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Clinical and genetic analysis of children with a dual diagnosis of Tourette syndrome and autism spectrum disorder

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ABSTRACT

Gilles de la Tourette Syndrome (TS) is a neurodevelopmental disorder that causes children to make repeated, brief involuntary movements or sounds. TS can be co-morbid with other neurodevelopmental disorders, including autism spectrum disorder (ASD). Clusters of biologically related genes have been associated with neurodevelopmental disorders, suggesting shared pathologies. However, the genetic contribution to TS remains poorly defined. We asked whether children with both TS and ASD differed clinically from children with ASD alone, and identified potentially deleterious genetic events in children with TS and ASD. We compared clinical data from 119 children with ASD and TS to 2603 children with ASD, all from the Simons Simplex Collection. We performed gene set enrichment analysis on *de novo* genetic events in children with both TS and ASD to identify candidate genes and pathways, and compared these genes and pathways with those previously identified in TS. Children with TS and ASD were diagnosed at an older age, had higher IQ scores, and had more restricted and repetitive behavior than children with ASD but not TS. Gene Ontology analysis revealed that proteins important for specific biological pathways, including regulation of calcium ion-dependent exocytosis, basement membrane organization, and visual behavior and learning, and specific cellular pathways, including basal lamina and ciliary transition zone, are enriched among genes with *de novo* mutations in children with TS and ASD. Clinical and genetic analysis of cohorts of affected children can help to determine the underlying pathophysiology of TS and other neurodevelopmental disorders.

1. Introduction

Gilles de la Tourette Syndrome (TS) is a neurodevelopmental disorder that causes children to make repeated, brief involuntary movements (motor tics) or sounds (vocal tics) (Dietrich et al., 2015; Georgitsi et al., 2016). TS is one of the most common childhood psychiatric disorders and occurs in about 0.8% of children. Children with TS often have other psychiatric disorders, such as obsessive-compulsive disorder, attention deficit hyperactivity disorder, or autism spectrum disorder (ASD). The lifetime prevalence of psychiatric co-morbidity in people with TS is estimated to be 86% (Cravedi et al., 2017; Hansen et al., 2018). As a group, childhood-onset neurodevelopmental disorders are present in up to 5% of children, with more boys than girls affected. The identification of candidate genes for neurodevelopmental disorders has been driven by advances in whole exome and whole genome sequencing (Iossifov et al., 2014; Parikshak et al., 2013; Sanders et al., 2015; Wright et al., 2015). Specific biological pathways are enriched among neurodevelopmental disorder candidate genes, such as neuronal cell adhesion, neuroactive ligand-receptor interaction, synaptic function, MAPK signaling, chromatin remodeling and cell cycle regulation (Berg and Geschwind, 2012; Fischbach and Lord, 2010; Geisheker et al., 2017; Krishnan et al., 2016; Lelieveld et al., 2017; Wen et al., 2016).

Although TS clusters in families with heritability estimated at ~0.77 (Browne et al., 2015; Mataix-Cols et al., 2015), few TS candidate genes have been identified. Where candidate genes have been identified, typically only one TS-affected individual or family was found to carry the genetic lesion (Abelson et al., 2005; Castellan Baldan et al., 2014; Georgitsi et al., 2016). Recent studies have identified TS candidate genes through studies of multiplex families (Eriguchi et al., 2017; Lawson-Yuen et al., 2008; Moya et al., 2013b; Sun et al., 2017; Verkerk et al., 2003) and of *de novo* copy number variants (CNVs) (Bertelsen et al., 2014; Fernandez et al., 2012; Huang et al., 2017; McGrath et al., 2014; Nag et al., 2013; Sundaram et al., 2010) or coding variants (Eriguchi et al., 2017; Willsey et al., 2017). Many TS candidate genes are involved in neurite outgrowth or similar processes during brain development. Pathway analyses also suggest roles for histaminergic signaling, ubiquitination, GABA receptor signaling, sphingolipid

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metabolism, and alpha-2 adrenergic receptor function in the pathology of TS. This is consistent with the idea that TS is a disorder of central nervous system development and function (Fernandez et al., 2012). Genetic factors likely intersect with environmental factors such as infections, auto-immunity, and pre- or perinatal complications to cause TS (Georgitsi et al., 2016). While current treatment is symptom-targeted, understanding of TS mechanisms will facilitate pharmacological therapies that are instead designed around pathogenesis specific to each affected individual.

