# Candidate Gene Associations to Withdrawn Behavior

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## Abstract

**BACKGROUND**—Social withdrawal is a core neuropsychiatric phenomenon in developmental psychopathology. Its presence predicts psychopathology across many domains, including depression, psychosis, autism, anxiety, and suicide. Withdrawn behavior is highly heritable, persistent, and characteristically worsens without intervention. To date, few studies have successfully identified genetic associations with withdrawn behavior, despite the abundance of evidence of its heritability. This may be due to reliance of categorical over dimensional measures of the behaviorally inhibited phenotype. The aim of this study is to identify associations between known psychiatric candidate genes and a dimensionally derived measure of withdrawn behavior.

**METHODS**—Genetic information was collected on 20 Single-nucleotide Polymorphisms (SNPs) from a custom-designed SNP chip and TAQMAN arrays of 4 Variable Number of Tandem Repeat (VNTR) genes for 551 individuals from 187 families. Linear mixed modeling was employed to examine the relationship between genotypes of interest and Child Behavior Checklist (CBCL) Withdrawn Behavior Subscale Score (WBS) while controlling for gender and age through multiple linear regressions.

**RESULTS**—Withdrawn behavior was highly associated with polymorphism rs6314 of the Serotonin Receptor 2A (*HTR2A*) [p = 0.009, estimate = 0.310 (bootstrap 95% CI 0.155 – 0.448), bootstrap p = 0.001)] and rs1800544 of the Alpha 2 Adrenergic (*ADRA2A*) [p = 0.001, estimate = - 0.310 (bootstrap 95% CI -0.479 – -0.126), bootstrap p = 0.001)] genes after correction for gender and age. The association between withdrawn behavior and *ADRA2A* was stronger for younger children.

**CONCLUSIONS**—*HTR2A* and *ADRA2A* genes are associated with withdrawn behavior. This reinforces the role of catecholaminergic genes in the heritability of withdrawn behavior.

## Keywords

Withdrawn Behavior; Child Behavior Checklist; Adult Self-Report; Behavioral Inhibition; Social Withdrawal

Social withdrawal is an observable, dimensional and core neuropsychiatric phenomenon in developmental psychopathology. Rarely associated with disruptive behavior, social withdrawal receives delayed clinical attention in the community, with a parallel

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underrepresentation in scientific literature. Earlier identification and characterization of withdrawn behavior (WB) has profound implications for predicting psychopathology across many domains, including depression (Goodwin, Fergusson, & Horwood, 2004; K. H. Rubin, Chen, McDougall, Bowker, & McKinnon, 1995), psychosis (Miller, Byrne, Hodges, Lawrie, & Johnstone, 2002), autism (Ooi, Rescorla, Ang, Woo, & Fung, 2010), anxiety (Aschenbrand, Angelosante, & Kendall, 2005; Kasius et al., 1997) and suicide (Ferdinand & Verhulst, 1995). WBs are heritable Hoekstra, Bartels, Hudziak, Van Beijsterveldt, & Boomsma, 2008) and longitudinal data have established that social withdrawal persists and worsens over time (Hofstra, Van der Ende, & Verhulst, 2000). This study proposes to expand the biological basis of this powerful and compelling trait through dimensional psychometric and genetic analysis of a substantial patient sample.

A biological basis for WBs such as shyness, inhibition and introversion has long been recognized. 10-15% of children as young as age two could be characterized by a shy, inhibited response to novelty, and those reaction patterns are preserved over time, correlated with autonomic activity, and serve as risk factors for social anxiety and avoidance in later life (Kagan, Reznick, & Snidman, 1988). Genetic factors have proven to be primary in the influence of WB in early childhood (Hoekstra et al., 2008) and adolescence. Estimates of heritability increase with age, reaching 45% by age 16 for both genders (Lamb et al., 2010). However, individual gene association with shyness or behavioral inhibition in children remains elusive. The strongest associations have been found in serotonin transporter 5-HTT and the dopamine receptors D4 and D2. The short allele variant of the serotonin transporter, 5-HTTLPR, has already been associated with depression, anxiety, neuroticism and harm avoidance (Lesch et al., 1996). Homozygosity for 5-HTTLPR was found to have a significant effect on WBs in children with ADHD (Zhao et al., 2005). The dopamine receptor D4 is functionally important in the inhibition of passive avoidance (Tarazi & Baldessarini, 1999). Three alleles, DRD4/48bp-repeat, 7-repeat and A1 have shown a statistical trend for association and linkage with WB (Marino et al., 2004). The D2 receptor also has potential to affect WB, as it is strongly implicated by animal models in the development of social attachments (Gingrich, Liu, Cascio, Wang, & Insel, 2000). The DRD2/Taq1 A1 allele has previously been discovered in association with schizoid and avoidant personality components (Blum et al., 1997).

