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A Genome-wide Scan in an Amish Pedigree with Parkinsonism

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Summary

The identification of familial Parkinson Disease (PD) genes is yielding important molecular pathogenetic insights. In an effort to identify additional PD genes, we studied an eight generation Amish pedigree with apparent autosomal dominant parkinsonism with incomplete penetrance. Phenotypic variability ranged from idiopathic PD to progressive supranuclear palsy (PSP), with the average age at onset 53 years (range of 39 to 74 years). We identified markers on chromosome 3 and 7 that were significant at a genome-wide level by parametric and nonparametric criteria, lod > 3 and non-parametric P-value <0.10, respectively. We also identified markers on chromosomes 10 and 22 with lod > 3. These data suggest that parkinsonism in this pedigree is genetically complex, with contributions from several loci.

Keywords

Parkinson's disease; progressive supranuclear palsy; parkinsonism; genetic linkage; Amish; population isolate

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, affecting approximately 120 per 100,000 individuals in the United States, with similar prevalence estimates worldwide (de Lau & Breteler 2006). The cause of PD is unknown, although older age and smoking habit appear to be established risk factors. 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), and frontotemporal dementia with parkinsonism (FTDP). Clinically, PD is characterized by the asymmetric onset of bradykinesia, resting tremor, muscular rigidity and later, impaired postural reflexes. While some clinical features help distinguish one form of parkinsonism from another, e.g., symmetric impairment, lack of resting tremor, early cognitive or behavioral changes, dysautonomia, and supranuclear palsy,

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Author Contributions Stephen Lee co-developed the project, conducted the ascertainment and genomic screen, and wrote the manuscript. Jacob McCauley and Deborah Murdock contributed to the analysis of the pedigree and microsatellite marker data. Yuki Bradford performed the genomic screen data analysis. Amy Crunk performed the ascertainment and maintained the pedigree data. Lynne McFarland assisted in the ascertainment. Lan Jiang performed data analysis. Tao Wang conducted the sequencing analysis. Nathalie Schnetz-Boutaud assisted in conducting the genomic screen. Jonathan Haines co-developed the project and provided overall guidance.

there is substantial overlap between each syndrome (Jankovic & Tolosa 2007), (Gelb, Oliver, & Gilman 1999).

A strategy for understanding the molecular pathogenesis of PD has been the identification and study of causative genes in rare families (Eriksen, Wszolek, & Petrucelli 2005). Thus far, seven genes have been identified in familial PD cases: α -synuclein (PARK1, PARK4), Parkin (PARK2), UCH-L1 (PARK5), PINK1 (PARK6), DJ-1 (PARK7), LRRK2 (PARK8), and ATP13A2 (PARK9) (Hardy et al. 2006), (Ramirez et al. 2006). Genetic studies conducted on multiplex families in Iceland, as well as smaller multiplex families from North America, have identified contributions from the *MAPT* gene that encodes *tau*, as well as additional loci on 1p32 (PARK10) and 2p36 (PARK11) (Scott et al. 2001), (Hicks et al. 2002), (Pankratz et al. 2002).

Advances in genetic approaches has generated increasing interest in the study of isolated populations derived from relatively few founders (Heutink & Oostra 2002). Genetic studies of population isolates have theoretical advantages, including the existence of well-ascertained multi-generational pedigrees, descended from fewer founders and spanning fewer generations (Arcos-Burgos & Muenke 2002). Moreover, the restricted geographical distribution tends to endow population isolates with greater environmental and phenotypic homogeneity. The Amish communities founded in Pennsylvania, Ohio, Indiana and Illinois represent such a population. By virtue of their strong cultural and religious beliefs, they represent a distinct and biologically isolated community, and are well recognized for their contributions to the genetics of Mendelian disorders (Patton 2005). Recent interest has focused on characterizing complex neurodegenerative disorders, including dementia and Parkinson's disease, in these Amish communities (Ashley-Koch et al. 2005), (Racette et al. 2002). In this report, we present the clinical characteristics of an eight generation pedigree of Amish descent and the results from a genomic screen.

