ARTICLE

Rare missense neuronal cadherin gene (*CDH2*) variants in specific obsessive-compulsive disorder and Tourette disorder phenotypes

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The recent finding that the neuronal cadherin gene *CDH2* confers a highly significant risk for canine compulsive disorder led us to investigate whether missense variants within the human ortholog *CDH2* are associated with altered susceptibility to obsessive-compulsive disorder (OCD), Tourette disorder (TD) and related disorders. Exon resequencing of *CDH2* in 320 individuals identified four non-synonymous single-nucleotide variants, which were subsequently genotyped in OCD probands, Tourette disorder probands and relatives, and healthy controls (total $N=1161$). None of the four variants was significantly associated with either OCD or TD. One variant, N706S, was found only in the OCD/TD groups, but not in controls. By examining clinical data, we found there were significant TD-related phenotype differences between those OCD probands with and without the N845S variant with regard to the co-occurrence of TD (Fisher's exact test $P=0.014$, OR = 6.03). Both N706S and N845S variants conferred reduced *CDH2* protein expression in transfected cells. Although our data provide no overall support for association of *CDH2* rare variants in these disorders considered as single entities, the clinical features and severity of probands carrying the uncommon non-synonymous variants suggest that *CDH2*, along with other cadherin and cell adhesion genes, is an interesting gene to pursue as a plausible contributor to OCD, TD and related disorders with repetitive behaviors, including autism spectrum disorders.

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INTRODUCTION

Obsessive-compulsive disorder (OCD) and Tourette disorder (TD) are chronic, severe neuropsychiatric disorders, commonly having an early age of onset and a significant genetic component as shown by family, twin, segregation and linkage studies.^{1–6} Compulsive, repetitive and tic/TD-like behaviors in rodent models have been associated with variants in single genes such as *Sapap3* and *Slitrk5*.^{7–10} Recently, single-nucleotide polymorphisms (SNPs) within the canine neuronal cadherin gene (*CDH2*) were shown to confer a significant risk for canine compulsive disorder (CCD).¹¹ CCD shares many similarities with OCD: (a) both are characterized by repetitive, time-consuming behaviors that cause distress and functional impairment; (b) both have at least partially genetic heritabilities; and (c) symptoms in both humans and dogs can be alleviated by behavioral therapy, administration of antidepressants or a combination of both therapies.^{12,13}

CDH2 belongs to the cadherin gene family of cell–cell adhesion molecules, which function in early brain morphogenesis, synaptogenesis and synaptic plasticity, including synaptic vesicle trafficking in glutamatergic neurons.^{14–17} Other cadherin genes, including *CDH8*, *CDH9* and *CDH10*, have recently been implicated in the etiology of autism spectrum disorders, which may also be characterized by repetitive and compulsive behaviors.^{18–20}

We hypothesized that variants in the human ortholog *CDH2* could confer susceptibility to OCD and OCD spectrum disorders such as TD. To test this, we exon-sequenced *CDH2* to identify non-synonymous SNPs in a sample of 160 healthy controls and 160 OCD probands from our National Institute of Mental Health (NIMH) Intramural Research Program's Laboratory DNA collection,²¹ and subsequently performed genotyping of identified putatively functional SNPs in a total of 1161 individuals, including OCD probands ($N=260$), TD probands and their relatives ($N=454$), and healthy controls ($N=447$).

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METHODS

Unrelated OCD probands ($N=260$) were evaluated with the Structured Clinical Interview for DSM-IV-TR (SCID), the Yale-Brown Obsessive Compulsive Scale (YBOCS) ratings and other measures as described previously.^{21,22} The mean $\pm$ SD of total YBOCS scores was 22.4 ± 0.5 , and there were no subgroup differences for the different OCD subgroups considered (including the OCD subgroups with identified *CDH2* variants). TD probands and relatives ($N=454$) were evaluated by an experienced child psychiatrist based upon TD-related rating scales, as described elsewhere.²³ Unrelated healthy volunteers ($N=447$) consisted of undergraduate students from a large public university who participated in a separate study of genes and personality in return for partial course credit; they were administered self-report scales for personality measures. Although the control group completed a battery of self-report questionnaires, we cannot completely rule out the occurrence of OCD or TD as they did not complete a formal diagnostic interview. Additional details on proband and control samples have been described previously.^{22–24} All studies were conducted under protocols approved by the Institutional Review Board at the NIMH Intramural Research Program (OCD probands), the Rutgers University Institutional Review Board (TD probands and relatives) and by the Human Subjects Committee at Florida State University (healthy controls). Written informed consent was obtained from all adult participants (or, at Rutgers, their legal guardians, with written assent for minors).