Within developmental psychopathology, hopes of simpler, direct gene-trait correlations are gone. The absence of large and significant results in genome-wide association studies examining known heritable phenotypes such as behavioral inhibition, has posed the problem of "missing heritability". Neuropsychiatric phenotypes may be particularly vulnerable due to a preference for categorical definitions, with dimensional approaches used rarely. This study offers the potential to reproduce previous findings and identify new genes' importance by examining a discreet number of known psychiatric candidate genes in a large sample measured by the Withdrawn Behavior Subscale Score (WBS) of the Adult Self-Report (ASR) and Child Behavior Checklist (CBCL), a dimensional measure of behavioral inhibition with established persistence (Hofstra et al., 2000), heritability (Hoekstra et al., 2008) and cross-culture generalizability (Heubeck, 2000). We anticipated associations between catecholaminergic genes and WB.

## Methods

### Subjects

Participants came from a family study conducted in the northeastern United States that was designed to examine genetic and environmental contributions to attention and aggression. Details of this sample are described in more detail elsewhere (Albaugh et al., 2010; Rettew, Althoff, Dumenci, Ayer, & Hudziak, 2008). Inclusionary criteria were: (1) proband child

between the ages of 6 and 18; (2) proband child living with at least one biological parent; and (3) proband child with at least one sibling between the ages of 6 and 18. Four target groups of probands were chosen based on the mother-rated CBCL. These groups included subjects with (1) $T$ scores > 67 on the attention problems (AP) scale and < 60 on the aggressive behavior (AG) scale; (2) $T$ scores > 67 on AG but < 60 on AP; (3) $T$ scores > 67 on both scales; and (4) $T$ scores < 60 on both scales. Siblings were not subject to any T score restrictions. The sample was almost exclusively Caucasian with an average score of 6.38 (SD= 2.15) on the Hollingshead SES scale (Hollingshead, 1975). Analysis was restricted to children with available genotypic data, which limited the sample of 551 individuals to 394 children (56% boys). The mean age of the children was 10.9 (SD=3.1, range = 5-18 see Table 2). All parents provided informed consent and all children provided assent. The data collection was approved by the Institutional Review Board.

## Measures

**Child Behavior Checklist—**Problem behavior over the preceding 6 months was measured with the CBCL/6-18, a 118-item questionnaire developed to measure problem behavior in children ages 6-18 (Achenbach & Rescorla, 2001) or the previous version of the CBCL/4-18 before the 6-18 version was available (Achenbach, 1991). CBCL items are scored on a three-point scale and load onto eight syndrome scales. The WBS (reliability 0.89, *Alpha* Coefficient 0.80) consists of the summation of eight items listed in Table 1. One of those items (item 5: "There is very little that he/she enjoys") was not on the CBCL 4-18, and was set to zero for those individuals in whom the 4-18 was used as per the recommendations of the ASEBA authors (Achenbach & Rescorla, 2001). Full scale scores on the WBS range from 0-16. All CBCL data is skewed towards the absence of symptoms (0), therefore square root transformation was employed to approximate a normal distribution.

**SNP and VNTR genotyping—**DNA was collected from buccal cells for consenting parents and assenting children via cotton gauze. Genomic DNA was isolated using a published procedure (Ehli, Lengyel-Nelson, Hudziak, & Davies, 2008). SNP genotyping for a panel of 64 polymorphisms was performed using TaqMan® OpenArray™ Technology according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). This is a custom-built array that includes 32 tagging SNPs for DNA fingerprinting and 32 SNPs suspected to be involved in various psychiatric disorders. Briefly, genomic DNA (2.5μl @ 50ng/μl) was premixed with 2X TaqMan® OpenArray™ Genotyping MasterMix. The resulting genomic DNA/PCR mix was loaded onto the OpenArray plates using the supplied autoloader. The OpenArray plates are pre-loaded by the manufacturer with forward and reverse Polymerase Chain Reaction (PCR) primers and fluorescently labelled probes/reporters (Vic and Fam) for allelic discrimination of the SNP alleles. Loaded OpenArray™ plates were inserted into a TaqMan® OpenArray™ Genotyping case with immersion fluid, sealed with glue, and prepared for PCR. PCR was performed on the Applied Biosystems 9700 Gold Flat-Block PCR machine using vendor supplied cycling conditions, which is specific for the OpenArray™ plates. The arrays were then imaged using the OpenArray™ NT imager. Genotype calling was performed using the OpenArray™ SNP Genotyping Analysis Software v1.0.3. Vectors/axis for each cluster were set using the non-template controls as a baseline and were drawn to best fit each cluster using a stringency (standard deviation from axis to be called) of 5 and a tolerance (standard deviation from nearest axis) of 3.8.

