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A Novel X-linked 4-Repeat Tauopathy with Parkinsonism and Spasticity

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Abstract

The parkinsonian syndromes comprise a highly heterogeneous group of disorders. Although 15 loci are linked to predominantly familial Parkinson's disease (PD), additional PD loci are likely to exist. We recently identified a multi-generational family of Danish and German descent in which five males in three generations presented with a unique syndrome characterized by parkinsonian features and variably penetrant spasticity for which X-linked disease transmission was strongly suggested (XPDS). Autopsy in one individual failed to reveal synucleinopathy; however, there was a significant 4-repeat tauopathy in the striatum. Our objective was to identify the locus responsible for this unique parkinsonian disorder. Members of the XPDS family were genotyped for markers spanning the X chromosome. Two-point and multipoint linkage analyses were performed and the candidate region refined by analyzing additional markers. A multipoint LOD_{max} score of 2.068 was obtained between markers DXS991 and DXS993. Haplotype examination revealed an approximately 20 cM region bounded by markers DXS8042 and DXS1216 that segregated with disease in all affected males and obligate carrier females and was not carried by unaffected at-risk males. To reduce the possibility of a false positive linkage result, multiple loci and genes associated with other parkinsonian or spasticity syndromes were excluded. In conclusion, we have identified a unique X-linked parkinsonian syndrome with variable

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spasticity and 4-repeat tau pathology, and defined a novel candidate gene locus spanning approximately 28 Mb from Xp11.2-Xq13.3.

Keywords

Genetic linkage; Parkinson's disease/parkinsonism; X-linked parkinsonism; X-linked spastic paraparesis; tauopathy

Introduction

The parkinsonian syndromes comprise a heterogeneous group of disorders characterized clinically by postural instability, rigidity, bradykinesia and resting tremor. Pathological features of classic Parkinson's disease (PD) include selective neurodegeneration of dopaminergic neurons in the *substantia nigra* and cytoplasmic Lewy bodies (LBs). Other parkinsonian diseases, such as progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) have predominant tau pathology. Mutations have been identified in a number of PD genes¹, and susceptibility loci²⁻⁸ and potentially protective alleles have been reported^{9, 10}. Other parkinsonian loci are likely to exist.

Linkage regions or genetic variation on the X-chromosome associated with late-onset PD (Xq21-25 and Xp11.23)^{4,11} or with neurodegenerative disorders that present with parkinsonian features (DYT3, Xq13 and FXTAS, Xq27.3)^{12,13}, have been reported. We identified a multi-generational family with a unique syndrome characterized by parkinsonism and variably penetrant spasticity in which X-linked disease transmission was strongly suggested (XPDS). We conducted whole-X-chromosome linkage studies to determine if the X-chromosome susceptibility locus overlaps with existing candidate regions or if a novel X-linked candidate region is responsible for XPDS.

Subjects and Methods

Subjects

Members from a four generation American family of Danish and German ethnic descent with a unique parkinsonian syndrome were evaluated at the Medical Genetics Clinic at the University of Washington (UW) Medical Center or the Neurology Clinics at the Puget Sound Veterans Affairs Hospital (T.D.B. or A.S.) and Oregon Health and Sciences University (J.N.) (Figure 1). Subjects gave informed consent for blood draw and DNA studies (protocols approved by the institutional review boards). Blood samples were obtained from nine family members, including all affected living males.

Neuropathology

Standard gross and microscopic neuropathologic evaluation was performed on the only autopsy case. Histologic stains included hematoxylin and eosin, Luxol Fast Blue, Bielschowsky, and Gallyas. Immunohistologic evaluation used the following antibodies: Ab peptide (6E10, Signet Labs, 1:400), alpha-synuclein (LB509, 1:1000 and syn 303, 1:500, gifts J.Q. Trojanowski), glial fibrillary acidic protein (polyclonal GFAP, Dako, 1:1000), tau (AT8, Endogen, 1:250; PHF-1, gift P. Davies, 1:10; RD3 and RD4, Upstate Cell Signaling Solutions, 1:800 and 1:80 respectively; Tau-2, Sigma, 1:500), SMI-31 (Sternberger Monoclonals, 1:4000), TDP43 (Protein Tech, 1:2000), and ubiquitin (Dako, 1:150).