Genomic DNA was extracted from whole blood obtained through venipuncture or from saliva samples (Oragene discs; DNA Genotek, Ottawa, ON, Canada). Exon sequencing was carried out in an initial subsample of 160 healthy controls and 160 OCD probands by the National Institutes of Health (NIH) Intramural Sequencing Center (NISC) as described previously.²⁵ These samples plus the remaining OCD probands, TD probands and relatives, and healthy controls were subsequently genotyped for the four non-synonymous *CDH2* variants identified by sequencing. Genotyping was performed using 5'-exonuclease TaqMan predesigned or custom assays under standard conditions: a total volume of 20 μ l and 20 ng of genomic DNA were amplified in the presence of 1 $\times$ PCR Master mix (Qiagen, Valencia, CA, USA) and 1 $\times$ TaqMan Assay (Applied Biosystems, Foster City, CA, USA; assay identification numbers and primer/probe sequences, as well as sequencing primer sequences are available from the corresponding author). Thermocycling conditions were as follows: 95 $^{\circ}$ C $\times$ 10 min, followed by 50 cycles (95 $^{\circ}$ C $\times$ 10 s, 60 $^{\circ}$ C $\times$ 30 s, fluorescence reading). The overall genotyping completion rate exceeded 97% for each assay. None of the SNPs deviated from Hardy–Weinberg equilibrium in OCD probands, TD probands and relatives, or controls as determined by contingency-table statistics (nominal $P>0.05$; data not shown). Duplicate samples (at least 10% of all samples, randomly chosen for each of the four SNPs) and no-template controls consistently yielded expected results. Statistical analyses were performed using Fisher's exact test with significance set at $P<0.05$ in two-sided analyses.

To begin to evaluate the functionality of the two *CDH2* variants of greatest interest, site-directed mutagenesis was used to generate the corresponding mutants for N706S and N845S using QuickChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA, USA) in the pCMV6-XL6 vector expressing human *CDH2* (Origene, Rockville, MD, USA). For the N706 variant, 5'-TCCAACGGG-3' was mutated to 5'-TCCAGCGGG-3'; for the N845S variant, 5'-GACAATGAC-3' was mutated to 5'-GACAGTGAC-3'. Bidirectional DNA sequence analysis was performed to confirm the mutagenesis procedure, as well as to discard any off effects on other regions of the constructs. HEK293 cells were grown and transfected under standard conditions. At 48 h after transfection, cells were harvested and protein extracts were obtained for western blot evaluations. Anti-N-cadherin was prepared by immunization of rabbits with the extracellular domain of N-cadherin, expressed and secreted into the media by HEK293 cells. Western blots were analyzed using ImageJ (NIH, Bethesda, MD, USA).

RESULTS

In the initial sample of healthy controls ($N=160$) and OCD probands ($N=160$), all 16 *CDH2* exons (Ref Seq NM_001792.3) were successfully sequenced, except for the first exon, which could not be amplified despite several primer re-designs. Four non-synonymous

Table 1 Summary of identified *CDH2* missense variants

Chromosome	Transcript	Protein	Reference	Variant	AA	
18 position	position	position	codon	codon	change	rs ID
(HG19)						
23847692	352	118	GCA	ACA	A to T	rs17445840
23837114	865	289	GTA	ATA	V to I	novel
23819054	2117	706	AAC	AGC	N to S	novel
23786302	2534	845	AAT	AGT	N to S	rs2289664

Abbreviations: AA, amino acid; HG19, human genome assembly 19.

