

ARTICLE

Intragenic deletions affecting two alternative transcripts of the *IMMP2L* gene in patients with Tourette syndrome

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Tourette syndrome is a neurodevelopmental disorder characterized by multiple motor and vocal tics, and the disorder is often accompanied by comorbidities such as attention-deficit hyperactivity-disorder and obsessive compulsive disorder. Tourette syndrome has a complex etiology, but the underlying environmental and genetic factors are largely unknown. *IMMP2L* (inner mitochondrial membrane peptidase, subunit 2) located on chromosome 7q31 is one of the genes suggested as a susceptibility factor in disease pathogenesis. Through screening of a Danish cohort comprising 188 unrelated Tourette syndrome patients for copy number variations, we identified seven patients with intragenic *IMMP2L* deletions (3.7%), and this frequency was significantly higher ($P=0.0447$) compared with a Danish control cohort (0.9%). Four of the seven deletions identified did not include any known exons of *IMMP2L*, but were within intron 3. These deletions were found to affect a shorter *IMMP2L* mRNA species with two alternative 5'-exons (one including the ATG start codon). We showed that both transcripts (long and short) were expressed in several brain regions, with a particularly high expression in cerebellum and hippocampus. The current findings give further evidence for the role of *IMMP2L* as a susceptibility factor in Tourette syndrome and suggest that intronic changes in disease susceptibility genes should be investigated further for presence of alternatively spliced exons.

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INTRODUCTION

Tourette syndrome (TS; MIM 137580) is a childhood onset chronic neurodevelopmental disorder characterized by multiple motor tics and at least one vocal/phonic tic.¹ TS is a complex disorder where several environmental and genetic components are likely to be involved in the disease etiology. About 88% of TS patients are diagnosed with comorbidities such as attention-deficit hyperactivity-disorder (ADHD), obsessive compulsive disorder/behavior (OCD/OCB), rage attacks, sleep difficulties, autism spectrum disorders (ASD) and affective disorders^{2,3} suggesting a shared background between these disorders.⁴ This notion is strengthened by finding of genes, such as *CHRNA7*, *CNTNAP2* and *NLGN4*, which are implicated in a wide variety of neurodevelopmental disorders, including TS and ASD.⁵

Although it is widely accepted that genetic factors have a significant role in TS etiology, only a few genes have been suggested to render susceptibility to TS and *IMMP2L* (inner mitochondrial membrane peptidase, subunit 2) located on chromosome 7q31 is one of these genes.⁵ The chromosome region 7q31 was first implicated as a

candidate locus for TS through identification of a constitutional chromosome translocation t(7;18)(q22;q22.3) in a family with OCD, motor tics and/or vocal tics; where the 7q22 breakpoint was localized between markers D7S515 and D7S522.⁶ In 2001, a boy with a *de novo* inverted duplication, dup(7)(pter-q31.1::q31.1-q22.1::q31.1-qter), presenting with vocal and motor tics was reported.^{7,8} The 7q31 breakpoint was within the region described by Boghosian-Sell and colleagues⁶ and disrupted a novel gene, *IMMP2L*.⁷ These two studies implicated 7q31 as a candidate TS region. Subsequently, a family-based association study was performed in a cohort of French-Canadian patients from Quebec, and a biased transmission of alleles from heterozygote parents to their TS offspring was found for markers D7S522, D7S523 and D7S1516 (located within *IMMP2L*).⁹ In a subsequent study, 39 TS patients were screened for intragenic sequence variations and 22 patients were further investigated for structural copy number variations (CNVs) involving *IMMP2L*, without finding any changes.¹⁰ Recent description of another patient with TS-like tics and a translocation, t(2;7)(p24.2;q31), where a 7.2-Mb microdeletion at the chromosome 7q31 breakpoint

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partly deleted *IMMP2L*, has again awakened the discussion about involvement of this gene in TS pathogenesis.¹¹

