RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

Meta-Analysis of Association Between Obsessive-Compulsive Disorder and the 3′ Region of Neuronal Glutamate Transporter Gene *SLC1A1*

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Additional supporting information may be found in the online version of this article.

Grant sponsor: Eli Lilly Canada Inc, Ontario Mental Health Foundation and the Obsessive-Compulsive Foundation; Grant sponsor: Novartis, Seaside Therapeutics, Roche, and Forest; Grant sponsor: Cyberonics and Medtronic.

Conflicts of interest: SE Stewart, C Mayerfeld, PD Arnold, JR Crane, C O'Dushlaine, JA Fagerness, JM Scharf, F Kassam, PR Moya, R Delorme, JL Kennedy, J Samuels, BD Greenberg, JT McCracken, AJ Fyer, MA Riddle, MA Grados, OJ Bienvenu, B Cullen, Y Wang, J Piacentini, YY Shugart, S Rasmussen, G Nestadt, DL Murphy, MA Jenike, EH Cook, DL Pauls, GL Hanna and CA Mathews declare no industry-related conflicts of interest. JR Wendland is now a full-time employee of F. Hoffmann-La Roche Ltd. MA Richter has received honoraria from Lundbeck and she is recipient of grant funding from Eli Lilly Canada Inc, Ontario Mental Health Foundation and the Obsessive-Compulsive Foundation. J Veenstra-VanderWeele receives research funding from Novartis, Seaside Therapeutics, Roche, and Forest. JA Knowles sits on the Scientific Advisory Committee for Next-Generation Sequencing of Life Technologies, Inc. and is a technical advisor to SoftGenetics, Inc.; he is on the Scientific Advisory Committee for Next-Generation Sequencing of Life Technologies, Inc. and is a technical advisor to SoftGenetics, Inc. SL Rauch participates in research funded by Cyberonics and Medtronic.

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Article first published online in Wiley Online Library
(wileyonlinelibrary.com): 18 April 2013
DOI 10.1002/ajmg.b.32137

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Manuscript Received: 22 October 2012; Manuscript Accepted: 15 January 2013

The neuronal glutamate transporter gene *SLC1A1* is a candidate gene for obsessive-compulsive disorder (OCD) based on linkage studies and convergent evidence implicating glutamate in OCD etiology. The 3′ end of *SLC1A1* is the only genomic region with consistently demonstrated OCD association, especially when analyzing male-only probands. However, specific allele associations have not been consistently replicated, and recent OCD genome-wide association and meta-analysis studies have not incorporated all previously associated *SLC1A1* SNPs. To clarify the nature of association between *SLC1A1* and OCD, pooled analysis was performed on all available relevant raw study data, comprising a final sample of 815 trios, 306 cases and 634 controls. This revealed weak association between OCD and one of nine tested *SLC1A1* polymorphisms (rs301443; uncorrected $P = 0.046$; non-significant corrected *P*). Secondary analyses of male-affecteds only (N = 358 trios and 133 cases) demonstrated modest association between OCD and a different SNP (rs12682807; uncorrected $P = 0.012$; non-significant corrected *P*). Findings of this meta-analysis are consistent with the trend of previous candidate gene studies in psychiatry and do not clarify the putative role of *SLC1A1* in OCD pathophysiology. Nonetheless, it may be important to further examine the potential associations demonstrated in this amalgamated sample, especially since the SNPs with modest associations were not included in the more highly powered recent GWAS or in a past meta-analysis including five *SLC1A1* polymorphisms. This study underscores the need for much larger sample sizes in future genetic association studies and suggests that next-generation sequencing may be beneficial in examining the potential role of rare variants in OCD.

Key words: obsessive-compulsive disorder; OCD; candidate gene; meta-analysis; *SLC1A1*; glutamate

How to Cite this Article:
Stewart SE, Mayerfeld C, Arnold PD, Crane JR, O'Dushlaine C, Fagerness JA, Yu D, Scharf JM, Chan E, Kassam F, Moya PR, Wendland JR, Delorme R, Richter MA, Kennedy JL, Veenstra-VanderWeele J, Samuels J, Greenberg BD, McCracken JT, Knowles JA, Fyer AJ, Rauch SL, Riddle MA, Grados MA, Bienvenu OJ, Cullen B, Wang Y, Shugart YY, Piacentini J, Rasmussen S, Nestadt G, Murphy DL, Jenike MA, Cook EH, Pauls DL, Hanna GL, Mathews CA. 2013. Meta-Analysis of Association Between Obsessive-Compulsive Disorder and the 3′ Region of Neuronal Glutamate Transporter Gene *SLC1A1*.
Am J Med Genet Part B 162B:367–379.

INTRODUCTION

Obsessive-compulsive disorder (OCD) is a debilitating anxiety disorder characterized by recurrent intrusive thoughts and/or rituals intended to mitigate anxiety [American Psychiatric Association, 2000]. OCD is a common psychiatric illness, with estimated lifetime prevalence rates ranging from 1% to 3% worldwide [Karno et al., 1988; Weissman et al., 1994; Kessler et al., 2005]. The genetic architecture of OCD is thought to be complex, [Cavallini et al., 1999; Nestadt et al., 2000a; Hanna et al., 2005a,b] but the specific mechanisms of this etiologic contribution have not yet been established. Despite numerous family, twin, and segregation analyses indicating substantial heritability of OCD, [Inouye, 1965; van Grootheest et al., 2005] most candidate gene findings have not been consistently replicated [Hemmings and Stein, 2006; Grados and Wilcox, 2007; Nestadt et al., 2010]. To date, gene selection for these studies has been guided by pathophysiological and pharmacological knowledge and therefore has focused primarily on the serotonergic and dopaminergic systems. However, among published OCD candidate gene studies, only those focusing on the glutamate transporter gene *SLC1A1* have demonstrated consistent evidence of

association [Hemmings and Stein, 2006; Grados and Wilcox, 2007; Nestadt et al., 2010].

Evidence suggests that abnormal glutamatergic neurotransmission may contribute to OCD pathophysiology [Pittenger et al., 2006, 2011; Wu et al., 2012]. Elevated glutamate concentrations have been found in the cerebrospinal fluid of OCD subjects, [Chakrabarty et al., 2005] and magnetic resonance spectroscopy investigations have revealed altered glutamatergic concentrations in the orbitofrontal white matter of adult patients with OCD [Whiteside et al., 2006] and in the anterior cingulate cortex of both pediatric and adult OCD subjects [Rosenberg et al., 2004; Yucel et al., 2008]. Positron emission tomography studies of OCD have shown increased glucose metabolism and blood flow in the cortico-striatal-thalamo-cortical (CSTC) circuit [Swedo et al., 1989; Rauch et al., 1994; Saxena and Rauch, 2000]. Moreover, two OCD rodent model studies have implicated glutamate-related genes, *SAPAP3* and *SLITRK5* [Welch et al., 2007; Mah, 2010]. Additionally, pharmacological evidence for the role of glutamatergic activity in OCD is emerging. Multiple studies have reported symptom improvement in OCD by augmenting with glutamate-modulating agents such as riluzole [Coric et al., 2005; Grant et al., 2007] or memantine [Aboujaoude et al., 2009; Stewart et al., 2010].

Located on chromosome 9p24, *SLC1A1* encodes the neuronal and epithelial high affinity glutamate transporter EAAC1, also called EAAT3, which is expressed in brain regions implicated in OCD, [Menzies et al., 2008] including the cerebral cortex, striatum, and thalamus [Kanai and Hediger, 2004]. In contrast to the other studied glutamate-related candidate genes such as *GRIK2* [Sampaio et al., 2011] and *BDNF* [Nicolini et al., 2009], *SLC1A1* is also a strong positional candidate. A whole genome linkage scan on 56 individuals from seven multigenerational OCD-affected families by Hanna et al. [2002] identified a linkage peak containing the 9p24 region (LOD 2.25), and a targeted replication study in this region on 50 multiplex or sibling pair pedigrees by Willour et al. [2004b] found suggestive evidence for linkage within 0.5 cM (<350 kb) of the original signal (HLOD 2.26). However, a subsequent full genome linkage scan primarily in sibling pairs did not find evidence for linkage in this region [Shugart et al., 2006b]. Of the known genes within this region, *SLC1A1* is one of only two with demonstrated expression in the brain [Smith et al., 1994].

To date, five candidate gene studies have reported associations between OCD and multiple single nucleotide polymorphisms (SNPs) and/or haplotypes within the 3′ region of *SLC1A1* [Arnold et al., 2006; Dickel et al., 2006; Stewart et al., 2007a; Shugart et al., 2009; Wendland et al., 2009] (see Table I). A recent study by Samuels et al. [2011] also found evidence of association with OCD in the 5′ region of *SLC1A1*. No negative candidate gene studies of *SLC1A1* in OCD have been published. Among the five studies examining the 3′ region of the gene, some of the analyzed SNPs overlapped, while others were unique across studies. The majority of SNP and haplotype associations reported in individual studies have not been replicated in other samples, and none of the *SLC1A1* polymorphisms has been confirmed as a susceptibility locus for OCD. Of the 18 SNPs analyzed from the 3′ region of *SLC1A1* in at least two published studies, only rs3780412 has demonstrated association as a single marker in more than one study sample (after correction for multiple testing [Dickel et al., 2006; Stewart et al., 2007a]).

