Published in final edited form as:

Ann Hum Genet. 2011 May ; 75(3): 351–358. doi:10.1111/j.1469-1809.2011.00643.x.

A Genome-wide Linkage Screen in the Amish with Parkinson Disease points to Chromosome 6

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Summary

Parkinson disease (PD) is a common complex neurodegenerative disorder with an underlying genetic etiology that has been difficult to dissect. Although some PD risk genes have been discovered, most of the underlying genetic etiology remains unknown. To further elucidate the genetic component, we have undertaken a genome-wide linkage screen in an isolated founder population of Amish living in the Midwestern United States. We performed tests for linkage and for association using a marker set of nearly 6,000 single nucleotide polymorphisms. Parametric multipoint linkage analysis generated a LOD score of 2.44 on chromosome 6 in the *SYNE1* gene, approximately 8 Mbp from the *PARK2* gene. In a different region on chromosome 6 (~67 Mbp from *PARK2*) an association was found for rs4302647 ($p < 4.0 \times 10^{-6}$), which is not within 300 kb of any gene. While the association exceeds Bonferroni correction, it may yet represent a false positive due to the small sample size and the low minor allele frequency. The minor allele frequency in affecteds is 0.07 compared to 0.01 in unaffecteds. Taken together, these results support involvement of loci on chromosome 6 in the genetic etiology of PD.

Introduction

Parkinson disease (PD) is the second most common neurodegenerative disorder after Alzheimer disease, affecting approximately 120 per 100,000 individuals in the United States, with similar prevalence estimates worldwide (1). Pathologically, PD is characterized by the loss of dopaminergic neurons and the presence of ubiquitin positive, intraneuronal cytoplasmic Lewy bodies in the substantia nigra pars compacta, and is distinct from other parkinsonian syndromes such as progressive supranuclear palsy (PSP), multiple system atrophy (MSA), Lewy Body dementia, and frontotemporal dementia with parkinsonism (FTDP). Clinically, PD is characterized by the asymmetric onset of bradykinesia, resting tremor, muscular rigidity and impaired postural reflexes. The underlying etiology of PD is unknown; however, several environmental factors appear to play a role, with smoking and caffeine suggested to be protective factors and pesticides to be risk factors(2–4).

In addition to environmental risk factors, there is strong evidence for genetic predisposition to PD. The genetic etiology for PD appears to be complex, involving several genes, only a few of which are currently known. Thus far, six genes have been identified and confirmed in rare familial PD cases: *PARK1/PARK4 (SNCA)*, *PARK2 (parkin)*, *PARK6 (PINK1)*, *PARK7 (DJ-1)*, and *PARK8 (LRRK2)*, and *PARK9 (ATP13A2)*(5). Although mutations in these genes have been found to account for fewer than 5% of all PD cases,(6) understanding the biological role of these genes has led to a renaissance in the study of Parkinson disease pathogenesis. Other identified but unconfirmed loci include *PARK3* (unknown gene), *PARK5 (UCH-L1)*, *PARK10* (unknown gene), *PARK 11* (candidate--*GIGYF2*), *PARK12* (unknown gene), *PARK13 (Omi/HTRA2)*, *PARK14 (PLA2G6)*, *PARK15 (FBXO7)*, and *SCA2*(5). Genetic studies conducted on multiplex families in Iceland(7) were the first to identify a locus on 1p32 (*PARK10*).

Candidate gene studies of regions identified through linkage scans have highlighted a number of susceptibility genes to explain the more sporadic forms of PD. Evidence suggests that parkin, *SNCA*, and *LRRK2* have may be involved in both familial and sporadic forms of PD(5). GBA is also a confirmed risk factor in sporadic forms of PD(8;9). In addition, associations to a handful of other genes including *FGF20*(10;11), *MAOB*(12–15), and *MAPT*(16;17) have replicated across multiple studies. Furthermore, other studies have detected associations between PD and mitochondrial haplogroups(18;19). The most recent attempts to identify PD genes have used genome-wide association studies (GWAS)(20–24), two of which have shown genome-wide significance in the *SNCA* gene and the *MAPT* region(23;24), providing further evidence for their involvement. Other GWAS and follow-up association studies have revealed candidate genes for the *PARK10* locus, with strong evidence for *HIVEP3*(25). Despite this growing list of loci, the vast majority of the PD related genetic risk remains unknown.

