factors, genetic factors, and interactions between the two, although we still understand only a small part of all the components in play.³⁻⁶ Many environmental factors have been implicated in PD, but only a few are consistent across studies (pesticides as a risk factor, smoking and caffeine as protective factors).^{3,7,8} In addition, an abundance of genetic risk factors have been identified, and many have been replicated and confirmed by subsequent studies. Loci have been identified that act under autosomal dominant (e.g. SNCA, LRRK2) and autosomal recessive (e.g. PRKN, DJ1, PINK1) models, and that function as strong risk factors for development of the disease and other associations with common polymorphisms (e.g.

variants in *GBA*, *MAPT*).^{4,9-11} At least 13 loci have been confirmed as PD-related loci and they have been identified through linkage, candidate gene, genome-wide association, and exome sequencing studies. These results are encouraging and exciting; however, there is still a large portion of the genetic component of PD that remains to be uncovered. In light of this, we have undertaken a study in an isolated founder population, from mid-western Amish communities in Ohio and Indiana, and conducted genome-wide association and linkage analyses to detect additional PD-related loci.

The Amish population is a promising population to study the genetic risk of PD for several reasons. The first PD-related loci were identified in genetically isolated founder populations.¹² We hypothesize that the genetics of PD may be less heterogeneous in the Amish due to the shared ancestry and minimal gene flow. Also, the Amish share a very homogenous lifestyle, including minimal caffeine and tobacco use, due to cultural and religious beliefs. This homogenous background may allow us to more easily detect genetic effects by reducing confounding effects of environmental factors. We have analyzed 798 individuals, who can all be connected into one 4,998-member pedigree. In this dataset we see a higher average kinship coefficient among pairs of affected individuals (.0177) compared to pairs of unaffected individuals (.0149). We performed a Wilcoxon rank-sum (Mann Whitney) test and found this difference to be statistically significant ($p < 1 \times 10^{-4}$), strongly suggesting that PD is heritable in the Amish and likely a valuable population to uncover future knowledge of the underlying genetics of PD.

CHAPTER II

METHODS

Subjects

Methods for ascertainment were reviewed and approved by the individual Institutional Review Boards of the respective institutions. Informed consent was obtained from participants recruited from Amish communities with which we have had established working relationship for over 10 years. These communities are in Elkhart, LaGrange, Adams, and surrounding Indiana counties, and Holmes and surrounding Ohio counties. To date we have enrolled over 2,200 Amish individuals with 32 of these diagnosed with PD.

Clinical Evaluation

The spectrum of clinical symptoms for this pedigree was previously described.^{13,14} A standardized interview for PD was conducted by a board-certified genetic counselor or genetic study research associate with participating individuals or a knowledgeable family informant. Individuals were screened for a history of encephalitis, dopamine-blocking medication exposure within one year before diagnosis, symptoms of normal pressure hydrocephalus (dementia, gait difficulty, and urinary incontinence), or a clinical course with unusual features suggestive of atypical or secondary parkinsonism. Individuals with a positive symptom history of PD and apparently unaffected individuals (mostly siblings) were personally examined by a board-certified neurologist with subspecialty training in movement disorders, and many have been examined more than

once to ensure the diagnoses are accurate over time. At these secondary interviews, participants were evaluated for a history of exposure to substances known or suspected to cause parkinsonism, including heavy metals and pesticides. Participants were classified as affected, unaffected or unknown, using published diagnosis criteria based on clinical history and neurologic examination.¹ Affected individuals had at least two cardinal signs of PD (resting tremor, bradykinesia, or rigidity) and no atypical features of parkinsonism. Individuals with unknown status had only one sign of PD, a history of atypical clinical features, or both. Unaffected individuals had no signs of PD. Age at onset was self-reported and defined as the age at which onset of the first symptom suggestive of PD was noted by the affected individual. Levodopa responsiveness was determined based on physician and patient observations. Individuals with uncertain symptom benefit or who never received levodopa therapy were classified as having an unknown response.