Both Dickel et al. [2006] and Stewart et al. [2007a] found evidence of association with rs3780412, although the effects were in opposite directions. Dickel et al. [2006] reported over-transmission while Stewart et al. [2007b] reported under-transmission of the major allele to affected male probands. Incidentally, Kwon et al. [2009] also found weak evidence of support for association between this SNP and atypical antipsychotic-induced OC symptoms (permuted $P = 0.07$).

Because the sample sizes of the five OCD 3′ *SLC1A1* candidate gene studies were small (N = 66–378 families), and also given the complex genetics of OCD, these ambiguous results are not surprising. Most recently, the first genome-wide association study (GWAS) of OCD has been published [Stewart et al., 2012], which did not identify significant association with any of the four examined *SLC1A1* SNPs, including rs2072657, rs301430, rs301434, and rs3780412. However, the GWAS notably did not examine several *SLC1A1* SNPs with previous reported association with OCD, and sex-specific analyses were not conducted. Moreover, a recent meta-analysis of OCD genetic association studies did not use raw genotype data to apply standard quality control thresholds across samples, and only examined five *SLC1A1* SNPs in primary analyses [Taylor, 2012].

In order to increase the statistical power to detect association between *SLC1A1* and OCD, and to detect the potential influence of publication bias or issues of multiple testing, the authors aggregated the raw data from the four published family-based association studies with unpublished data from genotyping performed by the OCD Mini-Collaborative, a subgroup of the International OCD Genetics Collaborative, consisting of research sites in Boston, Ann Arbor (Michigan), San Francisco, and Toronto. Analyses comprised an initial pooled analysis of this combined family-based association data, followed by a meta-analysis incorporating these results with the previously published results of a case–control association study. Secondary analyses were also performed to explore potential effects of gender on this association.

METHODS

Identification of Relevant Studies and Samples for Inclusion

Study selection procedure. Published studies examining the association between *SLC1A1* and OCD were originally identified via the MEDLINE/PubMed database using the terms ''OCD'' and ''*SLC1A1*,'' with a focus on the 3′ region of the gene. Additional published studies were identified through examination of the reference lists of the studies identified through MEDLINE. Unpublished datasets and raw genotype data for the pooled analysis were sought by contacting the authors of the published studies.

Study inclusion/exclusion. Studies were included in the pooled and/or meta-analyses if they had a type of control group (either unrelated controls or parents) and an OCD group, if SNPs within the 3′ region of the *SLC1A1* gene were examined and if the original authors agreed to provide raw genotype data. Excluded studies involved review articles and association studies that examined the

relationship of *SLC1A1* to non-OCD anxiety disorders. Data from *SLC1A1* SNPs in any published (or unpublished) linkage studies were not included in analyses.

Raw data extraction. Raw data from individual studies that was analyzed included phenotype information, SNP genotypes and minor allele frequencies.

Quality control. In order to obtain the most accurate assessment of data quality, the full datasets provided by the sites were used in the calculation of QC statistics. Genotype data from each site were cleaned using an iterative quality control (QC) process, whereby markers and subjects with excessive missing values (>50%) were dropped before the next round of QC standards were applied. In subsequent steps, SNPs were dropped for excessive missing values (>10%), low minor allele frequencies (<5%), high Mendel error rates (>5%), and apparent deviations from Hardy–Weinberg equilibrium (P-value $< 10^{-3}$). Subjects were removed for excessive missing values (>10%) and for high Mendel error rates (>5%). All QC steps were performed using PLINK [Purcell et al., 2007] (http://pngu.mgh.harvard.edu/purcell/plink/) for the pooled dataset. Additionally, QC checks were performed on the merged final dataset to ensure that Mendel error rates and Hardy–Weinberg P-values remained within acceptable ranges.

Statistical Analyses

We first conducted a pooled analysis of the family based data in PLINK using the transmission disequilibrium test (TDT) [Purcell et al., 2007]. We subsequently identified the common set of genotyped SNPs between family-based studies and case–control studies and then conducted single-marker analyses of these SNPs using PLINK. For the pooled analysis of family-based data, we conducted only single-marker association testing and did not examine haplotypes.

We then performed a meta-analysis using weighted z-scores to combine association results from the pooled family-based analysis and the case–control analysis [see de Bakker et al., 2008, for a review of the method]. The z-score was calculated from the chi-square and odds ratio test statistics provided in the PLINK output:

$$z_i = \sqrt{\chi_1^2} \quad \text{for chi-square testes with 1 df; } z > 0 \text{ if odds ratio} > 1, \text{ and } z < 0 \text{ if odds ratio} < 1$$

As the original studies included different groups of SNPs in their analyses, the sample size for each SNP test in this study was essentially unique. Thus, rather than weighting each original study (as is typically done in a meta-analysis) this study weighted each single-marker test. PLINK was used to obtain the number of complete trios (sample size) genotyped for each SNP. The four association tests performed in the case–control sample were given the same weight, as there was only one case–control study. Weighting was established using the Genetic Power Calculator [Purcell et al., 2003]. This online tool was used to obtain the non-centrality parameter (NCP) for each of these samples and to generate a theoretical equivalent "effective" case–control study with a 1:1 case:control ratio. The theoretical case–control sample with the NCP value closest to that of the actual sample was used to weight each single-marker association test as follows:

$$\text{Relative weight} = \sqrt{\frac{N_i}{N_{total}}}$$

where N_i is the theoretical equivalent case–control sample size and N_{total} is the sum of the theoretical sample sizes of the association tests performed for that SNP, that is the sum of theoretical equivalent weights of the family-based test and case–control test, when a case–control association test had been performed for that SNP.

The tests for the five SNPs genotyped exclusively in family-based studies did not require weighting, as no case–control results were available for meta-analysis. For the other four SNPs, weighted z-scores were summed to produce overall meta-analysis z-scores.

Chi-square values were generated by squaring the z-scores from the meta-analysis, and these chi-square values were then converted into P-values based on the normal distribution with one-degree of freedom. The results of the association tests for each of the nine polymorphisms were indicated as z-scores, P-values, and odds ratios with corresponding 95% confidence intervals. Odds ratios were calculated according to a fixed-effects model [Trikalinos et al., 2008]. To account for multiple testing of the nine examined SNPs, Bonferroni correction was conducted, and the required adjusted P-value was defined at 0.0056.

RESULTS

Study-Sample Descriptions

Samples included in this analysis were incorporated from one case–control study [Wendland et al., 2009] and four family-based studies [Arnold et al., 2006; Dickel et al., 2006; Stewart et al., 2007a; Shugart et al., 2009]. It should be noted that the sample from the study by Samuels et al. [2011] was the same as that by Shugart et al. [2009], but with genotyping of additional SNPs. Authors from each of these five identified studies agreed to provide original raw genotype data from their samples to enable the application of uniform quality control (QC) filters for the purpose of this study. Unpublished family-based data from the OCD Mini-Collaborative, which included samples from Boston, Ann Arbor (Michigan), San Francisco and Toronto, were also entered into the analyses.

In each original study, case or proband status was defined by the presence of a current diagnosis of Obsessive-Compulsive Disorder based on DSM-III-R or DSM-IV (Diagnostic and Statistical Manual of Mental Disorders), [American Psychiatric Association, 2000] criteria which were assessed via established psychiatric interviews. Subjects were recruited primarily from OCD and anxiety clinics, as described previously [Arnold et al., 2006; Dickel et al., 2006; Stewart et al., 2007a; Shugart et al., 2009; Wendland et al., 2009]. Subjects from these original cohorts were primarily Caucasian, although age and, notably, age-of-onset were variable. Only one study [Shugart et al., 2009] and the San Francisco site of the OCD Mini-Collaborative excluded probands with comorbid Tourette disorder, a common comorbidity estimated to occur in 10–30% of OCD cases [Geller et al., 1996; Ivarsson et al., 2008; Langley et al., 2010]. Previously unanalyzed genotype data collected through the OCD Mini-Collaborative were also included. After QC exclusions, the OCD Mini-Collaborative sample contributed 170 new complete

trios as well as 124 complete trios that had been previously included in one of the original five family-based association studies.

Study-Sample Genotyping and Verification

The platforms used for genotyping in the different published studies included TaqMan (TaqMan; Applied Biosystems; Foster City, California), Illumina GoldenGate and BeadArray system (Illumina; San Diego, CA), and Sequenom hME (Sequenom; MassARRAY; San Diego, CA). Genotyping performed by the OCD Mini-Collaborative was conducted on the Sequenom iPLEX platform (Sequenom; MassARRAY; San Diego, CA). In earlier studies performed by Arnold et al. [2006] and Dickel et al. [2006] the primary goal of SNP selection was to provide adequate coverage of *SLC1A1*. Subsequent studies by Stewart et al., Shugart et al., and Wendland et al. were designed, in part, to replicate the positive associations reported by these earlier studies, such that the majority of markers selected had been genotyped previously. In addition, Shugart et al. included SNPs from outside of the *SLC1A1* gene with potential regulatory properties, and Wendland et al. included SNPs predicting gene expression levels.