**VNTR genotyping—**Variable Number Tandem Repeat (VNTR) polymorphisms were genotyped in SERT (*5-HTTLPR*), DRD4 (48bp tandem repeat in exon III), DAT1 (40bp

VNTR in the 3' UTR), and DRD5 (di-nucleotide repeat 18.5kb upstream of txn start site) at the Avera Institute for Human Genetics (AIHG).

**SNP and VNTR quality control—**Six of the 64 SNP markers on the TaqMan® OpenArray™ panel did not pass quality control due to sub-optimal clustering (rs6265, rs1051312, rs1801260, rs1800545, rs1386497, and rs265981). Four of these markers were subsequently genotyped independently using Allelic Discrimination with Life Technologies TaqMan probes and primers (rs6265, rs1051312, rs1801260, and rs265981). 32 SNPs on the chip were for DNA fingerprinting purposes, had no suspected psychiatric function and were excluded from further analysis. SNPs specific to Alzheimer's Disease were also excluded. For SNPs, call rates were calculated for the total set of available samples/individuals. Hardy-Weinberg equilibrium (HWE) was calculated based on one sample of (unrelated) parents. All samples, where possible, were checked for Mendelian and sex errors. Of the total set, 1 SNP (rs1800955) showed an excessive Mendelian error (ME) rate (26.73%) and was excluded. All others had acceptable ME and HWE. 16 SNPs and 4 VNTRs were ultimately used.

## Statistical Analyses

**Data Preparation—**SNPs were coded into three-level variables based on the presence of one or two of the two alleles (0=allele 1 homozygote, 1=heterozygote, 2=allele 2 homozygote). VNTR coding was as follows: DAT1 (0=no 480-repeat, 1=one 480-repeat, 2=two 480-repeats), DRD4 (1=short[<=5 repeats]/short, 2=short/long[6-10 repeats], 3=long/long), DRD5 (0=no 148-repeat, 1=one 148-repeat, 2=two 148-repeats), SERT (1=short/short, 2=short/long, 3=long/long). For SERT, short was considered to be an S or Lg allele, and long was a La or XL allele.

**Main Effects of Genotype on Withdrawn Behavior—**All 16 SNPs and 4 VNTRs interrogating 15 genes were entered simultaneously into a single linear mixed model (LMM) in SPSS, with sex and mean-centered age as covariates, and the square-root transformed CBCL WBS as the dependent variable. LMM was used to account for the family structure of the data, and all SNPs and VNTRs were entered simultaneously to improve power and reduce risk for false positives that could result from multiple testing. Multi-level modeling of family structure was used with family as a random factor to account for nesting within a family. An additive model was assumed, and VNTRs and SNPs were entered as continuous variables. 1000 bootstrapping samples were used to obtain parameter estimates for fixed effects and to reduce the possible effects of population stratification by randomly removing, re-estimating, and replacing within family strata. An analysis-wide Bonferroni correction was applied, with 16 SNPs and 4 VNTRs requiring a significance level of 0.05/20, or 0.0025 in the overall model. Bootstrapping estimates of the effects are shown in Table 3, as are non-bootstrapped and bootstrapped estimates of the significance level for particular markers.

Once the overall model was run, the random effect of family relatedness was dropped and the model tested against the full model by examining the restricted log-likelihoods of the model. The fixed effects of sex and age were dropped separately from the model and tested against the full model. Fixed effects of each SNP and VNTR yielded were explored. We used a conservative metric for choosing significant fixed effects to explore using a Bonferroni correction. Only SNPs and VNTRs reaching a bootstrapped significance level of p<0.025 (two-tailed) were selected for follow-up analyses.