The severity of extrapyramidal signs and symptoms were evaluated by Hoehn-Yahr staging¹⁵ and the Unified Parkinson Disease Rating Scale (UPDRS-motor subscale) UPDRS-III¹⁶. When available, reports of brain imaging studies were reviewed to confirm the absence of hydrocephalus or vascular parkinsonism. Dementia was assessed by the memory-orientation-concentration test (Short-Blessed Test (SBT)).¹⁷ Diagnosis of progressive supranuclear palsy was determined from the NINDS-PSP International Workgroup clinical criteria.¹⁸

Genotyping

Genotyping and quality control (QC) in this dataset were performed as described previously.¹⁹ Briefly, genome-wide genotyping was performed on 830 DNA samples using the Affymetrix 6.0 GeneChip® Human Mapping 1 million array set (Affymetrix®),

Inc Santa Clara, CA). DNA for this project was allocated by the respective DNA banks at both the Hussman Institute of Human Genomics (HIHG) at the University of Miami and the Center for Human Genetics Research (CHGR) at Vanderbilt University. Genomic DNA was quantitated via the ND-8000 spectrophotometer and DNA quality was evaluated via gel electrophoresis. The genomic DNA (250ng/5ul) samples were processed according to standard Affymetrix procedures for the processing of the Affymetrix 6.0 GeneChip array. The arrays were then scanned using the GeneChip Scanner 3000 7G operated by the Affymetrix® GeneChip® Command Console® (AGCC) software. The data were processed for genotype calling using the Affymetrix® Power Tools (APT) software using the Birdseed calling algorithm version 2.0 Affymetrix®, Inc Santa Clara, CA.

Strict QC procedures were applied to both samples and SNPs to ensure the accuracy of our data prior to analyses. Sample QC included visualization of each DNA sample via agarose to ensure high quality samples prior to inclusion on the array and CEPH samples and duplicate samples plated across multiple arrays to check reproducibility across the arrays. Samples with call rates <95% were re-examined to ensure quality of genotypes; if the call rate for a sample was still <95% after this examination, we attempted to rerun the array with a new DNA sample. Nine samples failed at this point and were dropped due to low genotyping efficiency. The Anabaptist Genealogy DataBase (AGDB)²⁰ was used to determine the relationships between individuals; three samples were excluded because they did not connect with the rest of the samples and the relationship of these individuals could not be accounted for in analyses. Samples with genetic sex (based on X chromosome heterozygosity rates) contrary to reported sex were excluded from analyses (16 samples). Sex mismatches were not correlated with genotyping efficiency or sample quality (DNA source, date of

collection, or degradation). Three samples appeared aberrantly connected in the pedigree based on genotype data and were also excluded.

SNP QC comprised checking call rates and minor allele frequencies (MAF). 76,816 SNPs with call rates < 98% were dropped, along with 206,970 SNPs with MAF \leq 0.05. Due to the relatedness in this dataset we did not test SNPs for Hardy-Weinberg equilibrium. After this extensive QC, 798 samples and 622,812 SNPs were available for analysis. All samples are part of one 4,998-member pedigree with many consanguineous loops. The AGDB provided the pedigree information using an “all common paths” database query with all genotyped individuals.

Statistical Analysis

Association analysis was conducted using the Modified Quasi-Likelihood Score (MQLS)²¹ test for all SNPs. The MQLS test is analogous to a chi-square test for case-control data, but adjusts for correlations among individuals based on pairwise kinship coefficients estimated from the pedigree structure. The MQLS test can be run on all samples without dividing the pedigree, which offers a great advantage by incorporating all pedigree data into a single analysis. Allele frequencies adjusted for relationships are also calculated by this program. Association analysis included all individuals in the 4,998-member pedigree, including the 798 genotyped individuals (31 affected, 123 unaffected, 647 unknown individuals). To test the validity of the MQLS test in our pedigree, we performed simulation studies using this same pedigree structure and null data to assess the type 1 error rate using MQLS for association. The genomic inflation factor for this analysis is 1.04. Type 1 error rates were not inflated (Cummings et al., submitted).