An alternative approach that has generated increasing interest for identifying disease genes is the study of isolated populations derived from relatively few founders(26). Genetic studies of population isolates have multiple advantages, including the existence of well-ascertained multi-generational pedigrees descended from fewer founders and spanning fewer generations(27). Moreover, the restricted geographical distribution tends to endow population isolates with greater environmental and phenotypic homogeneity. The Amish communities of Ohio and Indiana represent such a population. By virtue of their strong cultural and religious beliefs, they represent a distinct and biologically isolated community. Studies in the Amish have made significant contributions to understanding the genetics of Mendelian disorders(28). Recent interest has focused on characterizing complex neurodegenerative disorders, including dementia and Parkinson disease, in Amish communities(29;30). We recently conducted a genome-wide microsatellite scan of an extended Midwestern Amish pedigree that implicated several PD candidate regions with significant linkage on chromosomes 3, 7, 10 and 22(31). We sought to confirm these results and to further characterize these regions of interest, by ascertaining additional members of this pedigree and conducting a genome-wide single-nucleotide polymorphism (SNP) based scan with much denser marker representation across the genome.

Materials and Methods

Subjects

Methods for ascertainment were reviewed and approved by the individual IRBs of the respective institutions. Informed consent was obtained from participants, recruited from the Amish communities in Elkhart, LaGrange, and surrounding Indiana counties, and Holmes and surrounding Ohio counties with which we have had established working relationships for over 10 years. A total of 647 genotyped individuals participated in this study: 33 affected

with PD, 99 unaffected, and 551 with an unknown diagnosis (Table 1). This dataset includes the 34 genotyped individuals from our previous linkage screen(31). All individuals diagnosed with parkinsonian syndromes other than PD, such as progressive supranuclear palsy (PSP), were classified as phenotypically unknown for the subsequent analyses.

Clinical Data

The spectrum of clinical symptoms for this pedigree was previously described(31). A standardized interview for PD was conducted by a board-certified genetic counselor 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. Participants were also evaluated for a history of exposure to substances known or suspected to cause parkinsonism, including heavy metals or pesticides. 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. Participants were classified as affected, unaffected or unknown, using published diagnostic criteria based on clinical history and neurologic examination(32). 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 1 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 was evaluated by Hoehn-Yahr staging(33) and the Unified Parkinson disease Rating Scale (UPDRS-motor subscale) UPDRS-III(34). 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))(35). Diagnosis of progressive supranuclear palsy was determined from the NINDS-PSP International Workgroup clinical criteria(36).

Genotyping

DNA samples were prepared from whole blood using standard methods (Puregene, Gentra Systems) and stored using a bar-coded system. Genotyping was done on 647 individuals and 6,008 markers using the Illumina Linkage Panel IVb. Duplicate quality control samples were placed both within and across DNA sample plates and equivalent genotypes were required for all quality control samples to ensure accurate genotyping. Genotyping call rates below 90% threshold (64 SNPs) were subsequently dropped before analysis. Following standard quality control procedures (37;38), 5,944 SNPs were analyzed.

Statistical Analysis

Linkage—Using the Anabaptist Genealogy Database (AGDB)(39;40), all of the familial relationships for the 647 individuals could be connected into a large, complex, 4932-member pedigree with a single founding couple. Because of the large size and substantial consanguinity of the pedigree, we used PedCut(41) to find an optimal set of sub-pedigrees including the maximal number of subjects of interest within a bit-size limit (24 in this study) conducive to linkage analysis. Each of the 12 sub-pedigrees ranged in size from 16 to 36 individuals. The number of genotyped individuals per sub-pedigree ranged from 2 to 13

individuals and included at least two PD affected individuals. Parametric and nonparametric multipoint linkage analysis based on the Lander-Green-Kruglyak algorithm was performed using Merlin (42). Parametric HLOD scores were computed assuming affecteds-only autosomal dominant and recessive models, and nonparametric calculations (LOD*) were computed using the NPL all and pairs statistics. The NPL all statistic looks for allele sharing among all affected individuals in each sub-pedigree, and the NPL pairs uses a pairwise approach to look for allele sharing among all affected pairs of individuals in a pedigree. For the parametric dominant model a disease allele frequency of 0.01 was used and penetrances of 0 for no copies of the disease allele and 0.0001 for one or two copies of the disease allele were used. For the parametric recessive model a disease allele frequency of 0.2 was used and penetrances of 0 for zero or one copy of the disease allele 0.0001 for two copies of the disease allele were used. These penetrances provide for an affecteds-only linkage analysis. SNP allele frequencies were estimated from the entire data set. Computations were done using the Advanced Computing Center for Research and Education (ACCRE) cluster at Vanderbilt University.