Some of the original genotyping runs included assays unrelated to *SLC1A1* as well as subjects affected by disorders other than OCD. After QC exclusions were made, the datasets were refined by excluding all data related to OCD-unaffected families (from family-based databases) and non-3′ *SLC1A1*-related SNPs. Because the QC thresholds used in this study differed slightly from those applied in the original studies, the post-QC datasets for meta-analysis inclusion were not identical to datasets analyzed in the original studies. To address this fact, attempts were made to replicate the original results by analyzing our post-QC datasets according to the methods—namely programs and settings—described in the published papers [FBAT (http://www.biostat.harvard.edu/∼fbat/default.htm), [Laird et al., 2000] PBAT (http://www.biostat.harvard.edu/∼clange/default.htm), [Lange et al., 2004] and TDT in Haploview (http://www.broadinstitute.org/haploview/haploview) [Barrett et al., 2005]. For each published study, concordance between original and new results was judged to be adequate (see Supplementary Table I).

Meta-Analysis SNPs

Of the SNPs passing QC thresholds described above, 18 were common to at least two of the published studies and/or the unpublished OCD mini-collaborative data. The SNPs genotyped in the five original studies are presented in Table I. Moreover, SNPs that were included in the recently published OCD GWAS [Stewart et al., 2012], and those included in a combined meta-analysis of multiple OCD candidate genes [Taylor, 2012], are identified. Genomic positions are those described in NCBI (National Center for Biotechnology Information) Build 36dbSNP Build 131/UCSC hg18hg19. Selection of SNPs for inclusion in this meta-analysis was based on whether a significant association, either as a single marker or as part of a haplotype, had been reported previously for the SNP and whether data were available from at least one additional study sample. Accordingly, nine SNPs were analyzed for this study. Data were coded in terms of forward strand orientation and then merged (with the exception of the case–control data) in PLINK [Purcell et al., 2007] using the default consensus calling setting. Among studies whose participants were later re-genotyped by the OCD Mini-Collaborative, overall concordance rates exceeded 99% in all cases. Additionally, QC checks were performed on the merged final dataset to ensure that Mendel error rates and Hardy–Weinberg *P*-values remained within the acceptable ranges.

The linkage disequilibrium (LD) pattern of the 9 SLC1A1 loci, which span a 22.5 kb region, was established using genotype data from individuals in this meta-analysis. Haplotype construction was performed in Haploview [Barrett et al., 2005]. The LD structure of these 9 SNPs is illustrated in Figure 1.

Meta-Analysis of Family-Based and Case–Control Single-Marker Associations

Four family-based association studies and one case–control study were included in this meta-analysis. Table II presents characteristics for each of the five studies. The total sample size for this meta-analysis included 815 complete trios and 306 cases with 634 ethnicity- and sex-matched controls. Males comprised 43.9% of OCD-affected probands (N = 358) and 43.5% of cases (N = 133). The original five studies had reported a combined total of 12 significant single-marker associations between *SLC1A1* polymorphisms and OCD. Among these SNPs, four were found to be associated exclusively in males, and only one was found to be associated in a global sample but not in the corresponding male subsample (see Table I).

In the present meta-analysis, one of the nine examined SNPs (rs301443) had a nominally significant uncorrected *P*-value ($P = 0.046$). This association did not survive Bonferroni correction for nine independent tests (required Bonferroni adjusted $P < 0.0056$) (see Table III).

Secondary Analyses by Gender Stratification

After stratifying the family and case–control datasets by gender of the affected proband, a secondary meta-analysis was performed on the male subsample. Only rs12682807 had a nominally significant uncorrected *P*-value ($P = 0.012$). This association did not survive multiple testing correction (see Table III).

DISCUSSION

To date, *SLC1A1* has been considered the strongest biological candidate gene for OCD based on positional linkage evidence [Hanna et al., 2002; Willour et al., 2004a; Nicolini et al., 2009], functional evidence connecting glutamate and OCD, and relatively consistent genetic association findings across samples. No other OCD candidate gene has demonstrated this level of supportive evidence. In the absence of replication at the polymorphism level, however, the association between *SLC1A1* and OCD has not been established. By integrating all relevant published and unpublished data, this study was able to provide an overall estimate of effect size for each of the nine *SLC1A1* polymorphisms that had been significantly associated with OCD in at least one previous study, and for which data were available from more than one site. Although each of these SNPs had demonstrated association with the OCD phenotype

TABLE I. 3' SLC1A1 Findings From Association, GWAS and Meta-Analysis OCD Studies

3' SLC1A1 SNPs investigated									
SNPs		**Original study findings included in meta-analysis**						**Other studies**	
SNP	**HG18 position**	**Arnold et al.**	**Dickel et al.**	**Stewart et al.**	**Shugart et al.**	**Wendland et al.**	**MiniCollaborative (unpublished)**	**GWAS**	**Taylor meta-analysis**
rs301430	chr9:4566680	x	Hm $P=0.003$	H perm $P=0.0043$; Hm perm $P=0.033$	x	H $P=0.04$; Hm $P=0.03$; E	x	x	x
rs3780412	chr9:4562480	x	Sm corr $P=0.036$	Sm perm $P=0.045$	x	x	x	x	x
rs3087879	chr9:4576808	S perm $P=0.22$; Sm perm $P=0.03$; H $P=0.003$; Hm $P=0.006$		x	x	H $P=0.04$; Hm $P=0.03$	x		x
rs301434	chr9:4572082	S perm $P=0.006$; Sm perm $P=0.01$; H $P=0.003$; Hm $P=0.006$	x	x	x	x	lost in QC	x	x
rs301979	chr9:4566851	x	Hm $P=0.003$	x	x		x		x
rs301435	chr9:4572843	x		x	x				
rs301443	chr9:4584919		x		S corr $P=0.017$		x		
rs12682807	chr9:4564022			H perm $P=0.0043$; Hm perm $P=0.033$			x		
rs2072657	chr9:4566451			H perm $P=0.0043$; Hm perm $P=0.033$			x	x	
rs2228622 (formerly s7856209)	chr9:4554432	x		Sm perm $P=0.045$	x		x		
rs7858819	chr9:4549892					H $P=0.04$; Hm $P=0.03$; E			
rs3933331	chr9:4379941					E	x		
rs1980943	chr9:4504246	x	x		x		x		
rs3780415	chr9:4545573	x			x		x		
rs10974625	chr9:4551596			x			x		
rs10814987	chr9:4473303		x		x				
rs10739062	chr9:4492848		x		x				
rs10115600	chr9:4509369		x		x				
rs3780413	chr9:4557353			x			x		
rs301440	chr9:4580305			x					
rs301437	chr9:4577560			x					

Single nucleotide polymorphisms (SNPs) listed by rs# include those examined in previous OCD studies of 3' SLC1A1. The base pair location for each SNP is listed in the column to the right of the SNP column (HG18 Position). The nine SNPs included in this meta-analysis are listed in the top nine rows. Five columns are provided for previously published study findings, one is provided for previously unpublished findings, one is provided for a published GWAS and one is provided for the only other OCD genotype meta-analysis. Blank cells within rows indicate the absence of that SNP from the related study. Cells with an 'x' indicate that the SNP was genotyped but that the *P*-value was >0.05. Cells with a *P*-value <0.05 are listed according to the type of related association test: S, significant single marker association; Sm, significant single marker association in males; H, part of significant haplotype; Hm, part of significant haplotype in males; corr *P*, significant single marker association with OCD after correction for multiple testing; perm *P*, significant single marker association with OCD after permutation testing; E, SLC1A1 eQTL (expression quantitative trait locus).