Linkage analyses were run using Merlin for autosomal chromosomes and MINX (Merlin in X) for the X chromosome.²² Due to the large size and substantial consanguinity of the pedigree, it was essential to cut the pedigree into smaller sub-pedigrees that were computationally feasible to analyze. To do this we used PedCut²³ to find an optimal set of sub-pedigrees with a bit size limit of 24 and a maximal number of subjects of interest in each pedigree. This procedure resulted in 10 sub-pedigrees (261 individuals, 85 genotyped) for analysis with an average of 8.5 genotyped individuals (3 genotyped affected) per sub-pedigree. (**Table 1**) Parametric two-point heterogeneity logarithm of the odds of linkage (HLOD) scores were computed using affecteds-only autosomal dominant and recessive models. Disease allele frequency was estimated at 1% under the dominant model and 20% under the recessive model. Under the dominant model, penetrances of 0 for no disease alleles and 0.0001 for one or two disease alleles were used. For the recessive model, penetrances of 0 for zero or one disease allele and 0.0001 for two disease alleles were used.

Table 1 Number of individuals by sub-pedigree in linkage analyses

Sub-ped	# genotyped (aff/unaff/unk)	# not genotyped (aff/unaff/unk)
1	3/8/2	0/1/2
2	3/3/1	1/1/12
3	3/6/0	0/0/12
4	3/4/1	0/0/17
5	3/4/1	0/0/14
6	3/4/1	0/0/23
7	3/5/2	0/0/23
8	3/4/1	0/0/19
9	3/3/1	0/0/27
10	3/4/0	0/0/24

The number of SNPs and samples in our dataset prevented us from running the entire dataset in multipoint linkage analysis due to the complexity of the pedigree and extensive computational time. Regions showing evidence for linkage, i.e. containing at least one two-point HLOD ≥ 3.0 , or with evidence for linkage (two-point HLOD ≥ 2.0) and association (MQLS $p < 1 \times 10^{-4}$) were followed up with parametric multipoint linkage analysis (also using Merlin). For the multipoint analyses, a seven megabase region surrounding each significant SNP(s) was used. If a clear LOD score peak was not observed in the results (e.g. the maximum LOD score was at one end, or the peak did not drop enough to define a 1-LOD-down support interval), we widened the region until we could define the complete peak. SNPs were pruned for linkage disequilibrium (LD) in each region so all pair-wise r^2 values were < 0.16 between SNPs.²⁴ The LD from the HapMap CEPH samples (parents only) was used for pruning. Initially, we chose to use this population for pruning because we were concerned that the consanguinity of the pedigree would cause us to overestimate LD between the SNPs and, subsequently, over-prune the data. Because the HapMap CEPH samples may not be an exact representation of LD in our Amish population, we further tested pruning using the data from this Amish dataset, but no significant difference was seen (data not shown). Because linkage analyses can be affected when breaking larger pedigrees into a series of smaller ones, we performed simulation studies assuming no linkage (e.g. null distribution) and using the same large pedigree structure and the same pedigree splitting method. We determined empirical cut-offs for significance in our linkage studies to maintain a nominal type I error rate. We found only 2.5% of the multipoint linkage scans generated a maximum HLOD > 3.0 (Cummings et al., submitted). Possible sub-haplotypes and co-inheritance were determined manually and using Merlin. HaploPainter²⁵ was used to draw sub-pedigrees with associated haplotypes.

Computations were performed using either the CHGR computational cluster or the Advanced Computing Center for Research and Education (ACCRE) cluster at Vanderbilt University. All map positions are listed in megabases (Mb) and refer to NCBI36/hg18, March 2006 positions.

CHAPTER III

RESULTS

Association Analysis

622,812 SNPs were analyzed. No SNPs met a genome-wide significance cutoff of $p < 5 \times 10^{-8}$. (**Figure 1**) However, 70 SNPs representing 35 regions were identified at $p < 1 \times 10^{-4}$ across 16 chromosomes. 16 of the 35 regions had at least two SNPs significant at this level. The three most significant SNPs are located on chromosomes 10q22.1 (*COL13A1*), 11q21 (*CCDC82*), and 15q25.1 (*TMC3*). (**Table 2**) All three SNPs are intronic.