Figure 1 Domain organization of N-cadherin (*CDH2*), with locations of variants found by exon sequencing in this study. The human *CDH2* gene located on human chromosome 18q11.2 and spans approximately 225 kb with several alternatively spliced transcripts. N-cadherin protein has tandemly repeated extracellular (EC) domains, a single pass transmembrane (TM) domain and a highly conserved cytoplasmic domain that links cadherins to the underlying cytoskeleton, in many cases via sequential binding of β -catenin to α -catenin and then to actin.^{15,27,28} S designates the signal peptide.

SNPs, Ala118Thr (A118T), Val289Ile (V289I), Asn706Ser (N706S) and Asn845Ser (N845S), were identified in *CDH2*, two of them (V289I and N706S) being novel variants (Table 1 and Figure 1). These four variants were chosen for follow-up genotyping in the ‘extended’ sample of OCD ($N=260$), TD probands and relatives ($N=454$), and healthy controls ($N=447$).

One of the novel variants, N706S, located between the extracellular domain EC5 and the transmembrane region of *CDH2* (Figure 1), occurred in three individuals: an OCD proband; a TD proband; and a sibling of a different, unrelated TD proband. The latter individual had motor and phonic tics, but did not meet full TD diagnostic criteria. Thus, N706S was found in 3/714 of the OCD/TD patients plus TD relatives sample and not in any of 447 controls; this difference was not statistically significant. Interestingly, these three individuals had unusual clinical features, as summarized in the Supplementary Material. In particular, the OCD proband with the N706S variant had extremely severe OCD (YBOCS rating of 32), rapid-cycling bipolar disorder as well as other distinctive features, including a family pedigree with multiple other neuropsychiatric problems, including schizophrenia (Supplementary Figure 1). *In silico* analysis using PMut predicted N706S as ‘pathologically relevant’.²⁶

The other novel variant, V289I, located in the extracellular domain EC2 of *CDH2* (Figure 1), was found in three individuals: a single TD proband who also had attention deficit hyperactivity disorder (ADHD) and polysubstance abuse; a single, unrelated TD proband who had OCD, ADHD and anorexia nervosa, and a single healthy control. No statistically significant differences were found for V289I.

The frequency of the N845S variant, located in the cytoplasmic domain of *CDH2* (Figure 1), was generally similar across the OCD probands (4.6%), TD probands (5.6%) and healthy control (4.3%) populations (NS). We then compared the OCD/TD probands with N845S to those without the variant. In the OCD subgroup ($N=260$) we found that of the 12 individuals (4.6%) with N845S, four (33.3%)

had coexisting TD diagnoses; in contrast, only 19 (7.7%) of those OCD probands without N845S ($N = 248$) had comorbid TD (Fisher's exact test $P = 0.014$, $OR = 6.03$). In considering the TD probands, 55% (5/9) of those with the N845S variant had OCD. In comparison, only 41% (62/153) of TD probands without N845S had OCD; this was not statistically significant.

The fourth variant, A118T, located in the propeptide region (Figure 1), was found in 10.4% of OCD probands, 6.1% of TD probands and 7.6% of controls (NS). This variant was not associated with any SCID-assessed diagnostic group.

In the total sample of OCD probands, TD probands and relatives, as well as the healthy control group, there were no other differences in other comorbid disorders or demographic variables between those with or without the four *CDH2* variants. Overall, among the TD probands, comorbid OCD or ADHD was diagnosed in 41% or 48%, respectively. Among the TD relatives, rates of TD, OCD or ADHD were 14%, 21% or 12%, respectively. Among the OCD probands, TD was present in 12% of the sample overall. (ADHD was not diagnostically evaluated in the OCD probands as it is not a component of the SCID adult evaluation.)

In the initial evaluations of the impact of the variants on N-cadherin functionality/activity, both N706S and N845S variants showed consistently, markedly reduced protein levels compared with wild-type *CDH2* when transfected in HEK293 cells (47% and 42%, respectively), as shown in Figure 2.