Association—To test for association, we implemented a novel approach, the Modified Quasi-Likelihood Score (MQLS) test (43). MQLS is analogous to a chi-square test, the most common approach for case-control data analysis with a binary trait, but it incorporates kinship coefficients to correct for correlated genotypes of all the pedigree relationships. The chi-square distribution is used to generate a p-value. The test was run on all 647 individuals at once without requiring division of the pedigree, and a population frequency of 0.01 was used to infer the probability of an unknown individual to be affected. The MQLS statistic cannot be applied to X chromosome data, which were, therefore, eliminated from the analysis. As with linkage analysis, computations were done using the ACCRE cluster.

Results

Multipoint Linkage

Parametric multipoint linkage analysis detected the greatest LOD score peak on chromosome 6 (152.51 Mbp) in the *SYNE1* gene, reaching a LOD score of 2.44 under the dominant model with a ± 1 -LOD-unit support interval from 143 Mbp to 154 Mbp. Sequence analysis of six randomly selected individuals (three affected and three unaffected) yielded no mutations or exonic deletions in the parkin gene (data not shown).

Other peaks with LOD scores ≥ 2.0 were found on chromosomes 19, 21, and 22 (Figure 1, Table 1). These same regions on chromosomes 6, 19, 21, and 22 contained the highest peaks for both dominant and recessive models of parametric and nonparametric linkage analyses. The dominant peak on chromosome 19 (rs648691) is ~4 Mbp wide (± 1 -LOD-unit support interval), and the dominant peak (which also overlaps a recessive peak) on chromosome 21 covers a ~15 Mbp region (± 1 -LOD-unit support interval). The recessive peak on chromosome 22, which covers the same region as the dominant peak, is ~6 Mbp wide. All other regions with a LOD score ≥ 1.0 are presented in supplemental data.

Association

The most significant MQLS p-value of 4×10^{-6} (Bonferroni threshold p-value = 8.41×10^{-6}) was calculated for rs4302647 on chromosome 6 (94.82 Mbp). Note that this is not in the same location as our reported linkage peak on chromosome 6 (152.51 Mbp). Fourteen additional SNPs had p-values < 0.001 (chr 1, 2, 3, 5, 8, 9, 10, and 22) (Table 2). The SNP on chromosome 22 (rs714027) is ~8 Mbp from our peak linkage region and ~7 Mbp from the linkage peak in our previous linkage screen in this population (31) (Table 3).

Discussion

We have found four regions of moderate linkage on chromosomes 6, 19, 21, and 22. Within the chromosome 6 region, the *PHACTR2* gene at 144 Mbp was found by Maraganore et al. to be nominally significant ($p=1.5 \times 10^{-5}$ in tier 2 results) and subsequently replicated by Wider et al. but with the opposite direction of the effect. Near the region at 160 Mbp lies the *SOD2* gene, shown to be significant in candidate gene studies(44–46), and at 162 Mbp lies the *PARK2* (parkin) gene. To our knowledge no previously replicated PD genes lie within the peak on chromosome 19. The peak on chromosome 21 is adjacent to the *PDXK* gene, which has been suggested as a candidate gene for PD by at least one previous study (47). Several studies have identified the *CYP2D6* gene, which lies under our chromosome 22 peak, as a PD candidate gene(48).

Our top association result (rs4302647) meets a Bonferroni significance criterion. However the minor allele frequencies in affecteds and unaffected were 0.07 and 0.01, respectively, so this result may be sensitive to small sample sizes because of the low minor allele frequencies. The closest gene, *TSG1*, a tumor suppressor gene, is located ~310 kb centromeric to this polymorphism. A PD GWAS study found a nominally significant ($p<5.6 \times 10^{-5}$, OR=1.39) SNP (rs4431442) about 5.5 Mbp away(22) from rs4302647. However, the closest SNP in our study to rs4431442 is rs2894891, at which an MQLS p-value of 0.67 was calculated. None of the other 14 SNPs with p-values <0.001 lie within 1 Mbp of the top results from the five PD GWAS published to date(20–24).

Intuitively using large isolated inbred pedigrees should increase the power to detect genetic effects, even with a relatively small overall number of genotyped individuals. However, it is not easy to quantify the actual power to find an association. Analytical solutions are not available, thus time and computationally infeasible simulation studies would be required to quantify power with this very large and complex pedigree.

While we did find some similarity when comparing our multipoint linkage and MQLS results to our previous microsatellite screen, the correspondence between the two studies is limited (Table 3). Multiple differences between the data sets of these two linkage screens could explain the limited consistency. For instance, the microsatellite screen used a smaller pedigree derived from the current pedigree. To deal with the complexity of the smaller pedigree, marriage loops were broken manually, whereas in the current analysis, PedCut was used to create smaller sub-pedigrees. Splitting of the pedigrees before linkage analysis, while necessary to be able to do the computations, clearly distorts the overall pedigree structure and gene flow pattern that can be analyzed. Our current study also includes more markers (~6,000 SNPs vs. 364 microsatellites), providing better coverage of the genome and allowing for significant results to be found that were missed in the previous screen.