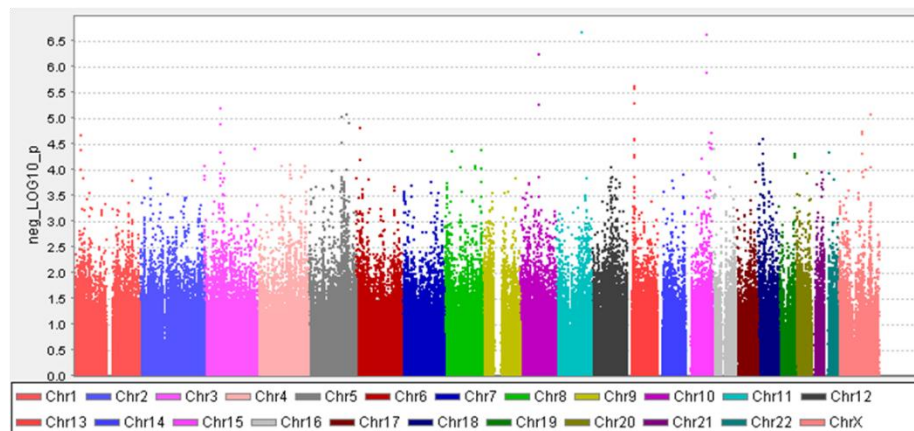


Figure 1 MQLS association results

Table 2 MQLS top results

Chr	SNP	Map (Mb)	MQLS p-value	Gene	Minor Allele	MAF Aff	MAF Unaff	MAF Ov
11q21	rs7118648	96.11	2.16×10^{-7}	CCDC82	G	0.21	0.03	0.03
15q25.1	rs3935740	81.63	2.31×10^{-7}	TMC3	T	0.29	0.08	0.08
10q22.1	rs17497526	71.58	5.50×10^{-7}	COL13A1	G	0.42	0.15	0.15

Linkage analysis

Five SNPs had a two-point HLOD > 3.0, all under a recessive model. (**Table 3**) All SNPs had an alpha = 1. The highest HLOD (3.67) was seen on chromosome 5q23.2 in an intron of the *MEGF10* gene, with a minor allele frequency (MAF) adjusted for relatedness of 0.08 in unaffecteds and 0.27 in affecteds. Two other SNPs in this gene had an HLOD \geq 2.5. Multipoint linkage analysis resulted in a peak HLOD of 1.81.

Table 3 Top linkage (two-point) results

Chr	SNP	Map (Mb)	HLOD (DOM)	HLOD (REC)	Gene	Minor Allele	MAF Aff	MAF Unaff	MAF Ov
5q23.2	rs17165041	126.70	2.51	3.67	MEGF10	G	0.28	0.08	0.08
9q21.33	rs1439054	86.46	2.44	3.01	-	G	0.26	0.10	0.09
10p14	rs2186063	10.53	2.29	3.48	-	T	0.46	0.18	0.18
10p12.31	rs1926693	22.47	2.11	3.14	-	G	0.16	0.06	0.06
19q13.12	rs16970293	40.59	2.80	3.57	LOC100128682	C	0.15	0.04	0.04

The next highest two-point HLOD was for rs16970293 on chromosome 19q13.12 in a non-coding gene (*LOC100128682*). Multipoint linkage analysis in this region resulted in a peak HLOD of 1.95 (recessive model). Other significant SNPs were located on chromosomes 9q21.33, 10p14, and 10p12.31, all in intergenic regions. The multipoint HLOD for chromosome 9 was less than 1.0, suggesting further investigation was unlikely to uncover new knowledge about PD. However, multipoint analysis of the regions on

chromosome 10 showed a strong linkage peak (dominant HLOD 4.35). (**Figure 2c, Table 4**)

Previously we identified linkage peaks in this population on chromosomes 6, 19, 21, and 22.¹³ In the current dataset with additional information, the two-point HLODs for these regions were between 1.5 and 1.99, and they did not meet our criteria for follow-up. However, because of the previous implication of these regions, we ran multipoint analyses. We did not see further evidence for linkage on chromosomes 19 and 22, but we did observe increased HLOD scores on chromosomes 6 (dominant HLOD 4.02) (**Figure 2b, Table 4**) and 21 (dominant HLOD 2.83).

