Published in final edited form as:

Psychiatr Genet. 2012 October ; 22(5): 256–260. doi:10.1097/YPG.0b013e328353fb63.

Brief Report: Functional Studies and Rare Variant Screening of *SLC1A1*/EAAC1 in Males with Obsessive-Compulsive Disorder

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Abstract

The neuronal glutamate transporter gene *SLC1A1*/EAAC1 is associated with obsessive-compulsive disorder (OCD) in several studies, with stronger association in males. Previous studies have primarily focused on common single nucleotide polymorphisms, rather than rare functional variants that are likely to have larger effects. We screened 184 males with OCD for rare variation in *SLC1A1* exons. No new coding variation was found. When combined with previous screens, only one *SLC1A1* amino acid variant has been detected in 841 subjects screened, less than for other neurotransmitter transporter genes (P = 0.0001). We characterized the function of the one *SLC1A1* missense variant previously reported in OCD, Thr164Ala (Wang *et al.*, 2009), finding that the Ala164 allele leads to decreased V_{max} and K_m (P < 0.0001) in transfected HEK cells. Further work will be necessary to understand the impact of this rare *SLC1A1*/EAAC1 Ala164 variant on neuronal function and circuitry relevant to OCD.

Keywords

Genetic; obsessive-compulsive disorder; glutamate; neurotransmitter; transporter

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INTRODUCTION

OCD linkage (Hanna *et al.*, 2002; Liang *et al.*, 2008; Veenstra-VanderWeele *et al.*, 2001; Willour *et al.*, 2004) and association studies (Arnold *et al.*, 2006; Dickel *et al.*, 2006; Kwon *et al.*, 2009; Shugart *et al.*, 2009; Stewart *et al.*, 2007; Wendland *et al.*, 2009) point to the neuronal glutamate transporter gene *SLC1A1*/EAAC1, with stronger association in males. Searches for rare genetic variants of larger effect have been fruitful in other childhood neuropsychiatric disorders, including Tourette's Syndrome (Abelson *et al.*, 2005; Ercan-Sencicek *et al.*, 2010). Although no common amino acid or splice site polymorphisms were detected in previous mutation screens of *SLC1A1* in OCD (Veenstra-VanderWeele *et al.*, 2001), Wang and colleagues (2009) detected one rare amino acid variant, Thr164Ala, in a single family with OCD. In this study, we screened for rare *SLC1A1* variants in the two populations of males with OCD in which association was first detected (Arnold *et al.*, 2006; Dickel *et al.*, 2006). To further assess the impact of *SLC1A1*/EAAC1 functional variation in OCD, we evaluated whether the Ala164 variant impacts transporter function in transfected HEK cells.

METHODS

Mutation Screen of *SLC1A1*

Mutation screen participants were ascertained at the University of Michigan (UM: 41 male subjects ascertained through child and adolescent probands) (Dickel *et al.*, 2006) and the Centre for Addiction and Mental Health, Toronto (CAMH: 143 primarily adult male subjects) (Arnold *et al.*, 2006), as previously described. All subjects gave informed consent to ongoing OCD studies, as approved by the UM Institutional Review Board and the CAMH Research Ethics Board. The mutation screen study was also approved by the Vanderbilt University Institutional Review Board as non-human subject research.

As described previously (Dickel *et al.*, 2006), the UM population was ascertained using either DSM-IIIR or DSM-IV criteria, based upon date of ascertainment. Probands younger than 18 years of age at the time of assessment were evaluated using either the Schedule for Affective Disorders and Schizophrenia for School Age Children-Epidemiologic Version (K-SADS-E) (Orvaschel, 1987) or the Schedule for Affective Disorder and Schizophrenia for School-Age Children—Epidemiologic Version–5 (Orvaschel, 1995). Probands and relatives 18 years and older were interviewed using either the Structured Clinical Interview for DSM-IIIR Axis I Disorders (Spitzer *et al.*, 1990; Spitzer *et al.*, 1992) or the Structured Clinical Interview for DSM-IV Axis I Disorders (First *et al.*, 1998). Interviews were supplemented with sections on OCD and tic disorders derived from the Schedule for Tourette and Other Behavioral Syndromes (Pauls and Hurst, 1991). The section on OCD included a series of questions modified to cover all the criteria for a DSM-IIIR or DSM-IV lifetime diagnosis of OCD and a checklist from the Yale-Brown Obsessive Compulsive Scale (Goodman *et al.*, 1989a; Goodman *et al.*, 1989b) modified to obtain information about the lifetime occurrence of specific obsessions and compulsions. Best-estimate lifetime diagnoses were made by two investigators using DSM-III-R or DSM-IV criteria. The UM population included only subjects of European origin who were ascertained during childhood, adolescence, and adulthood (median age at ascertainment 15, mean age 19.5, standard deviation 12.6, range

7–53 years old) using a recruitment strategy weighted toward early-onset OCD (median age of onset 8, mean age 8.5, standard deviation 4.2, range 3–27 years old), as described previously (Dickel et al., 2006).

