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Association between Autism Spectrum Disorder (ASD) in Individuals with Velo-Cardio-Facial (22q11.2 Deletion) Syndrome and PRODH and COMT Genotypes

Petya D. Radoeva[1], **Ioana L. Coman**[2], **Cynthia A. Salazar**[2], **Karen L. Gentile**[1], **Anne Marie Higgins**[2], **Frank A. Middleton**[1], **Kevin M. Antshel**[2], **Wanda Fremont**[2], **Robert J. Shprintzen**[3], **Bernice E. Morrow**[4], and **Wendy R. Kates**[2,*]

[1]Neuroscience and Physiology, SUNY Upstate Medical University, Syracuse, New York, USA

[2]Psychiatry and Behavioral Sciences, SUNY Upstate Medical University, Syracuse, New York, USA

[3]The Virtual Center for Velo-Cardio-Facial Syndrome, www.vcfscenter.com, Manlius, NY, USA

[4]Genetics, Albert Einstein College of Medicine, Bronx, New York, USA

Abstract

Velo-cardio-facial (VCFS; 22q11.2 deletion) syndrome is a genetic disorder that results from a hemizygous deletion of the q11.2 region on chromosome 22, and is associated with greatly increased risk for psychiatric disorders, including Autism Spectrum Disorder (ASD) and schizophrenia. There is emerging evidence for the involvement of catechol O-methyltransferase (COMT) and proline dehydrogenase (oxidase) 1 (PRODH) in the psychiatric phenotype of individuals with VCFS. Here, we tested the hypothesis that PRODH and COMT are associated with ASD in youth with VCFS. We found that individuals with VCFS and the low-activity alleles of both PRODH and COMT (rs4819756A and rs4680A) were more likely to present with ASD as compared to individuals with VCFS and the high-activity alleles of these genes ($p<0.05$; OR=6.0 (95% CI=1.27-28.26; N=87). Our results suggest that PRODH and COMT may interact to contribute to the ASD phenotype in individuals with VCFS.

Keywords

velo-cardio-facial syndrome; 22q11.2 deletion; proline dehydrogenase; catechol O-methyltransferase; autism spectrum disorder

Introduction

Velo-cardio-facial (VCFS; 22q11.2 deletion) syndrome is a CNV (copy number variation) genetic disorder, in which individuals possess only one copy of the q11.2 region on chromosome 22. It is associated with an increased risk for several psychiatric disorders.

* Correspondence concerning this article should be addressed to: W.R. Kates, Ph.D. Phone: +1 315 464-3270 Fax: +1 315 464-3297 katesw@upstate.edu Postal address: 750 East Adams Street, Syracuse, NY 13210.

Notably, up to 20% of individuals with VCFS have narrowly defined autism, about 14-50% have ASD (Antshel et al., 2007; Fine et al., 2005; Vorstman et al., 2006; Vorstman et al., 2009), and approximately 25% develop schizophrenia (Green et al., 2009; Murphy, 2002, Bassett & Chow, 2008).

The 22q11.2 region contains over 40 genes with diverse functions, several of which play roles in brain development, neurotransmitter levels, and myelination (Jungerius et al., 2008; Prasad et al., 2008), and haploinsufficiency of one or more of these genes has been hypothesized to contribute to the psychiatric phenotype in VCFS. There is a growing body of evidence suggesting that an interaction between two of the 22q11.2 genes- catechol O-methyltransferase (COMT) and proline dehydrogenase (oxidase) 1 (PRODH)- correlates with some of the psychiatric and cognitive phenotypes of VCFS. COMT is an enzyme that degrades catecholamines, including the neurotransmitter dopamine, while PRODH encodes a mitochondrial enzyme that degrades the amino acid proline, such that deficiency in PRODH can result in elevated proline levels (hyperprolinemia). Notably, a transcriptional and behavioral interaction between COMT and PRODH has been demonstrated in mice (Paterlini et al., 2005); and the combination of the low-activity allele of COMT and hyperprolinemia in individuals with VCFS has been associated with increased risk for psychosis (Raux et al., 2007) as well as deficits in smooth pursuit eye movements (Vorstman et al., 2009) and visual connectivity (Magnee et al., 2011).

Allelic variation in COMT and PRODH has been associated with variation in the activity levels of COMT and PRODH. One widely studied polymorphism in COMT is *Val*^{158}*Met* (rs4680), in which the “A” (methionine) allele results in lower COMT activity relative to the “G” (valine) allele (Tunbridge et al., 2006). Several functional SNPs have been reported in PRODH; rs4819756 (*R*^{185}*W*) has relatively high heterozygosity (0.536 for HapMap-CEU), and the “A” variant decreases the PRODH activity by about 45% (Bender et al., 2005).