MATERIALS AND METHODS

Recruitment and Genealogy

The study protocol was approved by the Institutional Review Board (IRB) of the Vanderbilt University Medical Center. Written, informed consent was obtained from all participants or their legal guardians. Individuals with parkinsonism were initially identified during ascertainment of an ongoing study of Alzheimer dementia in the index Amish community. With permission of individuals and the participating families, genealogical research was conducted using local Amish directories and family genealogy books. Recruitment of additional subjects was conducted by door-to-door interviews and by advertisement in the Amish community newspaper *The Budget*. The pedigree was constructed using Cyrillic 2.1 (<http://www.cyrillicsoftware.com>). The recruitment and ascertainment methods were previously applied successfully in an Alzheimer disease study in the Amish (Pericak-Vance et al. 1996), (Ashley-Koch et al. 2006).

Clinical Data

A standardized interview for PD was conducted by a board-certified genetic counselor on 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 as well as unaffected individuals were personally examined

by a board-certified neurologist with subspecialty training in movement disorders. Participants were classified as affected, unaffected or unclear, using published diagnostic criteria based on clinical history and neurologic examination (Gelb, Oliver, & Gilman 1999). Affected individuals had at least two cardinal signs of PD (resting tremor, bradykinesia, or rigidity) and no atypical features of parkinsonism. Individuals with unclear 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 (Hoehn & Yahr 1967) and the Unified Parkinson's Disease Rating Scale (UPDRS-motor subscale) UPDRS-III (Fahn, Elton, & members of the UPDRS Development Committee 1987). Brain-imaging studies were obtained from medical records. Dementia was assessed by the memory-orientation-concentration test (Short-Blessed Test (SBT)) (Katzman et al. 1983). Diagnosis of progressive supranuclear palsy was determined from the NINDS-PSP International Workgroup clinical criteria (Litvan et al. 1996).

Genotyping

DNA samples were prepared from whole blood using standard methods (Puregene, Gentra Systems) and stored using a bar-coded system. The genomic screen was conducted using a preselected set of 364 markers (almost all high quality tri- and tetra-nucleotide repeats) conducted in multiplex sets of two to three markers per sample. PCR was performed using standard thermal cycler protocols on 20 ng of DNA. PCR product was labeled using either pre-labeled fluorescent primers or post-PCR fluorescent staining (SybrGold, Molecular Probes) and detected employing an Hitachi FMBIO II.

Error Checking and Statistical Analysis

Markers were read in blinded fashion. Consistency checks were performed using PEDCHECK (O'Connell & Weeks 1998) and either rejected based on quality control, or accepted after re-reading and corrections. The complexity of the pedigree prohibited the calculation of exact lod scores. We instead calculated the parametric lod scores and nonparametric p-values, using approximation methods available in SIMWALK2 (Sobel & Lange 1996), (O'Connell & Weeks 1999) running on the Vanderbilt MultiProcessor Integrated Research Engine (VAMPIRE). Even with this approach and reducing the number of marriage loops from a total of 16 to 8, calculations for a single marker took between 3 and 6 hours of computer time. Marker allele frequencies were calculated from the Amish population under study (Table S1). Haplotypes were constructed manually using pedigree information and assigning phase on the basis of minimizing the number of recombinants.

Sequencing

Genomic sequence data was obtained from the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/>). GIGYF2 (TNRC15) mutation screening of exon and exon-intron boundaries containing at least 50 bp of flanking intronic sequences was performed by direct sequencing of PCR products (BigDye Terminator, Applied Biosystems, Inc.) generated from intronic primers designed using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Two affected subjects and one unaffected subject were sequenced and compared to GenBank data using Sequencher software (Genecodes, VA). Primer sequences not previously published are listed in Table S2. Sequencing of the Parkin gene was performed by direct sequencing of PCR products generated from previously published primers sequences except for exon 3 and 9 (Kitada et al. 1998).