Genotyping

DNA extracted from leukocytes or B-lymphoblastoid cell lines was used to amplify eighteen microsatellite markers spanning the X chromosome (panel 28 ABI-PRISM Linkage Mapping Set v 2.0, Perkin Elmer) at an average spacing of 10 cM. Alleles were resolved on an ABI PRISM 310 genetic analyzer running GENESCAN 2.1 software and analyzed with GenotyperR (v 2.5). To refine the candidate region, five additional Marshfield genetic markers (Xp: DXS8016, DXS8042 and Xq: DXS1217, DXS1196, DXS1216; Research Genetics Huntsville, AL) were genotyped and haplotypes were constructed¹⁴. Exclusion mapping of autosomal dominant familial spastic paraplegias (SPG) 3A and 4 and *FBXO7*, responsible for a parkinsonian-pyramidal syndrome¹⁵ were performed and analyzed as above.

Statistical analysis

Power analysis was performed with SLINK, a modified program of the LINKAGE package (v. 5.1)^{16, 17}. Two-point, multipoint, and haplotype analyses were performed with GENEHUNTER-IMPRINTING (v.1.3)^{18, 19}. Allele frequencies were obtained from CEPH. Sex-averaged map distances are available from Marshfield (<http://research.marshfieldclinic.org/genetics/>)²⁰.

Sequencing of XPDS candidate genes

XPDS candidate genes in the minimal linkage region were prioritized on the basis of expression patterns, function, or similarity to genes involved in neurodegenerative disorders, and sequenced using DNA from one affected male and one unaffected female carrier (IV-5 and III-12, Figure 1). SNPs were confirmed in affected male II-9 and female carrier III-2 by DNA sequencing. PCR primer pairs were designed to amplify candidate gene exons with at least 100 nt of flanking intronic sequence²¹. PCR products were treated with ExoSAP-IT (USB Biochemical) and sequenced with dye-terminator cycle technology (Big-Dye v. 3.1) on an ABI310 DNA analyzer.

Genes for known parkinsonian syndromes not in the region of the linkage signal were also evaluated. Sequencing of *MAPT*, *PARK2*, *LRRK2* and *SNCA* exons and gene dosage analysis of *PARK2* exons were performed in two affected males and two unaffected obligate carrier females^{21, 22}. The DSC3 single nucleotide polymorphism (SNP) associated with X-linked parkinsonism-dystonia (*DYT3*) was also sequenced²³.

Results

XPDS phenotype

The XPDS disease phenotype presents in the second through fifth decades with an onset range from 14 to 50 years. Age of disease onset varied between generations, with earlier ages of presentation in the most recent generation. Two persons had only a parkinsonian syndrome. Two and possibly three others first showed spasticity, followed by parkinsonian features. The disease followed a slowly progressive course with increasing resting tremor. Affected males in the first generation lived a normal life span.

Clinical characteristics of five affected males are summarized in Table 1. Two brothers (II-2 and II-9) had onset in their 50s of a typical parkinsonian syndrome consisting of cogwheel rigidity, resting tremor, and bradykinesia; neither developed spasticity. Rigidity and resting tremor were mildly asymmetrical in individual II-9 and moderately asymmetrical in individual II-2. Individual II-6, with the non-disease associated haplotype, died at age 82 and was clinically unaffected.

Individual III-8 gave a history that spasticity developed in his early 20s. He had the onset of a parkinsonian syndrome at age 44 with resting tremor, rigidity, and bradykinesia and reported a mild response to l-dopa. He had signs of spasticity with hyperactive tendon reflexes and Babinski responses. An MRI showed mild diffuse atrophy and disproportionate enlargement of lateral and 3rd ventricles compared to the extra-axial CSF spaces.

