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Association of *AADAC* Deletion and Gilles de la Tourette Syndrome in a Large European Cohort

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ABSTRACT

BACKGROUND: Gilles de la Tourette syndrome (GTS) is a complex neuropsychiatric disorder with a strong genetic influence where copy number variations are suggested to play a role in disease pathogenesis. In a previous small-scale copy number variation study of a GTS cohort ($n = 111$), recurrent exon-affecting microdeletions of four genes, including the gene encoding arylacetamide deacetylase (*AADAC*), were observed and merited further investigations.

METHODS: We screened a Danish cohort of 243 GTS patients and 1571 control subjects for submicroscopic deletions and duplications of these four genes. The most promising candidate gene, *AADAC*, identified in this Danish discovery sample was further investigated in cohorts from Iceland, the Netherlands, Hungary, Germany, and Italy, and a final meta-analysis, including a total of 1181 GTS patients and 118,730 control subjects from these six European countries, was performed. Subsequently, expression of the candidate gene in the central nervous system was investigated using human and mouse brain tissues.

RESULTS: In the Danish cohort, we identified eight patients with overlapping deletions of *AADAC*. Investigation of the additional five countries showed a significant association between the *AADAC* deletion and GTS, and a final meta-analysis confirmed the significant association ($p = 4.4 \times 10^{-4}$; odds ratio = 1.9; 95% confidence interval = 1.33–2.71). Furthermore, RNA in situ hybridization and reverse transcription-polymerase chain reaction studies revealed that *AADAC* is expressed in several brain regions previously implicated in GTS pathology.

CONCLUSIONS: *AADAC* is a candidate susceptibility factor for GTS and the present findings warrant further genomic and functional studies to investigate the role of this gene in the pathogenesis of GTS.

Keywords: AADAC, Association study, CNV, Copy number variation, Gilles de la Tourette syndrome, Neuropsychiatric disorder

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Gilles de la Tourette syndrome (GTS) (Mendelian Inheritance in Man: 137580) is a heterogeneous neuropsychiatric disorder characterized by the presence of chronic motor and vocal tics normally arising in childhood (1). The disorder often co-occurs with obsessive-compulsive disorder (OCD)/obsessive-compulsive behavior and attention-deficit/hyperactivity disorder (ADHD), but other behavioral problems, such as depression, anxiety, learning disabilities, and autism spectrum disorder (ASD), are also overrepresented among GTS patients (1). The prevalence of GTS ranges from .4% to 3.8% worldwide and male individuals are affected approximately four times more frequently than female individuals (2).

Twin and family studies suggest a strong genetic component for GTS susceptibility (3–5). Although multiple genes and chromosomal regions have been implicated in disease etiology, it has generally been difficult to replicate the findings (5), supporting the notion of GTS as a complex polygenic disorder. Furthermore, environmental factors are suggested to play a role in disease development (6) and GTS is thus accepted to be a multifactorial disorder with a largely unknown etiology.

Rare copy number variations (CNVs) play an important role in a number of neuropsychiatric disorders, including ASD and schizophrenia (7). To date, four genome-wide studies have investigated the possible role of CNVs in the pathogenesis of GTS (8–11). In one of these studies, Sundaram *et al.* (8) screened 111 GTS patients and 73 ethnically matched control subjects and identified four recurrent exon-affecting CNVs in patients that were not present in the control group or in the Database of Genomic Variants (DGV) by then. These CNVs

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included partial deletion of neurexin 1 (*NRXN1*) and α-T catenin (*CTNNA3*), genes that have previously been associated with ASD (12,13) and/or schizophrenia (14), as well as deletion of the entire fibrous sheath CABYR-binding protein (*FSCB*) gene and of the arylacetamide deacetylase (*AADAC*) gene, neither of which have previously been linked to a disorder. Although *AADAC* was deleted in three patients (2.7%) and none of the control subjects, it was not considered a possible GTS candidate gene, probably because its expression in neuronal tissues had never been demonstrated and the known function of the protein product could not readily be related to disease pathogenesis. In addition, the number of patients was too small to reach a statistically significant result.

In this study, we performed an initial CNV screening of 243 Danish GTS patients and 1571 Danish control subjects to investigate the preliminary findings of Sundaram *et al.* (8). As the *AADAC* deletion was the most promising candidate among these four CNVs, we carried out a follow-up study on a large cohort of GTS patients and nationality matched control subjects from five additional European countries. Furthermore, we investigated expression of *AADAC* in several GTS-related brain regions to provide further support for a role of this gene in disease pathogenesis.