The *IMMP2L* gene is the human homolog of *Imp2* of *Saccharomyces cerevisiae* and the transcript identified and published by Petek and colleagues⁷ has 6 exons, where the ATG start codon is within the second exon (NM_032549.3). *IMMP2L* is expressed in a wide range of tissues including the fetal and adult brain, but not in the adult liver and lung.⁷ Mammalian *IMMP1L* and *IMMP2L* constitute, like the yeast *Imp1* and *Imp2*, the subunits in the inner membrane peptidase complex, which catalyze the removal of the signal peptides required for the targeting of proteins from the mitochondrial matrix into the intermembrane space across the inner membrane.¹² The two subunits share a conserved core and they are both catalytically active, but process different substrates.^{13,14} Cytochrome c1 and mitochondrial glycerol-3-phosphate dehydrogenase 2 are substrates for *IMMP2L*, and *IMMP2L* mutations have been shown to impair signal peptide processing of both proteins.^{15,16} Both the substrates have important roles in energy metabolism and oxidation in the mitochondria. In recent studies, mitochondria isolated from the brain tissue of mice homozygous for an *Immp2l* mutation manifested hyperpolarization of mitochondrial inner membrane potential, increased superoxide ion generation and higher levels of ATP.¹⁶ Incomplete processing of *IMMP2L* substrates seems to result in a hyperactive mitochondrion with an increased production of superoxide¹⁶ and to promote the formation of a mitochondrial permeability transition pore which leads to release of pro-apoptotic proteins, and thereby activating cell death pathways.^{17,18}

In this study, we describe seven Danish TS patients with intragenic *IMMP2L* deletions, affecting one of two alternative *IMMP2L* transcripts (the previously described long transcript and a short alternative transcript with the two first exons residing within intron 3 of the long transcript). The deletions are significantly more frequent in the patients compared with the controls giving further evidence for *IMMP2L* as a TS susceptibility gene.

MATERIALS AND METHODS

Study subjects

The present cohort includes 188 unrelated TS patients (154 males and 34 females), who were recruited through the Herlev Tourette Clinic (Denmark) as part of a clinical study.³ Patients were examined using validated diagnostic instruments¹⁹ and clinical information about the parents of the patients with *IMMP2L* deletions was obtained through interviews. The study was approved by the Danish Institutional Review Board (H-KA-05118) and informed consent was obtained from each participant.

The control population includes material from 316 Danish individuals (203 males and 113 females) retrieved from the biobanks of the Kennedy Center and the Center for Neuropsychiatric Schizophrenia Research. Overt neurological disorders were ruled out for this cohort. Additional CNV calls from 1038 controls from the reference database genotyped on the Affymetrix CytoScan HD array platform were also included as control population (Affymetrix, Santa Clara, CA, USA).

Chromosome microarray

Chromosome microarray was initially carried out using Affymetrix Cyto-genetics Whole-Genome 2.7 M array (82 patients), but the manufacturer (Affymetrix) replaced it by a similar and more robust platform (CytoScan HD array), which was then used for the remaining patients (106 patients and 1038 Affymetrix controls). Both array platforms have comparable probe localization and density (~1 probe/kb) within the *IMMP2L* gene, and they are therefore not expected to introduce any bias in the pick-up rate of *IMMP2L* CNVs in the investigated individuals. Data were analyzed with ChAS software (Affymetrix) using the following filtering criteria: deletions >40 kb (a minimum of 30 markers) and duplications >40 kb (a minimum of 30 markers).

Quantitative PCR (qPCR)

Genomic DNA was extracted from peripheral blood using standard protocols and qPCR was carried out with Power SYBR Green PCR technology (Applied Biosystems, Foster City, CA, USA) using a 7500 Fast Real-time PCR system machine (Applied Biosystems). Each amplicon was quantified in triplicates and copy number estimates were derived by $\Delta\Delta C_T$ calculations using a copy number neutral *GAPDH* amplicon and control individuals.

Verification of the *IMMP2L* deletions was performed using qPCR amplicons targeting exon 3 and intron 3 of the long transcript (Figure 1). The same amplicons were used to investigate the parental origin of the deletions and to screen the control cohort of 316 Danish individuals. Validation and investigation of parental origin of all the other CNVs were also carried out using qPCR.

IMMP2L-deletion breakpoints were narrowed down with qPCR. The deletion junction fragments were amplified with long range PCR and sequenced in both directions.