Significant SNPs and VNTRs were examined independent of the other SNPs/VNTRs in additional LMMs to explore the individual main effects and interactions. In each LMM, the main effect of the SNP/VNTR on WB was estimated controlling for sex and mean-centered

age. Family was again used as a random effect and 1000 stratified bootstrapping runs were performed to estimate the significance of the model's fixed effects.

**Moderating Effects of Sex and Age**—For SNPs/VNTRs showing significant associations with WB, LMMs were conducted to test the interactions (1) between the SNP/VNTR and sex and (2) between the SNP/VNTR and age, again controlling for family structure with random effects and performing 1000 stratified bootstrapping samples to estimate fixed effects of the interactions.

## Results

Means and standard deviations on predictor and dependent variables are provided in Table 2. Withdrawn behaviour demonstrated skewness >1 (1.17) and therefore was square root transformed before being entered in the analysis.

The full model with random effects of family (family number) along with fixed effects of all SNPs and VNTRs demonstrated better fit than models with familial random effect removed (-RLL = 739.601 for full model and 756.126 for model removing genetic relatedness leading to p=$2.40 \times 10^{-4}$). Family relatedness was retained in subsequent LMMs.

Table 3 lists all of the SNPs and VNTRs tested in the full model, along with the estimates from the LMM and its 95% CI, and the bootstrapped and non-bootstrapped p-values. This model resulted in two SNPs with non-bootstrapped significance values less than the study-wide criterion of p<0.025. These SNPs, rs6314, a polymorphism in the *HTR2A* gene, and rs1800544, a polymorphism in the *ADRA2A* gene were then tested in models with SNP, sex, and age as fixed factors along with the family random effect included. Interactions with age and sex were tested in a separate analysis.

### HTR2A

In the follow-up model, the main effect of rs6314 was significant [p = 0.009, estimate = 0.310 (bootstrap 95% CI 0.155 – 0.448), bootstrap p = 0.001)] but neither sex, age nor their interactions with the marker were significant in the interaction model (all p's > 0.12). Children carrying the A allele (which codes for a missense mutation changing His to Tyr at locus 452 and, the *His*452*Tyr* polymorphism) had an average withdrawn score of 2.12, while those with the G/G genotype had an average score of 3.04 (the withdrawn behaviour scale ranged from 0 to 13 and 90% of the scores were between 0 and 6, with a mean of 2.9). A regression model not corrected for family structure demonstrates that this represents an effect size of a $R^2$ = 0.018 (i.e. this polymorphism explains 1.8% of the variance in WB in this sample).

### ADRA2A

In the follow-up model, the main effect of rs1800544 was significant [p = 0.001, estimate = - 0.310 (bootstrap 95% CI -0.479 – -0.126), bootstrap p = 0.001)] and the interaction of age × rs1800544 was significant [p = 0.035, estimate = 0.060 (bootstrap 95% CI -0.0022 – 0.110), bootstrap p = 0.017)] but the interaction of sex × rs1800544 was not (p = 0.135). Children carrying the G allele (which codes for either a C or a G at locus 1219 in the promoter of the gene, C-*1291G*) had an average withdrawn score of 2.35, while those with the C/C genotype had an average score of 3.23. Probing for effect size, a regression model not corrected for family structure demonstrates that this represents an effect size of a $R^2$ = 0.030 (i.e. this polymorphism explains 3.0% of the variance in this sample's WB). Probing for the age-related interaction, we isolated extreme ages by using +/- 1 SD above the mean age and looked at mean values. The difference in WBS was highest in the youngest children.

In fact, in the 52 children age 7 and under, being a C/C homozygote accounted for 15.4% of the variance in the WBS and for a 2.75-fold increase in the score (from 0.96 for the G-carriers to 2.64 for the C/C homozygotes). (Figure 1).

We also examined the additive role of carrying both risk genotypes by examining whether subjects carried no risk genotypes (i.e. being a *HTR2A* rs6314 C carrier G/G homozygote AND an *ADRA2A* rs1800544 G carrier C/C homozygote), carried 1 risk genotype (either *HTR2A* rs6314 G/G homozygote or *ADRA2A* rs1800544 C/C homozygote), or carried both risk genotypes (i.e. being a *HTR2A* rs6314 G/G homozygote AND an *ADRA2A* rs1800544 C/C homozygote). In a LMM model, there is a significant additive main effect [$p = 6.16 \times 10^{-6}$, estimate = 0.379 (bootstrap 95% CI 0.282 – 0.499), bootstrap p = 0.001]. A regression model not corrected for family structure demonstrated that this represents an effect size of a $R^2 = 0.054$ (i.e. this polymorphism explains 5.4% of the variance in this sample's WB) (Figure 2).

