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ARTICLE

PARK11 is not linked with Parkinson's disease in European families

Jürgen Prestel[1,10], Manu Sharma[1,10], Petra Leitner[1], Alexander Zimprich[1], Jenny R Vaughan[2], Alexandra Dürr[3], Vincenzo Bonifati[4,5], Giuseppe De Michele[6], Hasmet A Hanagasi[7], Matthew Farrer[8], Anne Hofer[1], Friedrich Asmus[1], Giampiero Volpe[6], Giuseppe Meco[4], Alexis Brice[3], Nicholas W Wood[2], Bertram Müller-Myhsok[9], Thomas Gasser*[,1] and The European Consortium on Genetic Susceptibility in Parkinson's Disease (GSPD)

[1]*Hertie-Institute for Clinical Brain Research, Department of Neurodegenerative Diseases, University of Tuebingen, Germany;* [2]*Department of Molecular Neuroscience, Institute of Neurology, Queen Square, London, UK;* [3]*INSERM U289 and Department of Genetics, Cytogenetics and Embryology, AP-HP, Salpetriere Hospital, Paris, France;* [4]*Department of Neurological Sciences, La Sapienza University, Rome, Italy;* [5]*Department of Clinical Genetics, Erasmus MC Rotterdam, The Netherlands;* [6]*Department of Neurological Sciences, Federico II University, Naples, Italy;* [7]*Department of Neurology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey;* [8]*Departments of Neurology and Neuroscience, Mayo Clinic Jacksonville, Jacksonville, FL 32224, USA;* [9]*Max-Planck-Institute of Psychiatry, Munich, Germany*

Parkinson's disease (PD) is a genetically heterogeneous disease. Recently, significant linkage has been reported to a 39.5 cM region on the long arm of chromosome 2 (2q36-37; *PARK11*) in North American Parkinson families under an autosomal dominant model of inheritance. We have performed a replication study to confirm linkage to this region in a European population. Linkage analysis in 153 individuals from 45 European families with a strong family history of PD did not show any significant LOD score in this region. Therefore, *PARK11* does not seem to play a major role for familial PD in the European population.
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Introduction

Parkinson's disease (PD) (MIM 168600) is the second most common neurodegenerative disorder after Alzheimer's disease. It affects 1.8% of the individuals, who are 65 years of age and older.[1] It is characterized by bradykinesia, rigidity, resting tremor and postural instability. Pathological hallmarks of PD involve the degeneration of dopaminergic neurons in the substantia nigra pars compacta and the formation of Lewy bodies.[2,3]

While the cause of PD is unknown, there is increasing evidence for a significant genetic component in idiopathic PD. Epidemiological studies showed that the risk of PD is at least doubled in first-degree relatives as compared with controls.[4] To date, six genes and several loci for monogenically inherited forms of PD – which account only for a small fraction of the diseases – have been identified or localized: mutations in the *parkin* gene[5] (*PARK2* (MIM 602544)), in the *PINK1* gene[6] (*PARK6* (MIM 605909)) and in the *DJ-1* gene[7] (*PARK7* (MIM 602533)) cause autosomal recessive early-onset parkinsonism, while missense mutations in the *α-synuclein* gene[8] (*PARK1* [MIM 168601]) and recently duplications and triplications of the wild-type *α-synuclein* locus[9,10] were found in a small number of families

*Correspondence: Professor Dr T Gasser, Hertie-Institute for Clinical Brain Research, Section for Neurodegenerative Diseases, University of Tuebingen, Hoppe-Seyler Str. 3, 72076 Tuebingen, Germany. Tel: +49 7071 29 86529; Fax: +49 7071 29 4839;
E-Mail: Thomas.Gasser@med.uni-tuebingen.de
[10]These two authors contributed equally to the work.
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Table 1 Characteristics of the families

Country	Number of families	Number of sibs: Affected all/genotyped	Number of sibs: Unaffected all/genotyped	% Male	% Female	Mean ± SD: Age at onset (years)
German	19	48/39	37/18	49.6	50.4	59.4 ± 8.3
English	11	35/24	44/24	46.3	53.7	58.3 ± 7.3
French	7	21/17	10/3	47.1	52.9	56.4 ± 12.8
Italian	6	18/14	14/8	47.8	52.2	52.8 ± 16.6
Turkey	2	8/6	18/0	52.0	48.0	61.0 ± 4.2
	45	130/100	123/53	48.2	51.8	57.6 ± 10.5

with autosomal dominant PD. The *UCH-L1* mutation (*PARK5* (MIM 191342)) has been reported in a single German family.[11] Recently, mutations in the *NR4A2* or *NURR1* gene (MIM 601828) were found in families with late-onset PD.[12] In addition, genetic studies have detected linkage to several chromosomal regions which might contain susceptibility loci for PD: *PARK3* (MIM 602404),[13] *PARK8* (MIM 607060),[14] *PARK9* (MIM 606693)[15] and *PARK10* (MIM 606852).[16]

Evidence for linkage to chromosome 2q36–37 (*PARK11* (MIM 607688)) was first detected in a sample of 160 families (170 affected sibling pairs) in a genome-wide screen.[17] An additional study was performed using a subset of the previous, but expanded sample, which included only pedigrees with a strong family history of PD: in an analysis of 65 families (77 sibling pairs) a maximum LOD score of 5.1 at the marker D2S206 on chromosome 2q36–37 was found using an autosomal dominant model of disease transmission.[18] Recently, Pankratz *et al*[19] confirmed their previous results using a further enlarged sample of 85 families (113 sibling pairs) with a strong family history of PD: they again reported a linkage to the 2q36–37 region (LOD score 4.9).