As described previously, the CAMH population was ascertained using DSM-IV criteria. All participants were assessed using age-appropriate versions of the Structured Clinical Interview for DSM-IV (First *et al.*, 1998; Hien *et al.*, 1994), and probands and affected relatives were assessed using age-appropriate versions of the Yale-Brown Obsessive Compulsive Scale (YBOCS) (Goodman *et al.*, 1989a; Goodman *et al.*, 1989b; Scahill *et al.*, 1997). Best-estimate lifetime diagnoses were made by two investigators using DSM-IV criteria. The CAMH population included primarily subjects of European origin (126 European, 3 Asian, 1 Hispanic, 7 Multiracial, and 5 who declined to respond) who were ascertained primarily during adulthood (median age 33, mean age 33.4, standard deviation 12.1, range 8–67 years old), as described previously (Arnold et al., 2006). Of the 107 CAMH subjects for whom age of onset was available, most had onset during childhood, adolescence, or early adulthood (median age of onset 12, mean 13.5, standard deviation 7.8, range 4–38).

SLC1A1 exons 1–12 were amplified as described previously (Veenstra-VanderWeele *et al.*, 2001), with a few changes. First, to improve amplification fidelity, 0.1 Units of Turbo Pfu were added to each PCR reaction. Second, 5% dimethyl sulfoxide (DMSO) was added to the PCR reactions for exon 1. Third, new exon 2 and 3 primers were designed (available on request). Samples were prepared following the manufacturer's protocol for heteroduplex screening on the Reveal (Transgenomic, Omaha, NE) polymorphism detection system (Li *et al.*, 2002). All samples with a heteroduplex pattern were sequenced on an Applied Biosystems 3730xl DNA Analyzer. Any samples with no confirmed SNP in one direction were sequenced in a second direction. Of note, the sensitivity of temperature gradient capillary electrophoresis (TGCE)-based polymorphism detection is estimated to be 90–97% (Gedge *et al.*, 2007), so our screening approach could have missed additional rare variants. Additionally, our TGCE-based screening only detected heterozygous individuals, so any homozygous rare variants would have been missed.

Functional Study of *SLC1A1*/EAAC1 Ala164 Variant

The Ala164 amino acid variant (Wang *et al.*, 2009) was introduced into an OriGene (Rockville, MD) plasmid containing the human *SLC1A1* cDNA sequence (Cat # SC117563, NM_004170.4) using the QuikChange Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA), following the manufacturer's guidelines (primers: sense 5'-cctgttttcagcagtacaaagctaagcgtgaagaagtgaagc-3' and antisense 5'-gcttcacttcttcacgcttagctttgtactgctgaaaacagg-3'), and confirmed by sequencing the full cDNA. Transient transfection of HEK293T cells with 0.25 μg plasmid DNA per well used FuGENE reagent (Roche Applied Science, Indianapolis, IN) in a 24-well TopCount plate (Perkin Elmer, Waltham, MA) at a density of 50,000 cells per 0.5 mL well. Cell protein content was normalized between genotypes (BCA, Thermo Fisher Scientific, Rockford, IL). Glutamate uptake kinetics was assessed as previously described (Lin *et al.*, 2001). Briefly, uptake of glutamate concentrations of 1, 5, 10, 50, 250, and 500 μM were assayed by scintillation

counts of tracer amounts of [^{3}H]glutamate (GE Healthcare, Pittsburgh, PA). Uptake values were corrected for non-specific uptake in Na^+-free media and normalized to the Thr164 uptake V_{max}. Michaelis-Menten curves were calculated using Prism 4.0 (GraphPad, LaJolla, CA).