Individual IV-5 had the onset of spastic paraparesis at age 14. He subsequently developed a parkinsonian syndrome that included resting tremor and bradykinesia. At age 30 a fluoro-DOPA PET scan showed bilateral decreased uptake in the putamen (R>L) with normal uptake in the caudate consistent with PD. He showed a mild response to l-dopa but with prominent dystonic dyskinesias. At age 31 bilateral electrodes were placed in the subthalamic nucleus for deep brain stimulation, which improved motor symptoms, including tremor. A few months later a series of generalized convulsions was controlled with mysoline and have not recurred. He tested negative for mutations in SCA1, 2, 3, 6, 7, and 8 and had normal long-chain fatty acids.

Individual IV-1 had the onset of marked spastic paraparesis at approximately age 26. At age 30 he had a stiff scissoring gait, hyperactive tendon reflexes, ankle clonus, cogwheel rigidity in his arms and resting tremor of the right hand. At age 36, parkinsonian features were more prominent including masked facies, asymmetric resting tremor of hands and feet, and cogwheel in his upper limbs. He had no bradykinesia or retropulsion. He now had bilateral Babinski reflexes as well as the previously noted signs of spasticity.

Neuropathology of XPDS

Neuropathology was performed on individual II-2 who died at age 86. Gross examination revealed a brain weight of 1235 g with mild ventriculomegaly. Microscopic examination revealed neurofibrillary tangle (NFT) pathology limited to the medial temporal lobe, consistent with a Braak stage of III²⁴. NFTs and neurites in the medial temporal lobe were immunopositive for all tau antibodies including RD3 and RD4 (Figure 3). Diffuse A β deposits were observed in the neocortex and limbic system, however neuritic plaques were only seen in the medial temporal lobe. The severity of plaques and tangle pathology was not sufficient to fulfill criteria for Alzheimer's disease (AD)²⁵. There was mild to moderate neuronal loss in the *substantia nigra* with rare NFT pathology and no LBs. Evaluation for β -synuclein failed to reveal pathologic lesions in the brainstem, limbic system, basal ganglia, or neocortex. TDP-43 immunostaining was negative.

Anterior portions of the striatum, including both putamen and caudate, demonstrated plaque-like structures that were Gallyas, AT8, PHF-1, and RD4 immunopositive, while RD3 and ubiquitin immunonegative. Notably, in the same case, the limited AD changes observed in the medial temporal lobe were immunopositive with RD3 (Figure 3H). Double immunolabeling with GFAP and AT8 revealed a close association of astrocytes and tau positive processes (Figure 3E), and individual glial cells had AT8 immunopositive processes (Figure 3F). Similar plaque-like structures were not observed in other regions including the neocortex and limbic system. SMI-31 (anti-neurofilament) immunostaining failed to reveal ballooned neurons in the amygdala, hippocampus, basal ganglia, and neocortex.

XPDS linkage analysis

Disease inheritance is consistent with X-linked transmission (Figure 1). All five affected males in three generations are related through unaffected females and none of the three sons of two affected males are affected. Assuming a disease frequency of 0.00001, 90% penetrance, and four alleles of equal frequency, a simulation study estimated a maximum two-point LOD score of 1.81 at recombination fraction, θ , of 0.00. Two-point analysis

obtained a maximum LOD score of 1.46 at DXS993. Multipoint analyses using 18 markers identified a candidate region delineated by DXS1214 (Xp sex averaged location [SAL] 33.54 cM) and DXS990 (Xq SAL 60.62 cM), and provided a maximum LOD score of 2.02. Inclusion of five additional markers narrowed the critical region to ~20 cM defined by DXS8042 (Xp SAL 37.87 cM; Xp11.2) and DXS1216 (Xq SAL 53.58 cM; Xq13.3). The XPDS disease haplotype was not present in the unaffected at-risk male (II-6). A final maximum LOD score of 2.068 was obtained between markers DXS991 and DXS993 (Figure 2). For an X-linked disorder, a LOD score >2 provides significant evidence in favor of linkage, with LOD~2 corresponding to $p \sim .05$.

