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ORIGINAL ARTICLE

BRD2 and TAP-1 genes and juvenile myoclonic epilepsy

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Abstract Juvenile myoclonic epilepsy (JME) is a genetically determined common subtype of idiopathic generalized epilepsy. Linkage of JME to the chromosomal region 6p21.3 has been reported. An association has been previously observed between JME and the positional candidate, 6p21.3 linked, *BRD2*. Another candidate in this region is the TAP-1 gene encoding the Transporter Associated with Antigen Processing. The aim of the present study is to determine whether these two genes modulate the vulnerability to JME. While no difference was observed in the allele and genotype frequencies of *BRD2* between JME and controls, an association was found between a *TAP-1* haplotype and JME, suggesting that this gene may be another 6p21.3 linked vulnerability factor to JME.

Keywords Juvenile myoclonic epilepsy · BRD2 · TAP-1 · Genetics · Association study

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Introduction

Juvenile myoclonic epilepsy (JME) is the most common form of idiopathic generalized epilepsy (IGE) that account for about 5–10% of all types of epilepsies [1]. It starts in adolescence and is mainly characterized by isolated myoclonic jerks on awakening that usually begin during adolescence. It is also highly drug-dependent, since 90% recurrence is reported after interruption of pharmacological treatment [2, 3].

Family and twin studies have provided strong evidence for a genetic contribution to JME [4]. In rare Mendelian JME, 15 loci have been suggested by linkage analysis [5]. Private mutations in two ion channel genes (α1-subunit of the GABAA receptor and chloride channel 2) and one non-ion channel (*myoclonin/EFHC1*) have been identified as putative JME genes [review in 5]. However, the genetics of JME is complex and involves probably the interaction of several genes with minor effect and environmental factors [4].

A first putative locus termed EJM1 (OMIM 254770), is on the human leukocyte antigen (HLA) region of chromosome 6p, and was significantly associated with HLA-DR13 [6]. However, several association studies suggested that other JME loci might be present on chromosome 6p (review in [5]). In 2003, Pal et al. [7] reported a linkage disequilibrium between single nucleotide polymorphisms (SNPs) and one haplotype within the *BRD2* (RING3) gene,

in chromosome 6p21.3, and JME. Four other studies, with mixed results, of IGEs or photoparoxysmal response, using *BRD2* polymorphisms, have been published to date [8–11].

Because of this inconsistencies and strong linkage disequilibrium within the 6p21.3 region, between HLA-DQ and HLA-DP, it is important to further explore this candidate gene, as well as other 6p21.3 positional candidates. Transporter Associated with Antigen Processing 1 (*TAP-1*) is one of these candidate genes. The TAP-1 (also known as RING4 or PSF1, OMIM 170260) gene belongs to a family of membrane transporters that possess an ATP binding cassette and 6–8 transmembrane domains [12]. The ATP-binding cassette transporter TAP translocates peptides from the cytosol to awaiting major histocompatibility complex (MHC) class I molecules in the endoplasmic reticulum. TAP is made up of the TAP1 and TAP2 (OMIM 170261) polypeptides.

TAP-1 gene comprises 11 exons and it is known to be polymorphic with several polymorphisms identified in different populations. To date, 13 nucleotide sequence variants have been described in *TAP-1* coding sequences [13–18]. Interestingly, Jackson and Capra [19] have reported an association between a polymorphism at the TAP-1 locus and insulin-dependent diabetes mellitus, a disease that seems to be associated with a higher risk for epilepsy [20].

The aim of the present study is to further explore *BRD2* SNPs and to determine whether two *TAP-1* functional polymorphisms—one located in exon 4, Ile333Val, and one in exon 10, Asp637Gly [12]—modulate the vulnerability to JME.

Methods

Subjects

The gene and allele frequencies gene were determined in a French sample comprising 154 healthy subjects and 159 unrelated patients with JME. Patients were 98 females and 61 men. They have a mean age of (28.3 ± 7.5 years) and were recruited as described previously [21] according to the classification of the International League Against Epilepsy [22]. Photoparoxysmal response or photosensitive epilepsy was established in 47 patients. The other 112 were tested and were negative.

Control subjects were 48 women and 106 men with a mean age of (37.2 ± 9.6 years). They were unrelated healthy blood donors. Anyone of them had evidence of any personal or family history of epilepsy. Control subjects and patients were selected from the same West European Caucasian, French-speaking population [21].

Molecular methods

BRD2 genotyping

We investigated three *BRD2* SNPs: one, rs3918149, in the promoter region, that displays the highest odd ratio in the study by Pal et al. [7] and two, rs516535 and rs206781, in the 3′ region, that showed the strongest association in the study of photoparoxysmal response [9]. The later were also significantly associated with JME in the study by Pal et al. [7] and were tested in [10]. Genotyping was carried out using ABI Taqman technology (details of Taqman probes are available on request).