Sequencing of reported putative and segregating mutations of the LRRK2 gene (Table S2) was performed under previously described conditions (Zimprich et al. 2004).

RESULTS

Clinical description

We obtained by word of mouth and community announcements the clinical history of approximately 200 individuals over age 65 in an Amish community in middle Ohio. Thirty-three individuals had a history of tremor or parkinsonism. Twenty-four individuals consented to participate in the study. Two were excluded on the basis of clinical findings consistent with essential tremor without parkinsonism. Another individual was excluded on the basis of advanced dementia and parkinsonism without additional clinical history.

Individuals who met the diagnostic criteria for *probable PD* were designated “PD” in Table 1 whereas individuals who met the NINDS-SPSP diagnostic criteria for progressive supranuclear palsy were designated “PSP” in Table 1 (Litvan, Agid, Calne, Campbell, Dubois, Duvoisin, Goetz, Golbe, Grafman, Growdon, Hallett, Jankovic, Quinn, Tolosa, & Zee 1996). Those with Probable PD had asymmetric onset of presenting symptoms that consisted rest tremor or stiffness. Focal dystonia, in the form of blepharospasm, was observed in one individual who was treated with levodopa. Individuals in this group who had been prescribed dopaminergic medications were responsive to medications. In those with prolonged disease duration, dyskinesias and other motor complications often developed. An environmental exposure history was obtained in all participants and most subjects in the community lived according to rural agrarian Amish traditions. One individual reported considerable exposure to pesticides although the specific type was unknown. Parkinsonian symptoms did not develop until many years after this exposure and its progressive nature and levodopa responsiveness suggested that this individual was also affected.

Disease severity as judged by the UPDRS motor subscale, showed a trend toward increasing severity with duration of illness. This trend was observed in the PD group even though the severity was partially masked by the symptomatic response to dopaminergic medications. In the PSP group, disease severity appeared much earlier in the course of illness. The correlation observed between disease severity and duration of illness suggests that parkinsonism is a progressive feature in this pedigree. Dementia was a rare feature in the PD group with only one subject with significant dementia after having the disease for 18 years. Two of the three subjects with probable PSP had Short-Blessed test scores compatible with dementia. None of the affected individuals had prominent visual hallucinations or cognitive fluctuations characteristic of diffuse Lewy body disease (DLB).

Brain imaging was obtained when available. One individual had a motor subcortical stroke that occurred several years after the onset of parkinsonism. A brain MRI of this individual showed no vascular or structural lesions that correlated with parkinsonian symptoms. A CT scan of one affected individual showed mild diffuse atrophy. In none of these cases was there observed selected frontal or temporal lobe atrophy suggestive of fronto-temporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), nor was there selective parietal lobe atrophy suggestive of corticobasal ganglionic degeneration (CBD).

Genealogical information was used to link the individuals into one large complex pedigree spanning eight generations (Figure 1). Where clinical histories were available for deceased individuals, parkinsonism was evident in some. No clear pattern of inheritance was evident. A sub-pedigree could be constructed from the individuals with PSP, tracing to a common ancestor across six generations. Age at onset information was limited for many historical cases and therefore anticipation could not be assessed (Table 1).

Simulation Study

Although simulation studies at best are a rough approximation, we sought to determine if this pedigree contained sufficient power for linkage analysis. The complexity of the pedigree required an approximation algorithm approach (Simwalk2) (Sobel & Lange 1996). It also required using a simplified diallelic marker to allow computation; such a simplification will underestimate power since the overall heterozygosity will be low. Initial lod scores were consistently lower using a recessive rather than a dominant model and was therefore the more conservative model to estimate power. Using 100 data replicates with a disease allele frequency of 0.05, an autosomal recessive inheritance with low penetrance, a marker allele frequency of 0.10 and a 5% recombination, we obtained a mean lod score of 3.65 (max 5.39) with excellent power (>95) to detect a lod score >3.0. The false positive rate was 0. In fact the highest lod score for an unlinked marker was 0.95 among any of the 100 runs.