METHODS AND MATERIALS

Subjects

In total, 1181 patients with GTS were assembled from six European countries: Denmark: 243 patients were recruited as part of a large clinical study at the Tourette Clinic in Glostrup (15) and diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) (16); Iceland: 466 patients were recruited from all over Iceland by deCODE Genetics in Reykjavik. Recruitment was based on either a clinical diagnosis using the ICD-10 Classification of Mental and Behavioral Disorders (17) ($n = 176$) or DSM-IV (18) ($n = 171$) or with an affirmative answer to the question: "Have you previously been diagnosed with Tourette syndrome?" ($n = 119$); Netherlands: 86 patients were recruited at the Department of Psychiatry at the University Medical Center Groningen (19) and diagnosed according to DSM-IV-TR (16) while 76 patients were collected at the Department of Psychiatry at VU University Medical Center Amsterdam and diagnosed using the Diagnostic Confidence Index (20) and the Yale Global Tic Severity Scale (21); Hungary: 109 patients were recruited as part of the efforts of a multinational consortium (The Tourette Syndrome Genetics–Southern and Eastern Europe Initiative [TSGeneSEE]) (22) and diagnosis was based on DSM-IV-TR (16); Germany: 108 patients were recruited from the Outpatient GTS Clinic of Hannover Medical School (23) and diagnosed according to DSM-IV-TR (16); Italy: 93 patients were recruited as part of the TSGeneSEE consortium (22) and diagnosis was based on DSM-IV-TR (16), Diagnostic Confidence Index (20), and Yale Global Tic Severity Scale (21). The gender ratios of the patients and the presence of OCD and ADHD are listed in Table 1.

A total of 118,730 nationality matched control subjects were assembled from the same six European countries: Denmark: DNA of 316 control subjects was retrieved from the biobanks of the Kennedy Center in Glostrup and 1571 control subjects were recruited through the Danish Donor Corps in the Copenhagen area (24); Iceland: 112,714 control subjects were recruited from all over Iceland as parts of various genetic programs at deCODE Genetics [a smaller subset of these individuals has been described before (24)]; Netherlands: 224 control subjects were recruited at the Donders Institute for Brain, Cognition and Behavior of the Radboud University Nijmegen Medical Center (25) and 2884 control subjects were collected as part of the Nijmegen Biomedical Study (26); Hungary: DNA from 96 healthy high school students was collected from the general Hungarian population; Germany: 199 control subjects were recruited from the Department of Transfusion Medicine, Hannover Medical School (23) and 512 control subjects were randomly selected from the general population of Munich (24); Italy: 51 control subjects were recruited as part of the TSGeneSEE consortium (22) and 163 individuals were recruited in South Verona for a genetic study on schizophrenia (24). To our knowledge, all the patients and control individuals were unrelated, except from a small subset of the Iceland cohort, which was accounted for in the statistical analyses. As the control individuals have been recruited in relation to different research projects, not all of them have been investigated specifically for GTS or other neurological disorders. However, whenever information about a neuropsychiatric phenotype was available, the individuals were excluded from the control cohorts. The gender ratios of the different control cohorts are given in Table 1.

All patient and control samples were investigated using DNA extracted from whole blood or buccal swabs. For all

Table 1. Phenotypic and Demographic Information About Patients and Control Individuals, *n* (%)

	Tourette Patients				Control Individuals	
Nationality	Male	Female	Comorbid ADHD	Comorbid OCD	Male	Female
Denmark	196 (81)	47 (19)	93 (38)	84 (35)	1279 (68)	608 (32)
Iceland	330 (71)	136 (29)	169 (36)	104 (22)	50,262 (45)	62,452 (55)
Netherlands	115 (71)	47 (29)	35 (22)	49 (30)	1564 (50)	1544 (50)
Hungary	91 (83)	18 (17)	41 (38)	11 (10)	58 (60)	38 (40)
Germany[a]	88 (82)	20 (18)	41 (38)	17 (16)	384 (54)	327 (46)
Italy	81 (87)	12 (13)	57 (61)	50 (65)	117 (55)	97 (45)
Total	901 (76)	280 (24)	436 (37)	315 (27)	53,664 (45)	65,066 (55)

ADHD, attention-deficit/hyperactivity disorder; OCD, obsessive-compulsive disorder.
[a]For 12 German patients, it was unknown whether they had any comorbidities.

Table 2. Numbers of Included Patients and Control Subjects, Numbers of Identified *AADAC* Deletions, and the Allelic Frequency of the Deletion in Each Cohort

	Patients	*AADAC* Deletion		Control Subjects	*AADAC* Deletion	
Nationality	*n*	*n*	%	*n*	*n*	%
Denmark	243	8	1.65	1887	44	1.17
Iceland	466	21	2.25[a]	112,714	2236[b]	.99
Netherlands	162	3	.93	3108	53	.85
Hungary	109	4	1.83	96	3	1.56
Germany	108	5	2.31	711	12	.84
Italy	93	2	1.08	214	2	.47
Total	1181	43	1.82	118,730	2340	.99

[a]For Icelandic patients with a clinically confirmed diagnosis and a self-reported diagnosis, the allelic frequency was 2.16% and 2.52%, respectively.