DISCUSSION

This is the first report on the exon sequencing of the neuronal cadherin gene *CDH2*, encoding N-cadherin in a large human sample and, to our knowledge, the first study of *CDH2* in any human disorder, other than *in vitro* studies of human cancer cells. Sequencing of *CDH2* confirmed the relatively low heterozygosity of two known missense variants, A118T and N845S. Further, we also identified two novel missense SNPs: N706S, located between the fifth extracellular domain and the transmembrane domain, and V289I within the second extracellular domain (Figure 1a). None of the four missense variants was significantly associated with OCD or TD diagnoses *per se*. Of interest, N706S occurred only in two unrelated OCD and TD probands and an unrelated TD proband's sibling (with motor and

phonic tics) and not in any of 447 controls, while N845S appears to be associated with OCD/TD-related subgroups.

Cadherins constitute a superfamily of adhesion molecules featuring an N-terminal tandem series of ectodomains, followed by a single anchoring transmembrane domain and a C-terminal cytoplasmic region (~150 amino acids) that links cadherins to the underlying cytoskeleton. In the case of *CDH2*/N-cadherin, this is via sequential binding of β -catenin to α -catenin and then through intermediates to actin.^{15,27,28} N-cadherin is required for critical brain processes, including long-term potentiation, pre- to post-synaptic adhesion, dendritic spine elongation – thereby regulating glutamate receptor trafficking and neuronal migration.^{29–32} A bioinformatic prediction of the multiple functional associations for *CDH2* is provided in Supplementary Figure 2.³³

The *CDH2* N845S variant lies in the highly conserved cytoplasmic domain. Loss of integrity of this domain leads to loss of adhesive function.^{34–40} N845 is located in the 'interaction region 2' of the extended region through which N-cadherin interacts with β -catenin.⁴¹ D846 forms a hydrogen bond with Y654 of β -catenin. Phosphorylation of Y654 by Src and other cytoplasmic kinases reduces the association of cadherins with β -catenin, resulting in dissociation of the cadherin-catenin complex. Thus, the N845S mutation in N-cadherin appears well placed to modulate cadherin– β -catenin interactions. However, there have been no site-directed mutagenesis studies before our initial study presented here, suggesting that a restricted amino-acid change resulting from the N845S variant might result in impaired N-cadherin expression and/or stability.

The N706S variant lies in the short region of *CDH2* connecting the extracellular domains of *CDH2* to the transmembrane segment (Figure 1). Our initial mutagenesis study presented here indicates that the N706S variant reduces *CDH2* expression and/or stability. This variant lies very close to the proposed cleavage site of *CDH2* by metalloproteinase ADAM10 (residues 714–715). The proteolytic cleavage by ADAM10 – as well as by PS1/ γ -secretase – is critically important for the roles of *CDH2* in cell adhesion and cell signaling.^{42,43} In addition, a prior study showed that induced single amino-acid changes that disrupted self-assembly of the transmembrane region reduced E-cadherin cell–cell adhesiveness.⁴¹ Thus, N706S, found in one OCD proband, an unrelated TD proband, and an unrelated TD proband's sibling with chronic tics, and in none of the 447 healthy controls in this study, may represent a very rare variant related to the complex OCD, TD as well as perhaps bipolar and other neuropsychiatric disorder phenotypes found in these individuals and at least one of their relatives. This finding is relevant in the case of TD, where bilineal transmission has been reported.⁶ Despite its lack of association, N706S seems an interesting variant to be followed up in larger cohorts.

The V289I variant lies in the EC2 ectodomain. Although EC1 has been documented to be critical to the adhesional/appositional functions of cadherins across cell–cell connections such as synapses, less seems to be known about the functional role of EC2.

As noted above, other members of the cadherin gene families have recently been found to be associated with autism spectrum disorders, in which repetitive behaviors are frequently observed.^{18–20,44,45} Protocadherins and other cadherins have also been studied as candidate risk genes, but generally in small samples (<100 patients) of schizophrenia, bipolar disorder and OCD patients.^{46–55} In addition, some cadherins have been specifically identified in genome-wide association scans of ADHD, addiction and neuroticism personality features.^{56–58} Of related interest, variants in *CDH2* and other cadherins have been widely found to

Figure 2 Western blots of HEK293 cells transfected with *CDH2* wild-type (WT), *CDH2* N706 mutant (M3) and *CDH2* N845S mutant (M4). Results are representative of a series of three independent transfections and western blot analyses.