RESULTS AND DISCUSSION

No new non-synonymous variants were detected in 184 male subjects with OCD. A number of known SNPs were identified, along with two novel polymorphisms (Table 1), neither of which occurs in a well-conserved region or interferes with any known transcription factor binding site by PROMO (Farre *et al.*, 2003) or TFBIND (Tsunoda and Takagi, 1999) analysis.

The uptake kinetics of the Ala164 *SLC1A1*/EAAC1 variant differed significantly from the wildtype (Thr164) (Figure 1, Michaelis-Menten Curve Fit, $P < 0.0001$). The V_{max} was lower for the Ala164 variant transporter than the wildtype (0.76 +/− 0.03 versus 1.0 +/− 0.03, with both genotypes normalized to the wildtype V_{max}). The K_m was also lower for the Ala164 variant transporter (83.7 +/− 13.0 μM versus 117.4 +/− 12.3 μM), indicating a higher affinity, such that the curves separate primarily at higher concentrations.

Our findings, when coupled with the previous mutation screen findings (Veenstra-VanderWeele *et al.*, 2001; Wang *et al.*, 2009), suggest that non-synonymous coding SNPs are very rare in *SLC1A1*, even in males showing evidence of association. In comparison with the serotonin transporter (*SLC6A4*) and vesicular monoamine transporter (*SLC18A2*) genes, which each show approximately 1% missense variants in the general population (Glatt *et al.*, 2001), *SLC1A1* shows significantly fewer missense variants (Fisher's exact $P = 0.0001$, 1 *SLC1A1* missense variant per 1120 OCD and 562 control chromosomes screened versus 21 *SLC6A4* or *SLC18A2* missense variants per 1800 control chromosomes, with 900 screened for each gene). Importantly, we did not screen *SLC6A4* or *SLC18A2* for missense variants in our sample, and so this comparison is indirect, but the degree of statistical significance is highly suggestive.

The paucity of *SLC1A1* missense variants suggests that activity of this transporter is physiologically crucial. Deleterious mutations in *SLC1A1* have only been reported in two families with dicarboxylic aminoaciduria, a rare disorder often associated with intellectual disability; although one affected man reportedly had excessive hand-washing that was never clinically evaluated (Bailey *et al.*, 2011). On the other hand, studies of mice lacking *Slc1a1* expression did not reveal any phenotypes that interfere with reproductive fitness. The initial study of EAAC1 knockout mice did identify reduced locomotor activity and dicarboxylic aminoaciduria (Peghini *et al.*, 1997). A follow-up study revealed increased neuronal degeneration over time due to oxidative stress in the absence of EAAC1-mediated cysteine uptake, necessary for the synthesis of glutathione (Aoyama *et al.*, 2006).

The change in transport kinetics observed with the *SLC1A1*/EAAC1 Ala164 variant was statistically significant in transfected cells, but it is hard to estimate the impact that it might have on neuronal function. Further studies of the response of the Ala164 variant to known

EAAC1 regulatory pathways (Fournier *et al.*, 2004; Gonzalez *et al.*, 2007; Ruggiero *et al.*, 2008; Waxman *et al.*, 2007) could clarify the mechanism of its altered function. Studies in neuronal or animal models would be necessary to understand the impact on synaptic function and neuronal pathways relevant to OCD.

If the functional *SLC1A1*/EAAC1 Ala164 variant does contribute to OCD susceptibility in this family, there are a few possible cellular or synaptic mechanisms. Prior studies have localized the EAAC1 protein to the postsynaptic side of glutamatergic synapses (Conti *et al.*, 1998). Its functional importance in these neurons may include modulation of glutamate concentrations, particularly in the perisynaptic space (Otis *et al.*, 2004; Scimemi *et al.*, 2009); uptake of glutamate as a substrate for GABA synthesis (Mathews and Diamond, 2003; Sepkuty *et al.*, 2002); and uptake of cysteine for glutathione synthesis (Aoyama *et al.*, 2006). Unlike the glial glutamate transporters, EAAC1 does not appear to play a major role in preventing glutamatergic neurotoxicity (Aoyama *et al.*, 2006). It is not clear how the altered function of EAAC1 conferred by the Ala164 variant would impact these functions within the brain circuitry that may mediate OCD (Radua and Mataix-Cols, 2009).