Most children with ASD have co-occurring conditions and symptoms (Cravedi et al., 2017; Hansen et al., 2018; Soke et al., 2018). About 3%–20% of children with TS have co-morbid ASD, while between 3% and 11% of children with ASD have TS (Baron-Cohen et al., 1999; Cravedi et al., 2017; Dietrich et al., 2015). The Simons Simplex Collection (SSC) is a collection of clinical and genetic data and biological samples from ~2700 individuals with ASD, and their parents and unaffected siblings (Fischbach and Lord, 2010). Of the SSC probands, 119 were also diagnosed with TS by a physician. TS and ASD could co-occur in an individual because of a single inherited or sporadic genetic mutation, two inherited or sporadic genetic mutations (one gene responsible for TS and a different gene responsible for ASD), or a combination of genetic mutations and multifactorial contributions. The purpose of this study was 1) to identify and clinically characterize probands in the SSC with a dual diagnosis of TS and ASD (TS/ASD) compared to those with ASD but not TS, 2) to analyze *de novo* genetic variants in TS/ASD probands including copy number variants, likely gene disrupting mutations, and missense mutations, and 3) to detect enrichment for biological pathways that could contribute to the pathogenesis of TS.

2. Material and methods

2.1. Analysis of clinical data

Use of SSC data from human subjects was approved by the Health Research Ethics Board-Biomedical Panel of the University of Alberta, Edmonton, Alberta, Canada. Comparisons were made between groups with TS ($N = 119$) or without TS ($N = 2603$), while probands with "suspected" tics ($N = 18$) were not included in either group. Statistical analyses were conducted using SAS Ver. 9.4 and IBM SPSS statistics Ver. 24 (IBM Corp.) We combined the SSC data in SPSS then used multivariable logistic regression to examine the differences between TS and non-TS probands, with diagnosis (TS vs non-TS) as an outcome and explanatory variables adjusted for age, sex, and IQ. A p-value ≤ 0.05 was considered statistically significant.

2.2. Analysis of genetic data

Genetic mutations in SSC probands were derived from data in "GPF: Genotype and Phenotype in Families" (www.iossifovlab.com/gpf/). Constraint scores defining tolerance to genetic mutation were derived from the default values in Exome Aggregation Consortium (ExAC) (Lek et al., 2016). Intolerance to mutation is assumed when the observed number of mutations (missense or likely gene disrupting) is significantly less than the number of mutations expected. Gene lists were analyzed by the PANTHER Overrepresentation Test (release 20170413) using a Bonferroni *post hoc* correction for multiple testing (Mi et al., 2017), and by the ClueGO Overrepresentation Test (v.2.5.0) with Bonferroni correction, using the 3.6.0 Biological/Molecular/Cellular Pathways (11.01.2018) and REACTOME Pathways (20.11.2017) in Cytoscape v.3.6.0 (Bindea et al., 2009).

3. Results

3.1. Identification of children with a dual diagnosis of TS and ASD

An extraordinary amount of clinical data has been collected on ~2700 children with ASD, their parents and at least one unaffected sibling who participated in the Simons Simplex Collection (Fischbach and Lord, 2010). The data include medical histories, biometrics, neonatal and pregnancy histories, and comprehensive autism-related psychological test results. Of these SSC probands, 119 (4.4%) had a physician-confirmed diagnosis of Tourette syndrome, a rate consistent with previous estimates of the diagnosis of TS within ASD cohorts (Baron-Cohen et al., 1999). Of these 119 children, 49 were diagnosed by a specialist, 22 by the primary care provider, and 48 by another physician. The ASD comparison group was 2603 SSC probands reported to have neither TS nor tics. We excluded 18 additional SSC probands from either category because they did not have a Tourette diagnosis, but did have tics "suspected". We then compared clinical characteristics of children with TS/ASD to those with ASD but not TS or tics.

3.2. Core phenotypes of children with TS/ASD dual diagnosis

We first investigated five phenotypes of ASD: age at diagnosis, verbal IQ, non-verbal IQ, the Autism Behavior Checklist (ABC) total score, and the Social Responsiveness Scale (SRS) T-score, parent reported (Constantino et al., 2003)(Table 1). These continuous variables were not normally distributed, so a Mann-Whitney U test was used to determine differences between groups. TS/ASD probands were, on average, 21 months older at the time of psychological testing than probands with ASD but not TS (TS/ASD median 10.7 years, range 4–17.9 *vs.* ASD 8.3 years, range 4–18, $p < 0.0001$). Both verbal IQ

Table 1
Age at ADOS, verbal IQ, nonverbal IQ, autism behavior checklist.

	TS/ASD	*ASD*
Age at ADOS administration (in years)		
N	119	2603
Mean (SD)	10.7 (3.4)	8.9 (3.5)
Median	10.7	8.3
IQR	4.8	5.1
Range	4.0–17.9	4.0–18.0
p-value*	< 0.0001	
Verbal IQ (VIQ)		
N	119	2602
Mean (SD)	84.2 (30.4)	77.7 (31.2)
Median	93	83
IQR	39	43
Range	11–140	5–167
p-value*	0.007	
Nonverbal IQ (NVIQ)		
N	119	2602
Mean (SD)	89.2 (23.0)	84.3 (26.3)
Median	92	89
IQR	32	35
Range	28–139	9–161
p-value*	0.05	
Autism Behavior Checklist		
N	119	2599
Mean (SD)	51.5 (23.9)	46.2 (25.8)
Median	48	43
IQR	33	38
Range	1–117	0–138
p-value*	0.01	

*Mann-Whitney U test comparing TS/ASD to ASD.