[b]A total of 10 Icelandic control subjects were homozygous for the deletion.

samples, collection was approved by local ethical boards of respective countries and informed written consent was obtained from all patients or their legal guardians. The total numbers of patients and control subjects from each population are shown in Table 2.

All cases and control subjects were carefully matched for ancestry to remove any potential bias introduced by population stratification. Ancestry information was based on the family name of the included individuals, as well as self-reported ancestry. For a subset of the subcohorts, principal component analysis was also performed to confirm ancestry (data not shown).

Genotyping

All patients and control individuals were genotyped for the *AADAC* deletion by employment of chromosome microarray, quantitative polymerase chain reaction (qPCR) or genome-wide genotyping (Table S1 in Supplement 1).

Chromosome microarray was carried out using Affymetrix CytoScan HD array or Affymetrix 2.7M array (Affymetrix, Santa Clara, California). Data were analyzed using Affymetrix Chromosome Analysis Suite software (ChAS) and the threshold was 5 markers for deletions and 10 markers for duplications. The data were interpreted with the aid of the University of California, Santa Cruz Human Genome Browser (http://genome.ucsc.edu;GRCh37/hg19assembly).

qPCR was used to screen a subset of the patients and control subjects for the *AADAC* deletion, as well as to verify all *AADAC* deletions identified by chromosome microarray in patients. See Supplement 1 and Table S2 in Supplement 1 for details.

Genome-wide genotyping using BeadChips was performed at deCODE Genetics using either Illumina HumanHap or Omni chips (Illumina, San Diego, California). Copy number detection was performed using the PennCNV algorithm (27), following the standard protocol using allele frequencies per batch from the samples typed on each genotyping array and adjusting for genomic waves (28) using guanine-cytosine model files generated specifically for each genotype assay. For all genotyping arrays, we excluded markers found within the genomic super-duplicate regions (29,30). Sample-based quality control was performed using the statistics calculated by PennCNV. Samples with BAF-Drift $>$ .01 or LLR-SD $>$ .3 were excluded. For the Illumina BeadChips, the threshold for detecting the *AADAC* deletion was also set to a minimum of five markers.

Statistical Analysis

Fisher's exact test was performed to examine the allelic association of the four recurrent exon-affecting CNVs reported by Sundaram *et al.* (8) with GTS in the Danish cohort.

Allelic association analysis of the *AADAC* deletion was also carried out for the Icelandic, Dutch, Hungarian, German, and Italian cohorts using Fisher's exact test. To account for the relatedness of the Icelandic cohort, dropdown simulations through the Icelandic genealogy were performed and an empirical *p* value was calculated from the fraction of simulations having a Fisher's exact *p* value less than the *p* value obtained using the observed allele counts. Results from all cohorts, except for the Icelandic cohort, were combined using the Mantel-Haenszel exact test, which was used to control for confounding due to population stratification. The results from Iceland were combined with those of the remaining cohorts using inverse variance weighted, fixed-effects meta-analysis.

Expression Analysis

Reverse transcription PCR was carried out on a panel of total RNA from 19 various brain regions of a normal human adult (Biochain, Newark, California) and all PCR products were Sanger sequenced (primer sequences are listed in Table S2 in Supplement 1).

In Situ Hybridization

In situ hybridization was performed on paraffin embedded human cerebellum sections (Capital Biosciences, Rockville, Maryland) and on 10-micron sections of snap-frozen brains of adult BALB/c mice and E15.5 embryos (31). See Supplement 1 for details.

RESULTS

We investigated the frequency of the four exon-affecting CNVs (of the *NRXN1*, *CTNNA3*, *FSCB*, and *AADAC* genes) reported by Sundaram *et al.* (8) in 243 Danish patients with GTS and 1571 Danish control subjects. We identified eight patients with an $\sim$36 kilobase (kb) deletion encompassing the entire *AADAC* gene (Figure 1A), while an exon-affecting *CTNNA3* deletion was detected in a single patient and none of the patients had exon-affecting *NRXN1* or *FSCB* deletions (Table S3 in Supplement 1). Despite a modest *p* value of .38 (odds ratio [OR] = 1.4; 95% confidence interval [CI] = .65–3.04), the *AADAC* deletion was the most promising candidate CNV for further investigations. A subsequent follow-up screening of 938 patients with GTS and 116,843 population-based control subjects from Iceland, the Netherlands, Hungary, Germany, and Italy was performed to further investigate the association of the *AADAC* deletion and GTS. The Mantel-Haenszel exact test was used to estimate the combined association in the additional five populations and showed a statistically significant association ($p = 4.6 \times 10^{-4}$; OR = 2.1; 95% CI = 1.37–3.07).