There are important limitations to our screen for uncommon variants in *SLC1A1*. First, recent studies of rare copy number variants in other neuropsychiatric disorders such as schizophrenia (Consortium, 2008; Stefansson *et al.*, 2008) have identified rare variants of large effects with frequency of <0.5%, and it is unlikely we would detects variants of this frequency in our sample of 184 patients. Second, our heteroduplex screening approach may have missed additional rare variants (Gedge *et al.*, 2007). Third, we did not screen a control group in this study to identify the rate of *SLC1A1* variants in the general population. Finally, this screen was limited to the *SLC1A1* exons and flanking intronic regions.

In conclusion, our screen revealed no new amino acid variants in *SLC1A1* in males with OCD, with the only two novel variants occurring in intronic regions unlikely to affect transcription. In contrast, the previously published Ala164 variant showed a significant impact on function in transfected cells. The decrease in transport that we found for this variant is in the opposite direction of the increased *SLC1A1* expression observed for the rs301430 synonymous coding SNP C allele (Wendland *et al.*, 2009), which leaves open the question of whether increased or decreased *SLC1A1*/EAAC1 function may be implicated in OCD. Further work may clarify this relationship, including analysis of lymphoblastoid cell lines from patients with OCD or analysis of mice with altered but not ablated EAAC1 function.

Acknowledgements:

The authors would like to thank Suma Jacob, Diane Koram, Kristin Chadha, and Olga Likhodi for technical assistance. We would like to thank Randy Blakely, Edwin H. Cook, Jr, and Michael Boehnke for mentorship and advice. This work was supported, in part, by a NARSAD Young Investigator Grant (JV), NIH grants MH081066 (JV), an NIH grant to the Vanderbilt Institute for Clinical and Translational Research (RR024975), NIH grants MH01065 and MH58376 (GLH), a Type B grant from the Ontario Mental Health Foundation (JLK, MAR, PDA), and operating grant MOP-38077 (JLK, MAR, PDA).

Sources of support: This work was supported, in part, by NIH grants MH081066 (JV), an NIH grant to the Vanderbilt Institute for Clinical and Translational Research (RR024975), NIH grants MH01065 and MH58376 (GLH), a Type B grant from the Ontario Mental Health Foundation (JLK, MAR, PDA), and operating grant MOP-38077 (JLK, MAR, PDA).

References

Abelson JF, Kwan KY, O'Roak BJ, Baek DY, Stillman AA, Morgan TM, Mathews CA, Pauls DL, Rasin MR, Gunel M, Davis NR, Ercan-Sencicek AG, Guez DH, Spertus JA, Leckman JF, St Dure L, Kurlan R, Singer HS, Gilbert DL, Farhi A, Louvi A, Lifton RP, Sestan N, State MW. (2005). Sequence variants in SLITRK1 are associated with Tourette's syndrome. Science 310:317–20. [PubMed: 16224024]

Aoyama K, Suh SW, Hamby AM, Liu J, Chan WY, Chen Y, Swanson RA. (2006). Neuronal glutathione deficiency and age-dependent neurodegeneration in the EAAC1 deficient mouse. Nat Neurosci 9:119–26. [PubMed: 16311588]

Arnold PD, Sicard T, Burroughs E, Richter MA, Kennedy JL. (2006). Glutamate transporter gene SLC1A1 associated with obsessive-compulsive disorder. Arch Gen Psychiatry 63:769–76. [PubMed: 16818866]

Bailey CG, Ryan RM, Thoeng AD, Ng C, King K, Vanslambrouck JM, Auray-Blais C, Vandenberg RJ, Broer S, Rasko JE. (2011). Loss-of-function mutations in the glutamate transporter SLC1A1 cause human dicarboxylic aminoaciduria. J Clin Invest 121:446–53. [PubMed: 21123949]

Consortium IS. (2008). Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature 455:237–41. [PubMed: 18668038]

Conti F, DeBiasi S, Minelli A, Rothstein JD, Melone M. (1998). EAAC1, a high-affinity glutamate tranporter, is localized to astrocytes and gabaergic neurons besides pyramidal cells in the rat cerebral cortex. Cereb Cortex 8:108–16. [PubMed: 9542890]

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 63:778–85. [PubMed: 16818867]

Ercan-Sencicek AG, Stillman AA, Ghosh AK, Bilguvar K, O'Roak BJ, Mason CE, Abbott T, Gupta A, King RA, Pauls DL, Tischfield JA, Heiman GA, Singer HS, Gilbert DL, Hoekstra PJ, Morgan TM, Loring E, Yasuno K, Fernandez T, Sanders S, Louvi A, Cho JH, Mane S, Colangelo CM, Biederer T, Lifton RP, Gunel M, State MW. (2010). L-histidine decarboxylase and Tourette's syndrome. N Engl J Med 362:1901–8. [PubMed: 20445167]