## Discussion

We demonstrate here the first positive associations of rs6314 in the HTR2A gene and rs1800544 in the ADRA2A promoter with quantitatively-derived WB in children, a phenotype which may be associated with many developmental trajectories, including autism, depression, suicidality, and psychotic disorders. In this sample, children homozygous for the HTR2A reference genotype (G/G) demonstrate significantly higher WBS in this sample, as did those with the C/C genotype of ADRA2A.

The molecular consequence of the *His*452*Tyr* polymorphism is well understood. The polymorphism affects the coding for the cytosol tail of a G protein-coupled receptor, substituting a neutral amino acid with a basic one at the C-terminus (Erdmann et al., 1996). The results in smaller peak amplitude of intracellular calcium ion mobilization and a longer time course for receptor response, decreasing the activation levels of 2nd messengers phospholipases C&D and their cascade effects (Hazelwood & Sanders-Bush, 2004). Serotonergic stimulation yields a blunted receptor response with this polymorphism (Ozaki et al., 1997), suggesting its role in WB.

There are neuroanatomical consequences of the amino acid change associated with this polymorphism. *His*452*Tyr* has been correlated with reduced volumes of grey matter in the left hippocampus, and reduced white matter in the superior and middle temporal gyri bilaterally, as well as in the left inferior temporal gyrus (Filippini et al., 2006). The left hippocampus is associated with the processing of novelty (Strange, et al., 1999), learning and verbal memory (Milner, 1972). While the causal relationship is unclear, decreased volume in the left hippocampus has been linked to both depression, a common disorder associated with withdrawn behavior, and schizophrenia, a disorder commonly associated with withdrawn behavior (von Gunten, et al., 2000; Zierhut, et al., 2013). The superior temporal gyrus plays a role in auditory processing, language (Bigler, et al., 2007), and the perception of the emotional content of human facial expressions (Radua, et al., 2010). It has been associated with the hallucinations and disordered thought of schizophrenia, with anatomic abnormalities preceding the onset of symptoms (Matsumoto, et al., 2001). The middle temporal gyrus serves in language comprehension (Turken & Dronkers, 2011), with abnormalities associated clinically with depression (Ma, et al., 2012) and schizophrenia (Onitsuka, et al., 2004). The left inferior temporal lobe gyrus' functions include semantic processing (Binder, et al., 2000) and it too is implicated in both depression (Kennedy, et al., 2006) and schizophrenia (Onitsuka, et al., 2004).

As a functional polymorphism in the serotonin system with effects in neuroanatomical volumes, *His*452*Tyr* has become a repeated target for the investigation of the genetics of emotional, behavioral and cognitive phenomena. Nonetheless, many neuropsychiatric investigations using categorical phenotypic definitions have failed to identify any significant associations. Only negative data currently exists associating *His*452*Tyr* with depression (Minov et al., 2001), suicide attempts in depression (Du et al., 1999), borderline personality disorder (Ni et al., 2006), and temperament (Kusumi et al., 2002) and there are conflicting data for bipolar disorder (Etain et al., 2004; McAuley et al., 2009), psychosis (Fanous et al., 2004; Mata et al., 2004), schizophrenia (Erdmann et al., 1996; K. Melkersson & Hulting, 2009), ADHD (Guimaraes et al., 2007; Heiser et al., 2007), and autism (Guhathakurta et al., 2009).

There have been positive associations of this polymorphism with adolescent onset antisocial personality disorder and its rule-breaking subtype (as opposed to aggression) (Burt & Mikolajewski, 2008), affective symptoms in schizophrenia (Fanous et al., 2004), hippocampal response to novelty and tendency to judge novel as familiar (Schott et al., 2011), and episodic long term memory (Wagner et al., 2008), age dependency of memory effects (Papassotiropoulos et al., 2005),

It is germane that the only positive diagnostic finding associated with this polymorphism is the Axis II condition of antisocial personality. With this single exception, inquiries directed at categorical diagnosis of psychiatric conditions have consistently yielded negative or mixed results, and no Axis I disorder has consistently been associated with the genotype. Other positive findings are limited to pervasive affective symptoms, nuances of metabolism or measurement of memory. Our study sought to examine the association of genes associated with a dimensionally measured phenotypic phenomenon, WB. The CBCL, seeking to cluster reported traits by their simple empiric co-occurrence in nature, may better capture the relevance of this polymorphism to neuropsychiatry.