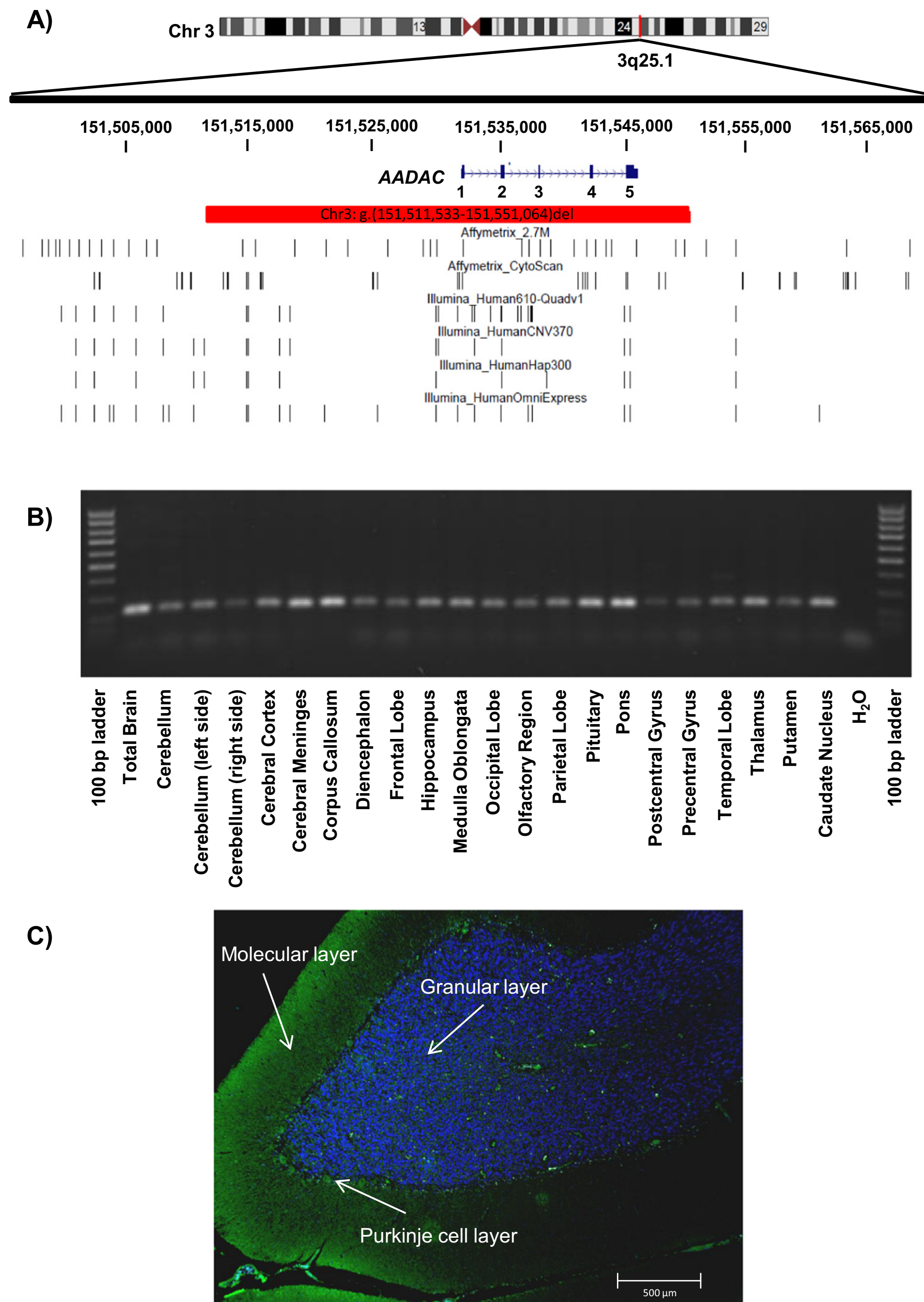

Figure 1. Genomic location of the *AADAC* deletion and expression of *AADAC* in human brain regions. **(A)** Genomic region showing the *AADAC* gene and indicating the exact location of the deletion (in red) identified in both patients and control subjects of all populations. The positions of the markers within the genomic region for each of the employed Affymetrix and Illumina array platforms are indicated below the deletion. **(B)** *AADAC* expression in various human brain regions. Using reverse transcription polymerase chain reaction, *AADAC* expression was investigated in a panel containing 19 different brain regions. Complementary DNA was synthesized according to the manufacturer's instructions using .5 μg total RNA, random primers (Promega, Madison, Wisconsin), and SuperScript III reverse transcriptase (Invitrogen, San Diego, California). **(C)** *AADAC* expression in adult human cerebellum. Using fluorescent in situ hybridization, *AADAC* expression was identified in the molecular layers of the human cerebellum. bp, base pair.