be associated with various cancers^{16,59,60} and, specifically, upregulated *CDH2* has been associated with transepithelial spreading of melanoma and pancreatic cancer together with rapid recurrence of cancer.^{61–63}

In summary, although *CDH2* is an attractive candidate gene based on the CCD study findings,¹¹ the present results suggest that these *CDH2* variants are not disease-causing by themselves. Further studies are needed to clarify if N706S and N845S, identified in OCD and TD subgroups, may or may not be risk factors of interest in OCD/TD when investigated in larger cohorts. Also, future experiments are underway to better characterize the impact of N706S and N845S on N-cadherin functionality.

There are several limitations to this study. Our strategy was directed exclusively toward non-synonymous variants in *CDH2*, as they provide a 'fast-track' for functional characterization and interpretation of findings. However, genetic variation leading to a disorder might not necessarily be located in protein coding regions; it is known that very distant regulatory elements affecting gene expression can have a role in the etiology of disorders. Importantly, an overwhelming majority of human genetic variation comes from non-coding variants.⁶⁴ The relatively small sample size available for examination of the very rare *CDH2* variants (N706S and V289I) and for N845S in OCD and TD subphenotypes calls for major replication studies before drawing conclusions. Also, the healthy controls provided only allele frequencies and not complete phenotypic information. Finally, gene–gene and gene–environment interactions are relevant to accurate genotype–phenotype associations. In particular, there is some evidence for environmental contributions to OCD onset, OCD severity and other features of OCD, including possible contributions from psychological trauma, head trauma and autoimmune reactions.⁶⁵ Further research using larger numbers of samples from rigorously phenotyped affected and control individuals will be helpful to evaluate the validity of increased risk for compulsive and related disorders conferred by *CDH2* and other cadherins in neuropsychiatric disorders.

CONFLICT OF INTEREST

JRW is a Senior Principal Scientist, Pharma Research and Early Development at F Hoffmann-La Roche Ltd. None of the other authors has anything to disclose.