Farre D, Roset R, Huerta M, Adsuara JE, Rosello L, Alba MM, Messeguer X. (2003). Identification of patterns in biological sequences at the ALGGEN server: PROMO and MALGEN. Nucleic Acids Res 31:3651–3. [PubMed: 12824386]

First M, Spitzer R, Gibbon M, Willaims J. (1998). Structured Clinical Interview for DSM-IV Axis I Disorders. New York, NY: Biometrics Research, New York State Psychiatric Institute.

Fournier KM, Gonzalez MI, Robinson MB. (2004). Rapid trafficking of the neuronal glutamate transporter, EAAC1: evidence for distinct trafficking pathways differentially regulated by protein kinase C and platelet-derived growth factor. J Biol Chem 279:34505–13. [PubMed: 15197183]

Gedge F, McDonald J, Phansalkar A, Chou LS, Calderon F, Mao R, Lyon E, Bayrak-Toydemir P. (2007). Clinical and analytical sensitivities in hereditary hemorrhagic telangiectasia testing and a report of de novo mutations. J Mol Diagn 9:258–65. [PubMed: 17384219]

Glatt C, DeYoung J, Delgado S, Service S, Giacomini K, Edwards R, Risch N, Freimer N. (2001). Screening a large reference sample to identify very low frequency sequence variants: comparisons between two genes. Nature Genetics 27:435–438. [PubMed: 11279528]

Gonzalez MI, Krizman-Genda E, Robinson MB. (2007). Caveolin-1 regulates the delivery and endocytosis of the glutamate transporter, excitatory amino acid carrier 1. J Biol Chem 282:29855–65. [PubMed: 17715130]

Goodman W, Price L, Rasmussen S, Mazure C, Delgado P, Heninger G, Charney D. (1989a). Yale-Brown Obsessive Compulsive Scale: II. validity. Archives of General Psychiatry 46:1012–1016. [PubMed: 2510699]

Goodman W, Price L, Rasmussen S, Mazure C, Fleischmann R, Hill C, Heninger G, Charney D. (1989b). The Yale-Brown Obsessive Compulsive Scale: I. development, use, and reliability. Archives of General Psychiatry 46:1006–1011. [PubMed: 2684084]

Hanna G, Veenstra-Vander Weele J, Cox N, Boehnke M, Himle J, Curtis G, Leventhal B, Cook E. (2002). Genome-wide linkage analysis of families with obsessive-compulsive disorder ascertained through pediatric probands. American Journal of Medical Genetics (Neuropsychiatric Genetics) 114:541–552. [PubMed: 12116192]

Hien D, Matzner FJ, First MB, Spitzer RL, Gibbon M, Williams JBW. (1994). Structured Clinical Interview for DSM-IV-Child Edition. New York, NY: Columbia University.

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 66:1233–41. [PubMed: 19884611]

Li Q, Liu Z, Monroe H, Culiat CT. (2002). Integrated platform for detection of DNA sequence variants using capillary array electrophoresis. Electrophoresis 23:1499–511. [PubMed: 12116161]

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 B Neuropsychiatr Genet 147B:1000–2. [PubMed: 18286588]

Lin CI, Orlov I, Ruggiero AM, Dykes-Hoberg M, Lee A, Jackson M, Rothstein JD. (2001). Modulation of the neuronal glutamate transporter EAAC1 by the interacting protein GTRAP3–18. Nature 410:84–8. [PubMed: 11242046]

Mathews GC, Diamond JS. (2003). Neuronal glutamate uptake Contributes to GABA synthesis and inhibitory synaptic strength. J Neurosci 23:2040–8. [PubMed: 12657662]

Orvaschel H (1987). Schedule for Affective Disorders and Schizophrenia for School-Aged Children--Epidemiologic Version: K-SADS-E, 4th ed. Philadelphia, PA: Medical College of Pennsylvania.

Orvaschel H (1995). Schedule for Affective Disorder and Schizophrenia for School-Age Children-Epidemiologic Version-5. Ft Lauderdale, Fla: Nova Southeastern University.