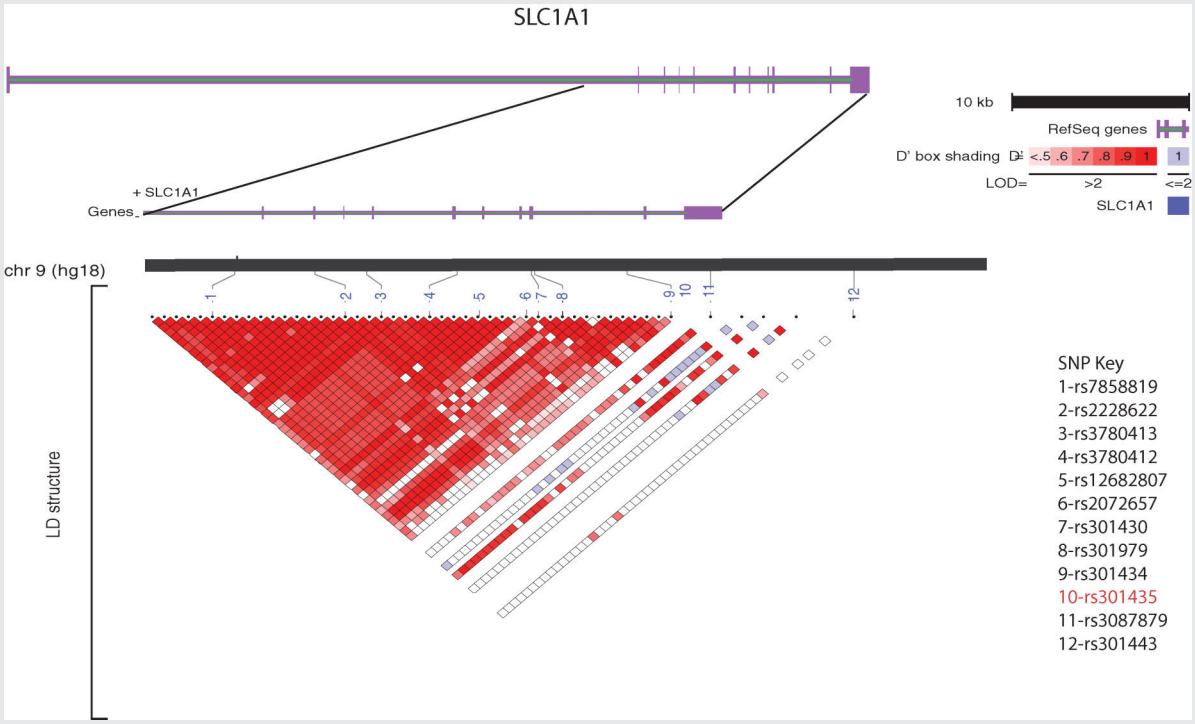

FIG. 1. SLC1A1 SNPs included for meta-analysis and their previously reported single marker or haplotype OCD associations. Single marker associations: SNP 4 — Dickel et al., 2006, Stewart et al., 2007; SNP 9 — Arnold et al., 2006; SNP 11 — Arnold et al., 2006; SNP 12 — Shugart et al., 2009. Haplotype associations: SNPs 5, 6, 7 — Stewart et al., 2007; SNPs 7, 8 — Dickel et al., 2006; SNPs 7, 11 — Wendland et al., 2009; SNPs 9, 11 — Arnold et al., 2006.

in one or more of the original studies, most of these associations were no longer significant when data were combined across studies. This meta-analysis found only two nominally significant associations—rs301443 in the global sample ($P = 0.046$, fixed-effects pooled OR = 1.17) and rs12682807 in the male sample ($P = 0.012$, fixed-effects pooled OR = 1.65)—among the nine examined *SLC1A1* polymorphisms and OCD.

The greatest strength of this meta-analysis lies in its sample size. It represents a significant increase in power as compared to previous studies. The smallest of the original *SLC1A1* studies contained 66 families [Stewart et al., 2007a] and the largest contained 378 families [Shugart et al., 2009]. The combined sample for this meta-analysis included 815 affected offspring trios and 306 cases with 634 controls. Thus, power to detect an association was markedly increased by combining these samples. The only comparable study, a meta-analysis of polymorphisms from multiple candidate genes in OCD [Taylor, 2012], included five of the *SLC1A1* polymorphisms that were examined by us but excluded data from the large study by Shugart et al. [2009] and also excluded rs3014443 (our major finding). Taylor reported a nominally significant association with the 3′ SNP rs3087879, which was not found in our meta-analysis.

Additionally, we were able to conduct a pooled analysis, as authors of all five of the original studies generously agreed to provide their original, pre-QC genotype data. Pooled analyses produce similar results to meta-analyses [Lin and Zeng, 2010], however they have the advantages of allowing for more consistent quality control and facilitating secondary analyses [Lin and Zeng, 2010; Consortium, 2012]. The previously mentioned meta-analysis by Taylor [2012] was limited by using only data reported in earlier publications, thus necessitating exclusion of studies for which there was insufficient data to compute odds ratios.

Furthermore, our pooled analysis was generally robust against potential bias from population stratification because most of the data were from family-based association studies. Lastly, all sites contributing data to our study used well-validated phenotyping methods based upon standard DSM criteria, decreasing the risk for a dilution effect emerging from misdiagnosis and inappropriate study inclusion.

A secondary analysis was conducted on males to test for a sex-specific association between *SLC1A1* and OCD, given reports of this in all but one of the original published studies, including all four family-based association studies [Arnold et al., 2006; Dickel et al., 2006; Stewart et al., 2007a; Shugart et al., 2009]. The majority of reported single-marker associations in *SLC1A1* were driven by males, which is consistent with the sex differences observed in phenotypic presentations of OCD. The course of the disorder is more chronic in males [Ravizza et al., 1997; Bogetto et al., 1999], who are also more likely to experience onset during childhood. Males are also more likely to present with comorbid conditions such as tic disorder or attention-deficit/hyperactivity disorder [Fischer et al., 1996; Lensi et al., 1996; Bogetto et al., 1999; Zohar et al., 1999;

TABLE II. Characteristics of Previous 3′ SLC1A1 Candidate Gene Studies

Study	Design	Number of participants	Race/ethnicity	Number of affected males (%)	Mean OCD onset (SD)	Diagnostic criteria	# of SNPs analyzed	Analysis method
Wendland et al. [2009]	Case–control	325 OCD probands, 662 matched controls	Caucasian	139 (43%)	14 y (9.6)	DSM-IV	6	PLINK
Arnold et al. [2006]	Family-based	157 families	96% Caucasian	60 (38%)	14.4 y (9.20)	DSM-IV	9	Single-locus in FBAT; TDT in Haploview
Stewart et al. [2007]	Family-based	66 Families	Not provided	47 (72.3%)	7.9 y (5.9)	DSM-IV	13	FBAT
Shugart et al. [2009]	Family-based	378 Families	97% Caucasian	325 (36%) (Note: #s are for total study population)	9.3 y	DSM-IV	13	PBAT
Dickel et al. [2006]	Family-based	71 Families	Not provided	25 (35%)	Two groups ascertained: 8.8 y (3.9) 8 y (3.7)	DSM-III-R or DSM-IV	9	Single-locus in FBAT; TDT in Haploview

Matsunaga et al., 2000; Tukel et al., 2004]. Segregation analyses have also revealed evidence of sexual dimorphism in the inheritance of OCD [Nestadt et al., 2000a; Hanna et al., 2005a]. However, the present meta-analysis provided limited evidence of a sex-specific association between *SLC1A1* and OCD. While the total combined OCD-affected sample was less than half (43.8%) male, resulting in a significant reduction in power for the secondary analysis, this meta-analysis is nevertheless the most comprehensive exploration of sex effects on the *SLC1A1*-OCD association to date.

The largely negative results of this meta-analysis are not entirely unexpected, given the findings from previous individual studies. Only three of the nine markers included in this meta-analysis were reported to be significant, either as a single marker or as part of a haplotype, in more than one of the previous studies. The only SNP that had been positively associated with *SLC1A1* as a single marker in more than one study was rs3780412, and this SNP was not significant in either the global or male-specific analysis of the present meta-analysis. This study does not, therefore, clarify the findings from previous association studies involving *SLC1A1* and OCD. The lack of association suggests that previous findings may have been false positive results. However, other limitations of this study may also explain the lack of an association in this meta-analysis. First, despite the relatively large sample size compared to earlier, single site studies, this meta-analysis is admittedly small relative to current guidelines for study size. The Psychiatric GWAS Consortium Coordinating Committee [2009] reported that the majority of recent significant association findings involve alleles with genotypic relative risks of 1.1–1.4, and that detection of such risk alleles may require samples of between 8,000 and 20,000 cases [Cichon et al., 2009].

Second, heterogeneity at both the genetic and phenotypic level may have impeded our ability to detect an association. Sample size considerations are especially relevant in light of the genetic heterogeneity thought to underlie OCD [Liang et al., 2008]. For example, while the first linkage scan for OCD [Hanna et al., 2002; Willour et al., 2004b] reported a linkage signal on chromosome 9p24 that was subsequently replicated [Willour et al., 2004a], subsequent linkage studies using primarily sibling pairs [Shugart et al., 2006a; Hanna et al., 2007; Ross et al., 2011; Mathews et al., 2012] failed to detect this signal. Furthermore, in the linkage study by Willour et al. [2004a] the group noted that only 59% of their pedigrees had demonstrated evidence for linkage with the 9p24 candidate region. Linkage studies are robust to allelic heterogeneity [Risch, 2000] but not to locus heterogeneity, and both forms of heterogeneity will contribute to poor replication across association studies.