Materials and methods

We have performed a replication study in a set of European sib pair families to verify the linkage at 2q36–37 in a European population. In all, 45 families were selected for this study. We included families with a strong family history of PD, defined according to the same criteria regarding family history as used by Pankratz *et al*: the families had at least four first-, second- or third-degree relatives reported to have PD, or they included an affected sibling pair who also had a parent reportedly diagnosed with PD. Of our 45 families, 15 included at least one affected individual with an age of onset $\leqslant 50$ years. The diagnosis of PD in the index patients was established according to the UK Parkinson's Disease Society Brain Bank criteria.[20] After appropriate informed consent was obtained, blood samples had been drawn from the individuals for DNA extraction. The characteristics of the families are described in Table 1.

Pankratz *et al* reported a significantly linked region between marker D2S126 and D2S125 spanning a distance of 39.5 cM. We have selected only that region for analysis where the highest LOD score was reported. Six markers (D2S2382, D2S126, D2S396, D2S206, D2S338, D2S125) with an average spacing density of 9.4 cM were used for analysis. These six dinucleotide repeat markers with an average heterozygosity of 82% were genotyped on chromosome 2. Marker order and genetic distances between the markers were obtained from the sex-averaged genetic map from Marshfield Genetic Laboratories. PCR amplification was performed for each marker in a 10-μl reaction using 20 ng of genomic DNA, 2 pM of each primer, 0.2 mM of each dNTP, 1 μl 10 × PCR buffer (containing 15 mM $MgCl_2$), 0.5 or 1 mM $MgCl_2$ and 0.3 U of Taq DNA polymerase (Taq PCR Core Kit, Qiagen). Amplification conditions were as follows: preincubation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C or 60°C for 30 s and extension at 72°C for 40 s and final extension for 2 min at 72°C. In all, 1 μl of the PCR product was added to 20 μl of formamide containing the GeneScan-500 ROX size standard. The products were separated by capillary electrophoresis using an ABI PRISM 3100-*Avant* Genetic Analyzer (Applied Biosystems). The genotypes were determined by using GeneScan version 3.7. Mendelian inconsistencies in the genotypic data were checked by using the program PedCheck.[21]

In order to evaluate the power of our sample, a simulation study was performed by using the SLINK program.[22] It showed that our sample size is sufficient for finding significant evidence for linkage, with an average maximum LOD score of $Z = 2.8$. Two-point LOD scores were calculated using the MLINK programme of LINKAGE software package.[23] The mode of inheritance was set as autosomal dominant with a disease allele frequency of 0.005. Marker allele frequencies were based on all individuals genotyped. The penetrance was set at 40% for $\leqslant 50$ years and at 80% for > 50 years of age. The phenocopy rate

Table 2 Results of the parametric and nonparametric analysis: multipoint LOD scores

		LOD score 45 families		*LOD score 36 families*	
Marker	*Distance (cM)*[c]	*MLOD*[a]	*NPL*[b]	*MLOD*[a]	*NPL*[b]
D2S2382	0	−5.78	0.45	−5.8	0.28
D2S126	7.6	−3.01	0.6	−3.61	0.48
D2S396	19.4	−5.48	0.24	−3.39	0.27
D2S206	27.29	−7.01	0.13	−5.01	0.18
D2S338	37.04	−3.38	0.15	−2.74	0.18
D2S125	47.13	−6.13	0.07	−4.71	0.08

[a]MLOD = multipoint maximum parametric lod score.
[b]NPL = multipoint nonparametric linkage score, S_{all}.
[c]Distances in Haldane cM.

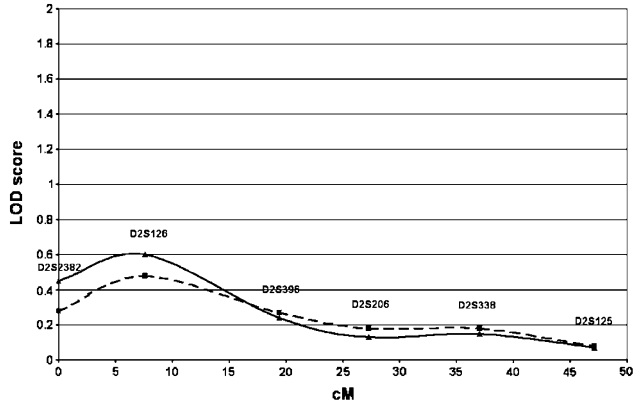

Figure 1 Results of the multipoint nonparametric linkage analysis. Multipoint LOD score graph for 45 families (solid line) and 36 families (dashed line).

