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Multiple rare *SAPAP3* missense variants in trichotillomania and OCD

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Obsessive–compulsive disorder (OCD) and the spectrum of associated conditions, such as trichotillomania (TTM), Tourette syndrome and body dysmorphic disorder, affect about 2–4% of the world population. 1,2 Clinically OCD spectrum disorders are characterized by persistent intrusive thoughts (obsessions), repetitive actions (compulsions) and excessive anxiety. Although heritability studies in OCD have shown a 3–12 times increased risk for first-degree relatives and twin studies revealed higher concordance amongst monozygotic twins (80–90%) compared to dizygotic twins (47–50%), the identification of the underlying risk-conferring genetic variation by means of classic genetic association studies has proven to be difficult.3

Recently, it has been shown that mice deficient of the postsynaptic synapse-associated protein 90 (SAP90)/postsynaptic density-95 (PSD95)-associated protein 3 (*SAPAP3*, also known as *Dlgap3*) develop an OCD-like phenotype, which includes compulsive grooming and increased anxiety. Interestingly, the phenotype of *Sapap3* knock-out mice can be rescued by administering selective serotonin reuptake inhibitors.[4]

We hypothesized that rare variants in the human orthologue *SAPAP3* could contribute to disorders in the OCD spectrum. To test this, we resequenced *SAPAP3* in three case populations, including 77 unrelated TTM probands collected at Duke University, 44 OCD with TTM probands from National Institute of Mental Health (NIMH), and 44 OCD cases without TTM from NIMH.[5,6] Controls were 48 OCD spectrum-negative subjects from NIMH6 and a psychiatric comparison sample of 138 subjects screened for depression but not specifically for OCD from Duke University.7 A board-certified psychiatrist saw all patients and controls and diagnoses met *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. criteria. Samples were collected under approved institutional review board protocols. In 165 cases and 178 controls the complete coding region and flanking intronic sequence of *SAPAP3* was

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resequenced using standard capillary sequencing methods (Applied Biosystems, Foster City, CA, USA).

We detected seven novel nonsynonymous heterozygous variants, with all but A189V occurring only once (Table 1; Figure 1). Thus, in total, heterozygous *SAPAP3* variants were present in 4.2% of diagnosed TTM/OCD patients, but only in 1.1% of controls (two changes in Duke control samples, with one developing depression subsequent to entry into the study). The majority of changes presented missense mutations; one variant was an in-frame insertion of five amino acids, A148insGPAGA. *In silico* analysis of the missense variants applying PMut and PolyPhen predicted two, or three, respectively, variants as of functional relevance (Table 1). The remaining polymorphisms were considered benign, including the two changes detected in controls. Further, we genotyped 6 of the identified variants in the TTM/OCD subjects in an additional sample of 281 OCD cases and in 751 general population controls.[6] R13C and P606T were found in one control each, whereas A189V was present in three controls. This suggests that these specific variants are not by themselves disease-causing abnormalities, but still leaves open the possibility that an aggregate of susceptibility variants may prove contributory to disease, as suggested for some other disorders including autism as well as OCD. The combined analyses of 2766 alleles showed that all changes are very rare, with minor allele frequencies between 0.00036 (T523K, K910R) and 0.002 (A189V).

Available pedigrees from TTM/OCD mutation carriers were enriched for a diverse set of psychiatric conditions, including panic disorder, attention deficit hyperactivity disorder (ADHD), depression, bipolar disorder and substance abuse as well as OCD spectrum disorders (details are given in Supplementary Figure 1 and Supplementary Table 1). This situation is quite typical for psychiatric genetic studies and complicates allele segregation studies. Cosegregation of genotype and phenotype is also confounded by phenotypic penetrance rates, limited psychometric instruments and assortative mating. Thus, we consider it more significant to study the combined mutation load of SAPAP3 comparing cases to controls. Similar approaches were recently adopted by other studies.[8] We observed a significant case–control association in our moderately sized sample (Fisher's one-sided exact test $P = 0.045$). With generally still limited abilities to determine functional consequences of genetic variants, we speculate that the predicted moderate functional consequences (Table 1) are not detrimental for protein function but rather increase susceptibility for OCD spectrum behavior, possibly through permissive or epistatic interactions with additional genetic and environmental factors. A recent study estimated that up to 70% of low-frequency missense alleles in humans have mildly deleterious effects.[9] The excess of rare mildly deleterious variants in any OCD risk gene could be promoted by an inefficient evolutionary selection against OCD risk alleles, which is supported by the high OCD spectrum frequency of 2–4% in the population, early disease onset and normal reproductive fitness.