(VIQ) and non-verbal IQ (NVIQ) were significantly higher in the children with TS/ASD (VIQ, TS/ASD median 93, range 11–140 *vs.* ASD 83, range 5–167, $p = 0.007$ and NVIQ TS/ASD 92, range 28–139 *vs.* ASD 89, range 9–161, $p = 0.05$). The ABC total score was significantly higher in the TS/ASD group compared to ASD alone (TS/ASD median 48, range 1–117 *vs.* ASD 43, range 0–138, $p = 0.01$) suggesting more challenging behavior in the TS/ASD cohort. These differences in core phenotypes between groups were all statistically significant (p-value < 0.05, Mann-Whitney U test). However, no difference between groups was detected using the SRS T-score (parent-reported) (TS/ASD 81 *vs.* ASD 79, not significant).

We next examined a specific measure of restricted and repetitive behaviors (RRBs) associated with ASD (Bodfish et al., 2000), because repetitive behaviors are one feature of TS (Cath et al., 2000). The Repetitive Behavior Scale-Revised (RBS-R) measures stereotyped behavior, self-injurious behavior, compulsive behavior, ritualistic behavior; sameness behavior, and restricted behavior on a Likert scale. We also examined the Standardized Autism Diagnostic Observation Schedule (ADOS) Social and Communication subscale, as the social behavior network is postulated to be disrupted in TS (Albin, 2018). These continuous variables were analyzed using a Mann–Whitney U test because of lack of normality of the data. TS/ASD probands scored higher on the RBS-R (TS/ASD median 29, range 1–93 *vs.* ASD 24, range 0–105, $p = 0.008$) indicating that behavior occurs and is a more severe problem in probands with the dual diagnosis. There were too few probands in the TS category to determine whether the increased restricted and repetitive behaviors were driven by particular subscales within the RBS-R. Although there is evidence to suggest a negative correlation between NVIQ and restricted and repetitive behavior in the ASD population (Bishop et al., 2006), there were too few probands in the TS/ASD category to determine whether this correlation was also present in the dual diagnosis subcohort. On the ADOS social and communication subscale, TS/ASD probands had lower average scores (TS/ASD median 11, range 4–23 *vs.* ASD 13, range 5–24, $p = 0.001$) suggesting slightly less impairment in this domain (Table 2).

A binomial logistic regression was performed to ascertain the effects of age and sex on VIQ, NVIQ, ABC, restrictive and repetitive, and social and communication scores. All variables were still significantly different between groups after adjusting for age, for sex, or for both (sex was not statistically significant in the models) (Table 3). Variables from the bi-variate binomial logistic regressions (all of which were statistically significant) were added in multivariable logistic regression, and ones which were not statistically significant were removed from it.

Table 2
Repetitive behavior scale-revised and ADOS: Social and communication.

	Repetitive Behavior Scale-Revised	
	TS/ASD	*ASD*
N	119	2601
Mean (SD)	32.0 (20.2)	27.0 (17.2)
Median	29.0	24.0
IQR	28.0	22.0
Range	1–93	0–105
p-value*	0.008	
	ADOS: Social and Communication	
	TS/ASD	*ASD*
N	119	2603
Mean (SD)	12.1 (4.0)	13.4 (4.2)
Median	11	13
IQR	6	7
Range	4–23	5–24
p-value*	0.001	

*Mann-Whitney U test.

Table 3
Binomial logistic regression: variables adjusted for sex and age.