Here, our goal was to evaluate the association between COMT (rs4680), PRODH (rs4819756) and ASD in youth with VCFS. We chose to study ASD specifically, because there were no previous studies of the additional genetic risk factors for ASD in individuals with VCFS. Furthermore, COMT and PRODH had been previously associated with ASD and autistic features in the non-VCFS population (Guilmatre et al., 2009; Limprasert et al., 2008). Based on the non-VCFS (Limprasert et al., 2008), and VCFS (Raux et al., 2007; Vorstman et al., 2009) literature evaluating the effects of COMT and PRODH, we hypothesized that participants with VCFS and ASD will be more likely to carry low-activity alleles of both COMT and PRODH relative to individuals with VCFS without ASD.

Methods

Our participants were part of an ongoing longitudinal study of VCFS (Antshel et al., 2007; Kates et al., 2007; Kates et al., 2011), and are a subsample of a larger group of individuals with molecularly-confirmed VCFS. The study was approved by the SUNY Upstate Medical University Institutional Review Board. The current report includes only participants for whom we have obtained phenotypic information from the Autism Diagnostic Interview, Revised (ADI-R), and genotypic information on two polymorphisms: rs4680 and/or

rs4819756, in COMT and PRODH respectively. The subjects in this study were 42 females (aged 7 to 21; Mean (SD)=13.5(3.5)) and 45 males (aged 6 to 19; Mean (SD)=13.8(3.4) at the time of the ADI-R. The race of the participants was Caucasian (N=84) or Asian (N=2).

The ASD phenotypes were characterized using the Autism Diagnostic Interview, Revised (ADI-R) (Lord et al., 1994). ADI-R was conducted by two clinical psychologists, certified in the reliable administration of the ADI-R for research purposes, with the caregivers of the individuals with VCFS (Antshel et al., 2007). Each participant was classified as having ASD if they met criteria for qualitative impairments in reciprocal social interaction, and for deficits in either communication or repetitive behaviors and stereotyped patterns (or both) as previously described (Antshel et al., 2007; Kaufmann et al., 2004). In many aspects the ASD phenotype in individuals with VCFS is similar to ASD in the general population (Antshel et al., 2007; Kates et al., 2007). In the current report, 20 out of 87 participants (23%) met criteria for ASD.

Genotyping of rs4680 and rs4819756 was conducted as follows: rs4680 was genotyped by sequencing or the ABI PRISM 5′ nuclease assay TaqMan® as described previously (Coman et al., 2010), and rs4819756 was amplified via PCR, using the following primers: 5'-AGGGGTATAGCCAGCAAAGAG-3' and 5'-GGCCTTTGTTAAGGAGACTGC-3' (see the Supplementary Methods for further details, Supplemental Digital Content 1, http://links.lww.com/PG/A114). To evaluate the association between the SNPs and ASD, we conducted Fisher's exact tests and calculated odds ratios and confidence intervals. Based on previous literature suggesting an interaction between COMT and PRODH (Paterlini et al., 2005; Raux et al., 2007), we evaluated the interaction between rs4680 and rs4819756 by comparing the ASD phenotype of subjects who had low-activity alleles of *both* PRODH and COMT (A-A) vs. participants who had the high-activity alleles (G-G) of both genes (rs4680-rs4819756).

Results

The allele distributions with respect to ASD diagnosis are given in Table 1. There were no significant main effects of PRODH or COMT on ASD diagnosis (Fisher's Exact test: $p>0.05$). However, COMT and PRODH showed an interaction, such that individuals with VCFS and low-activity alleles of both COMT and PRODH (rs4680A and rs4819756A) were more likely to have ASD, as compared to individuals with VCFS and the high-activity alleles of COMT and PRODH: Fisher's Exact test: $p<0.05$; OR=6.0 (95% CI=1.27-28.26) (Table 1). When the two non-Caucasian individuals were excluded from analysis, these results remained significant (Fisher's Exact test: $p<0.05$; OR=5.8 (95% CI= 1.22-27.14).

Discussion

Our findings contribute to a growing literature on effects of PRODH and COMT on psychiatric symptoms in individuals with VCFS, suggesting that the low-activity alleles of both genes are associated with ASD in this patient population. There are several mechanisms by which high proline concentrations could affect brain functioning. Delwing and colleagues (2003) explored effects of proline on the rat brain, and found that high

proline levels induce oxidative stress. Higher levels of proline can also alter glutamatergic transmission by potentiating excitatory transmission at hippocampal synapses (Paterlini et al., 2005), and PRODH-deficient mice have alterations in levels of neurotransmitters (glutamate, GABA and/or aspartate) and deficits in sensorimotor gating (Gogos et al., 1999). COMT is an enzyme that degrades catecholamines, and affects prefrontal dopaminergic levels and functioning (Tunbridge et al., 2006).