Sequence analysis of *IMMP2L*

Exons and flanking intron sequences were investigated by Sanger sequencing using genomic DNA.

Transcription studies

Reverse transcription PCR was carried out using peripheral blood leukocytes from TS patients, total RNA from various brain regions of a normal human adult (Biochain, Newark, CA, USA), human universal reference total RNA (Clontech, Palo Alto, CA, USA) and human whole-brain total RNA (Stratagene, La Jolla, CA, USA). cDNA was synthesized from 0.5 μ g total RNA using SuperScript III reverse transcriptase (Invitrogen, San Diego, CA, USA).

In situ hybridization

In situ hybridization was performed on eight-micron sections of snap-frozen or formalin-fixed, paraffin-embedded (FFPE) brains from adult, 7 days old (D7) and embryonic (E16.5 or E15.5) BALB/c mice as previously published²⁰ with some modifications: Hybridizations were carried out overnight at 47 °C and the tyramide signal amplification was detected using tyramide signal amplification Plus Fluorescein system for frozen sections or tyramide signal amplification DNT kits for FFPE sections (Perkin Elmer, Skovlunde, Denmark). The oligonucleotide probes were 5'- and 3'-FITC labeled and targeted the long (5'-ACAATGTCACCACGGTGTACTTCAAAATTCCTCACTT-3') (exon 3) or the short (5'-ATGCACTTCTTCAATCTTAGGCTAACTCTTCTCGGTTGGC-3') (exon 3b) transcripts of human *IMMP2L* (TAG Copenhagen, Copenhagen, Denmark). The same probes were used against mouse and human sections. Animals were handled in accordance with the guidelines of the European Communities directive of 86/609/EEC. Paraffin-embedded human corpus callosum sections were purchased from Capital Biosciences (Rockville, MD, USA). Images were obtained using an Olympus Macroview Microscope (MVX10) equipped with an Olympus FIII View and ColorView II camera and Cell P AnalySIS software (Olympus Denmark, Ballerup, Denmark).

RESULTS

Identification and characterization of *IMMP2L* deletions

Screening of 188 unrelated TS patients for CNVs resulted in identification of intragenic *IMMP2L* deletions in seven patients (P1–P7) (Figure 1, Table 1). In three patients, we found an additional CNV (duplication), which may contribute to the phenotype (Table 1). Other likely benign CNVs (>40 kb) are listed in Supplementary Table S1.

All the *IMMP2L* deletions were at the 5'-end of the gene and ranged in size from 49 to 331 kb. In four patients (P3–P6), the deletions did not include any coding exons of the long *IMMP2L* transcript (NM_001244606.1), but were within intron 3. We speculated whether this intron included alternatively spliced exons; and using Ensemble database, we identified a shorter *IMMP2L* transcript (ENST00000450877) with two unique exons (designated 3a and 3b). The translation start site was within exon 3a, and the short transcript