Analysis of known PARK loci and Parkinson Genes

To evaluate the possibility that PD in this pedigree was linked to known PARK loci, we genotyped microsatellite markers surrounding PARK1 through PARK8 and found that none of the markers returned significant lod scores (*not shown*). We also sequenced the Parkin gene, exons containing putative and segregating mutations in LRRK2, GIGYF2 (TNRC15), the PARK11 candidate gene GIGYF2 (TNRC15) on chromosome 1 and the MAPT gene, frequently associated with progressive supranuclear palsy, did not reveal any mutations that were associated with affection status (Table S2).

Genomic Screen

To test the possibility that genetic loci were linked to parkinsonism in this pedigree, we performed a genome-wide scan at a density of ~10 cM. While the genome scan was being performed, one of the subjects originally identified as PD developed clinical features consistent with PSP. We also ascertained two additional subjects who met diagnostic criteria for possible PSP. It was not clear whether these subjects should be classified as unaffected individuals representing phenocopies of the phenotype, or if they were affected individuals representing a broader phenotype with phenotypic variability. We therefore analyzed the data under two models, classifying PSP as either unaffected or affected (Table 2). Two markers, D7S2195 and D10S2325, showed lod > 3 irrespective of affection status of the PSP individuals. Two additional markers, D3S1764 and D22S425 showed lod > 3 under the model classifying PSP as affected. Using additional nonparametric test statistics optimized for additive models (Entropy and NPL-PAIR), only D3S1764 and D7S2195 were significant ($p < 0.10$) under the model classifying PSP as affected. These results suggest that the markers on chromosome 3 and 7 were the most promising but that the status of the other markers could not be excluded. Multipoint analysis was not possible because of the extreme complexity of the pedigree.

Haplotype analysis was conducted to further characterize the identified regions of interest (Table 3). Interestingly, there was a definite trend where the number of unaffected became much less common as the haplotype moved across the peak marker for chromosomes 3, 10, and 22. The most interesting haplotypes were D3S1764-D3S1744, D10S189-D10S2325-D10S1423, and D22S425-D22S1685 where there were substantial differences in the ratio of affected vs. unaffected. These results suggest that genes contributing to the affected status lie between these respective markers.

DISCUSSION

In this paper, we describe an extended Amish pedigree with apparent autosomal dominant parkinsonism with variable penetrance. Simulation analysis under conservative estimates (autosomal recessive model) indicated that this pedigree was adequately powered to detect

linkage with a low false positive rate. The genome-wide scan identified markers on chromosomes 3, 7, 10 and 22 with parametric lod score > 3 . While false positive lod scores due to pedigree errors are possible, we consider this unlikely. In support of this, Pollin *et al.* found that genealogical information of the Anabaptist Genealogy Database to be reliable, with nearly all deviations from the founder haplotypes (at least in the male lineage) to be consistent with mutation rates rather than errors (Pollin *et al.* 2008). Although less sensitive, nonparametric analysis was significant for markers on chromosome 3 and 7 ($p < 0.10$). Haplotype analysis supported the existence of genes between markers D3S1764-D3S1744, D10S189-D10S1423, and D22S425-D22S1685, respectively, and suggested the contribution of multiple genes in this pedigree.

None of these markers were reported in previous whole genome linkage studies. Scott *et al.* reported a mlod of 1.62 for D3S2460 (135 cM) in levodopa-nonresponsive PD (Scott, Nance, Watts, Hubble, Koller, Lyons, Pahwa, Stern, Colcher, Hiner, Jankovic, Ondo, Allen, Jr., Goetz, Small, Masterman, Mastaglia, Laing, Stajich, Slotterbeck, Booze, Ribble, Rampersaud, West, Gibson, Middleton, Roses, Haines, Scott, Vance, & Pericak-Vance 2001) and Pankratz *et al.* reported a lod 1.3 for D10S1208 (63cM) in a whole genome scan of multiplex PD negative for parkin mutations (Pankratz, Nichols, Uniacke, Halter, Rudolph, Shults, Conneally, & Foroud 2002). Chromosome 22 (D22S685, 37 cM) was previously reported to contain a QTL for age at onset (Li *et al.* 2002). None of these previously reported markers are located near the markers we have identified in this study.