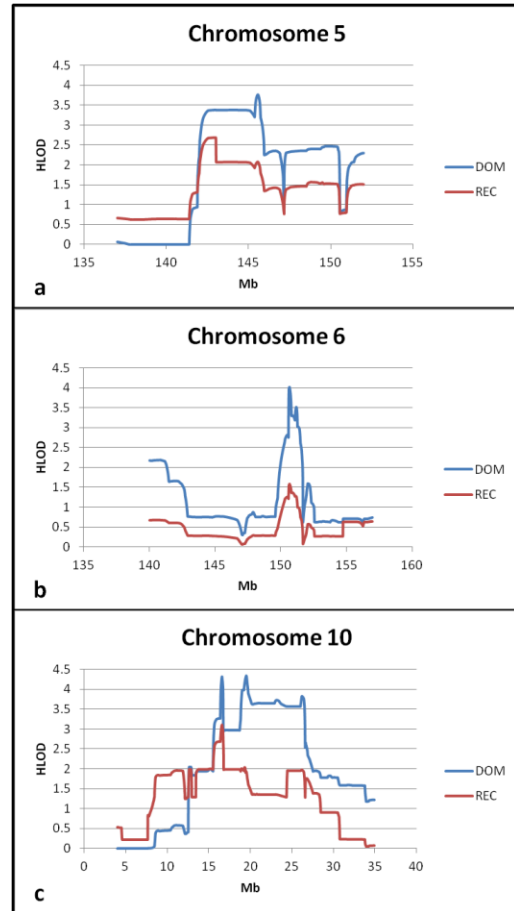


Figure 2 Multipoint HLOD scores for significant regions

Table 4 Most significant multipoint linkage results (HLOD > 3)

Chr	Map (Mb)	Peak HLOD	Model	Alpha	1-LOD down SI (Mb)
5q32	145.56	3.77	DOM	0.69	(142.08, 146.89)
6q25.1	150.70	4.02	DOM	0.69	(150.59, 151.35)
10p12.33	19.50	4.35	DOM	1.00	(16.40, 26.55)

Overlap

To integrate the knowledge gained from performing linkage and association analyses, we looked at the overlap between the two. Six SNPs in five regions showed evidence of linkage (two-point HLOD ≥ 2.0) and association (MQLS $p < 1 \times 10^{-4}$). (**Table 5**) Alpha values for the HLOD scores were equal to one. Two regions are in genes (*FHIT*, *MEGF10*), the others are intergenic. Multipoint linkage analyses were run for each of these regions. The region on chromosome 5q23.2 was analyzed as described above. Regions at 3p14.2, 4q34.2, and 18q11.2 showed no further evidence for linkage based on multipoint analysis. However, chromosome 5q31.3 showed greater evidence for linkage with an HLOD of 3.77 under a dominant model. (**Figure 2a, Table 5**)

Table 5 Overlapping linkage (two-point) and association results

Chr	SNP	Map (Mb)	HLOD (DOM)	HLOD (REC)	MQLS p-value	Gene	Minor Allele	MAF Aff	MAF Unaff	MAF Ov
3p14.2	rs41355450	60.09	2.19	2.62	4.56×10^{-5}	FHIT	C	0.33	0.12	0.12
4q34.2	rs6848215	177.72	1.55	2.35	8.16×10^{-5}	-	A	0.77	0.47	0.46
5q23.2	rs17165041	126.70	2.51	3.67	2.97×10^{-5}	MEGF10	G	0.28	0.08	0.08
5q23.2	rs17673147	126.70	2.04	2.71	9.48×10^{-6}	MEGF10	A	0.23	0.06	0.07
5q31.3	rs17403174	142.93	1.66	2.10	9.59×10^{-5}	-	C	0.26	0.09	0.09
18q11.2	rs299238	18.56	1.55	2.28	4.67×10^{-5}	-	T	0.75	0.45	0.45