XPDS critical region

Human genomic sequence for the DXS8042-DXS1216 critical region is available as five contigs (NT_079573, NT_086939, NT_011638, NT_011630, and NT_011669) spanning ~28.5 Mb that includes an ~3 Mb gap encompassing the pericentromeric region. A comprehensive gene retrieval in this region (UCSC Browser March 2006 and NCBI Map Viewer Build 36.1) identified ~370 RefSeq genes, including multiple splice variants per gene. With splice variants represented as a single gene ~200 candidate genes remain, including 29 known disease loci and the G-, melanoma-, and P-antigen-family clusters and a synovial sarcoma breakpoint clusters. In addition, 118 hypothetical genes, twelve microRNAs, and one small nuclear RNA are annotated in the critical region. The observation of earlier onset in more recent generations, one feature of anticipation, raises the possibility of a nucleotide repeat expansion mechanism for XPDS. The XPDS candidate region harbors 174 simple trinucleotide repeats (range 8-90.7 repeat units) where 36 repeats are found in genes (31 intronic, 3 in UTRs and in one exon each in *SHROOM4* and *RBM10*). Disruptions in *SHROOM4* are linked to Stocco dos Santos X-linked mental retardation syndrome. *RBM10* contains an imperfect GGA repeat in exon 3 that is not expanded in individuals III-12 and IV-5 (Figure 1) and no other coding sequence change was detected.

Fifty-eight other candidate genes within the XPDS critical region were excluded due to lack of expression in the brain or classification as disease genes for distinctly different disorders. Seventeen candidate genes were selected on the basis of central nervous system expression and other characteristics (Table 2). Proteins that function within the ubiquitin/proteasome, mitochondrial, or oxidative stress pathways were given high priority. All exons and splice junctions of these genes were sequenced. Polymorphisms in dbSNP were identified in *UBA1* (rs11558783), *USP11* (rs10126669), *SYN* (rs2294219), and *TIMM17B* (rs1128363). A novel A→G nucleotide change encoding an R38Q amino acid substitution in *GRIPAP1* was identified in one carrier female and was not present in either affected male. No other non-polymorphic changes were found.

Candidate gene analysis outside the linkage region

Although a LOD score of 2.068, the maximum possible for this family's structure, provides approximately 95% confidence for the X-chromosome locus we identified, a 5% possibility remains that the disease gene lies elsewhere. Given that the sequence analyses of positional candidates had not yielded a causative mutation, we believed it to be prudent to rule out known genes that cause similar parkinsonian phenotypes. The disease-single-nucleotide change 3 (DSC3) in exon 4 of the multiple transcript system that is diagnostic for X-linked dystonia-parkinsonism (*DYT3*) was ruled out by targeted analysis in affected male IV-5. Several autosomal genetic risk factors for parkinsonism were evaluated by DNA sequencing of the same subject: *PARK2*, *MAPT*, *SNCA*, and *LRRK2*²⁶. No pathogenic mutations were identified in these genes. No dosage variations were identified in *PARK2*. *MAPT* SNP analysis revealed that affected subjects III-8 and IV-5 had H1/H2 haplotypes, while the

remaining family members had H1/H1 haplotypes. Linkage to *FBXO7* and *PLA2G6* (22q12-q13), atlastin (SPG3, 14q11) and spastin (SPG4, 2p22) were excluded.

Discussion

We have described a unique parkinsonian syndrome, XPDS, mapping to Xp11.2-Xq13.3. Affected males present with a neurologic disease in which everyone eventually develops parkinsonian features with variable spasticity. XPDS is unlike idiopathic PD because of prominent spasticity and a lack of LB pathology and is unlike classic hereditary spastic paraplegia because of the co-occurrence of parkinsonian features.

We identified an ~20 cM region on the X-chromosome of shared genotypes among affected males, providing significant evidence for linkage ($p .05$). It is an unlikely alternative that an autosomal locus is responsible for the disease (with reduced penetrance in females) or that the more classic PD cases are disease phenocopies given the transmission pattern in the family of affected males in three generations related through unaffected females and the absence of mutations in genes responsible for three common autosomal parkinsonian disorders. Detailed analyses that ruled out the potential involvement of nine autosomal PD-associated genes strengthens our confidence in the X-chromosome linkage findings; however, until the X-linked disease gene and mutation are identified the possibility of a false positive linkage finding remains.