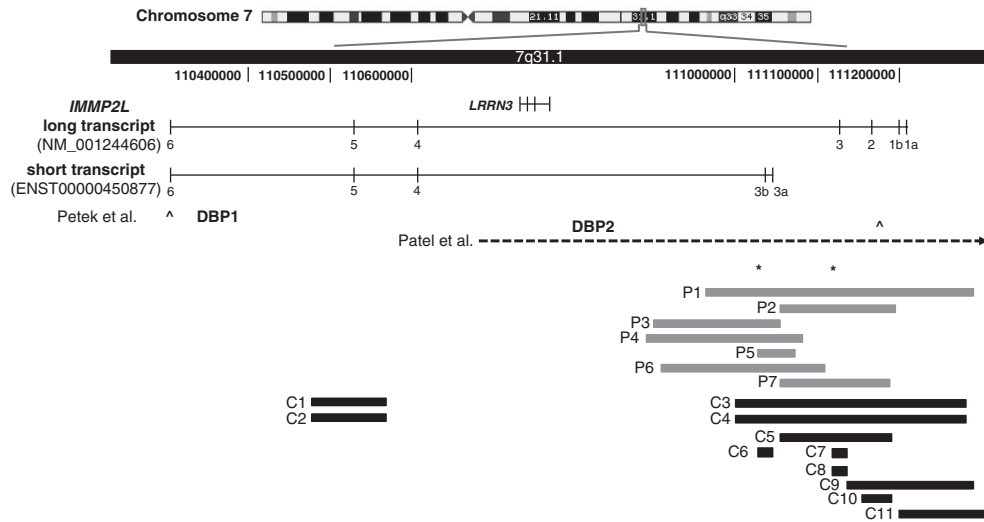


Figure 1 Structural variations within the *IMMP2L* transcripts. (Genomic region showing the long (NM_001244606.1) and the short (ENST00000450877) transcripts of *IMMP2L*, and the structural variations identified in this study or by others. DBP1 and DBP2 indicate the duplication breakpoints and the open arrows indicate the amplicons (corresponding to exon 6 and exon 1b/IVS1a) used to screen for *IMMP2L* deletions (Petek *et al*⁷ and Petek *et al*,¹⁰ respectively). Horizontal dashed-arrow shows the 7.2-Mb deletion described by Patel *et al*.¹¹ Horizontal gray blocks indicate the seven intragenic deletions (P1–P7) identified in Tourette patients in this study. The three deletions identified in the Danish background population (C6–C8) and the eight deletions identified in the Affymetrix CytoScan HD reference database (C1–C5 and C9–C11) are shown as horizontal black blocks. Asterisks represent localization of the qPCR amplicons.

Table 1 Overview of the deletions encompassing *IMMP2L*, identified in 7 out of 188 unrelated TS patients

Patient	Sex	Symptom	Deletion	Deletion size (kb)	Exons deleted	Parental origin	Parental phenotype	Additional deletions/duplications (CNVs)
P1 TS101 6972466	M	TS ADHD	chr7.hg19:g.110.959.798 _111.290.945delins CATACTACAGGACTTACAACTTACAA	331	1a, 1b, 2, 3, 3a, 3b	Pat	Pat: dyslexia, temper. Mat: unaffected	
P2 TS71 6962812	M	TS ADHD	chr7.hg19:g.111.051.907 _111.199.868del	148	2, 3	Mat	Pat: ADHD symptoms Mat: tics, OCB	
P3 TS131 6982155	M	TS ADHD Asperger	chr7.hg19:g.(?_110.892.600) _(111.055.300_?)del	~163	3a, 3b	Mat	Pat: unaffected Mat: unaffected	chr1.hg19:g.(?_231711404)_(231814354_?) dup 102 kb paternal duplication which includes <i>DISC1</i> partially
P4 TS133 6982192	M	TS OCD	chr7.hg19:g.110.889.476 _111.080.538delins ACACCTACTGCTCA	191	3a, 3b	Pat	Pat: OCB, stuttering Mat: unaffected	chr4.hg19:g.(?_188104036)_(189795272_?) dup 1.7 Mb paternal duplication which includes <i>ZFP42</i> , <i>TRIML1</i> , <i>TRIML2</i> , <i>LOC339975</i> , <i>LOC401164</i>
P5 TS32 6952838	M	TS	chr7.hg19:g.111.029.575 _111.079.376del	49	3a, 3b	NA	Pat: unaffected Mat: unaffected	chr15.hg19:g.(?_32019187)_(32439298_?) dup 420 kb duplication which includes <i>OTUD7A</i> and <i>CHRNA7</i> partially
P6 TS36 8942118	M	TS ADHD OCD	chr7.hg19:g.110.904.173 _111.113.977delinsCA	210	3a, 3b	Pat	Pat: stubbornness Mat: unaffected	
P7 TS126 8932009	M	TS ADHD	chr7.hg19:g.(?_111.054.100) _(111.197.400_?)del	~143	2, 3	Pat	Pat: unaffected Mat: unaffected	

Abbreviations: del, deletion; dup, duplication; M, male; mat, mater/mother; NA, not analyzed as parental DNA was not available; pat, pater/father. Patient identities (P1–P7) are followed by family numbers and encrypted patient identification codes.

included exons 3a, 3b and exons 4–6 (common with the long transcript).