*C-1291G* lies in the promoter region of the $_{2A}$-adrenoceptor gene, and is thought to result in an increased density of $_{2A}$-adrenoceptors with subsequent increased activity of the sympathetic nervous system (Lario et al., 1997). Previous studies have associated the C allele with internalizing symptoms of the anxiety, affective, schizoid, and somatization diagnostic groups (Comings, Gonzalez, Cheng Li, & MacMurray, 2003) as well as risk for suicide (Fukutake et al., 2008). In contrast, the G allele, associated with less WB in this study, has been linked to more externalizing disorders including ADHD (e.g., Comings, 2001; Roman et al., 2003), oppositional defiant disorder and conduct disorder (Lario et al., 1997), and temperamental traits of lower harm avoidance and higher novelty seeking (de Cerqueira et al., 2011). Adolescent girls with a history of maltreatment show a reverse of this association pattern, with the C allele becoming more associated with ADHD symptoms (Kiive, Kurrikoff, Maestu, & Harro, 2010). Our results substantiate the dominant findings that associate the C allele with internalizing behaviors, and mark the first direct association between the C allele and a dimensional measure of WB. Our results further implicate a developmental mediator to the allele's effect on WB, as the ADRA2A polymorphism's association with WB is relatively profound at ages 7 and under, with a lower association in the older children. Multiple disorders share the withdrawn behavior phenotype. These disorders vary in age of onset, with anxiety and autism, for example, emerging during early childhood, while depression and psychosis typically delying their presentations until after puberty. Perhaps this finding implicates the ADRA2A polymorphism as specific to these early emerging disorders, with *His*452*Tyr* serving a generic contributing role to withdrawn behaviors. The effects of both polymorphisms, rs6314 in the HTR2A gene and rs1800544 in the ADRA2A promoter, have an additive effect in association with WB.

This study is also significant for what it did not find. Of the 15 genes examined using 16 SNPS and 4 VNTRS, only two genes demonstrated a significant association with WB using this approach. The fact that these genes account for 1.8% (5HTR2A) and 3.0% (ADRA2A) of the variance demonstrates that the study was powered to detect only relatively large genetic effects. For participants age 7 and under, ADRA2A accounted for 15.4% of the variance. Additively, the genotypes account for up to 5.4% of variance in WB across all ages. This is possibly because we took such a conservative approach to correcting for multiple comparisons to avoid false positives. It is also possible that a dimensionally based measurement of withdrawn behavior is better suited to detect some polymorphism effects over others.

There are likely other smaller associations contained within the data. Findings may also have been limited by the small sample size and a sample that was selected for aggression and attention problems. This selection for aggression may further explain why some genes previously associated with WB, DRD4 and 5-HTTLPR, did not reach significance in this study. A sample specifically selected for the presence of withdrawn behavior may have reproduced past findings in addition to strengthening or adding to the findings presented here. It is likely that these systems would also be involved if tested in a larger or different sample to address concerns related to population stratification. Given that this sample was almost exclusively Caucasian, population stratification is less likely, even if one considers the chance of latent subpopulations (Hutchison, Stallings, McGeary, & Bryan, 2004).

## Conclusion

Overall, the association of WB with the *His452Tyr* and *C-1291G* polymorphisms has implications for the concept of social withdrawal as an identifiable core neuropsychiatric phenomenon, the developmental selectivity for genetic impact on social withdrawal and for the role of employing genetic studies on instruments that have an empirically supported, dimensional paradigm. These results, as they are examined longitudinally and interpreted in conjunction with other methods of analysis (D. H. Rubin, Althoff, Walkup, & Hudziak, 2012), may further inform our knowledge of the developmental trajectories of children displaying WB.

## Acknowledgments

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## ABBREVIATIONS

| | |
|---|---|
| **SNPs** | Single Nucleotide Repeats |
| **VNTR** | Variable Number of Tandem Repeats |
| **CBCL** | Child Behavior Checklist |
| **ASR** | Adult Self Report |
| **WB** | Withdrawn Behavior |
| **WBS** | Withdrawn Behavior Subscale Score |
| **HTR2A** | Serotonin Receptor 2A |
| **ADRA2A** | Alpha 2A Adrenergic Receptor |

**Key points**

- Social withdrawal is an observable, dimensional and core neuropsychiatric phenomenon in developmental psychopathology.