CHAPTER IV

DISCUSSION

The region on chromosome 5q31.3 has been linked to PD in several previous studies, although this study is the first to implicate this region in the Amish population. It was initially identified in an analysis of 174 families by Scott et al in 2001²⁶, and this is the seventh study that has implicated this region since that time.²⁷⁻³² Foroud et al combined samples from two of these studies into one dataset for a more powered linkage analysis and analyzed 20 microsatellite markers across 79 cM in this region and found no additional evidence of linkage.³³ However, these two studies each identified the 5q peak *after* removing families with other PD-linked loci (*parkin*, chromosome 2)^{27,32}, but the combined study appears to have used all families and did not condition for either of these two loci. Association studies published to-date have not identified a marker in this region.^{34,35} In light of previous analyses of this region, we propose that a more in-depth study of this region in this Amish population and in other familial datasets may prove enlightening. There are many genes in this region. Of particular interest is synphilin-1 in this region³⁶⁻³⁸, which encodes a protein that interacts with alpha-synuclein, the main component of Lewy bodies. No study has conclusively identified an allele significantly associated with PD in this gene. However, most studies have analyzed synphilin-1 only for idiopathic PD; it is possible it could play a role in familial PD.

The most significant linkage region we identified was on chromosome 10p12.31. There are several candidate genes in this region: *RAB18*, which has been linked to Warburg Micro syndrome, a developmental disorder with brain abnormalities³⁹,

SLC39A12, a zinc transporter; and *ITGA8*, an integrin receptor expressed in the brain. *ITGA8* was recently associated with PD in a large meta-analysis.⁹

This region on chromosome 10 was also identified in an earlier linkage analysis we performed using microsatellite markers in a subset of the current dataset.¹⁴ It did not show up in the initial two-point genome-wide linkage scan we performed here, but this may be due to variances in coverage. The current study is the most in-depth of the studies we have performed for PD in this population.

The region on chromosome 6 was only identified in multipoint linkage in this study because it was previously implicated in the Amish.¹³ This suggests that there may be other linked loci in this population that we missed because the two-point HLOD scores were not high enough to meet our criteria for follow-up. Ideally, we would analyze all markers across the genome that we have genotyped using multipoint linkage analysis. At the current point in time, however, this is not feasible due to time and computational requirements.

Table 6 Peak LOD scores by sub-pedigree for the most significant multipoint linkage regions

Chr	Sub-ped 1	Sub-ped 2	Sub-ped 3	Sub-ped 4	Sub-ped 5	Sub-ped 6	Sub-ped 7	Sub-ped 8	Sub-ped 9	Sub-ped 10
5	0.2904	-1.4653	0.0437	2.1626	1.5572	-0.9193	-0.1998	0	1.1993	0.9418
6	0.2862	0.6731	-0.2512	-0.9409	-0.1427	-0.9279	1.078	0.8798	3.0017	0.9414
10	0.2904	-0.0658	1.45	2.1581	-0.143	0.4807	1.1975	0	1.3069	-0.4649

Of the 10 sub-pedigrees we analyzed in multipoint linkage analysis, between three and five showed evidence for linkage ($LOD \geq 0.5$) in each of the four significant regions (**Table 6, Figure 3**). While there was some overlap, the set of sub-pedigrees linked to each region varied, and two sub-pedigrees did not link to any of the regions (sub-pedigrees one and six). This may be the result of the relatively small size (and

hence power) of the sub-pedigrees, or a greater level of heterogeneity for PD loci in the Amish than previously suspected. Drawings of sub-pedigrees 4 and 9 with possible haplotypes at the peak HLOD scores on chromosomes 5 and 6, respectively, are shown in supplementary data (Figures 4, 5).