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- 6 Mathews CA, Grados MA: Familiality of Tourette syndrome, obsessive-compulsive disorder, and attention-deficit/hyperactivity disorder: heritability analysis in a large sib-pair sample. *J Am Acad Child Adolesc Psychiatry* 2011; **50**: 46–54.
- 7 Shmelkov SV, Hormigo A, Jing D et al: Slitrk5 deficiency impairs corticostriatal circuitry and leads to obsessive-compulsive-like behaviors in mice. *Nat Med* 2010; **16**: 598–602, 591p following 602.
- 8 Welch JM, Lu J, Rodriguez RM et al: Cortico-striatal synaptic defects and OCD-like behaviours in Sapap3-mutant mice. *Nature* 2007; **448**: 894–900.
- 9 Taylor JL, Rajbhandari AK, Berridge KC, Aldridge JW: Dopamine receptor modulation of repetitive grooming actions in the rat: potential relevance for Tourette syndrome. *Brain Res* 2011; **1322**: 92–101.
- 10 Swerdlow NR, Sutherland AN: Preclinical models relevant to Tourette syndrome. *Adv Neurol* 2006; **99**: 69–88.
- 11 Dodman NH, Karlsson EK, Moon-Fanelli A et al: A canine chromosome 7 locus confers compulsive disorder susceptibility. *Mol Psychiatry* 2010; **15**: 8–10.
- 12 Overall KL, Dunham AE: Clinical features and outcome in dogs and cats with obsessive-compulsive disorder: 126 cases (1989–2000). *J Am Vet Med Assoc* 2002; **221**: 1445–1452.
- 13 Moon-Fanelli AA, Dodman NH, Famula TR, Cottam N: Characteristics of compulsive tail chasing and associated risk factors in Bull Terriers. *J Am Vet Med Assoc* 2011; **238**: 883–889.
- 14 Goodwin M, Yap AS: Classical cadherin adhesion molecules: coordinating cell adhesion, signaling and the cytoskeleton. *J Mol Histol* 2004; **35**: 839–844.
- 15 Shapiro L, Love J, Colman DR: Adhesion molecules in the nervous system: structural insights into function and diversity. *Annu Rev Neurosci* 2007; **30**: 451–474.
- 16 Suriano G, Seixas S, Rocha J, Seruca R: A model to infer the pathogenic significance of CDH1 germline missense variants. *J Mol Med* 2006; **84**: 1023–1031.
- 17 Reichardt LF: N-cadherin and integrins: two receptor systems that mediate neuronal process outgrowth on astrocyte surfaces. *Neuron* 2008; **60**: 398–399.
- 18 Kroisel PM, Windpassinger C, Wagner K et al: De novo translocation t(5;18)(q33.1;q12.1) associated with autistic disorder. *Am J Med Genet A* 2004; **129A**: 98–100.
- 19 Pagnamenta AT, Khan H, Walker S et al: Rare familial 16q21 microdeletions under a linkage peak implicate cadherin 8 (CDH8) in susceptibility to autism and learning disability. *J Med Genet* 2010.
- 20 Wang K, Zhang H, Ma D et al: Common genetic variants on 5p14.1 associate with autism spectrum disorders. *Nature* 2009; **459**: 528–533.
- 21 Wendland JR, DeGuzman TB, McMahon F, Rudnick G, Detera-Wadleigh SD, Murphy DL: SERT Ileu425Val in autism, Asperger syndrome and obsessive-compulsive disorder. *Psychiatr Genet* 2008; **18**: 31–39.
- 22 Wendland JR, Kruse MR, Cromer KR, Murphy DL: A large case-control study of common functional SLC6A4 and BDNF variants in obsessive-compulsive disorder. *Neuropsychopharmacology* 2007; **32**: 2543–2551.
- 23 Heiman GA, King RA, Tischfield JA: New Jersey Center for Tourette Syndrome sharing repository: methods and sample description. *BMC Med Genomics* 2008; **1**: 58.
- 24 Wendland JR, Kruse MR, Murphy DL: Functional SLITRK1 var321, varCDfs and SLC6A4 G56A variants and susceptibility to obsessive-compulsive disorder. *Mol Psychiatry* 2006; **11**: 802–804.
- 25 Biesecker LG, Mullikin JC, Facio FM et al: The ClinSeq Project: piloting large-scale genome sequencing for research in genomic medicine. *Genome Res* 2009; **19**: 1665–1674.
- 26 Ferrer-Costa C, Gelpi JL, Zamakola L, Parraga I, de la Cruz X, Orozco M: PMUT: a web-based tool for the annotation of pathological mutations on proteins. *Bioinformatics* 2005; **21**: 3176–3178.
- 27 Kobielak A, Fuchs E: Alpha-catenin: at the junction of intercellular adhesion and actin dynamics. *Nat Rev Mol Cell Biol* 2004; **5**: 614–625.
- 28 Shapiro L, Weis WI: Structure and biochemistry of cadherins and catenins. *Cold Spring Harb Perspect Biol* 2009; **1**: a003053.
- 29 Bozdagi O, Wang XB, Nikitczuk JS et al: Persistence of coordinated long-term potentiation and dendritic spine enlargement at mature hippocampal CA1 synapses requires N-cadherin. *J Neurosci* 2010; **30**: 9984–9989.
- 30 Kawauchi T, Sekine K, Shikanai M et al: Rab GTPases-dependent endocytic pathways regulate neuronal migration and maturation through N-cadherin trafficking. *Neuron* 2010; **67**: 588–602.
- 31 Tanaka H, Shan W, Phillips GR et al: Molecular modification of N-cadherin in response to synaptic activity. *Neuron* 2000; **25**: 93–107.
- 32 Nuriya M, Hagan RL: Regulation of AMPA receptor trafficking by N-cadherin. *J Neurochem* 2006; **97**: 652–661.
- 33 Jensen LJ, Kuhn M, Stark M et al: STRING 8 – a global view on proteins and their functional interactions in 630 organisms. *Nucleic Acids Res* 2009; **37**: D412–D416.
- 34 Hirano S, Kimoto N, Shimoyama Y, Hirohashi S, Takeichi M: Identification of a neural alpha-catenin as a key regulator of cadherin function and multicellular organization. *Cell* 1992; **70**: 293–301.
- 35 Nagafuchi A, Takeichi M: Cell binding function of E-cadherin is regulated by the cytoplasmic domain. *EMBO J* 1988; **7**: 3679–3684.
- 36 Oyama T, Kanai Y, Ochiai A et al: A truncated beta-catenin disrupts the interaction between E-cadherin and alpha-catenin: a cause of loss of intercellular adhesiveness in human cancer cell lines. *Cancer Res* 1994; **54**: 6282–6287.
- 37 Ozawa M, Kemler R: Altered cell adhesion activity by pervanadate due to the dissociation of alpha-catenin from the E-cadherin/catenin complex. *J Biol Chem* 1998; **273**: 6166–6170.