Variable	*p-value*	*Odds ratio*	*95% CI for odds ratio*	
			Lower limit	*Upper limit*
Age at diagnosis (in years)	< 0.0001	1.13	1.08	1.19
Verbal IQ (rescaled by 10)	0.03	1.07	1.01	1.14
Nonverbal IQ (rescaled by 10)	0.049	1.08	1.002	1.16
ABC total score (rescaled by 10)	0.03	1.08	1.01	1.16
RBS-R (rescaled by 10)	0.002	1.16	1.05	1.27
ADOS Social and Communication	0.002	0.93	0.88	0.97
Variable adjusted for age	***p-value***	***Odds ratio***	***95% CI for odds ratio Lower limit***	***Upper limit***
Verbal IQ (rescaled by 10)	0.03	1.07	1.01	1.13
Nonverbal IQ (rescaled by 10)	0.04	1.08	1.01	1.16
ABC total score (rescaled by 10)	0.004	1.11	1.03	1.19
RBS-R (rescaled by 10)	0.001	1.18	1.07	1.3
ADOS Social and Communication	0.01	0.94	0.9	0.99
Variable adjusted for sex	***p-value***	***Odds ratio***	***95% CI for odds ratio Lower limit***	***Upper limit***
Age at diagnosis (in years)	< 0.0001	1.14	1.08	1.19
Verbal IQ (rescaled by 10)	0.03	1.07	1.01	1.14
Nonverbal IQ (rescaled by 10)	0.06	1.07	0.997	1.16
ABC total score (rescaled by 10)	0.03	1.08	1.01	1.16
RBS-R (rescaled by 10)	0.002	1.16	1.05	1.27
ADOS Social and Communication	0.002	0.93	0.88	0.97
Variable adjusted for sex and log-transformed age	***p-value***	***Odds ratio***	***95% CI for odds ratio Lower limit***	***Upper limit***
Verbal IQ (rescaled by 10)	0.04	1.06	1.004	1.13
Nonverbal IQ (rescaled by 10)	0.052	1.07	1.001	1.16
ABC total score (rescaled by 10)	0.003	1.11	1.04	1.19
RBS-R (rescaled by 10)	0.001	1.18	1.07	1.3
ADOS Social and Communication	0.01	0.94	0.9	0.99

Several different methods were tested and they all converge to the same final model. As with bi-variable model, age did not pass Box-Tidwell assumption (linearity of the age with respect to the logit of the dependent variable) so it was log-transformed. In the final model, higher age at diagnosis (odds ratio 3.7), higher RBS-R score (odds ratio 1.2) and lower ADOS Social and Communication score (odds ratio 0.93) were all associated with the TS diagnosis (Table 4). These preliminary findings suggest a complex phenotype that includes physician-diagnosed tics characteristic of Tourette (by definition) and a diagnosis of

Table 4
Multivariable logistic regression.

Variable	***p-value***	***Odds ratio***	***95% CI for odds ratio***	
			Lower limit	***Upper limit***
Age at diagnosis (in years, log transformed)	< 0.0001	3.73	2.26	6.25
RBS-R (rescaled by 10)	0.0002	1.2	1.09	1.31
ADOS Social and Communication	0.005	0.93	0.89	0.98

ASD. The more frequent and more severe behaviors in the areas of restricted and repetitive behaviors, and a better score in the area of social and communication were associated with the TS diagnosis even after adjusting for the fact that TS/ASD probands were diagnosed almost two years later, on average, compared to probands with ASD alone. We could not determine whether the TS diagnosis was made before or after the ASD diagnosis in this cohort.

3.3. Genetic analysis of probands with a TS/ASD dual diagnosis

We accessed copy number variant and whole exome data from 119 SSC probands with TS/ASD through the Simons Foundation resource "GPF: Genotype and Phenotype in Families". Some probands had more than one *de novo* genetic event. Eighteen probands carried 20 *de novo* copy number variants (Supplementary Table 1a). Five of these probands had either a duplication (n = 3) or deletion (n = 2) on chromosome 14q11.2, but this recurrent CNV was also found in siblings and so is not pathogenic. Otherwise, the CNV regions did not overlap among probands. We used the CNV information in ExAC (Lek et al., 2016; Ruderfer et al., 2016) and literature searches to determine the likelihood that each CNV was pathogenic for ASD or TS. Seven of the CNVs (1.6 Mb duplication at 1q21.1, *DOCK7* deletion at 1p31.3, 1.6 Mb deletion at 3q29, 5.4 Mb duplication at 6p11, 56 kb deletion at 7q36, 5.9 Mb duplication at 20q11, and XYY syndrome) had previously been associated with ID, ASD or contained a single ID-causing gene. Deletions that include *NRXN1* and duplications that include *CNTN6* are found in ~1 in 100 people with TS (Huang et al., 2017), but were not found in the SSC TS/ASD probands.