Otis TS, Brasnjo G, Dzubay JA, Pratap M. (2004). Interactions between glutamate transporters and metabotropic glutamate receptors at excitatory synapses in the cerebellar cortex. Neurochem Int 45:537–44. [PubMed: 15186920]

Pauls DL, Hurst CR. (1991). Schedule for Tourette and Other Behavioral Syndromes (Adult or Child Form, Version C1). New Haven, CT: Child Study Center, Yale University School of Medicine.

Peghini P, Janzen J, Stoffel W. (1997). Glutamate transporter EAAC-1-deficient mice develop dicarboxylic aminoaciduria and behavioral abnormalities but no neurodegeneration. Embo J 16:3822–32. [PubMed: 9233792]

Radua J, Mataix-Cols D. (2009). Voxel-wise meta-analysis of grey matter changes in obsessive-compulsive disorder. Br J Psychiatry 195:393–402. [PubMed: 19880927]

Ruggiero AM, Liu Y, Vidensky S, Maier S, Jung E, Farhan H, Robinson MB, Sitte HH, Rothstein JD. (2008). The endoplasmic reticulum exit of glutamate transporter is regulated by the inducible mammalian Yip6b/GTRAP3–18 protein. J Biol Chem 283:6175–83. [PubMed: 18167356]

Scahill L, Riddle MA, McSwiggin-Hardin M, Ort SI, King RA, Goodman WK, Cicchetti D, Leckman JF. (1997). Children's Yale-Brown Obsessive Compulsive Scale: reliability and validity. J Am Acad Child Adolesc Psychiatry 36:844–52. [PubMed: 9183141]

Scimemi A, Tian H, Diamond JS. (2009). Neuronal transporters regulate glutamate clearance, NMDA receptor activation, and synaptic plasticity in the hippocampus. J Neurosci 29:14581–95. [PubMed: 19923291]

Sepkuty JP, Cohen AS, Eccles C, Rafiq A, Behar K, Ganel R, Coulter DA, Rothstein JD. (2002). A neuronal glutamate transporter contributes to neurotransmitter GABA synthesis and epilepsy. J Neurosci 22:6372–9. [PubMed: 12151515]

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 B Neuropsychiatr Genet.

Spitzer R, Williams J, Gibbon M, First M. (1990). Structured Clinical Interview for DSM-III-R (SCID). Washington, DC: American Psychiatric Press, Inc.

Spitzer RL, Williams JB, Gibbon M, First MB. (1992). The Structured Clinical Interview for DSM-III-R (SCID). I: History, rationale, and description. Arch Gen Psychiatry 49:624–9. [PubMed: 1637252]

Stefansson H, Rujescu D, Cichon S, Pietilainen OP, Ingason A, Steinberg S, Fossdal R, Sigurdsson E, Sigmundsson T, Buizer-Voskamp JE, Hansen T, Jakobsen KD, Muglia P, Francks C, Matthews PM, Gylfason A, Halldorsson BV, Gudbjartsson D, Thorgeirsson TE, Sigurdsson A, Jonasdottir A, Bjornsson A, Mattiasdottir S, Blondal T, Haraldsson M, Magnusdottir BB, Giegling I, Moller HJ, Hartmann A, Shianna KV, Ge D, Need AC, Crombie C, Fraser G, Walker N, Lonnqvist J, Suvisaari J, Tuulio-Henriksson A, Paunio T, Toulopoulou T, Bramon E, Di Forti M, Murray R, Ruggeri M, Vassos E, Tosato S, Walshe M, Li T, Vasilescu C, Muhleisen TW, Wang AG, Ullum H, Djurovic S, Melle I, Olesen J, Kiemeney LA, Franke B, Sabatti C, Freimer NB, Gulcher JR, Thorsteinsdottir U, Kong A, Andreassen OA, Ophoff RA, Georgi A, Rietschel M, Werge T, Petursson H, Goldstein DB, Nothen MM, Peltonen L, Collier DA, St Clair D, Stefansson K. (2008). Large recurrent microdeletions associated with schizophrenia. Nature 455:232–6. [PubMed: 18668039]

Stewart SE, Fagerness JA, Platko J, Smoller JW, Scharf JM, Illmann C, Jenike E, Chabane N, Leboyer M, Delorme R, Jenike MA, Pauls DL. (2007). Association of the SLC1A1 glutamate transporter gene and obsessive-compulsive disorder. Am J Med Genet B Neuropsychiatr Genet 144B:1027–33. [PubMed: 17894418]

Tsunoda T, Takagi T. (1999). Estimating transcription factor bindability on DNA. Bioinformatics 15:622–30. [PubMed: 10487870]

Veenstra-VanderWeele J, Kim S-J, 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. Molecular Psychiatry 6:160–167. [PubMed: 11317217]

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. (2009). A screen of SLC1A1 for OCD-related alleles. Am J Med Genet B Neuropsychiatr Genet.