In five patients (P1, P2 and P4–P6), we characterized the deletions at base-pair level. In three families (P1/TS101, P2/TS71 and P4/TS133), the deletion was inherited from a parent with a neuropsychiatric feature, whereas in three cases it was inherited from an unaffected parent (P3/TS131, P6/TS36 and P7/TS126) (Table 1). The genomic location of all *IMMP2L* deletions has been submitted to the Leiden Open Variation Database.

Frequency of *IMMP2L* deletions

Using two qPCR amplicons (in exon 3 and intron 3), we identified three deletions in a cohort of 316 individuals representing the Danish background population (0.9%). One deletion involved only the short transcript (C6) and two deletions involved only the long transcript (C7 and C8) (Figure 1). The frequency of *IMMP2L* deletions in our TS cohort is 3.7% (7/188) and this is significantly higher than that of the background population ($P = 0.0447$, two-tailed Fisher's exact test).

To avoid a potential bias, we investigated the presence of CNVs along the entire gene among 1038 controls from the Affymetrix CytoScan HD reference database. *IMMP2L* deletions affecting the long transcript were present in eight controls (C1–C5 and C9–11) (0.8%) and two of these (C3 and C4) also included the short *IMMP2L* transcript (Figure 1). The frequency of *IMMP2L* deletions in the TS cohort is thus significantly higher than that observed in 1038 controls ($P = 0.0039$; Fisher's exact test).

DNA sequence analyses

None of the seven patients had small base-pair changes of the non-deleted *IMMP2L* allele. Furthermore, we sequenced the remaining 174 patients for mutations within exons 3a and 3b without detecting any pathogenic variations. We did not include the other exons as these were previously screened for mutations in 39 TS patients by Petek and colleagues,¹⁰ without detecting any mutations.

Other alternative transcripts of *IMMP2L*

In this study, we could not amplify the entire transcript (NM_032549.3), which was predicted to have a 440-bp long exon 1.7 On the other hand, we could amplify the entire NM_001244606.1 transcript and we showed that this transcript had two 5' non-coding exons of 54 and 74 bp (designated exon 1a and 1b, respectively). Throughout the text we use NM_001244606.1 as the long *IMMP2L* transcript.

Further studies using different combinations of primers showed that both the long (NM_001244606.1) and the short (ENST00000450877) transcripts had two isoforms, with and without exon 5, respectively. All these transcripts had open-reading frames. Several other smaller transcripts were also identified, but none of these had a reasonable open-reading frame that would be translated to a functional protein.

Predicted protein encoded by the short *IMMP2L* transcript

The 157 amino-acid protein product of the short transcript does not contain an N-terminal transmembrane domain (TMpred) and is likely to be a soluble protein with an average hydropathicity of 0.71 (SOSUI). The structurally important substrate-binding cleft NX₅S-motif of the long *IMMP2L* transcript and the highly conserved C-terminal¹² are present in the short isoform.