TS/ASD probands also carried *de novo* coding variants, including 15 likely gene disrupting (LGD) variants (nonsense, frameshift, or splice site) and 80 other coding variants (77 missense, 2 in-frame deletion, and 1 stop-lost, Supplementary Tables 1b and 1c). These represent sequence changes (mutations) in the child with ASD that are not present in either of their parents. These sequence changes may or may not be pathogenic, that is contribute to their phenotype of ASD or TS. Estimates are that 43% of *de novo* LGD mutations (in ~400 genes) and 13% of *de novo* missense mutations contribute to 9% and 12% of ASD diagnoses, respectively, in simplex cases. Some probands had two or more *de novo* mutations. Only one gene (*URB1*) was mutated *de novo* in more than one proband. We analyzed the 95 *de novo* coding variants, located in 94 genes, in three ways. First, genes that are constrained for coding variation may be more likely to be dominant disease genes (Lek et al., 2016). The ExAC database provides a metric for this constraint and assigns a probability score (pLI) of being tolerant or intolerant to either LGD or missense mutations. Using this metric, 34 of the 94 genes carrying *de novo* coding variants were deemed intolerant to either LGD or missense mutations. Second, variants were sorted into either likely deleterious, likely neutral, or of unknown consequences to the encoded protein using the PROVEAN (Protein Variation Effect Analyzer) prediction algorithm (Choi et al., 2012). By definition, all 15 LGD variants are predicted to be deleterious to the encoded protein. As well, 36 missense or small insertion/deletion variants were predicted to be deleterious to the encoded protein (34 variants) or of unknown consequences to the encoded protein (2 variants). Third, we analyzed two databases of genes implicated in NDDs (the Simons Foundation SFARI autism susceptibility gene database (gene.sfari.org) and the Geisinger Developmental Brain Disorder Genes Database (geisingeradmi.org/care-innovation/studies/dbd-genes/) (Gonzalez-Mantilla et al., 2016) and also performed literature searches to determine whether any genes with *de novo* variants are plausible or confirmed NDD susceptibility genes (Luo et al., 2018). Six variants are in TS candidate genes: *de novo* missense mutations were identified in *FN1*, *URB1*, *AMBRA1*, *RUFY2*, and *STAB1* in probands in the Tourette International Collaborative Genetics (TIC-Genetics) and Tourette Syndrome Association International Consortium on Genetics (TSAICG) cohorts (Willsey et al., 2017), and a child with sporadic TS carried a missense mutation in *RICTOR* in a previous study (Eriguchi et al., 2017). Sixteen variants are in NDD susceptibility genes in the SFARI and/or Geisinger gene list (Suppl. Tables 2-3): *AMBRA1*, *CACNA1G, CHD7*, *GIGYF2*, *GRIK5*, *KIRREL3, OFD1*, *MED13L*, *PHF2*, *RAB2A*, *SLC25A39, SLITRK5, SUV420H1/KMT5B*, *USP7, ZNF407,* and *ZNF559*. Another 14 genes were identified as plausible developmental disorder susceptibility genes through literature searches: *ADD3, CHRND*, *DDR2*, *FN1, GTF2IRD1, LAMB2, NEB*, *NOTCH1*, *PEX11B, PKHD1, RPGRIP1L, SLX4*, *SPECC1L*, and *ZAK/MAP3K20*. Altogether, 9 of 15 genes carrying *de novo* LGD variants and 25 of 80 genes carrying other coding variants in TS/ASD probands had been previously identified as TS, ASD or NDD candidate genes.

We next inspected 94 genes carrying *de novo* variants for potential functional overlap (Suppl. Table 4). Interestingly, 21 genes containing variants encode membrane-associated proteins, 12 genes encode proteins important for cell-cell adhesion or communication, 8 genes encode proteins that act in G-protein signaling and 4 genes encode regulators or components of centrioles or cilia. To more systematically assess whether the genes containing *de novo* variants cluster in specific biological pathways, we performed a pathway analysis on the entire set of 94 genes using the enrichment analysis tool ClueGO and data from the Gene Ontology (GO) Consortium (Bindea et al., 2009). ClueGO visualizes the non-redundant biological terms for large clusters of genes in a functionally grouped network. ClueGO identified significant enrichment of genes encoding proteins in specific pathways, including biological pathways (regulation of calcium ion-dependent exocytosis, glomerulus development, positive regulation of fibroblast proliferation, hindlimb morphogenesis, basement membrane organization, astrocyte differentiation, visual behavior and visual learning, 14 genes, $P < 0.01$), cellular pathways (ciliary transition zone, basal lamina/basement membrane, 7 genes, $P < 0.01$), and molecular pathways