Marked heterogeneity of the OCD phenotype has also been consistently identified as a formidable challenge in genetic studies of the disorder. The present meta-analysis combined early-onset OCD cases with adult-onset OCD cases, despite the assertion that genetic etiology of early-onset OCD may be distinct [Walitza et al., 2010]. In a large family study of OCD, Nestadt et al. [2000b] found that earlier age at onset was strongly associated with familiality and saw no evidence of familiality when age at onset was older than 17 years. Additionally, a meta-analysis of twin studies in OCD performed by van Grootheest et al. [2005] found dramatically higher heritability in the early-onset form of the disorder, from 0.45 to 0.65, whereas heritability in adult-onset cases ranged from

TABLE III. Meta-analysis Results for Global and Male-only Samples

SNP		MAF				Global sample numbers					Z-scores		OR		Global meta-analysis results					
rs#	**Location**	**Cases**	**Ctrls**	**Hap map**	**Allele**	**# Trios**	**# Cases:ctrls**	**Trio T:U**	**Trio wt.**	**c:c wt.**	**Trios**	**c:c**	**Trios**	**c:c**	**Meta-z**	**χ^2**	***P*-value**	**OR**	**L 95%CI**	**U 95%CI**
rs3780412	Intron	0.45	0.446	0.425 C	C/T	813	306:634	420: 425	657	412	−0.172	0.251	0.988	1.025	0.021	0.000	0.983	1.411	−31.22	34.04
rs12682807	Intron	0.097	0.088	0.133 C	C/A	747	0	129: 109	604	0	1.297		1.183		1.297	1.681	0.195	1.183	0.929	1.437
rs2072657	intron	0.308	0.313	0.317 G	G/T	728	0	278: 285	589	0	−0.295		0.975		−0.295	0.087	0.768	0.975	0.810	1.141
rs301430	Syn coding	0.285	0.284	0.325 C	C/T	805	306:634	325: 320	651	412	0.197	0.538	1.016	1.06	0.489	0.239	0.625	1.455	−0.048	2.958
rs301979	Intron	0.294	0.313	0.292 G	G/C	798	0	325: 341	645	0	−0.62		0.953		−0.62	0.384	0.535	0.953	0.801	1.105
rs301434	intron	0.457	0.456	0.392 T	T/C	641	306:634	323: 329	518	412	−0.235	−1.795	0.982	0.837	−1.370	1.878	0.171	1.290	0.926	1.654
rs301435	Intron	0.468	0.458	0.347 C	C/T	569	0	291: 296	460	0	−0.206		0.983		−0.206	0.043	0.836	0.983	0.821	1.145
rs3087879	3′ UTR	0.337	0.344	0.325 C	C/G	792	306:634	359: 363	640	412	−0.149	−2.217	0.989	0.792	−1.503	2.260	0.133	1.267	0.958	1.575
rs301443	**Intron**	**0.327**	**0.310**	**0.258 C**	**C/G**	**778**	**0**	**351: 300**	**629**	**0**	**1.999**		**1.17**		**1.999**	**3.995**	**0.046**	**1.17**	**1.016**	**1.324**

SNP		MAF				Male-only sample numbers					Z-scores		OR		Male-only meta-analysis results					
rs#	**Location**	**Cases**	**Ctrls**	**Hap map**	**Allele**	**# Trios**	**# Cases:ctrls**	**Trio T:U**	**Trio wt.**	**c:c wt.**	**Trios**	**c:c**	**Trios**	**c:c**	**Meta-z**	**χ^2**	***P*-value**	**OR**	**L 95%CI**	**U 95%CI**
rs3780412	Intron	0.436	0.450	0.425 C	C/T	357	133:290	171: 191	289	182	−1.051	−0.033	0.895	0.995	−0.844	0.712	0.399	1.320	0.675	1.964
rs12682807	**Intron**	**0.104**	**0.088**	**0.133 C**	**C/A**	**326**	**0**	**66:40**	**264**		**2.525**		**1.65**		**2.525**	**6.377**	**0.012**	**1.65**	**1.261**	**2.039**
rs2072657	Intron	0.301	0.309	0.317 G	G/T	313	0	114: 120	253		−0.392		0.95		−0.392	0.154	0.695	0.95	0.694	1.206
rs301430	Syn coding	0.293	0.28	0.325 C	C/T	350	133:290	156: 142	283	182	0.811	1.190	1.099	1.212	1.377	1.897	0.168	1.616	0.933	2.298
rs301979	Intron	0.297	0.306	0.292 G	G/C	347	0	131: 145	281		−0.843		0.903		−0.843	0.710	0.399	0.903	0.667	1.140
rs301434	Intron	0.455	0.457	0.392 T	T/C	243	133:290	128: 127	197	182	0.063	−0.940	1.008	0.870	−0.606	0.367	0.544	1.329	0.409	2.250
rs301435	Intron	0.459	0.462	0.347 C	C/T	215	0	113: 116	174		−0.198		0.974		−0.198	0.039	0.842	0.974	0.715	1.233
rs3087879	3′ UTR	0.332	0.343	0.325 C	C/G	348	133:290	148: 156	282	182	−0.459	−1.563	0.949	0.781	−1.337	1.787	0.181	1.229	0.927	1.531
rs301443	Intron	0.330	0.312	0.258 C	C/G	340	0	163: 134	275		1.683		1.216		1.683	2.832	0.092	1.216	0.988	1.444

Single nucleotide polymorphisms (SNPs) listed by rs# included in meta-analysis. In the columns to the right, minor allele frequencies (MAF) are presented for our case samples, for control samples and as reported in HapMap. The minor and major alleles are presented as oriented to the forward strand. Characteristics of the sample size for each SNP are presented including the number of affected offspring trios (trios) as calculated in PLINK, the number of cases and controls (case:control). The ratio of transmitted to untransmitted minor alleles for trios are presented as the count of transmitted minor alleles: Count of untransmitted minor alleles. Weightings for the trio and case–control samples are provided in the columns to the right. Calculated values for the final meta χ^2 and *P*-values are included, in addition to odds ratios (OR) for trios (as calculated in PLINK), for the case–controls samples (c:c) and for the entire sample (meta-OR). The lower and upper limits of the 95% confidence interval (CI) are in the columns to the far right. The rows at the top of the table describe findings for the global sample, and those at the bottom describe findings for the males-only sample.
Bolded rows indicate final meta-analysis $P < 0.05$.

0.27 to 0.47. The results of this meta-analysis also suggest the potential importance of subphenotypic considerations for future genetic studies of OCD. Factor analytic studies have repeatedly demonstrated that OCD is comprised of discrete but overlapping symptom dimensions [Mataix-Cols et al., 2005; Stewart et al., 2007b; Stewart et al., 2008]. It has been proposed that specific symptom dimensions may be more closely tied to underlying genetic etiologic factors than the entire phenotypic spectrum of the disorder, such that different candidate genes may be responsible for phenotypic expression of distinct symptom dimensions [Pato et al., 2002]. Ideally, a meta-analysis of OCD would stratify by symptom dimensions for secondary analyses. However, the present study would not have been adequately powered to detect *SLC1A1* association with putative age-of-onset or symptom-based OCD subphenotypes [Wendland et al., 2009; Samuels et al., 2011].

In addition to the sample size and heterogeneity, a third potential limitation of this study is the particular analytic method selected. Numerous analytic approaches exist for combining case–control and family-based data [Infante-Rivard et al., 2009], including extensions of likelihood models and combinations of distinct estimates. For the latter category, which was employed in this meta-analysis, two separate analyses were first performed; using a chi-square test in the case–control dataset and a TDT in the merged trio dataset. Kazeem and Farrall [2005] described the method of integrating case–control and TDT studies using odds ratios, which can be weighted since the logarithm of the odds ratio is asymptotically normally distributed. Nicodemus [2008] described some of the computational packages designed to carry out such analyses, but for the current meta-analysis we determined that the easiest and most reliable method of combining the estimates of disease-marker association was the weighted *z*-score, as described by de Bakker et al. [2008]. The *z*-score can be easily derived from the chi-square and odds ratio, both of which were provided in the PLINK output. Because the odds ratio was not provided in the FBAT output, PLINK was a much more attractive program than FBAT for analyzing the family-based data. While this study employed a standardized approach across all analyses, a different approach could have been used, which may have altered the results slightly. This slight lack of precision is particularly relevant to the present study, in which markers are demonstrating only modest association to the disease phenotype.

Finally, the current meta-analysis of *SLC1A1* is premised on potentially incorrect assumptions about its underlying genetic architecture. We examined only common polymorphisms, when it may be the case that multiple rare variants within *SLC1A1* contribute to the OCD phenotype. Detecting such rare variants would require a sequencing approach. To date, studies searching for rare variation in SLC1A1 [Veenstra-VanderWeele et al., 2001, 2012; Wang et al., 2010] have been limited in scope to coding regions and have primarily used capillary-based screening methods, rather than targeting the entire gene via next-generation sequencing methods. Also, if there is substantial allelic heterogeneity for *SLC1A1* and OCD, haplotype [Lambert et al., 2012] or gene-based [Huang et al., 2011; Bacanu, 2012] analytic methods that consider combined effects of SNPs may be more likely to identify associations. Finally, it is possible that insertion/deletion or copy number variants were contributors to the previously identified OCD associations with *SLC1A1*. It is interesting in this regard that our strongest findings was for rs301443, which lies within a small deletion detected by Dickel et al. [2006].

In conclusion, the glutamate transporter gene *SLC1A1* has been a very promising candidate gene for OCD, supported by various lines of biological evidence in addition to multiple genetic association studies. However, this meta-analysis does not support a strong role for common *SLC1A1* risk variants in OCD etiology, nor does it convincingly establish a role for gender in the overall OCD risk liability of *SLC1A1* polymorphisms. On the other hand, it is possible that the signals observed in this analysis are legitimate and represent a true association between *SLC1A1* and OCD. Regardless, it is important to note that the results do not undermine the potential contribution of glutamatergic dysregulation to OCD pathology. Investigating the roles of glutamatergic systems in OCD remains warranted and is relevant to both clinical practice and future research. The results from this study demonstrate the need for larger collaborative samples and subphenotyping, and highlight the potential for false-positive findings, or at least the need for cautious interpretation of positive findings, with candidate gene studies. If heterogeneity of the *SLC1A1* gene comprises both common and rare variants, then next-generation sequencing may also be productive in future studies of *SLC1A1* and OCD [Wang et al., 2010; Bailey et al., 2011].