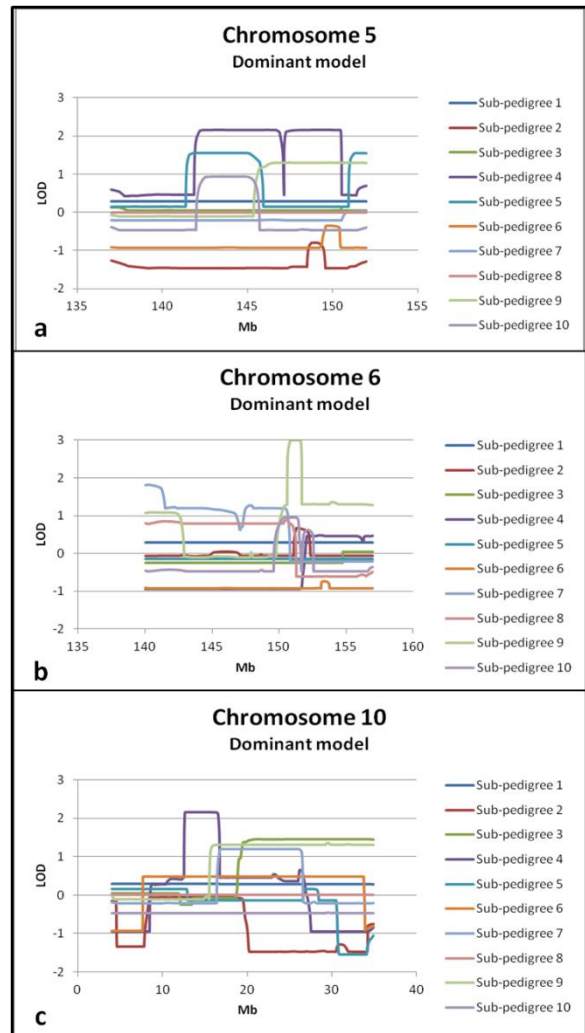


Figure 3 LOD scores by sub-pedigree for with multipoint HLOD > 3.0

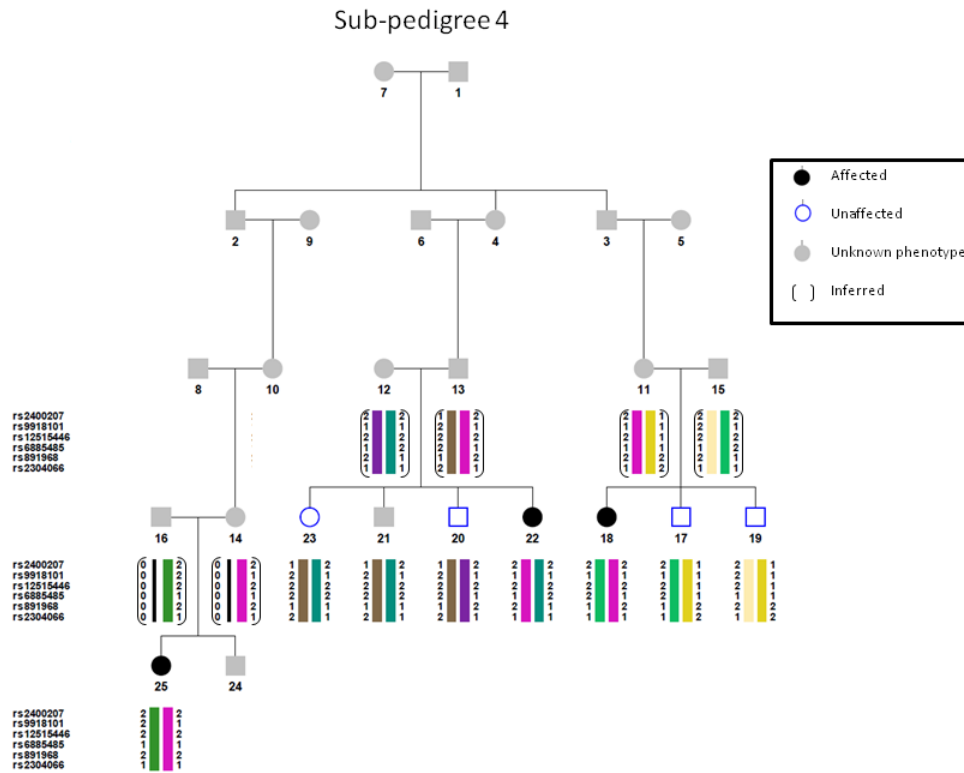


Figure 4 Possible haplotypes and co-inheritance pattern on chromosome 5

Sub-pedigree 4 is shown with possible haplotype combinations for the peak region of co-inheritance on chromosome 5. The six SNPs in this haplotype are at the location of the maximum HLOD score. Genders have been randomized to protect privacy.

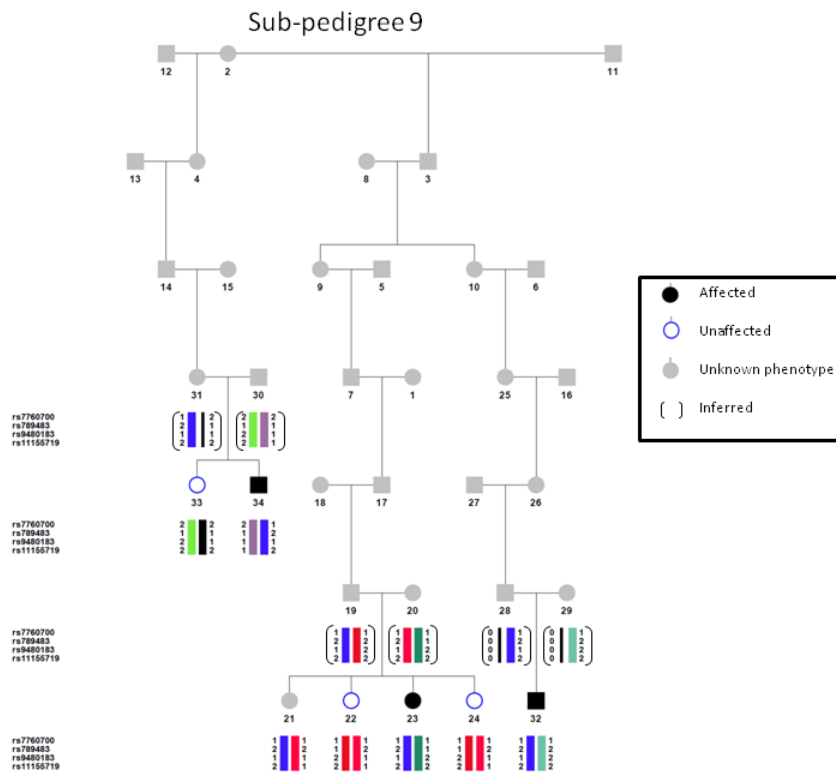


Figure 5 Possible haplotypes and co-inheritance pattern on chromosome 6

Sub-pedigree 9 is shown with possible haplotype combinations for the peak region of co-inheritance on chromosome 6. The four SNPs in this haplotype are at the location of the maximum HLOD score. Genders have been randomized to protect privacy.

CHAPTER V

CONCLUSIONS

Simulation studies suggest the HLOD scores we have observed on chromosomes 5, 6, and 10 are unlikely to be false positives. In each case, there is additional evidence that a true locus could exist in that region. Although we were somewhat surprised to find significant evidence of linkage to multiple loci, we are encouraged that the strong linkage signals represent true loci. We suggest that this population is a prime candidate for follow-up in these regions due to the homogenous environment and a more homogenous genetic background than the general population. These benefits and the large size of the family studied may allow us to more easily detect and decode a true effect in these regions. We suggest an in-depth study of the Amish population particularly to investigate the importance of chromosome 5q31 as a risk locus, as this is a more genetically and environmentally homogenous study population and may lead to more conclusive decisions in this region.

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