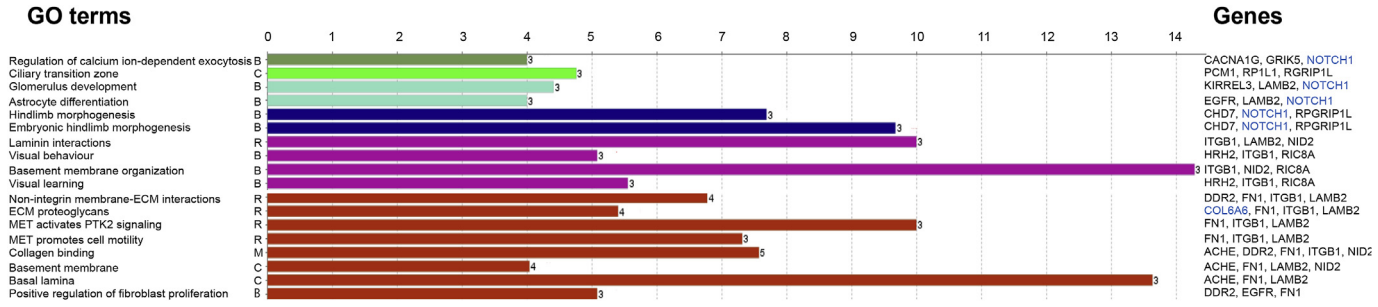


Fig. 1. Gene Ontology (GO) Consortium terms enriched in the set of 94 genes containing *de novo* variants in TS/ASD probands. The histogram shows GO terms identified for each set of genes. Bars represent the percentage of genes identified per term compared to the total number of genes in each term. Colors of bars represent grouped functions. Number beside bars represents number of genes identified in each pathway, and genes are listed for each term identified. Genes in blue font carry *de novo* LGD mutations in TS/ASD probands. GO term categories identified include: B, Biological Process; M, Molecular Function; C, Cellular Component; R, Reactome Pathway. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(collagen binding, 5 genes, $P < 0.001$) (Fig. 1). Genes important for interactions with the extracellular matrix and cell motility were enriched within the "Reactome" (Fabregat et al., 2018) suite in ClueGo (Fig. 1). In total, 17 genes participate in molecular, cellular, biological or Reactome pathways that are enriched among *de novo* mutated genes in TS/ASD probands. The pathway analysis confirms the over-representation of genes encoding cell surface, membrane-associated, and ciliary proteins among the TS/ASD candidate genes.

We next performed a narrower pathway analysis, excluding 27 genes that were considered tolerant to mutation and where the variant was predicted to be neutral, leaving 67 genes. ClueGO identified significant enrichment of genes encoding proteins in a subset of the pathways identified with the whole set of 94 genes, including biological pathways (regulation of calcium ion-dependent exocytosis, *CACNA1G, GRIK5, NOTCH1*, and hindlimb morphogenesis, *CHD7, NOTCH1, RPGRIP1L*), cellular pathways (ciliary transition zone, *PCM1, RP1L1, RPGRIP1L*), and molecular pathways (collagen binding, *ACHE, DDR2, ITGB1*).

Last, we performed a pathway analysis in which we combined a set of 38 genes previously identified as TS candidate genes through a combination of family studies, exome sequencing in TS cohorts, and candidate genes selected for their action in dopaminergic and serotonergic pathways with the set of 94 genes in which *de novo* variants were found in probands with both TS and ASD (Supplementary Tables 1-2). This analysis revealed an enrichment for genes important for the broad category of receptor-mediated signaling processes originating at the cell membrane and neural responses (*ACHE, CACNA1G, CHRND, EGFR, GIGYF2, GRIK5, NOTCH1, RIC8A, SLC8A2, SLC17A1, SLITRK5* joining 19 previous TS candidate genes in this functional group) overlapping with cell-cell communication or adhesion and extracellular matrix/basement membrane (*COL6A6, DDR2, FN1, ITGB1, KIRREL3, LAMB2, NID2, NOTCH1, RIC8A* joining 7 previous TS candidate genes in this functional group) (Fig. 2, Suppl. Fig. 1).

In summary, we performed multiple gene ontology pathway analyses, in which we considered 94 genes with *de novo* mutations in TS/ASD probands, then limited analysis to 67 intolerant genes carrying deleterious mutations, or combined 94 new TS/ASD candidate genes with 38 previously identified TS candidate genes. These analyses consistently pointed to an enrichment for genes encoding proteins important for cell-cell communication or adhesion, or to receptor-mediated signaling processes originating at the cell membrane as candidate genes for TS/ASD.