ACKNOWLEDGMENTS

The authors are would like to express their utmost gratitude to the OCD-affected families who participated in this research. In addition, they would like to thank the International OCD Foundation (IOCDF) for their role in establishing the IOCDF Genetics Collaborative, as well as other individuals who played roles in assisting this study, including Dianne Hezel and Rhonda Ellwyn. This work was supported by an American Academy of Child and Adolescent Psychiatry (AACAP) Early Investigator Research Grant, an Anxiety Disorders Association of America (ADAA) Junior Investigator Research Grant, funding from the British Columbia Provincial Health Services Administration, the University of British Columbia and a Michael Smith Foundation Clinical Research Scholar Award to SES. The original studies were supported by funding from the National Institutes of Health (NS16648 and MH073250 to DLP; K20 MH 01065 and R01 MH 58376 to GLH; R01-MH-50214 to GN; K08-MH081066 to JVV; Intramural Research Program of the National Institute of Mental Health (DLM)), the International Obsessive Compulsive Disorder Foundation (PDA and GLH), the Ontario Mental Health Foundation (Type B grant to PDA), the Canadian Institutes for Health Research (MOP-38077 to PDA), National Alliance on Research in Schizophrenia and Depression (Young Investigator awards to PDA and CAM) and the Judah and McIngvale Foundations. The views expressed in this manuscript do not necessarily represent the views of the NIMH, NIH, HHS, or the United States Government.

REFERENCES

Aboujaoude E, Barry JJ, Gamel N. 2009. Memantine augmentation in treatment-resistant obsessive-compulsive disorder: An open-label trial. J Clin Psychopharmacol 29(1):51–55.

American Psychiatric Association. 2000. Diagnostic criteria from DSM-IV-TR. Washington, D.C: American Psychiatric Association. Vol. xii,p 370.

Arnold PD, Sicard T, Burroughs E, Richter MA, Kennedy JL. 2006. Glutamate transporter gene SLC1A1 associated with obsessive-compulsive disorder. Arch Gen Psychiatry 63(7):760–769.

Bacanu SA. 2012. On optimal gene-based analysis of genome scans. Genet Epidemiol 36(4):333–339.

Bailey CG, Ryan RM, Thoeng AD, Ng C, King K, Vanslambrouck JM, Auray-Blais C, Vandenberg RJ, Bröer S, Rasko JE. 2011. Loss-of-function mutations in the glutamate transporter SLC1A1 cause human dicarboxylic aminoaciduria. J Clin Invest 121(1):446–453.

Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 21(2):263–265.

Bogetto F, Venturello S, Albert U, Maina G, Ravizza L. 1999. Gender-related clinical differences in obsessive-compulsive disorder. Eur Psychiatry 14(8):434–441.

Cavallini MC, Pasquale L, Bellodi L, Smeraldi E. 1999. Complex segregation analysis for obsessive compulsive disorder and related disorders. Am J Med Genet 88(1):38–43.

Chakrabarty K, Bhattacharyya S, Christopher R, Khanna S. 2005. Glutamatergic dysfunction in OCD. Neuropsychopharmacology 30(9):1735–1740.

Cichon S, Craddock N, Daly M, Faraone SV, Gejman PV, Kelsoe J, Lehner T, Levinson DF, Moran A, Sklar P, Sullivan PF. 2009. Genomewide association studies: History, rationale, and prospects for psychiatric disorders. Am J Psychiatry 166(5):540–556.

Consortium MDDWGotPG. 2012. A mega-analysis of genome-wide association studies for major depressive disorder. Mol Psychiatry Epub ahead of print.

Coric V, Taskiran S, Pittenger C, Wasylink S, Mathalon DH, Valentine G, Saksa J, Wu YT, Gueorguieva R, Sanacora G, Malison RT, Krystal JH. 2005. Riluzole augmentation in treatment-resistant obsessive-compulsive disorder: An open-label trial. Biol Psychiatry 58(5):424–428.

de Bakker PI, Ferreira MA, Jia X, Neale BM, Raychaudhuri S, Voight BF. 2008. Practical aspects of imputation-driven meta-analysis of genome-wide association studies. Hum Mol Genet 17(R2):R122–R128.

Dickel DE, Veenstra-VanderWeele J, Cox NJ, Wu X, Fischer DJ, Van Etten-Lee M, Himle JA, Leventhal BL, Cook EH Jr, Hanna GL. 2006. Association testing of the positional and functional candidate gene SLC1A1/EAAC1 in early-onset obsessive-compulsive disorder. Arch Gen Psychiatry 63(7):778–785.

Fischer DJ, Himle JA, Hanna GL. 1996. Age and gender effects on obsessive-compulsive symptoms in children and adults. Depress Anxiety 4(5): 237–239.

Geller DA, Biederman J, Griffin S, Jones J, Lefkowitz TR. 1996. Comorbidity of juvenile obsessive-compulsive disorder with disruptive behavior disorders. J Am Acad Child Adolesc Psychiatry 35(12):1637–1646.

Grados M, Wilcox HC. 2007. Genetics of obsessive-compulsive disorder: A research update. Expert Rev Neurother 7(8):967–980.

Grant P, Lougee L, Hirschtritt M, Swedo SE. 2007. An open-label trial of riluzole, a glutamate antagonist, in children with treatment-resistant obsessive-compulsive disorder. J Child Adolesc Psychopharmacol 17(6): 761–767.

Hanna GL, Fingerlin TE, Himle JA, Boehnke M. 2005a. Complex segregation analysis of obsessive-compulsive disorder in families with pediatric probands. Hum Hered 60(1):1–9.

Hanna GL, Fischer DJ, Chadha KR, Himle JA, Van Etten M. 2005b. Familial and sporadic subtypes of early-onset obsessive-compulsive disorder. Biol Psychiatry 57(8):895–900.

Hanna GL, Veenstra-VanderWeele J, Cox NJ, Boehnke M, Himle JA, Curtis GC, Leventhal BL, Cook EH Jr. 2002. Genome-wide linkage analysis of families with obsessive-compulsive disorder ascertained through pediatric probands. Am J Med Genet 114(5):541–552.

Hanna GL, Veenstra-Vanderweele J, Cox NJ, Van Etten M, Fischer DJ, Himle JA, Bivens NC, Wu X, Roe CA, Hennessy KA, Dickel DE, Leventhal BL, Cook EH. 2007. Evidence for a susceptibility locus on chromosome 10p15 in early-onset obsessive-compulsive disorder. Biol Psychiatry 62(8):856–862.

Hemmings SM, Stein DJ. 2006. The current status of association studies in obsessive-compulsive disorder. Psychiatr Clin North Am 29(2):411–444.

Huang H, Chanda P, Alonso A, Bader JS, Arking DE. 2011. Gene-based tests of association. PLoS Genet 7(7):e1002177.

Infante-Rivard C, Mirea L, Bull SB. 2009. Combining case–control and case-trio data from the same population in genetic association analyses: Overview of approaches and illustration with a candidate gene study. Am J Epidemiol 170(5):657–664.

Inouye E. 1965. Similar and dissimilar manifestations of obsessive-compulsive neuroses in monozygotic twins. Am J Psychiatry 121:1171–1175.

Ivarsson T, Melin K, Wallin L. 2008. Categorical and dimensional aspects of co-morbidity in obsessive-compulsive disorder (OCD). Eur Child Adolesc Psychiatry 17(1):20–31.

Kanai Y, Hediger MA. 2004. The glutamate/neutral amino acid transporter family SL C1: Molecular, physiological and pharmacological aspects. Pflugers Arch 447(5):469–479.

Karno M, Golding JM, Sorenson SB, Burnam MA. 1988. The epidemiology of obsessive-compulsive disorder in five US communities. Arch Gen Psychiatry 45(12):1094–1099.

Kazeem GR, Farrall M. 2005. Integrating case–control and TDT studies. Ann Hum Genet 69(Pt3):329–335.

Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE. 2005. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 62(6):617–627.

Kwon JS, Joo YH, Nam HJ, Lim M, Cho EY, Jung MH, Choi JS, Kim B, Kang DH, Oh S, Park T, Hong KS. 2009. Association of the glutamate transporter gene SLC1A1 with atypical antipsychotics-induced obsessive-compulsive symptoms. Arch Gen Psychiatry 66(11):1233–1241.

Laird NM, Horvath S, Xu X. 2000. Implementing a unified approach to family-based tests of association. Genet Epidemiol 19(Suppl1):S36–S42.