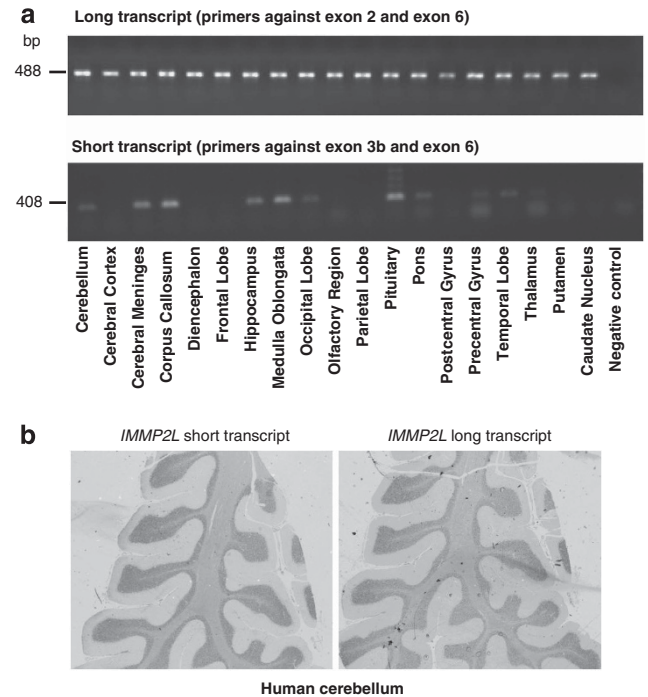


Figure 2 Expression studies of the *IMMP2L* transcripts in human brain. (a) The expression profile of long (above) and short (below) *IMMP2L* transcripts in 19 human brain regions using reverse transcription PCR. The long transcript is expressed in all regions, whereas the short transcript is expressed in the cerebellum, meninges, corpus callosum, hippocampus, medulla oblongata, occipital lobe, pituitary, pons, precentral gyrus, temporal lobe and thalamus. (b) Expression of the short and long transcripts of *IMMP2L* in adult human cerebellum showing expression especially in the granular layer and the medullar region.

Tissue-expression profiles of the long and the short *IMMP2L* transcripts

We investigated tissue-expression profiles of the long (primers against exons 2 and 6) and the short transcript (primers against exons 3b and 6) to gather further evidence of their involvement in the central nervous system pathology implicated in TS. Both transcripts were present in human total RNA and total brain RNA, whereas the short transcript was not expressed in blood leukocytes. Studies with 19 different human brain regions showed that the long transcript was expressed in all the regions whereas the short transcript was expressed in the cerebellum, meninges, corpus callosum, hippocampus, medulla oblongata, occipital lobe, pituitary, pons, precentral gyrus, temporal lobe and thalamus (Figure 2a).

In situ hybridization studies

In the whole-mouse embryo sections (E16.5), expression of the short transcript was more specific to the brain compared with the long transcript (RNA-*In situ* hybridization studies, data not shown). In the brain sections of the mouse embryos (E15.5), both transcripts were widely expressed at a moderate level (Figure 3a). In the 7 days old mice, both transcripts gave strong signals in the cerebellum and hippocampus (Figure 3b). On the sagittal sections of the adult mice brain both transcripts gave strong signals in the cerebellum and the hippocampus/dental gyrus, and the short transcript was also expressed in the olfactory bulb (Figure 3c). On the coronal sections, expression was detected in the hippocampus/dental gyrus, together with hypothalamic nuclei, fornix, cingulum, superior colliculus,