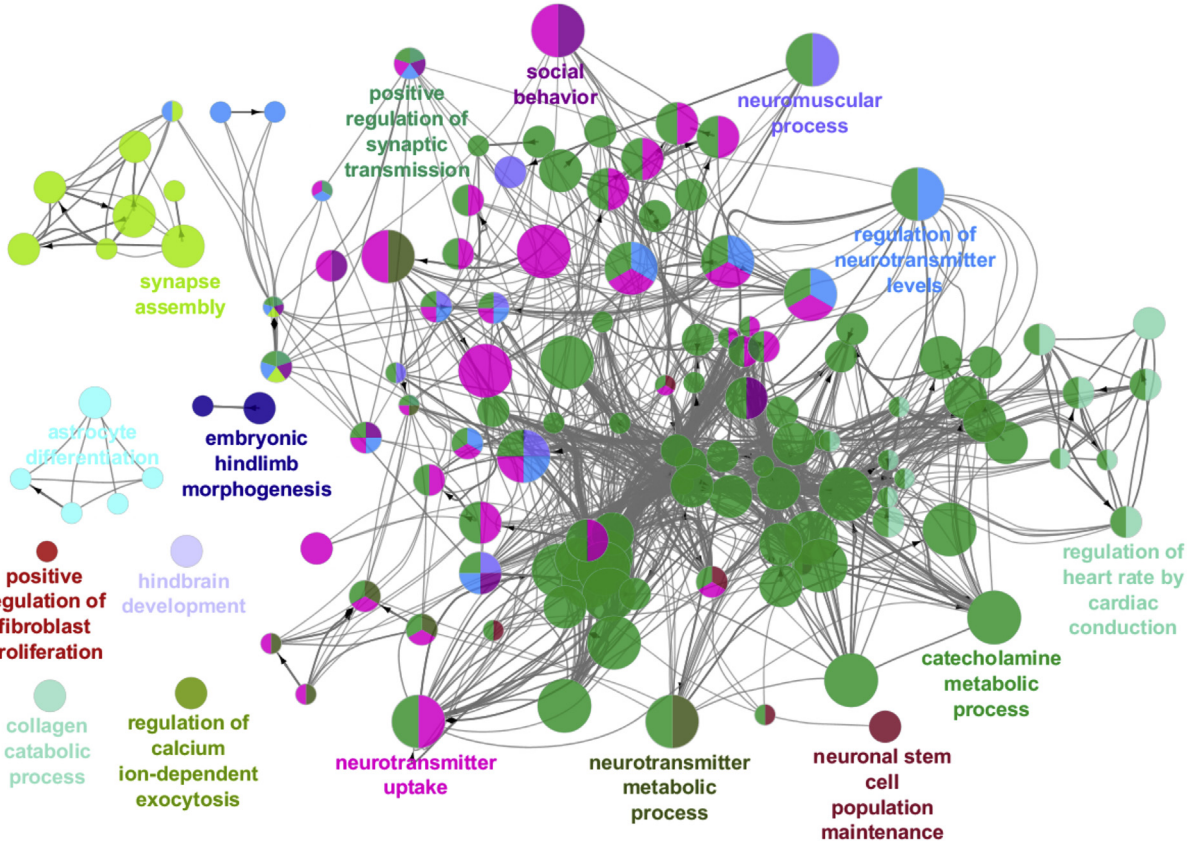
4. Discussion

Our analysis focused on a cohort of children who carry a dual diagnosis of TS and ASD. This cohort differs from the children in the SSC who have ASD alone in that, on average, they were diagnosed later, have IQ that is ~6 points higher, and have a more severe score in the area of restricted and repetitive behavior. Interestingly, a recent meta-analysis showed that having co-occurring conditions/symptoms influenced the age at which the ASD diagnosis was made in a series of ~1800 4- and 8-year olds with an ASD diagnosis, although TS was not included as a co-occurring condition in this analysis (Soke et al., 2018). Children with co-occurring attention deficit hyperactivity disorder, anxiety, oppositional defiant disorder, and aggressive behaviors were evaluated for ASD at a later age than children without these specific conditions (Soke et al., 2018). The clinical part of our study is limited by the fact that the TS diagnosis was made by a physician but there is no information about criteria used for the diagnosis. Information about the type of tic present (motor or vocal, or combination of the both), severity, and duration is not available. The finding of an elevated RBS-R score in the TS/ASD probands is confounded by the fact that this measure could be capturing tics as well as ASD-related restricted and repetitive behaviors in these probands with TS (Cohen et al., 2013). The ASD probands range from 3 to 18 years of age, and the median age of onset of TS symptoms is 7 years. Therefore, some of ASD probands in the "non-TS" category (likely < 2%) could have gone on to develop TS. This inclusion of a small number of TS probands among the 2603 non-TS probands could have a small effect on the outcomes reported. There are also site-specific differences in the assessments of children in the SSC (Lord et al., 2012), although these differences are assumed to add equivalent variability to both the TS/ASD and ASD groups. Last, parents and siblings have been tested and do not have neurological conditions. The SSC is thus depleted for heritable forms of TS. Concordance of these findings in a replication cohort is important to validate our results.