We expected to see better overlap between our most strongly associated MQLS and linkage results. As stated previously, differences in pedigree manipulation lead to differences in linkage results. The same problem could also explain the lack of overlap between our significant linkage results and the strongest MQLS results since MQLS analysis does not require pedigree manipulation. It is also possible that these differences may reflect the different types of signals these methods were designed to detect. Linkage analysis tests for the co-segregation of two or more loci on a chromosome with limited recombination between them. In doing so, linkage analysis detects shared genomic regions between related individuals with the same phenotype. MQLS corrects for the relationships between related individuals and tests for association between an allele and the phenotype (i.e. if an allele is more common among affected individuals compared to unaffected individuals). Strong association results could be due to a protective effect, while affecteds-only linkage analysis

would not be as powerful to detect such an effect. Association analysis is typically more sensitive in detecting smaller genetic effects in the population as a whole, while linkage analysis is better at finding large genetic effects or alleles causing disease in a small number of related individuals.

Our results support the hypothesis that the genetic etiology of Parkinson disease is complex and most likely involves multiple genes. The genetically heterogeneous nature of PD could be due to phenotypic heterogeneity, and, therefore, clustering individuals based on more specific phenotypic characteristics is a worthwhile direction for future studies with larger sample sizes. Our study reveals several potential chromosomal regions and loci for genetic susceptibility for PD requiring further investigation to determine their true significance. Our results provide additional evidence for the involvement of some previously implicated PD genes, with the strongest evidence, from both linkage and association results for potential PD loci on chromosome 6.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the family participants and community members for graciously agreeing to participate, making this research possible. This work was funded by a grant from the Michael J. Fox Foundation and NIH grants AG019085 (JLH, MPV) and AG019726 (WKS). Additional work was performed using the Vanderbilt Center for Human Genetics Research Core facilities: the Genetic Studies Ascertainment Core, the DNA Resources Core, and the Computational Genomics Core.

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Figure 1.
Chromosomal views of the multipoint linkage results of chromosomes containing the highest LOD score peaks

Table 1

Dataset description for this study

	Number	Male(Female)	Average Age of Onset	Average Age of Exam
Affecteds	33	22(11)	56	76
Unaffecteds	99	44(55)	-	76
Unknowns	515	228(286)	-	79

Highest LOD score (≥ 2.0) peaks for multipoint linkage analysis (Region determined by +1-LOD-unit support interval), locations based on NCBI Build 36.1

Table 2

				Parametric		Nonparametric			
Chr	Map (Mpb)	SNP	Gene	DOM	REC	Npall	Nppairs	Region	PD Genes in Region
6	152.51	rs718527	SYNE1	2.44	1.05	1.60	1.38	143-154	PHACTR2
21	32.16	rs1041756	-	2.44	2.00	1.77	1.66	25-40	
19	55.38	rs648691	-	2.41	1.78	1.82	1.67	53-57	
22	42.65	rs929090	-	1.89	2.17	1.94	1.88	37-43	CYP2D6

Table 3

Most strongly associated p-values from MQLS analysis for association to PD with minor allele frequencies calculated by MQLS to adjust for pedigree relationships, based on dbSNP build 130

Chr	SNP	Map (Mbp)	Minor Allele	Overall Minor Allele Frequency	Affecteds Allele Frequency	Unaffecteds Allele Frequency	MQLS p-value
6	rs4302647	94.82	T	0.01	0.07	0.01	4.00×10^{-6}
5	rs903391	43.2	C	0.23	0.46	0.23	6.50×10^{-5}
14	rs799474	34.59	C	0.44	0.16	0.45	8.60×10^{-5}
3	rs1516348	0.18	C	0.44	0.70	0.44	9.90×10^{-5}
9	rs1412340	31.61	A	0.23	0.44	0.22	3.52×10^{-4}
12	rs2070628	123.92	C	0.0008	0.0101	0.001	4.97×10^{-4}
2	rs1978371	15.6	C	0.37	0.63	0.36	5.34×10^{-4}
22	rs714027	28.9	C	0.47	0.71	0.46	5.86×10^{-4}
1	rs1578215	17.86	A	0.11	0.27	0.10	5.94×10^{-4}
8	rs2162229	81.21	A	0.41	0.17	0.41	6.08×10^{-4}
8	rs717340	81.21	C	0.40	0.17	0.41	7.39×10^{-4}
10	rs4751909	116.19	A	0.39	0.16	0.39	9.47×10^{-4}