Previous studies have presented evidence for an interaction between PRODH and COMT (Karayiorgou & Gogos, 2004; Paterlini et al., 2005; Vorstman et al., 2009). In PRODH-deficient mice, COMT was shown to be transcriptionally upregulated, likely as a compensatory response secondary to PRODH deficiency and resulting dopaminergic hyperactivity (Paterlini et al., 2005). Furthermore, inhibition of COMT (by tolcapone) in PRODH-deficient mice induced working memory and sensorimotor gating (PPI) deficits (Paterlini et al., 2005). Notably, both PPI (Perry et al., 2007) and working memory deficits (Bennetto et al., 1996; Joseph et al., 2005) have been reported in individuals with autism and youth with VCFS (Antshel et al., 2008; Sobin et al., 2005). A recent VCFS study found abnormalities in smooth pursuit eye movements in children who had both high levels of proline and the low-activity allele of COMT (Vorstman et al., 2009). Thus, individuals with VCFS with the low-activity COMT allele may be unable to compensate sufficiently for the dopaminergic hyperactivity resulting from PRODH deficiency (Karayiorgou & Gogos, 2004; Vorstman et al., 2009).

Intriguingly, other genetic syndromes with alterations in neurotransmission (including increased dopaminergic signaling) have been associated with ASD. For example, dopamine overproduction is one of the core features in Timothy syndrome (Pasca et al., 2011). The latter syndrome is caused by a single gene mutation in CACNA1C (L-type calcium channel Cav1.2), and has high ASD prevalence (Splawski et al., 2004). It has been postulated that that altered neuronal transmission may be one of the underlying mechanisms contributing to disconnection within critical brain circuits (fronto-striatal, fronto-temporal and fronto-parietal), in turn resulting in ASD (Geschwind, 2011).

While our current study found association between COMT, PRODH and ASD in VCFS, it is possible that the ASD phenotype in our sample may be a marker of additional psychiatric problems. It has been reported that relative to VCFS-affected individuals without autism, those with VCFS and autism have a significantly higher prevalence of comorbid psychiatric disorders (Antshel et al., 2007) and prodromal symptoms for schizophrenia (Vorstman et al., 2006), though the relationship and degree of overlap between ASD and schizophrenia in VCFS has recently been a topic of debate. Future follow-up studies of our participants could address the question whether the low-activity alleles of PRODH and COMT are associated with psychosis (in addition to the association with ASD) and/or other psychiatric disorders (such as ADHD, anxiety disorders, and intellectual disability) in this patient sample. At this point, it is not clear whether these effects may be specific to the diagnosis of ASD as opposed to a more complex VCFS sub-phenotype.

While our current findings are intriguing, and our study is amongst the largest genetic studies of a psychiatric phenotype in VCFS thus far (Bassett et al., 2007; Raux et al., 2007),

replication of our results in independent VCFS samples is essential. Yet, it is a challenge to obtain a large number of participants with VCFS, given that the prevalence of the disorder is estimated to be between 1 in 2,000 and 1 in 7,000 (Shprintzen, 2005). In order to find 87 participants (as in our current study), in theory, one would have to screen between 174,000 and 609,000 individuals from the general population. Therefore, the importance of future collaborative studies across multiple VCFS centers cannot be overstated. Confirmation of our results by others can open new horizons for unique, targeted interventions in this patient population, perhaps specifically aimed at modulating proline and catecholamine levels in susceptible individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Genotypes of PRODH (rs4819756), COMT (rs4680), and PRODH-COMT (rs4819756-rs4680) vs. ASD diagnosis in individuals with VCFS.

SNP (Gene)	ASD diagnosis	Allele Count (% of diagnostic group)		Odds Ratio (CI)	P-Value
rs4819756 (PRODH)		***A***	***G***	2.5 (0.86, 6.96)	0.113
	ASD	11 (58)	8 (42)		
	no ASD	23 (36)	41 (64)		
rs4680 (COMT)		***A***	***G***	2.2 (0.80, 6.03)	0.193
	ASD	11 (55)	9 (45)		
	no ASD	24 (36)	43 (64)		
rs4819756-rs4680 (PRODH-COMT)		***A-A***	***G-G***	6.0 (1.27, 28.26)	***0.041***
	ASD	6 (32)	4 (21)		
	no ASD	6 (9)	24 (38)		

P-values for the Fisher's exact test are given. For rs4819756-rs4680, only the A-A and G-G genotypes are included in the table, and therefore the percentages do not add up to 100. Abbreviations: ASD: autism spectrum disorder; CI: confidence interval: COMT: catechol O-methyltransferase; PRODH: proline dehydrogenase (oxidase) 1.