Overall, we found that 3.6% of the patients and 2.0% of the control individuals were carriers of the deletion. The total numbers of patients and control individuals with deletion of *AADAC* and the allele frequency of the deletion in each cohort are given in Table 2. None of the investigated continental European individuals were homozygous for the deletion, but

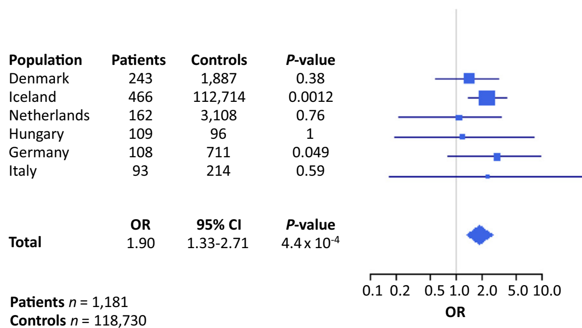

Figure 2. Forest plot showing association of *AADAC* with Gilles de la Tourette syndrome across the six European cohorts. For each cohort, the number of investigated patients and control subjects as well as the p values are given. The odds ratios (ORs) are represented by squares (the size of which is proportional to the weights used in the meta-analysis) and the horizontal lines represent the 95% confidence interval (CI) for each population. The Mantel-Haenszel exact test and inverse variance-weighted fixed-effects meta-analysis were used to combine association evidence across cohorts. The OR, 95% CI, and p value are given for the meta-analysis and the diamond represents the result of the combined meta-analysis.

among the Icelandic control subjects, 10 homozygous individuals were observed. However, the Icelandic control cohort was by far the largest ($n = 112{,}714$) and this finding is to be expected for a population in Hardy-Weinberg equilibrium. In the final meta-analysis of all 1181 investigated patients and 118,730 control subjects from the six countries (Denmark, Iceland, the Netherlands, Hungary, Germany, and Italy), the association between deletion of the *AADAC* gene and the GTS phenotype was statistically significant ($p = 4.4 \times 10^{-4}$; OR = 1.9; 95% CI = 1.33–2.71) (Figure 2).

A very limited number of the DNA samples originated from buccal swaps; however, there was no significant difference in the detection rate of the *AADAC* deletion between DNA obtained from blood samples or buccal swaps.

The distribution of comorbidities among patients with the *AADAC* deletion was comparable with that of the combined patient cohorts, although it is worth noting that the frequency of GTS-only was 11% higher among the deletion carriers (Figure S1A in Supplement 1). The gender distributions among the patients with the *AADAC* deletion and among the combined patient cohorts were not significantly different (Figure S1B in Supplement 1). Thus, the association of the *AADAC* deletion with GTS does not appear to be gender specific. Therefore, the difference in the gender ratios between patients and control subjects is unlikely to contribute to the significant findings of this study (Table 1).

In a large Icelandic sample of genotyped trios ($n = 34{,}726$), we found the *AADAC* deletion in 722 of the offspring. In all the instances, the deletion was transmitted. Thus, the CNV may have arisen as a single event, and in keeping with this finding, a single nucleotide polymorphism (SNP) marker (chr3:151,551,059), which is approximately 19 kb distal to the *AADAC* gene, is in strong linkage disequilibrium ($R^2 = .98$) with the *AADAC* deletion in the Icelandic population. However, it is worth mentioning that this SNP was not identified among the eight Danish deletion carriers, and although it might be used as a genotyping marker in the Icelandic population, it cannot automatically be used in other populations, e.g., the SNP is not found in the 1000 Human Genome project (32). In further support for the hypothesis that the deletion has arisen as a single event, we mapped the deletion breakpoints in several individuals from each of the investigated populations, including both patients and control subjects, using PCR (primer sequences are available in Table S2 in Supplement 1). The breakpoints of all of these deletions were exactly the same, spanning a 39,532 base pair region at chromosome position chr3:151,511,533-151,551,064 (University of California, Santa Cruz, Feb. 2009 GRCh37/hg19 release) (Figure 1A).

Expression Analysis

We investigated tissue-specific expression of the *AADAC* transcript using a panel of total RNA from 19 different regions of human adult brain to provide evidence for the involvement of *AADAC* in the central nervous system. Expression of *AADAC* was observed in all the investigated regions, with particularly high expression in corpus callosum, pituitary, pons, cerebral meninges, cerebral cortex, thalamus, and caudate nucleus (Figure 1B).