Lambert JC, Grenier-Boley B, Harold D, Zelenika D, Chouraki V, Kamatani Y, Sleegers K, Ikram MA, Hiltunen M, Reitz C, Mateo I, Feulner T, Bullido M, Galimberti D, Concari L, Alvarez V, Sims R, Gerrish A, Chapman J, Deniz-Naranjo C, Solfrizzi V, Sorbi S, Arosio B, Spalletta G, Siciliano G, Epelbaum J, Hannequin D, Dartigues JF, Tzourio C, Berr C, Schrijvers EM, Rogers R, Tosto G, Pasquier F, Bettens K, Van Cauwenberghe C, Fratiglioni L, Graff C, Delepine M, Ferri R, Reynolds CA, Lannfelt L, Ingelsson M, Prince JA, Chillotti C, Pilotto A, Seripa D, Boland A, Mancuso M, Bossù P, Annoni G, Nacmias B, Bosco P, Panza F, Sanchez-Garcia F, Del Zompo M, Coto E, Owen M, O'Donovan M, Valdivieso F, Caffara P, Scarpini E, Combarros O, Buée L, Campion D, Soininen H, Breteler M, Riemenschneider M, Van Broeckhoven C, Alpérovitch A, Lathrop M, Trégouët DA, Williams J, Amouyel P. 2012. Genome-wide haplotype association study identifies the FRMD4A gene as a risk locus for Alzheimer's disease. Mol Psychiatry Epub ahead of print.

Lange C, DeMeo D, Silverman EK, Weiss ST, Laird NM. 2004. PBAT: Tools for family-based association studies. Am J Hum Genet 74(2):367–369.

Langley AK, Lewin AB, Bergman RL, Lee JC, Piacentini J. 2010. Correlates of comorbid anxiety and externalizing disorders in childhood obsessive compulsive disorder. Eur Child Adolesc Psychiatry 19(8):637–645.

Lensi P, Cassano GB, Correddu G, Ravagli S, Kunovac JL, Akiskal HS. 1996. Obsessive-compulsive disorder. Familial-developmental history, symptomatology, comorbidity and course with special reference to gender-related differences. Br J Psychiatry 169(1):101–107.

Liang KY, Wang Y, Shugart YY, Grados M, Fyer AJ, Rauch S, Murphy D, McCracken J, Rasmussen S, Cullen B, Hoehn-Saric R, Greenberg B, Pinto A, Knowles J, Piacentini J, Pauls D, Bienvenu O, Riddle M, Samuels J, Nestadt G. 2008. Evidence for potential relationship between SLC1A1 and a putative genetic linkage region on chromosome 14q to obsessive-compulsive disorder with compulsive hoarding. Am J Med Genet Part B 147B(6):1000–1002.

Lin DY, Zeng D. 2010. Meta-analysis of genome-wide association studies: No efficiency gain in using individual participant data. Genet Epidemiol 34(1):60–66.

Mah AK. 2010. SLITRK5, a protein that links striatal deficits to OCD-like behaviours in mice. Clin Genet 78(4):350–352.

Mataix-Cols D, Rosario-Campos MC, Leckman JF. 2005. A multidimensional model of obsessive-compulsive disorder. Am J Psychiatry 162(2): 228–238.

Mathews CA, Badner JA, Andresen JM, Sheppard B, Himle JA, Grant JE, Williams KA, Chavira DA, Azzam A, Schwartz M, Reus VI, Kim SW, Cook EH, Hanna GL. 2012. Genome-wide linkage analysis of obsessive-compulsive disorder implicates chromosome 1p36. Biol Psychiatry 72(8):629–636.

Matsunaga H, Kiriike N, Matsui T, Miyata A, Iwasaki Y, Fujimoto K, Kasai S, Kojima M. 2000. Gender differences in social and interpersonal features and personality disorders among Japanese patients with obsessive-compulsive disorder. Compr Psychiatry 41(4):266–272.

Menzies L, Chamberlain SR, Laird AR, Thelen SM, Sahakian BJ, Bullmore ET. 2008. Integrating evidence from neuroimaging and neuropsychological studies of obsessive-compulsive disorder: The orbitofronto-striatal model revisited. Neurosci Biobehav Rev 32(3):525–549.

Nestadt G, Grados M, Samuels JF. 2010. Genetics of obsessive-compulsive disorder. Psychiatr Clin North Am 33(1):141–158.

Nestadt G, Lan T, Samuels J, Riddle M, Bienvenu OJ III, Liang KY, Hoehn-Saric R, Cullen B, Grados M, Beaty TH, Shugart YY. 2000a. Complex segregation analysis provides compelling evidence for a major gene underlying obsessive-compulsive disorder and for heterogeneity by sex. Am J Hum Genet 67(6):1611–1616.

Nestadt G, Samuels J, Riddle M, Bienvenu OJ III, Liang KY, LaBuda M, Walkup J, Grados M, Hoehn-Saric R. 2000b. A family study of obsessive-compulsive disorder. Arch Gen Psychiatry 57(4):358–363.

Nicodemus KK. 2008. Catmap: Case–control and TDT meta-analysis package. BMC Bioinformatics 9:130.

Nicolini H, Arnold P, Nestadt G, Lanzagorta N, Kennedy JL. 2009. Overview of genetics and obsessive-compulsive disorder. Psychiatry Res 170(1):7–14.

Pato MT, Pato CN, Pauls DL. 2002. Recent findings in the genetics of OCD. J Clin Psychiatry 63(Suppl6):30–33.

Pittenger C, Bloch MH, Williams K. 2011. Glutamate abnormalities in obsessive compulsive disorder: Neurobiology, pathophysiology, and treatment. Pharmacol Ther 132(3):314–332.

Pittenger C, Krystal JH, Coric V. 2006. Glutamate-modulating drugs as novel pharmacotherapeutic agents in the treatment of obsessive-compulsive disorder. NeuroRx 3(1):69–81.

Purcell S, Cherny SS, Sham PC. 2003. Genetic power calculator: Design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19(1):149–150.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. 2007. PLINK: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81(3):559–575.

Rauch SL, Jenike MA, Alpert NM, Baer L, Breiter HC, Savage CR, Fischman AJ. 1994. Regional cerebral blood flow measured during symptom provocation in obsessive-compulsive disorder using oxygen 15-labeled carbon dioxide and positron emission tomography. Arch Gen Psychiatry 51(1):62–70.

Ravizza L, Maina G, Bogetto F. 1997. Episodic and chronic obsessive-compulsive disorder. Depress Anxiety 6(4):154–158.

Risch NJ. 2000. Searching for genetic determinants in the new millennium. Nature 405(6788):847–856.

Rosenberg DR, Mirza Y, Russell A, Tang J, Smith JM, Banerjee SP, Bhandari R, Rose M, Ivey J, Boyd C, Moore GJ. 2004. Reduced anterior cingulate glutamatergic concentrations in childhood OCD and major depression versus healthy controls. J Am Acad Child Adolesc Psychiatry 43(9): 1146–1153.

Ross J, Badner J, Garrido H, Sheppard B, Chavira DA, Grados M, Woo JM, Doo P, Umaña P, Fournier E, Murray SS, Mathews CA. 2011. Genome-wide linkage analysis in Costa Rican families implicates chromosome 15q14 as a candidate region for OCD. Hum Genet 130(6):795–805.

Sampaio AS, Fagerness J, Crane J, Leboyer M, Delorme R, Pauls DL, Stewart SE. 2011. Association between polymorphisms in GRIK2 gene and obsessive-compulsive disorder: A family-based study. CNS Neurosci Ther 17(3):141–147.

Samuels J, Wang Y, Riddle MA, Greenberg BD, Fyer AJ, McCracken JT, Rauch SL, Murphy DL, Grados MA, Knowles JA, Piacentini J, Cullen B, Bienvenu OJ III, Rasmussen SA, Geller D, Pauls DL, Liang KY, Shugart YY, Nestadt G. 2011. Comprehensive family-based association study of the glutamate transporter gene SLC1A1 in obsessive-compulsive disorder. Am J Med Genet Part B 156B(4):472–477.

Saxena S, Rauch SL. 2000. Functional neuroimaging and the neuroanatomy of obsessive-compulsive disorder. Psychiatr Clin North Am 23(3): 563–586.

Shugart YY, Samuels J, Willour VL, Grados MA, Greenberg BD, Knowles JA, McCracken JT, Rauch SL, Murphy DL, Wang Y, Pinto A, Fyer AJ, Piacentini J, Pauls DL, Cullen B, Page J, Rasmussen SA, Bienvenu OJ, Hoehn-Saric R, Valle D, Liang KY, Riddle MA, Nestadt G. 2006a. Genomewide linkage scan for obsessive-compulsive disorder: Evidence for susceptibility loci on chromosomes 3q, 7p, 1q, 15q, and 6q. Mol Psychiatry 11(8):763–770.

Shugart YY, Samuels J, Willour VL, Grados MA, Greenberg BD, Knowles JA, McCracken JT, Rauch SL, Murphy DL, Wang Y, Pinto A, Fyer AJ, Piacentini J, Pauls DL, Cullen B, Page J, Rasmussen SA, Bienvenu OJ, Hoehn-Saric R, Valle D, Liang KY, Riddle MA, Nestadt G. 2006b. Genomewide linkage scan for obsessive-compulsive disorder: Evidence for susceptibility loci on chromosomes 3q, 7p, 1q, 15q, and 6q. Mol Psychiatry 11(8):763–770.