The XPDS locus does not overlap with any known X-chromosome PD or spasticity locus. Pelizaeus-Merzbacher disease, X-linked adrenoleukodystrophy, the XDP locus^{23, 27}, Fragile-X tremor/ataxia syndrome¹¹ and PARK12²⁸ all map distal to XPDS. Thus, XPDS is a novel parkinsonian syndrome with a unique gene locus.

Pathological findings in the one autopsied case failed to reveal β -synuclein immunopositive inclusions or neurites that are generally observed in PD. However, there were plaque-like structures observed in the striatum that were positive with silver stain, and tau and GFAP immunopositive. These aggregates of tau positive glial processes had the appearance of astrocytic plaques, a frequent finding in CBD²⁹. Consistent with CBD the astrocytic plaque processes were immunopositive with the 4-repeat (4R), but not the 3-repeat (3R), tau antibody suggesting this was a 4R tauopathy. Unlike CBD, we did not observe astrocytic plaques in other brain regions, nor did we observe CBD-associated pathologic changes such as ballooned neurons, neuropil threads in white matter, or oligodendroglial tau pathology. Thus, neuropathologically this appears to be a distinct 4R tauopathy. Similar to CBD and PSP, there was a predominance of the H1 haplotype in this family³⁰.

Two autosomal recessive syndromes share features of both parkinsonism and pyramidal track signs. These are Kufor-Rakeb and a parkinsonian-pyramidal syndrome with mutations in *ATP13A2* and *FBXO7*, respectively^{31, 32}. In addition to different inheritance patterns these disorders have earlier onset and more severe findings than in the present family. They may represent the disorder described by Davison (1954) with one autopsy showing nerve cell loss in the *globus pallidus* and *substantia nigra* without LBs and pallor of the pyramidal tracks³³.

The XPDS critical region contains ~200 candidate genes and 118 hypothetical genes. We selected 17 candidate genes for sequencing, including a ubiquitin-related gene (*UBQLN*), two mitochondrial-related genes (*NDUFB11* and *TIMM17B*), two protein kinases (*CASK* and *PCTK1*), and two G-protein coupled receptors (*GPR34* and *GPR173*). The remaining candidate genes were selected for high CNS expression levels. Within the time frame of this study five candidate genes were published as causing distinctly different diseases (*CASK*, *UBA1*, *PORCN*, *SYN*, and *EFNB1*; Table 2). No disease-associated mutations or

polymorphisms were identified in these genes. Screening of seven additional genes, based on CNS expression is underway including *CRSP2* (cofactor for SP1 transcriptional activation), *DDX3X* (putative RNA helicase), *RGN* (senescence marker protein), *RBM10* (RNA binding motif protein), *USP11* (ubiquitin specific protease), *PPP1R3F* (protein phosphatase inhibitory subunit), and *MAOB*, because of previous linkage studies suggesting it is a PD susceptibility locus³⁴. Although brain-specific micro-RNAs have been implicated in neurodegeneration³⁵, the micro-RNAs that map to the critical region are implicated in erythropoietic, immunologic, and oncogenic pathways and are unlikely to cause XPDS. Our analyses have only ruled out exon deletions for those exons that demonstrated SNP heterozygosity. Array comparative genomic hybridizations may be used to exclude copy number variants and additional screening for triplet repeat expansions and PD-associated SNPs (genome wide association studies) may be employed. Ascertainment of additional XPDS family members may further refine the critical region and aid in identifying the disease gene.

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Figure 1. The four-generation pedigree of the XPDS family

A diagonal line denotes individuals who are deceased. Haplotypes are shown for all pedigree members from whom DNA was obtained. The apparent disease-related haplotype region is boxed. Haplotype markers are listed to the right of the genotype for subject II-9 and approximate distances between markers are listed to the right. Secondary genotyping defined the critical region between markers DXS8042 and DXS1216; DX1216 is located between markers DXS991 and DXS986.