The parkinsonian phenotype in this pedigree includes three cases of PSP. Although usually considered clinically and pathologically distinct from PD, the inclusion of the PSP cases in our analysis did not adversely affect lod scores, and suggests the presence of phenotypic heterogeneity, which has been observed in other genetic forms of parkinsonism, including α -synuclein, LRRK2, and parkin gene mutations (Nishioka *et al.* 2006), (Fuchs *et al.* 2007), (Whaley *et al.* 2006), (Papapetropoulos *et al.* 2006), (Kunishige *et al.* 2004). We did not observe linkage to markers associated with α -synuclein, LRRK2 or Parkin. Sequencing of known segregating LRRK2 mutations and the entire Parkin coding region excludes the role of these genes. Compound heterozygosity for autosomal recessive genes, such as DJ1 and PINK1, could result in an apparent autosomal dominant inheritance pattern on account of cosegregation across several meioses. Although not feasible computationally, a joint linkage analysis across these two regions would address this possibility.

Racette *et al.* described a large Old Order Amish pedigree with multi-incident PD (Racette, Rundle, Wang, Goate, Saccone, Farrer, Lincoln, Hussey, Smemo, Lin, Suarez, Parsian, & Perlmutter 2002), (Racette *et al.* 2006). To our knowledge, our pedigree is not related to any members of this pedigree. Initial screening of their pedigree also suggested that genetic factors are likely to be responsible for PD in their population but the results of a genomic screen have not been reported. Interestingly, ascertainment of other Amish communities have revealed considerable variation in the prevalence of PD, suggesting the possibility of familial clustering and genetic risk for PD in this Amish community (Scott, W., personal communications). Due to social and cultural beliefs, autopsy studies are rarely conducted in this population, thus pathological confirmation has been difficult to obtain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* 2004;vol. 44(no 4):601–607. [PubMed: 15541309]

Figure 1.

Pedigree of extended family with parkinsonism. Arrowhead denotes the proband. Single asterisks (*) denote individuals included in the genomic screen. Double asterisks (**) denote PSP. Shaded figures represent affected individuals confirmed by examination and/or medical records. Not all unaffected individuals are displayed. Diamonds denote de-identified gender status. (Lange & Lange 2004)

Table 1
Clinical Features of subjects included in the genomic screen
 Individuals with essential tremor or with only one clinical feature of parkinsonism were excluded.

ID	Clinical Diagnosis ¹	Age at Exam	Age at Onset	Disease duration	Clinical features ²	Hoehn-Yahr ³	Notes
1	PD	79	55	24	RT, BK, Rg, As, LDR	III	Significant Pesticide/herbicide exposure
2	PD	66	47	19	RT, BK, Rg, As, LDR	IV	Proband. CT head showed mild diffuse cerebral atrophy
3	PD	71	53	18	RT, BK, As, LDR	III	mildly impaired upgaze, blepharospasm noted
4	PD	71	53	18	BK, Rg, As, LDR	IV	
5	PD	74	57	17	RT, BK, Rg, As	IV	
6	PD	73	58	15	RT, BK, Rg, As, LDR	III	Onset preceded recent stroke by many years
7	PD	64	53	11	RT, Rg, As, LDR	III	
8	PD	57	50	8	RT, Rg, As, LDR	I	
9	PD	79	73	6	As, BK, LDR, Rg	II	
10	PD	80	75	5	RT, BK, Rg, As, LDR	II	
11	PD	70	65	5	RT, BK, Rg, As	II	
12	PD	71	68	3	BK, Rg, LDR	II	
13	PSP	79	68	11	RT, BK, Rg, As	n/a	Pseudobulbar affect
14	PSP	68	63	5	BK, Rg, PI	n/a	Downgaze paresis
15	PSP	63	61	2	BK, Rg, PI	n/a	Initially included as Probable PD but developed downgaze paresis