In Situ Hybridization Studies

In murine embryonic (E15.5) brain, the *AADAC* transcript was ubiquitously expressed at a moderate level (Figure 3A). In the adult mouse, expression was also observed in most of the brain regions (data not shown) with particularly high levels in the cerebellum, hippocampus, olfactory bulb, cerebral cortex, pons, and medulla (Figure 3B). These results support in situ hybridization data provided by the Allen Brain Atlas (33). Using a coronal section of the adult mouse brain, expression was observed especially in the molecular layer and the Purkinje cell layer of the cerebellum (Figure 3C). The very high expression in the Purkinje cell layer was especially obvious when using a transverse section of cerebellar folia (Figure 3D). No signal was observed when performing negative control experiments (Figure S2 in Supplement 1).

In the human cerebellum, the *AADAC* transcript was highly expressed in the molecular and Purkinje cell layers of the cerebellar cortex, while limited staining was observed in the granular layer (Figure 1C).

DISCUSSION

In this study, we investigated four exon-affecting CNVs previously identified in a small GTS cohort (8). Although the *NRXN1* gene has been associated with GTS in several studies (8,10), our initial screening of the Danish cohort indicated *AADAC* as the strongest candidate among these four genes tested. In the follow-up study of the Icelandic, Dutch, Hungarian, German, and Italian cohorts, the association was shown to be statistically significant and this was also the case when including the Danish results in a meta-analysis.

Deletions of *AADAC* have been reported in DGV and are also observed in our ethnically matched control subjects, including 10 homozygous Icelandic control subjects without phenotype information; however, the frequency of the deletion is significantly higher among patients than control subjects ($p = 4.4 \times 10^{-4}$; OR = 1.9; 95% CI = 1.33–2.71) even after