- Withdrawn behavior predicts psychopathology across many domains, including depression, psychosis, autism, anxiety, and suicide.

- Withdrawn behavior is highly heritable, persistent, and characteristically worsens without intervention.

- Despite evidence of heritability, association of withdrawn behavior to particular genes has been elusive.

- Using the Child Behavior Checklist as a dimensional measure of withdrawn behavior, this study finds that withdrawn behavior is highly associated with polymorphism rs6314 of the Serotonin Receptor 2A (HTR2A) and rs1800544 of the Alpha 2 Adrenergic (ADRA2A) genes.
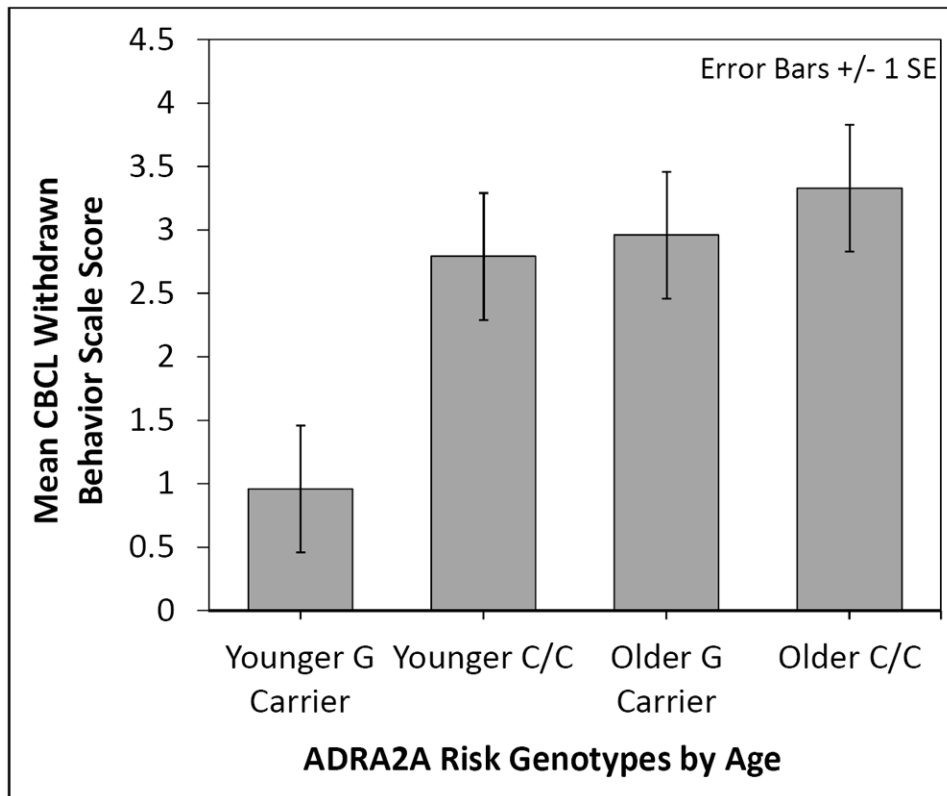
**Figure 1.**
The ADRA2A risk genotype increases scores on the withdrawn behavior scale primarily at younger ages.
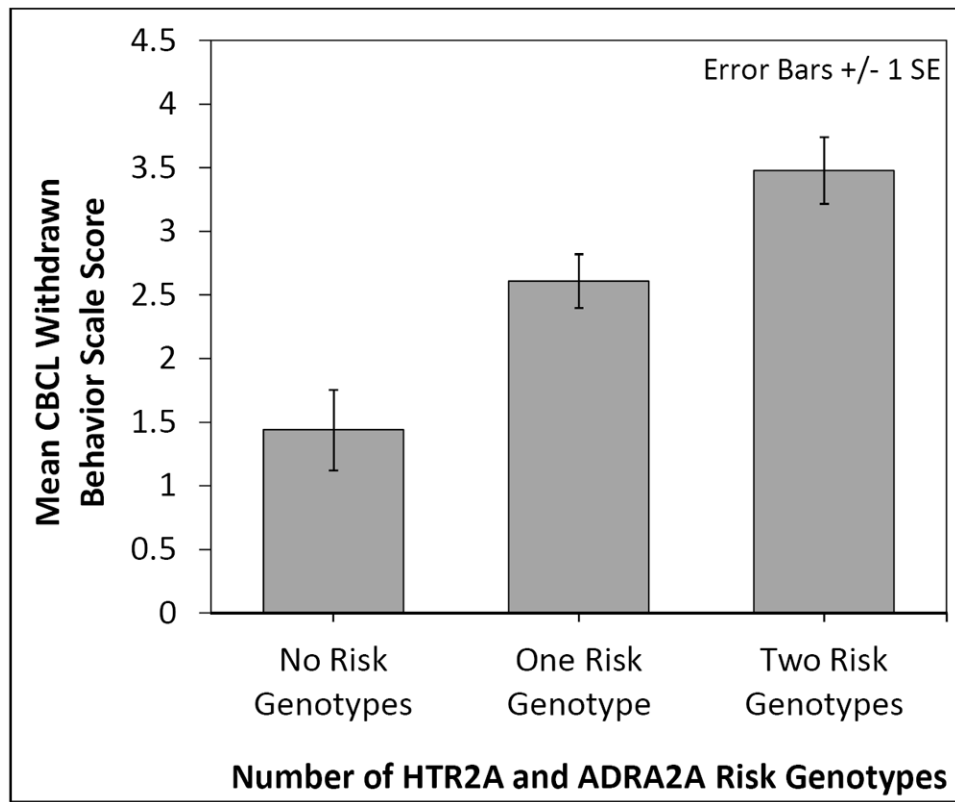
**Figure 2.**
Increased scores on the withdrawn behavior scale are associated with the number of risk genotypes in HTR2A and ADRA2A SNPs.