Genotyping of the TAP-1 dimorphism

The polymorphisms within *TAP-1* were analyzed using Polymerase Chain Reaction and Single-Strand Conformation Polymorphism (SSCP) analyses. Amplification reactions for the SSCP analyses for both polymorphisms contained 1 μg of DNA, 200 μM of each dNTP, 150 μM $MgCl_2$, 0.2 μM of primers, 2 Units of Taq polymerase and 1× Taq buffer, in a total volume of 48 μl. Primers were: TAP-1(Ile333Val) (F) CTGAGTGATTCTCTGAGTGAG and (R) CATTTTCCC ACCTTCTTGGG; TAP-1 (Asp637Gly): (F) TGTGGCTA TACCGTTCTCATC and (R) GGATAAGTACACACGGT TTCC. PCR cycling conditions included 3 minutes initial denaturizing step at 94°C, followed by 29 cycles of 30 s at 92°C, 30 s at appropriate annealing temperature—58°C for both Ile333Val and Asp637Gly—and 30 s at 72°C for extension step. Amplification products are resolved on TBE 1× 4–6% polyacrylamide denaturing gels, which are run for 3 h at 300 V. Fragments' sizes were 161 bp for Ile333Val and 133 bp for Asp637Gly.

Statistical analysis

Two-tailed Chi-square or Fisher's exact tests were used to compare allelic and genotypic distributions between the JME and control groups. Linkage disequilibrium and haplotypes frequencies were estimated using the PHASE and HAPLOVIEW programs [23, 24]. Since we compared genotypic and allelic distributions of five polymorphic markers between JME patients and controls, a correction for multiple testing was required. For a Bonferroni correction on the p values for interaction, we therefore used $p = 0.05/5 = 0.01$ as a threshold for significance.

Results

Genotype and alleles frequencies of all *BRD2* and *TAP-1* markers in the different groups were in Hardy–Weinberg

Table 1 *BRD2* and *TAP-1* alleles in Juvenile Myoclonic patients and controls

Allele	Controls [*N* (%)]	JME [*N* (%)]	OR [(95% CI)]	*P* value
BRD2				
rs3918149_A	38 (12.34)	37 (11.63)	0.99 (0.61–1.61)	0.79
rs516535_C	123 (39.93)	124 (38.99)	0.96 (0.70–1.32)	0.81
rs206781_T	200 (64.93)	197 (61.95)	1.13 (0.82–1.57)	0.44
TAP-1 Ile333Val				
333Val	58 (18.83)	62 (19.50)	1.04 (0.69–1.58)	0.83
TAP-1 Asp637Gly				
637Asp	250 (81.17)	259 (81.45)	1.02 (0.67–1.55)	0.92
TAP-1 haplotypes				
333Ile-637Asp	239 (77.60)	231 (72.64)	0.77 (0.53–1.10)	0.15
333Val-637Gly	47 (15.26)	34 (10.69)	0.65 (0.41–1.07)	0.09
333Val-637Asp	11 (3.57)	28 (8.80)	2.61 (1.27–5.33)	0.007
333Ile-637Gly	11 (3.57)	25 (7.86)	2.30 (1.11–4.77)	0.02

JME Juvenile myoclonic epilepsy

equilibrium. We found no significant differences between the allelic (Table 1) and genotyping (data not shown) distributions of all three *BRD2* SNPs between JME and control groups. No difference was also observed in the subsample of patient with photoparoxysmal response or photosensitive epilepsy (data not shown). No gender effect was found.

Similarly, no difference was observed between the allele distributions of both TAP-1 SNPs (Table 1). As previously reported [12], we found incomplete linkage disequilibrium between the two functional *TAP-1* SNPs, the haplotypes 333Ile-637Asp and 333Val-637Gly being more frequent than calculated in both controls and patients. Interestingly, the two rare haplotypes, 333Val-637Asp and 333Ile-637Gly, are more frequent in JME patients than in controls: 2.61, 95%CI (1.27–5.33), uncorrected $p = 0.007$ and 2.30, 95%CI (1.11–4.77), uncorrected $p = 0.02$, respectively) (Table 1). After correction for multiple testing the association with 333Val-637Asp remains significant ($pc = 0.04$).

Discussion

This study was first designed to attempt replication of the association between a promoter *BRD2* SNP, rs3918149, and JME observed in a family-based association study [7] and replicated in small samples from British (34 JME and 256 controls) and Irish (57 JME, 227) origins [11]. Our negative result is in line with those reported with the same variant in other populations (German, Australian, and Indian) [11]. We also did not replicate the association found between two 3′*BRD2* SNPs and photoparoxysmal response [9].

Power to detect association at the level of $\alpha = 0.01$ in this study was around 0.80 if genotype relative risks were 6 as reported by Pal et al. [7]. But the power falls with lower genotype relative risks (less than 0.50 with relative risks lower than 2.0). Therefore, our study may be underpowered in these figures. However, it must be emphasized that the two positive results were observed in small samples [11], while the negative ones were found in the largest ones ([11] and the present study).