¹ PD = Parkinson's disease, PSP = progressive supranuclear palsy

² RT = resting tremor, BK = bradykinesia, Rg = rigidity, LDR = levodopa responsive, As = asymmetry of onset

³ Hoehn and Yahr scores were obtained in the off status when possible.

Table 2

Genomic screen results

PSP as unaffected						
Marker	Map (cM)	Parametric LOD scores		Empirical P-values of nonparametric marker allele sharing statistics		
		AD ¹	AR ²	BLOCKS ³	MAX-TREE ⁴	ENTROPY ⁵
D3S1764	153	2.81	2.13	0.228	0.315	0.178
D7S2195	155	3.50	2.22	0.154	0.247	0.116
D10S2325	33	4.09	1.99	0.256	0.365	0.209
D22S425	14	1.95	1.06	0.370	0.531	0.320
PSP as affected						
Marker	Map (cM)	Parametric LOD scores		Empirical P-values of nonparametric marker allele sharing statistics		
		AD ¹	AR ²	BLOCKS ³	MAX-TREE ⁴	ENTROPY ⁵
D3S1764	153	3.60	2.42	0.123	0.083	0.065
D7S2195	155	3.88	2.63	0.110	0.153	0.073
D10S2325	33	3.09	1.84	0.246	0.220	0.172
D22S425	14	3.06	1.32	0.328	0.390	0.266

¹ Parametric and nonparametric linkage analysis results from SIMWALK2 (Lange and Lange, 2004)

² Parametric and nonparametric linkage analysis results from SIMWALK2 (Lange and Lange, 2004)

³ Parametric and nonparametric linkage analysis results from SIMWALK2 (Lange and Lange, 2004)

⁴ Parametric and nonparametric linkage analysis results from SIMWALK2 (Lange and Lange, 2004)

⁵ Parametric and nonparametric linkage analysis results from SIMWALK2 (Lange and Lange, 2004)

⁶ Parametric and nonparametric linkage analysis results from SIMWALK2 (Lange and Lange, 2004)

¹ Parametric linkage analysis under an autosomal dominant model

² Parametric linkage analysis under an autosomal recessive model

³ Nonparametric linkage analysis test statistic optimized for recessive models.

⁴ Nonparametric linkage analysis test statistic optimized for dominant models.

⁵ Nonparametric linkage analysis test statistic optimized for additive models.

⁶ Nonparametric linkage analysis test statistic optimized for additive models.

Table 3
Haplotype analysis
Comparison of the observed haplotype frequency in affected (A) and unaffected (U) individuals and their relative ratio (A/U) where (n) is the maximum number of possible haplotypes being compared.

Marker	Haplotype		A	U	A/U	n
D3S4523	1					
D3S2322	4	4	0.38	0.40	0.93	15
D3S1764		4	0.25	0.17	1.50	19
D3S1744		4	0.21	0.05	4.38	26
D3S1763			0.08	0.10	0.88	29
D7S1804	4					
D7S2202	4	4	0.13	0.10	1.31	26
D7S2195		14	0.21	0.17	1.25	28
D7S3070		4	0.17	0.20	0.83	30
D7S3058			0.04	0.10	0.44	35
D10S1435	3					
D10S189	2	2	0.25	0.31	0.81	17
D10S2325		6	0.13	0.02	5.25	25
D10S1423		3	0.13	0.02	5.25	25
D10S1426		3	0.17	0.12	1.40	19
D22S420	4					
D22S425		6	0.17	0.17	1.00	25
D22S1685		4	0.13	0.02	5.25	18
D22S689		5	0.08	0.07	1.17	14