**Table 1**

Items from the CBCL 6-18 Withdrawn/Depressed Scale

5. There is very little that he/she enjoys

42. Would rather be alone than with others

65. Refuses to talk

69. Secretive, keeps things to self

75. Too shy or timid

102. Underactive, slow moving or lacks energy

103. Unhappy, sad or depressed

111. Withdrawn, doesn't get involved with others

**Table 2**

Mean Scores (SD) and Distribution on Age and Withdrawn Behavior Scores for Males and Females

|  | Males | Females |
|---|---|---|
| n | 220 | 174 |
| Age SD | 11 (3) | 10.7 (3.1) |
| Withdrawn Behavior Score SD | 3 (2.8) | 2.6 (2.9) |

**Table 3**

Results from overall linear mixed model of 20 markers from 15 genes on withdrawn behavior scale controlling for family structure.

| Variable | Estimate [bootstrap 95% CI] | p-value | bootstrap p-value |
|---|---|---|---|
| Intercept | 0.128 [-1.002, 1.441] | .874 | .830 |
| COMT (rs4680) | 0.054 [-0.049, 0.181] | .542 | .360 |
| DRD2 (rs1800497) | 0.09 [-0.03, 0.267] | .423 | .268 |
| DBH (rs1611115) | 0.125 [-0.089, 0.262] | .292 | .186 |
| DBH (rs2519152) | 0.038 [-0.139, 0.172] | .702 | .630 |
| NET (rs998424) | 0.115 [-0.403, 0.403] | .664 | .605 |
| NET (rs3785157) | 0.345 [-0.142, 0.673] | .196 | .118 |
| **ADRA2A (rs1800544)** | **-0.508 [-0.715, -0.351]** | **$2.5 \times 10^{-4}$** | **.001** |
| ADRA2A (rs553668) | -0.298 [-0.589, -0.047] | .088 | .032 |
| HTR1B (rs6296) | -0.039 [-0.205, 0.113] | .699 | .628 |
| **HTR2A (rs6314)** | **0.435 [0.21, 0.601]** | **.001** | **.001** |
| TPH2 (rs1843809) | -0.052 [-0.19, 0.125] | .644 | .516 |
| DAT (rs2652511) | -0.136 [-0.224, 0.012] | .131 | .047 |
| DAT (rs40184) | 0.248 [0.136, 0.413] | .010 | .004 |
| DRD4 (rs3758653) | 0.112 [-0.056, 0.275] | .340 | .188 |
| DRD1 (rs265981) | 0.034 [-0.055, 0.183] | .714 | .632 |
| BDNF (rs6265) | 0.216 [0.073, 0.43] | .033 | .032 |
| 5-HTTLPR | 0.007 [-0.157, 0.133] | .939 | .925 |
| DAT VNTR | 0.135 [-0.072, 0.375] | .319 | .231 |
| DRD4 VNTR | -0.122 [-0.311, 0.066] | .348 | .225 |
| DRD5 VNTR | -0.039 [-0.279, 0.123] | .774 | .739 |