Waxman EA, Baconguis I, Lynch DR, Robinson MB. (2007). N-methyl-D-aspartate receptor-dependent regulation of the glutamate transporter excitatory amino acid carrier 1. J Biol Chem 282:17594–607. [PubMed: 17459877]

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 66:408–16. [PubMed: 19349310]

Willour VL, Yao Shugart Y, Samuels J, Grados M, Cullen B, Bienvenu OJ 3rd, Wang Y, Liang KY, Valle D, Hoehn-Saric R, Riddle M, Nestadt G. (2004). Replication study supports evidence for linkage to 9p24 in obsessive-compulsive disorder. Am J Hum Genet 75:508–13. [PubMed: 15272418]

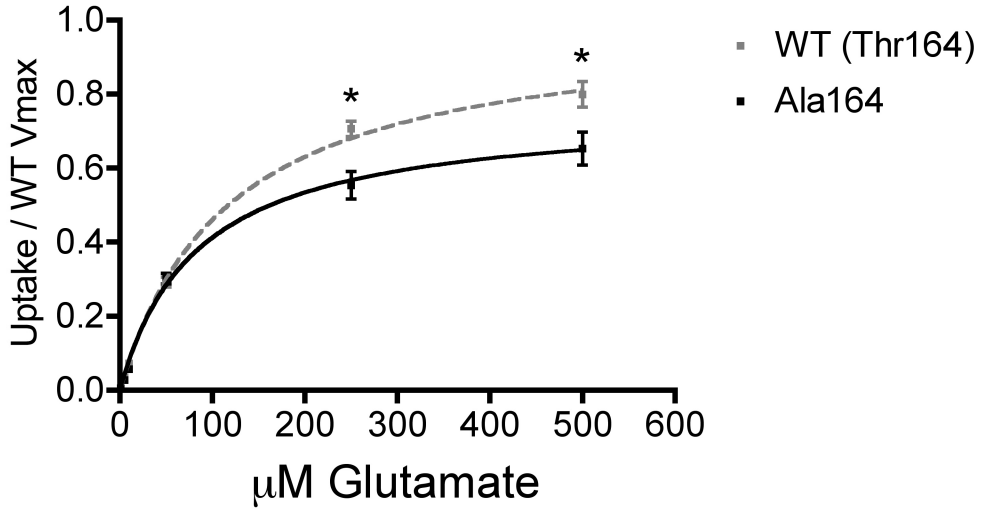

Figure 1:

Michaelis-Menten curve of glutamate uptake in Human Embryonic Kidney (HEK) cells transfected with wildtype (Thr164) and Ala164 *SLC1A1*/EAAC1 cDNA construct. Specific glutamate uptake per μg protein and minute is shown after normalization to the wildtype V_{max}. Each data point corresponds to the mean and standard error of the mean of 9 individual transfected wells across 3 separate experiments, except for the 250 μM data points, which correspond to 6 individual transfected wells across 2 separate experiments. The Michaelis-Menten curves differed significantly between genotypes ($F_{2,98} = 23.12$, $P < 0.0001$). The V_{max} of the *SLC1A1*/EAAC1 Ala164 variant was decreased to 0.76 +/− 0.03 (s.e.m.) of the wildtype V_{max}. The K_m of the Ala164 variant was decreased to 83.7 +/− 12.3 μM versus 117.4 +/− 13.03 μM for the wildtype. * $P < 0.001$ by Bonferroni post-test.

Table 1

SLC1A1 polymorphisms identified by sequencing in males with obsessive–compulsive disorder

Exon	Polymorphism	Heterozygous Subjects
1	rs12002726	17
4	CCCA[T]TCAC insertion at UCSC 4,564,237	1
4	rs2228622	66
4	G→C at UCSC 4,564,522	1
5	rs73383440	1
5	rs7022772	2
9	rs301429	1
9	rs41279547	2
10	rs301430	55
10	rs1471786	15
10	rs2072657	30