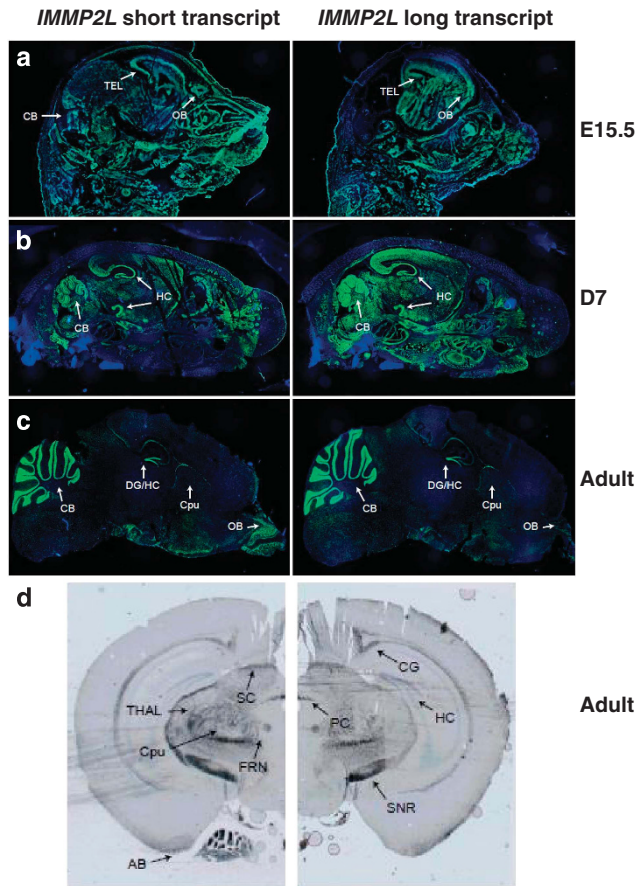


Figure 3 Expression of the short and long transcripts of *IMMP2L* in mouse brain sections. Sagittal sections from (a) mouse embryo (E15.5); (b) mouse at age of seven days (D7); and (c) adult mouse are hybridized with long (exon 3) and short (exon 3b) *IMMP2L* transcripts using fluorescence *in situ* hybridization. In the embryo, a general expression of both the transcripts were observed, whereas in the D7 mouse, and especially the adult mouse, the signals were mainly localized to the hippocampus/dentate gyrus and the cerebellum. (d) The coronal sections (Bregma -2.92) of adult mouse were hybridized with both transcripts using DNP chromogenic detection. SC, superior commissure; CG, cingulum; PC, posterior commissure; TH, thalamus; CP, caudate putamen; FRN, fornix; HC, hippocampus; SNR, substantia nigra; AM, amygdala; DG, dentate gyrus, CB, cerebellum; OB, olfactory bulb; TEL, telencephalon.

posterior commissure, substantia nigra, amygdala, as well as putamen and thalamus (Figure 3d).

In the human cerebellum, both transcripts were highly expressed in the granular layer of the cerebellar cortex and some staining was also obtained in the medulla composed of the white matter (Figure 2b).

DISCUSSION

In this study, we investigated a cohort of 188 unrelated TS patients and identified seven *IMMP2L* deletions (Figure 1). Four deletions included only two exons (3a and 3b), which were not part of the long *IMMP2L* transcript (NM_001244606.1). This resulted in identification of an alternative transcript (ENST00000450877), where the ATG start codon was within exon 3a. Both transcripts were expressed in several brain regions. This study underlines that intronic deletions should be investigated further for presence of alternative transcription start sites, alternative exons and regulatory sequences.

Partial deletions within *IMMP2L* have not previously been associated with TS and deletions of this gene have been observed in control populations. However, the deletion frequency was significantly higher in the present TS cohort (3.7%) compared with the background population (0.9%) and the Affymetrix reference cohort (0.8%). The use of the Danish background population as a control cohort enabled us to minimize the population stratification bias between patients and controls, whereas the Affymetrix reference cohort ensured to eliminate a potential bias produced by different detection methods (chromosome microarray and qPCR). The present results, therefore, suggest that intragenic deletions of *IMMP2L* may be genetic risk factors in TS pathogenesis. In a previous study, *IMMP2L* deletions were not detected among 22 TS patients, but this may be due to the small size of this cohort or the localization of the amplicons used.¹⁰ The type of *IMMP2L* mutations found in TS patients is chromosome aberrations or deletions, but not small base-pair changes. This suggests that haploinsufficiency of one or more *IMMP2L* transcripts, rather than structural changes in the protein structure (eg, due to base-pair substitutions), is associated with TS susceptibility. However, screening of larger TS cohorts for small base-pair changes in *IMMP2L* is warranted to support this hypothesis.

IMMP2L has previously been associated with autism^{21,22} and ADHD.²³ Furthermore, in the Decipher database, deletions and duplications spanning *IMMP2L* are reported in patients with developmental delay and ASD. Notably, among the seven families investigated in this study, five index patients have ADHD and one has Asperger syndrome. These findings, together with the previous studies, suggest that *IMMP2L* may be one of the shared genetic factors between neurodevelopmental disorders. However, it is likely that *IMMP2L* deletions are not fully penetrant as underlined by the finding of deletions in unaffected parents (Table 1) and in control populations. Investigation of further and larger TS cohorts is necessary to understand the role of this gene in the pathogenesis of TS and other neurodevelopmental disorders.