Figure 2. Multipoint linkage analysis performed with GENEHUNTER-IMPRINTING using 23 markers spanning the X chromosome.

Figure 3. Immunostaining characteristics of plaque-like pathology in the striatum of the XPDS autopsied case

AT8, Gallyas, and RD4 (4-repeat tau specific antibody) stain plaque-like pathology in the striatum (A–D). Double immunolabeling with GFAP for astrocytes and AT8 demonstrate close association of glia and tau pathology (E) and double labeling of glial processes (F, arrow). Immunostaining with the 3-repeat tau specific antibody RD3 fails to label this pathology in the striatum (G), although age-associated neurofibrillary tangle and neuritic plaque pathology in the hippocampus was immunopositive (H).

Table 1

Clinical characteristics of the affected XPDS subjects.

Pedigree No.	II-2	II-9	III-8	IV-1	IV-5
Gender	M	M	M	M	M
Age at onset, years Mean, 39.2 ± 16.8 Range, 14–58	54	58	44	26	14
Age: death (D) or current, years: Mean, 59.8 ± 20.3 Range, 36–86	D 86	78	61	36	38
Cogwheel rigidity	+	+	+	+	+
Resting tremor	+	+	+	+	+
Bradykinesia	+	+	+	–	+
Masked facies	+	+	–	+	+
Hyperactive tendon response	–	–	+	+	+
Babinski sign	?	–	+	+	+/-
Medication	l-dopa: 2,000 mg/day	seligiline: 5 mg/day	l-dopa	–	l-dopa
Miscellaneous	Columbia parkinsonism score=22 (70 yo); UPDRS score=16 (76 yo); Moderate memory loss late in disease.	UPDRS score=18 (66 yo); Brain CT age 58: normal.	–	normal MRI (brain/spinal cord)	+ Fluoro-dopa PET; normal brain MRI; seizure disorder; nystagmus; deep brain stimulation.

Table 2

Candidate genes screened in the XPDS critical region.

Gene	Accession #	start-end coordinates	Gene function/DISEASE
GPR34	NM_005300	41433169-41441474	G-protein coupled receptor, highly expressed in brain, up-regulated in activated microglia
FUNDC1	NM_173794	44267848-44287160	Expressed in brain, function unknown
CHST7	NM_019886	46318135-46342781	Sulfotransferase, low levels of expression in brain
NDUFB11	NM_019056	46886562-46889381	NADH dehydrogenase (ubiquinone) 1 beta
PCTK1	NM_033018	46962575-46974336	Protein kinase, highly expressed in brain
TIMM17B*	NM_005834	48635675-48640370	Translocase of inner mitochondrial membrane
KCND1	NM_004979	48703582-48743719	Potassium voltage-gated channel, highly expressed in brain
GRIPAP1*	NM_020137	48715077-48743619	Highly expressed in nervous system, may regulate AMPA receptor distribution
SYP	NM_003179	48931208-48943605	Neuronal integral membrane protein in small synaptic vesicles
TMEM29	NM_014138	52944586-52954308	Transmembrane protein 29, highest expression in brain
GPR173	NM_018969	53095230-53123967	G-protein coupled receptor, highly expressed in brain, super-conserved
UBQLN2	NM_013444	56606797-56610102	N-terminal ubiquitin-like domain C-terminal ubiquitin-associated domain
CASK	NM_003688	41264287-41667212	MENTAL RETARDATION AND MICROCEPHALY
UBA1*	NM_153280	46935142-46959471	SPINAL MUSCULAR ATROPHY, INFANTILE X-LINKED
PORCN	NM_203476	48252314-48264146	FOCAL DERMAL HYPOPLASIA
SYN1*	NM_006950	47316244-47364200	RETT SYNDROME
EENB1	NM_004429	67965555-67982754	CRANIOFRONTONASAL SYNDROME

* Coding sequence single nucleotide polymorphisms identified.