We considered the hypothesis that these inconsistencies are because the causal variant lies outside *BRD2*, since linkage disequilibrium is high across the region containing this gene. Several other coding regions have been unsuccessfully sequenced in the *BRD2* region by Pal et al. [11]. We therefore decided to test a positional candidate that has not been considered in previous studies, *TAP-1*. A preliminary, unpublished, study performed in a sample of Tunisian IGE patients suggested an association with this gene (S. Layouni, MSc thesis). The present results suggest that two *TAP-1* haplotypes are susceptibility factors to JME. This association now requires further replication in carefully phenotyped and closely matched populations.

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References

1. Janz D (1985) Epilepsy with impulsive petit mal (juvenile myoclonic epilepsy). Acta Neurol Scand 72:442–452
2. Dreifuss FE (1989) Juvenile myoclonic epilepsy: characteristics of a primary generalized epilepsy. Epilepsia 30(Suppl 4):S1–S7
3. Grünewald RA, Panayiotopoulos CP (1993) Juvenile myoclonic epilepsy. Arch Neurol 50:597–598
4. Turnbull J, Lohi H, Kearney JA et al (2005) Sacred disease secrets revealed: the genetics of human epilepsy. Hum Mol Genet 14(Spec 2):2491–2500
5. Delgado-Escueta A (2007) Advances in genetics of juvenile myoclonic epilepsies. Epilepsy Cur 7(3):61–67
6. Greenberg DA, Delgado-Escueta AV, Widelitz H et al (1988) Juvenile myoclonic epilepsy (JME) may be linked to the BF and

HLA loci on human chromosome 6. Am J Med Genet 31:185–192
7. Pal DK, Evgrafov OV, Tabares P (2003) BRD2 (RING3) is a probable major susceptibility gene for common juvenile myoclonic epilepsy. Am J Hum Genet 73(2):261–270
8. Greenberg DA, Durner M, Keddache M et al (2000) Reproducibility and complications in gene searches: linkage on chromosome 6, heterogeneity, association, and maternal inheritance in juvenile myoclonic epilepsy. Am J Human Genet 66:508–516
9. Lorenz S, Kirsten PT, Gehrmann A et al (2006) Association of BRD2 polymorphisms with photoparoxysmal response. Neurosci Lett 400:135–139
10. de Kovel CG, Pinto D, de Haan GJ (2007) Association analysis of BRD2 (RING3) and epilepsy in a Dutch population. Epilepsia 48(11):2191–2192
11. Cavalleri GL, Walley NM, Soranzo N et al (2007) A multicenter study of BRD2 as a risk factor for juvenile myoclonic epilepsy. Epilepsia 48:706–712
12. van Endert PM, Lopez MT, Patel SD et al (1992) Genomic polymorphism, recombination, and linkage disequilibrium in human major histocompatibility complexencoded antigen-processing genes. Proc Nat Acad Sci USA 89:11594–11597
13. Colonna M, Bresnahan M, Bahram S et al (1992) Allelic variants of the human putative peptide transporter involved in antigen processing. Proc Natl Acad Sci USA 89:3932–3936
14. Powis S, Tonks S, Mokridge I et al (1993) Alleles and haplotypes of the MHC-encoded ABC transporters TAP1 and TAP2. Immunogenetics 37:373–380
15. Powis S, Rosenberg W, Hall M et al (1993) TAP1 and TAP2 polymorphism in celiac disease. Immunogenetics 38:345–350
16. Shi L, Yang G, Fu Y et al (1997) Human TAP1 polymorphisms detected by denaturing gradient gel electrophoresis. Tissue Antigens 49:421–426
17. Tang J, Freedman DO, Allen S et al (2001) TAP1 polymorphisms in several human ethnic groups: characteristics, evolution, and genotyping strategies. Hum Immun 62:256–268
18. Lajoie J, Zijenah LS, Faucher MC et al (2003) Novel TAP1 polymorphisms in indigenous Zimbabweans: their potential implications on TAP function and in human diseases. Hum Immun 64:823–829
19. Jackson DG, Capra JD (1993) TAP1 alleles in insulin-dependent diabetes mellitus: a newly defined centromeric boundary of disease susceptibility. Proc Nat Acad Sci USA 90:11079–11083
20. O'Connell MA, Harvey AS, Mackay MT, Cameron FJ (2008) Does epilepsy occur more frequently in children with type 1 diabetes? J Paediatr Child Health 44(10):586–589
21. Guipponi M, Thomas P, Girard-Reydet C et al (1997) Lack of association between juvenile myoclonic epilepsy and GABRA5 and GABRB3 genes. Am J Med Genet B Neuropsychiatr Genetics 74(2):150–153
22. Commission On Classification And Terminology of The International League against Epilepsy (1989) Proposal for revised clinical and electroencephalographic classification of epileptic seizures. Epilepsia 30:389–399
23. Stephens M, Smith N, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. Am J Human Genet 68:978–989
24. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265