- 1 Hettema JM, Neale MC, Kendler KS: A review and meta-analysis of the genetic epidemiology of anxiety disorders. *Am J Psychiatry* 2001; **158**: 1568–1578.
- 2 Pauls DL: The genetics of obsessive-compulsive disorder: a review. *Dialogues Clin Neurosci* 2010; **12**: 149–163.
- 3 Rasmussen SA, Tsuang MT: The epidemiology of obsessive compulsive disorder. *J Clin Psychiatry* 1984; **45**: 450–457.
- 4 Worbe Y, Mallet L, Golmard JL et al: Repetitive behaviours in patients with Gilles de la Tourette syndrome: tics, compulsions, or both? *PLoS One* 2010; **5**: e12959.
- 5 Felling RJ, Singer HS: Neurobiology of tourette syndrome: current status and need for further investigation. *J Neurosci* 2011; **31**: 12387–12395.

- 38 Shimoyama Y, Nagafuchi A, Fujita S *et al*: Cadherin dysfunction in a human cancer cell line: possible involvement of loss of alpha-catenin expression in reduced cell–cell adhesiveness. *Cancer Res* 1992; **52**: 5770–5774.
- 39 Watabe M, Nagafuchi A, Tsukita S, Takeichi M: Induction of polarized cell–cell association and retardation of growth by activation of the E-cadherin–catenin adhesion system in a dispersed carcinoma line. *J Cell Biol* 1994; **127**: 247–256.
- 40 Takeichi M: The cadherins: cell–cell adhesion molecules controlling animal morphogenesis. *Development* 1988; **102**: 639–655.
- 41 Huber O, Kemler R, Langosch D: Mutations affecting transmembrane segment interactions impair adhesiveness of E-cadherin. *J Cell Sci* 1999; **112**: Part 23 4415–4423.
- 42 Reiss K, Maretzky T, Ludwig A *et al*: ADAM10 cleavage of N-cadherin and regulation of cell–cell adhesion and beta-catenin nuclear signalling. *EMBO J* 2005; **24**: 742–752.
- 43 Uemura K, Kihara T, Kuzuya A *et al*: Characterization of sequential N-cadherin cleavage by ADAM10 and PS1. *Neurosci Lett* 2006; **402**: 278–283.
- 44 Dibbens LM, Tarpey PS, Hynes K *et al*: X-linked protocadherin 19 mutations cause female-limited epilepsy and cognitive impairment. *Nat Genet* 2008; **40**: 776–781.
- 45 Morrow EM, Yoo SY, Flavell SW *et al*: Identifying autism loci and genes by tracing recent shared ancestry. *Science* 2008; **321**: 218–223.
- 46 Soronen P, Ollila HM, Anttila M *et al*: Replication of GWAS of bipolar disorder: association of SNPs near CDH7 with bipolar disorder and visual processing. *Mol Psychiatry* 2010; **15**: 4–6.
- 47 Durand CM, Kappeler C, Betancur C *et al*: Expression and genetic variability of PCDH11Y, a gene specific to *Homo sapiens* and candidate for susceptibility to psychiatric disorders. *Am J Med Genet B* 2006; **141B**: 67–70.
- 48 Giouzezi M, Williams NA, Lonie LJ, DeLisi LE, Crow TJ: ProtocadherinX/Y, a candidate gene-pair for schizophrenia and schizoaffective disorder: a DHPLC investigation of genomic sequence. *Am J Med Genet B* 2004; **129B**: 1–9.
- 49 Lachman HM, Petroulo OA, Pedrosa E, Novak T, Nolan K, Stopkova P: Analysis of protocadherin alpha gene deletion variant in bipolar disorder and schizophrenia. *Psychiatr Genet* 2008; **18**: 110–115.