Shugart YY, Wang Y, Samuels JF, Grados MA, Greenberg BD, Knowles JA, McCracken JT, Rauch SL, Murphy DL, Rasmussen SA, Cullen B, Hoehn-Saric R, Pinto A, Fyer AJ, Piacentini J, Pauls DL, Bienvenu OJ, Riddle MA, Liang KY, Nestadt G. 2009. A family-based association study of the glutamate transporter gene SLC1A1 in obsessive-compulsive disorder in 378 families. Am J Med Genet Part B 150B(6):886–892.

Smith CP, Weremowicz S, Kanai Y, Stelzner M, Morton CC, Hediger MA. 1994. Assignment of the gene coding for the human high-affinity glutamate transporter EAAC1 to 9p24: Potential role in dicarboxylic aminoaciduria and neurodegenerative disorders. Genomics 20(2):335–336.

Stewart SE, Fagerness JA, Platko J, Smoller JW, Scharf JM, Illmann C, Jenike E, Chabane N, Leboyer M, Delorme R, Jenike MA, Pauls DL. 2007a. Association of the SLC1A1 glutamate transporter gene and obsessive-compulsive disorder. Am J Med Genet Part B 144B(8):1027–1033.

Stewart SE, Jenike EA, Hezel DM, Stack DE, Dodman NH, Shuster L, Jenike MA. 2010. A single-blinded case–control study of memantine in severe obsessive-compulsive disorder. J Clin Psychopharmacol 30(1):34–39.

Stewart SE, Rosario MC, Baer L, Carter AS, Brown TA, Scharf JM, Illmann C, Leckman JF, Sukhodolsky D, Katsovich L, Rasmussen S, Goodman W, Delorme R, Leboyer M, Chabane N, Jenike MA, Geller DA, Pauls DL. 2008. Four-factor structure of obsessive-compulsive disorder symptoms in children, adolescents, and adults. J Am Acad Child Adolesc Psychiatry 47(7):763–772.

Stewart SE, Rosario MC, Brown TA, Carter AS, Leckman JF, Sukhodolsky D, Katsovitch L, King R, Geller D, Pauls DL. 2007b. Principal components analysis of obsessive-compulsive disorder symptoms in children and adolescents. Biol Psychiatry 61(3):285–291.

Stewart SE, Yu D, Scharf JM, Neale BM, Fagerness JA, Mathews CA, Arnold PD, Evans PD, Gamazon ER, Osiecki L, McGrath L, Haddad S, Crane J, Hezel D, Illman C, Mayerfeld C, Konkashbaev A, Liu C, Pluzhnikov A, Tikhomirov A, Edlund CK, Rauch SL, Moessner R, Falkai P, Maier W, Ruhrmann S, Grabe HJ, Lennertz L, Wagner M, Bellodi L, Cavallini MC, Richter MA, Cook EH, Kennedy JL, Rosenberg D, Stein DJ, Hemmings SM, Lochner C, Azzam A, Chavira DA, Fournier E, Garrido H, Sheppard B, Umaña P, Murphy DL, Wendland JR, Veenstra-Vanderweele J, Denys D, Blom R, Deforce D, Van Nieuwerburgh F, Westenberg HG, Walitza S, Egberts K, Renner T, Miguel EC, Cappi C, Hounie AG, Conceição do, Rosário M, Sampaio AS, Vallada H, Nicolini H, Lanzagorta N, Camarena B, Delorme R, Leboyer M, Pato CN, Pato MT, Voyiaziakis E, Heutink P, Cath DC, Posthuma D, Smit JH, Samuels J, Bienvenu OJ, Cullen B, Fyer AJ, Grados MA, Greenberg BD, McCracken JT, Riddle MA, Wang Y, Coric V, Leckman JF, Bloch M, Pittenger C, Eapen V, Black DW, Ophoff RA, Strengman E, Cusi D, Turiel M, Frau F, Macciardi F, Gibbs JR, Cookson MR, Singleton A, Hardy J, Crenshaw AT, Parkin MA, Mirel DB, Conti DV, Purcell S, Nestadt G, Hanna GL, Jenike MA, Knowles JA, Cox N, Pauls DL, Consortium ftNABE, Database ftUBE. 2012. Genome-wide association study of obsessive-compulsive disorder. Mol Psychiatry Epub ahead of print.

Swedo SE, Schapiro MB, Grady CL, Cheslow DL, Leonard HL, Kumar A, Friedland R, Rapoport SI, Rapoport JL. 1989. Cerebral glucose metabolism in childhood-onset obsessive-compulsive disorder. Arch Gen Psychiatry 46(6):518–523.

Taylor S. 2012. Molecular genetics of obsessive-compulsive disorder: A comprehensive meta-analysis of genetic association studies. Mol Psychiatry Epub ahead of print.

Trikalinos TA, Salanti G, Zintzaras E, Ioannidis JP. 2008. Meta-analysis methods. Adv Genet 60:311–334.

Tukel R, Polat A, Genc A, Bozkurt O, Atli H. 2004. Gender-related differences among Turkish patients with obsessive-compulsive disorder. Compr Psychiatry 45(5):362–366.

van Grootheest DS, Cath DC, Beekman AT, Boomsma DI. 2005. Twin studies on obsessive-compulsive disorder: A review. Twin Res Hum Genet 8(5):450–458.

Veenstra-VanderWeele J, Kim SJ, Gonen D, Hanna GL, Leventhal BL, Cook EH Jr. 2001. Genomic organization of the SLC1A1/EAAC1 gene and mutation screening in early-onset obsessive-compulsive disorder. Mol Psychiatry 6(2):160–167.

Veenstra-VanderWeele J, Xu T, Ruggiero AM, Anderson LR, Jones ST, Himle JA, Kennedy JL, Richter MA, Hanna GL, Arnold PD. 2012. Functional studies and rare variant screening of SLC1A1/EAAC1 in males with obsessive-compulsive disorder. Psychiatr Genet 22(5): 256–260.

Walitza S, Wendland JR, Gruenblatt E, Warnke A, Sontag TA, Tucha O, Lange KW. 2010. Genetics of early-onset obsessive-compulsive disorder. Eur Child Adolesc Psychiatry 19(3):227–235.

Wang Y, Adamczyk A, Shugart YY, Samuels JF, Grados MA, Greenberg BD, Knowles JA, McCracken JT, Rauch SL, Murphy DL, Rasmussen SA, Cullen B, Pinto A, Fyer AJ, Piacentini J, Pauls DL, Bienvenu OJ, Riddle M, Liang KY, Valle D, Wang T, Nestadt G. 2010. A screen of SLC1A1 for OCD-related alleles. Am J Med Genet Part B 153B(2):675–679.

Weissman MM, Bland RC, Canino GJ, Greenwald S, Hwu HG, Lee CK, Newman SC, Oakley-Browne MA, Rubio-Stipec M, Wickramaratne PJ, et al. 1994. The cross national epidemiology of obsessive compulsive disorder. The Cross National Collaborative Group. J Clin Psychiatry (Suppl 55):5–10.

Welch JM, Lu J, Rodriguiz RM, Trotta NC, Peca J, Ding JD, Feliciano C, Chen M, Adams JP, Luo J, Dudek SM, Weinberg RJ, Calakos N, Wetsel WC, Feng G. 2007. Cortico-striatal synaptic defects and OCD-like behaviours in Sapap3-mutant mice. Nature 448(7156):894–900.

Wendland JR, Moya PR, Timpano KR, Anavitarte AP, Kruse MR, Wheaton MG, Ren-Patterson RF, Murphy DL. 2009. A haplotype containing quantitative trait loci for SLC1A1 gene expression and its association with obsessive-compulsive disorder. Arch Gen Psychiatry 66(4): 408–416.

Whiteside SP, Port JD, Deacon BJ, Abramowitz JS. 2006. A magnetic resonance spectroscopy investigation of obsessive-compulsive disorder and anxiety. Psychiatry Res 146(2):137–147.

Willour VL, Yao Shugart, Samuels Y, Grados J, Cullen M, Bienvenu B, Wang OJ III, Liang Y, Valle KY, Hoehn-Saric D, Riddle R, Nestadt MG. 2004a. Replication study supports evidence for linkage to 9p24 in obsessive-compulsive disorder. Am J Hum Genet 75(3):508–513.

Willour VL, Yao Shugart Y, Samuels J, Grados M, Cullen B, Bienvenu OJ, Wang Y, Liang KY, Valle D, Hoehn-Saric R, Riddle M, Nestadt G. 2004b. Replication study supports evidence for linkage to 9p24 in obsessive-compulsive disorder. Am J Hum Genet 75(3):508–513.

Wu K, Hanna GL, Rosenberg DR, Arnold PD. 2012. The role of glutamate signaling in the pathogenesis and treatment of obsessive-compulsive disorder. Pharmacol Biochem Behav 100(4):726–735.

Yucel M, Wood SJ, Wellard RM, Harrison BJ, Fornito A, Pujol J, Velakoulis D, Pantelis C. 2008. Anterior cingulate glutamate–glutamine levels predict symptom severity in women with obsessive-compulsive disorder. Aust N Z J Psychiatry 42(6):467–477.

Zohar J, Gross-Isseroff R, Hermesh H, Weizman A. 1999. Is there sexual dimorphism in obsessive-compulsive disorder? Neurosci Biobehav Rev 23(6):845–849.