was assumed as 2%. Multipoint parametric and nonparametric analysis was carried out by using SimWalk 2 version 2.89.[24] The sib transmission/disequilibrium test (S-TDT) was used to observe the transmission of alleles among affected sibs.[25]

In the 15 families, that included at least one affected individual with an age of onset $\leqslant$50 years, a marker in intron 7 of the *parkin* gene (D6S305) was genotyped in order to identify families more likely to have a mutation in this known PD-susceptibility gene. Linkage analysis was repeated excluding those families showing possible linkage to D6S305.

Results

We did not obtain any significant LOD score in the parametric analysis, nor in the nonparametric analysis: the results are shown in Table 2, Figure 1 and in the online information (see supplementary information: Tables 3 and 4). The highest LOD score (0.6) was found in the multipoint nonparametric analysis at marker D2S126 (Table 2, Figure 1).

We did not observe any significant *z* score in the S-TDT, which showed that none of the marker alleles is associated with the disease. The results of the S-TDT are shown in the supplementary information (Table 5). In our families, we could not perform the TDT test, because parental genotypes were not available. There is probably not much loss of power, when the parents are not genotyped as in our case, given the fact that affected sibs as well as unaffected sibs are genotyped.

Linkage to marker D6S305 in the *parkin* gene could not be excluded in nine of the 15 families, that included at least one affected individual with an age of onset $\leqslant$50 years. Excluding these nine families, we did not obtain any significant LOD score in the parametric nor nonparametric linkage analysis of the remaining 36 families. Again, the highest LOD score (0.48) was found in the nonparametric analysis at marker D2S126.

Discussion

The studies by Pankratz *et al* showed a significant linkage of PD to 2q36–37 in a North American population. The sample of Pankratz *et al*[17,19] was primarily Caucasian (94%), although Hispanics (5%) also participated. Interestingly, the Hispanic families in the sample provided a substantial portion of the linkage evidence.[19] We could not find a significant linkage to this region in our European families.

The discrepancy of the results between both studies might be explained by the different population. However, none of the other PD genome-wide linkage studies in the last years have reported evidence of linkage to chromosome 2q: DeStefano *et al*[26] included affected sibling pairs mainly from the United States and also from Canada, Germany and Italy, Scott *et al*[27] analysed white families from the United States and Australia, while Hicks *et al*[16] performed a scan on Icelandic families.

Pankratz *et al* reported a significant LOD score both in a sample with and without *parkin* mutations. The inclusion of the families with *parkin* mutations resulted in a higher LOD score, but the LOD score remained clearly significant in the sample without *parkin* mutations.[18,19] We repeated our linkage analysis excluding nine families, in which we could not rule out linkage to marker D6S305 in the *parkin* gene. Excluding these families did not change our overall results, indicating that there is no specific contribution of this subset of families to our results. It is also unlikely that the parkinsonism in these nine families is caused by *parkin* mutations, because we included only families compatible with autosomal dominant inheritance in our study and mutations in the *parkin* gene cause autosomal recessive parkinsonism (with the exception of very few families, in whom the contribution of the *parkin* mutation is still somewhat controversial).

A possible linkage of our families to other dominant PD loci such as PARK3 and PARK8 was not subject of this study and cannot be excluded.

The mean age of onset in our PD families (57.6 ± 10.5 years) was similar to the mean age at onset in the studies of Pankratz *et al*: 58.0 ± 12.2 years,[18] 58.3 ± 12.0 years.[19] This indicates that the discrepancy of the results between the studies by Pankratz *et al* and our study cannot be explained by a different age at onset of PD in the population.

We genotyped the same six markers as Pankratz *et al* in the region, where the highest LOD score was reported. Thus, the discrepancy of the results between the studies cannot be explained by a different marker density. Employing a denser set of markers would most probably not affect our overall results.

It may be argued that the original study by Pankratz *et al* overestimated the linkage, so that the true effect conferred by the PARK11 locus is smaller, and therefore escaped detection in our sample. We did not find a significant LOD score in our analysis. The highest LOD score of our study occurred in the nonparametric analysis at marker D2S126 (LOD score 0.6) and is far away from significance. The marker D2S126 is nearly 20 cM apart from D2S206, where the highest LOD score was reported by Pankratz *et al*.

In summary, our study did not provide evidence of a susceptibility locus for Parkinson's disease at 2q36–37 in our families. Therefore, *PARK11* does not seem to play a major role for familial PD in the European population. A susceptibility locus at 2q36–37 may be a rare form, occurring in specific populations.

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Electronic-database information

The URLs for data presented herein are as follows

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/ (for the chromosome 2q genetic map).

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for PD, PARK1, PARK2, PARK3, PARK5, PARK6, PARK7, PARK8, PARK9, PARK10, PARK11, NR4A2).

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com.ejhg)