- 50 Pedrosa E, Stefanescu R, Margolis B *et al*: Analysis of protocadherin alpha gene enhancer polymorphism in bipolar disorder and schizophrenia. *Schizophr Res* 2008; **102**: 210–219.
- 51 Vincent JB, Noor A, Windpassinger C *et al*: Characterization of a de novo translocation t(5;18)(q33.1;q12.1) in an autistic boy identifies a breakpoint close to SH3TC2, ADRB2, and HTR4 on 5q, and within the desmocollin gene cluster on 18q. *Am J Med Genet B* 2009; **150B**: 817–826.
- 52 Cherlyn SY, Woon PS, Liu JJ, Ong WY, Tsai GC, Sim K: Genetic association studies of glutamate, GABA and related genes in schizophrenia and bipolar disorder: a decade of advance. *Neurosci Biobehav Rev* 2010; **34**: 958–977.
- 53 Ivleva EI, Morris DW, Moates AF, Suppes T, Thaker GK, Tamminga CA: Genetics and intermediate phenotypes of the schizophrenia – bipolar disorder boundary. *Neurosci Biobehav Rev* 2010; **34**: 897–921.
- 54 Johnson C, Drgon T, McMahon FJ, Uhl GR: Convergent genome wide association results for bipolar disorder and substance dependence. *Am J Med Genet B* 2009; **150B**: 182–190.
- 55 Maier W: Common risk genes for affective and schizophrenic psychoses. *Eur Arch Psychiatry Clin Neurosci* 2008; **258** (Suppl 2): 37–40.
- 56 Lasky-Su J, Neale BM, Franke B *et al*: Genome-wide association scan of quantitative traits for attention deficit hyperactivity disorder identifies novel associations and confirms candidate gene associations. *Am J Med Genet B* 2008; **147B**: 1345–1354.
- 57 Terracciano A, Sanna S, Uda M *et al*: Genome-wide association scan for five major dimensions of personality. *Mol Psychiatry* 2010; **15**: 647–656.
- 58 Redies C, Hertel N, Hubner CA: Cadherins and neuropsychiatric disorders. *Brain Res* 2012; **1470**: 130–144.
- 59 Bex G, van Roy F: Involvement of members of the cadherin superfamily in cancer. *Cold Spring Harb Perspect Biol* 2009; **1**: a003129.
- 60 Guilford P, Humar B, Blair V: Hereditary diffuse gastric cancer: translation of CDH1 germline mutations into clinical practice. *Gastric Cancer* 2010; **13**: 1–10.
- 61 Liu ZJ, Xiao M, Balint K *et al*: Notch1 signaling promotes primary melanoma progression by activating mitogen-activated protein kinase/phosphatidylinositol 3-kinase-Akt pathways and up-regulating N-cadherin expression. *Cancer Res* 2006; **66**: 4182–4190.
- 62 Qi J, Chen N, Wang J, Siu CH: Transendothelial migration of melanoma cells involves N-cadherin-mediated adhesion and activation of the beta-catenin signaling pathway. *Mol Biol Cell* 2005; **16**: 4386–4397.
- 63 Nakajima S, Doi R, Toyoda E *et al*: N-cadherin expression and epithelial–mesenchymal transition in pancreatic carcinoma. *Clin Cancer Res* 2004; **10**: 4125–4133.
- 64 Altshuler D, Durbin RM, Abecasis GR *et al*: A map of human genome variation from population-scale sequencing. *Nature* 2010; **467**: 1061–1073.
- 65 Murphy DL, Moya PR, Wendland JR, Timpano KR: *Genetic Contributions to Obsessive-Compulsive Disorder (OCD) and OCD-Related Disorders*. Cambridge, UK: Cambridge University Press, 2012.

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