In addition to the *IMMP2L* deletions, three patients have CNVs, which have previously been associated with neuropsychiatric disorders (Table 1). One patient with TS only (P5) has a duplication including the *CHRNA7* gene (15q13.3) and duplications of this region have been observed across a range of neuropsychiatric phenotypes including intellectual disability, ASD and ADHD.^{24–28} It is therefore possible that both the *IMMP2L* deletion and the *CHRNA7* duplication contribute to the disease pathogenesis in this patient. In another patient (P4) with TS and OCD both the *IMMP2L* deletion and the 4q35.2 duplication are inherited from the father with OCB and stuttering. This duplication includes five genes, and CNVs of *TRIML1*, *TRIML2* and *LOC401164* have been observed among ASD patients.²⁹ It is thus likely that both CNVs contribute to the disease pathogenesis. In the third patient (P3) with TS, ADHD and Asperger syndrome, the *IMMP2L* deletion and the partial *DISC1* duplication are inherited from phenotypically normal parents (mother and father, respectively). *DISC1* has been implicated in several psychiatric disorders,³⁰ including ADHD,³¹ and may contribute to the patients symptoms. Finding of these additional CNVs in TS patients with *IMMP2L* deletions supports the notion of a complex pattern of inheritance in TS, such as oligogenic heterozygosity or epistatic interactions.

Mitochondrial dysfunction has been associated with a range of human disorders, including neuropsychiatric disorders.³² A defective *IMMP2L* may therefore be related to the pathogenesis of neuropsychiatric disorders, including TS, due to a hyperactive mitochondrion with increased production of superoxide through a defective processing of *IMMP2L* substrates, such as cytochrome c1 and glycerol-3-phosphate

dehydrogenase 2, leading to apoptosis. It is also possible that a defective *IMMP2L* leading to an unbalance in mitochondria function may be a risk factor affecting myelination. In support of this hypothesis, it has recently been reported that mitochondria dysfunction through oxidative stress, perturbed calcium homeostasis and release of pro-apoptotic factors could compromise myelinogenesis.³³ Both *IMMP2L* transcripts show high expression in the cerebellum, which is involved in TS pathogenesis.^{34,35} Mitochondria dysfunction at the cellular level and cerebellum involvement at the anatomic level are also related to other movement disorders such as dystonia and ataxia.^{36,37} It is therefore plausible that an unbalanced mitochondria function through a defective cerebellar *IMMP2L* may contribute to tic formation. However, further studies are necessary to understand the involvement of mitochondria dysfunction due to a deficient *IMMP2L* in TS.

Different isoforms of *IMMP2L* may have differential roles in development and in different brain regions. In mouse embryo (E16.5), the short transcript is mainly expressed in the brain whereas the long transcript shows an ubiquitous expression (data not shown). In the E15.5 and D7 mouse both transcripts are expressed throughout the brain, whereas in the adult mouse both transcripts are mainly expressed in the hippocampus/dental gyrus and cerebellum (Figures 3a and b). In human adult brain (RNA panel), the long transcript shows an overall expression, whereas the short transcript shows a higher expression in the cerebellum, hippocampus and corpus callosum, regions which are shown to be involved in TS pathogenesis.³⁸ It is thus possible that differences in the expression of the alternative *IMMP2L* transcripts in specific brain regions due to the deletions, may lead to TS susceptibility.³⁹

Noradrenergic drugs, such as clonidine, are used in the treatment of patients with tics and ADHD, but evidence for the effect of this medicine is limited.⁴⁰ Notably, clonidine has recently been associated with improvement in tic symptoms and overall well-being of a patient with a chromosome aberration involving *IMMP2L*.⁴¹ Further studies are warranted to investigate whether clonidine supplement is treatment of choice in patients with a nonfunctional *IMMP2L* gene.

The present findings together with previous studies support that genomic rearrangements affecting *IMMP2L* may be one of the predisposing factors involved in TS and overlapping neurodevelopmental disorders. Investigation of other larger cohorts for *IMMP2L* deletions and functional studies of both transcripts are necessary to gain a better insight into the role of this gene in disease pathogenesis.

WEB RESOURCES

UCSC Genome Browser, Human Genome Database Build hg19, February 2009 (<http://www.genome.ucsc.edu>).

Database of Genomic Variants (DGV), <http://projects.tcag.ca/cgi-bin/variants/gbrowse/hg19>.

Decipher database, Database of Chromosomal Imbalance and Phenotype in Humans; <http://decipher.sanger.ac.uk>.

Ensembl database, <http://www.ensembl.org/index.html>.

TMpred, http://www.ch.embnet.org/software/TMPRED_form.html.

SOSUI, http://bp.nuap.nagoya-u.ac.jp/sosui/sosui_submit.html.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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