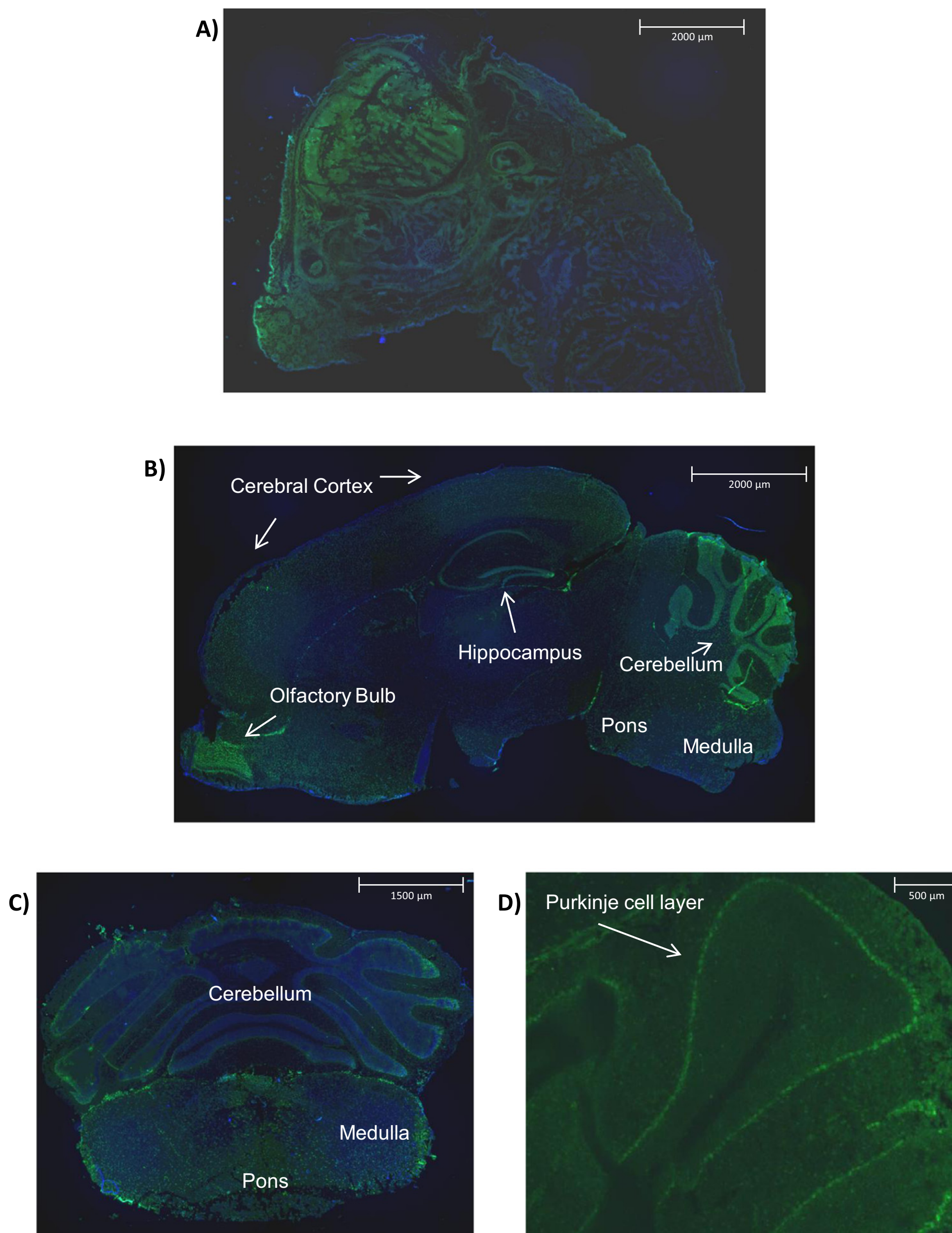

Figure 3. Expression of *AADAC* in mouse brain sections. Sagittal sections from **(A)** mouse embryo (E15.5) and **(B)** adult mouse and **(C)** coronal section from adult mouse hybridized with probes for exon 2 of the mouse *Aadac* gene using fluorescence in situ hybridization. Fluorescein isothiocyanate signals are green and DAPI stains the DNA blue. In the embryo, the gene was ubiquitously expressed, whereas expression was localized to the cerebellum, olfactory bulb, hippocampus, cerebral cortex, and pons in the adult mouse. **(D)** Sagittal section of cerebellar folia in adult mouse showing particularly high expression in the Purkinje cell layer. Please note that only fluorescein isothiocyanate signals are shown in the figure.

Bonferroni correction for multiple testing ($p = 1.8 \times 10^{-3}$). Our result is the first support for a significant association between *AADAC* and GTS, suggesting that this gene plays a role in the pathogenesis of GTS. The finding of *AADAC* deletions among the general population, including subjects with complete knockout of the gene, may indicate incomplete penetrance of the deletion, variable expression, or presence of other prerequisite genetic or environmental factors. Furthermore, many of the control subjects were recruited for different research projects not related to neuropsychiatric disorders and we cannot exclude that some of the deletion carriers are affected with GTS, chronic tics, or other related disorders.

Besides the study by Sundaram *et al.* (8), three other studies have investigated the role of CNVs in GTS (9–11); however, none of these have reported finding of *AADAC* deletions. A plausible explanation may be that these studies predominantly focused on rare (<1%) and/or large CNVs, which would have excluded the *AADAC* deletion. Thus, while McGrath *et al.* (11) and Nag *et al.* (10) only investigated or reported CNVs larger than 500 kb, respectively, Sundaram *et al.* (8) investigated CNVs as small as 10 kb. In addition, Fernandez *et al.* (9) only included rare CNVs, defined if less than 50% of its length overlapped CNVs present at ≥1% frequency in the March 2010 update of the DGV database. Sundaram *et al.* (8) used an older version of DGV to define rare

CNVs, where the *AADAC* deletion was not yet identified in a significant number of cases. Therefore, re-examination of these data may give further support to the present finding.

Except for the Danish and Icelandic patients, all the patient cohorts were screened for the deletion using a single qPCR primer pair within the first exon of *AADAC*, while the majority of control individuals were screened using chip genotyping, which includes several SNP probes within the gene. qPCR is a valid method for verification of chromosome microarray findings and all the *AADAC* deletions identified with chromosome microarray could be verified with qPCR using exon 1 and exon 5 primers. As the breakpoints of all the deletions, which were fine mapped across the different nationalities, were identical, there should be no bias in the pickup rate using the different array platforms, as they all had sufficient coverage within the deletion region (Figure 1A). The lower frequency of *AADAC* deletions in the patient cohorts from the Netherlands and Italy can therefore probably not be explained by the detection method but is more likely a consequence of the relatively small sample sizes, which make them more sensitive to chance variation. As the Illumina HumanHap300 and HumanCNV370 platforms have a lower probe density within the deletion region compared with the other arrays, we investigated whether these platforms are more sensitive to probe failure. The deletion detection frequency was .018 and .020 among the Icelandic control subjects using Illumina HumanHap300 and HumanOmniExpress, respectively. Likewise, the detection frequency was .023 and .015 among the Dutch control subjects using a merge of the Illumina HumanHap300 and HumanCNV370 and HumanOmniExpress, respectively. Thus, the lower-density platforms employed to investigate the control subjects do not appear to have a lower detecting frequency. Furthermore, for the Danish control cohort, the deletion detection frequency was .019 and .028 using qPCR and Illumina HumanOmniExpress, respectively. This indicates that the sensitivity of the different array platforms used to investigate the control subjects is not lower than the sensitivity of qPCR used for several patient cohorts. Overall, this illustrates that the significant association of the *AADAC* deletion with GTS is not likely to be due to different detection methods.