In summary, on the background of an intriguing Sapap3-OCD mouse model we suggest that the present data support a role for *SAPAP3* in TTM and OCD. Expansion of our approach and modeling of rare genetic variants in *SAPAP3* will be essential to further test this hypothesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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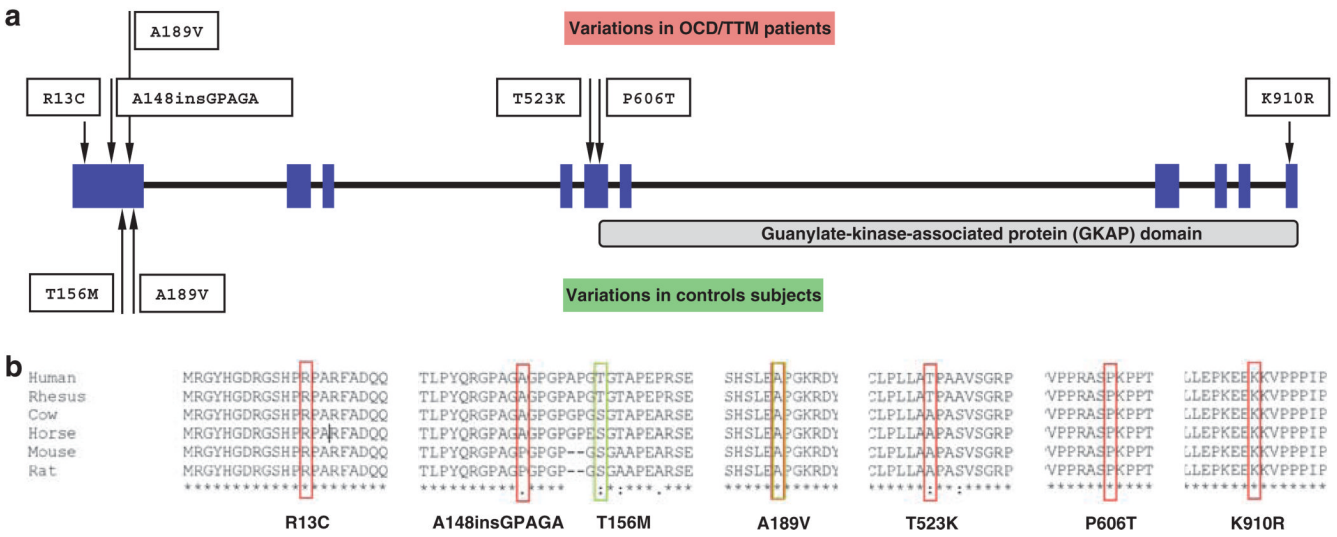

Figure 1.

Identified rare nonsynonymous polymorphisms in synapse-associated protein 90/postsynaptic density-95-associated protein 3 (SAPAP3). (**a**) Schematic of SAPAP3, which consists of 10 coding exons (blue boxes). Seven rare changes were identified in trichotillomania and obsessive–compulsive disorder (OCD) patients (upper part), but only two in controls (lower part). Most mutations fell into exon 1; however, three changes affected the conserved Guanylate-kinase-associated protein (GKAP) domain. (**b**) SAPAP3 is highly conserved between species (~97% identical amino acids between human and mouse). Accordingly the identified rare changes affected conserved residues. Red boxes—OCD cases, green boxes—controls.

Table 1

Identified rare variants in *SAPAP3* and predicted functional relevance

Detected nonsynonymous variants	Analyzed samples				Prediction algorithms	
	121 Duke TTM and NIMH OCD *w* TTM	44 NIMH OCD *w/o* TTM	48 NIMH controls	130 Duke controls	PMut[a]	PolyPhen[a]
R13C; c.38C > T	1	0	0	0	Pathological	Possibly damaging
A148insGPAGA; c.441_442ins GGGCCAGCAGGGGCA	0	1	0	0	NA[b]	NA[b]
T156M; c.467C > T	0	0	0	1	Neutral	Benign
A189V; c.566C > T	1	1	0	1	Neutral	Benign
T523K; c.1569-70CC > AA	1	0	0	0	Pathological	Possibly damaging
P606T; c.1816C > A	0	1	0	0	Neutral	Possibly damaging
K910R; c.2728A > G	1	0	0	0	Neutral	Benign
Combined allele frequencies	7/330 TTM and OCD (2.1%)		2/356 controls (0.56%)			

Abbreviations: NA, not applicable; NIMH, National Institute of Mental Health; OCD, obsessive–compulsive disorder; TTM, trichotillomania.

[a] PMut:http://mmb2.pcb.ub.es:8080/PMut; PolyPhen:http://genetics.bwh.harvard.edu/pph.

[b] Prediction of the effects of in/dels is not possible with PMut and PolyPhen, but a functional effect is likely for a five amino acid insertion.