One could also speculate that phenotypic differences across the study samples, e.g., differences in the frequencies of comorbidities across the populations, could affect the results. However, although the frequency of comorbid ADHD was much lower among the Dutch patients compared with the other populations, we did not observe an overrepresentation of GTS patients with comorbid ADHD among the deletion carriers, which might have explained the low frequency of deletions among the Dutch patients. On the contrary, more patients with GTS-only were observed among the deletion carriers, suggesting that the *AADAC* deletion is specifically associated with the GTS phenotype and not with one of the comorbidities, although this difference was not significant. In support for the suggested GTS specificity of the *AADAC* deletion, genome-wide CNV studies performed for OCD and ADHD have not reported finding of CNV regions overlapping with the *AADAC* deletion (11,34–39). However, further studies on patients with GTS-only, ADHD-only, and OCD-only are warranted to give further answers to these questions.

A final concern when performing case-control studies is the risk of population stratification. The Mantel-Haenszel test does not assume that the deletion has the same frequency in the different populations investigated, hereby protecting against false positives caused by differences in deletion frequency among these populations. The Mantel-Haenszel test does, however, not protect against false positives caused by within-population differences in ancestry between cases and control subjects; but we believe that these differences are unlikely, given the efforts made to ensure uniform ancestry within each population.

In all the control cohorts, the deletion frequencies were similar, except for the Hungarian and Italian cohorts, but the number of investigated individuals in these two cohorts was small and the deletion frequency estimates were therefore prone to fluctuations. Considering the similar deletion frequencies across the different countries, the deletion is likely to have arisen from a single event thousands of years ago. In further support for this hypothesis, the deletion is not flanked by segmental duplications known to drive nonallelic homologous recombination giving rise to recurrent CNVs (40) but rather appears to have arisen through a seemingly random event. In addition, the deletion was always inherited from either of the parents among the 722 Icelandic trios, and the breakpoints were identical for both patients and control subjects of all investigated nationalities when mapped.

The function of AADAC in the brain and nervous system is unknown. In this study, we detected expression of *AADAC* in mouse and human brain regions (such as hippocampus, corpus callosum, and caudate nucleus), which are implicated in GTS pathogenesis (41), giving further support for a role of *AADAC* in GTS etiology. Notably, expression of *AADAC* was particularly high and specific in the Purkinje cell layer of the human cerebellum. The role of Purkinje cells in the pathogenesis of GTS has not been investigated to date, but abnormal or dysfunctional Purkinje cells have been implicated in other neurodevelopmental and movement disorders such as ASD, ataxia, and dystonia (42–45). The cerebellum has previously been implicated in GTS at the anatomical level by imaging studies, where regional reductions in cerebellar volume correlated with the degree of tic severity (46) and cerebellar activity was shown to be increased at tic initiation (47). It is thus possible that aberrant activity of Purkinje cells and a dysfunctional cerebellum may play a role in GTS pathogenesis and should be investigated further.

AADAC is suggested to be involved in neutral lipid lipolysis, detoxification, and drug metabolism (48) and the gene has previously been shown to be expressed in the liver, small intestine, adrenal glands, and pancreas (49). Possibly owing to this knowledge, *AADAC* has not previously been regarded as an attractive candidate gene for GTS. However, other drug-metabolizing enzymes of the liver are shown to be expressed in the brain, where they are suggested to be involved in the metabolism of neurotransmitters and neurosteroids (50). One example is the suggested involvement of the cytochrome P450 enzymes CYPD in dopamine metabolism (51) as indicated by expression in dopaminergic cells of the rat substantia nigra (52), co-localization with the dopamine transporter (53), and association of *CYP2D6* genetic variants with Parkinson disease (54) and Alzheimer disease (55). Our finding of *AADAC* expression in the brain suggests that its protein product may

play a role in the central nervous system; however, whether it is involved in the synthesis or metabolism of neurotransmitters or has another function is currently unknown. Identification of endogenous substrates of the AADAC enzyme in the brain is therefore warranted, as are functional studies investigating the consequences of the *AADAC* deletion at the cellular level in neuronal tissues.

In conclusion, we have identified a statistically significant association of the deletion of *AADAC* with GTS using a large cohort of patients and population-based control subjects from six European countries. This finding indicates that *AADAC* could be one of the genetic factors that play a role in the pathogenesis of GTS. Demonstration of *AADAC* expression in various brain tissues gives further support for its involvement in normal neuronal function. However, investigations of additional patient cohorts are needed to replicate the identified association between *AADAC* and GTS, and functional studies are warranted to investigate the role of its enzyme product in the human brain.

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ARTICLE INFORMATION

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