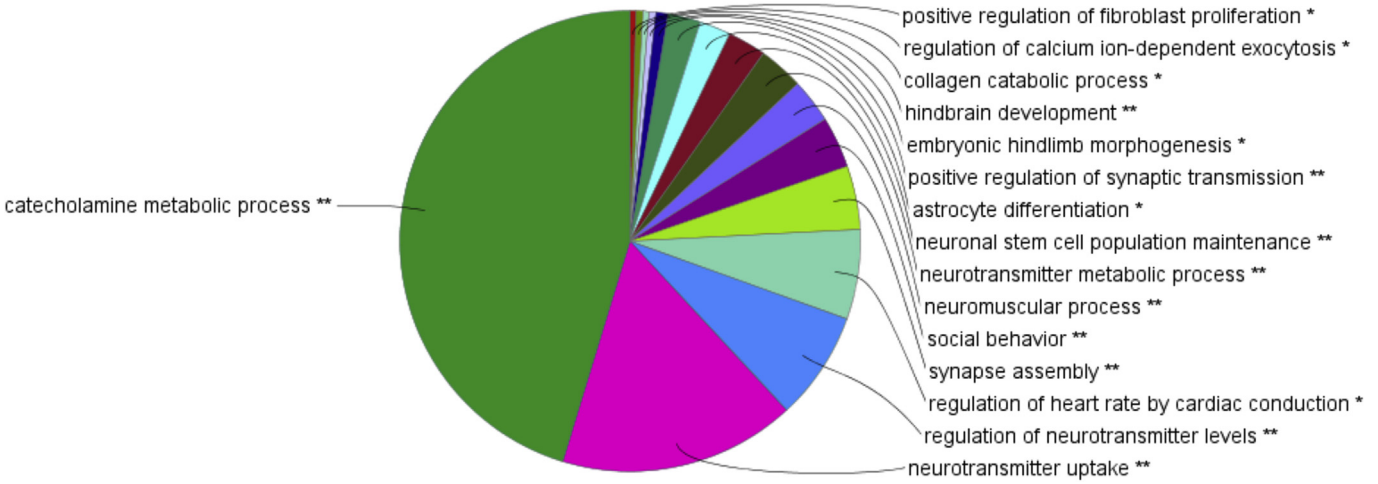
Candidate genes that have been suggested to contribute to TS encode, for example, proteins in the serotonin and dopamine pathways (e.g. *SLC6A4* (Moya et al., 2013b)), or mutated in animal models (*CHD2* (Moya et al., 2013a)) (Supplementary Table 2). Other studies have implicated genes such as *CNTN6*, *IMMP2L*, *NRXN1*, and *SLITRK1* as TS candidate genes through studies of chromosomal variants (Abelson et al., 2005; Alexander et al., 2016; Bertelsen et al., 2014; Fernandez et al., 2012; Huang et al., 2017; Karagiannidis et al., 2012; McGrath et al., 2014; Nag et al., 2013; Sundaram et al., 2010), *CNTNAP2, HDC*, *NLGN4*, *PNKD,* and *TBC1D7* through genome-wide association or studies of multiplex families (Alexander et al., 2016; Lawson-Yuen et al., 2008; Sun et al., 2017; Tsetsos et al., 2016; Verkerk et al., 2003), and *CELSR3, FN1, NEK10*, *NIPBL*, *RICTOR, STRIP2, TNRC6A* and *WWC1* by exome sequencing (Eriguchi et al., 2017; Willsey et al., 2017). The TIC consortium screened 325 TIC Genetics trios and 186 TSAICG trios by whole exome sequencing and estimated that 12% of clinical cases carry a *de novo* damaging variant mediating their risk of TS and that ~400 genes are vulnerable to these variants. According to this estimate, up to 14 of the 94 genes carrying *de novo* variants in the SSC TS/ASD probands could contribute to the risk of TS.

TS candidate genes appear to cluster in categories like cell-cell contact and plasma membrane-associated processes that control cell-cell communication, synaptic transmission and plasticity, and receptor-mediated intracellular signaling. This finding is consistent with the previous identification of *NRXN1* and *CNTN6* as TS candidate genes (Huang et al., 2017). *NRXN1* encodes a cell surface receptor that binds neuroligins, while *CNTN6* encodes a neuronal cell adhesion molecule. Other cell surface proteins implicated in TS include the cell adhesion protein *NLGN4* (Lawson-Yuen et al., 2008), the neuronal transmembrane protein neurexin-like protein *CNTNAP2* (Verkerk et al., 2003), serotonin transporter *SLC6A4* (Moya et al., 2013b), neuronal cadherin *CDH2* (Moya et al., 2013a), the laminin-related cell adhesion protein *NTN4* (Padmanabhuni et al., 2016), the non-classic cadherin transmembrane receptor *CELSR3*, and the cell adhesion protein fibronectin 1 encoded by *FN1*. The *de novo* variant in *FN1* in a TS/ASD proband supports its nomination as a likely TS risk gene (Willsey et al., 2017); *FN1* mutations can also cause skeletal dysplasia or glomerulopathy (Lee et al., 2017). A TS/ASD proband carries a *de novo* missense mutation in the neurotrophin receptor gene *SLITRK5*, which is highly related to *SLITRK1,* a TS candidate gene (Abelson et al., 2005; Alexander et al., 2016; Karagiannidis et al., 2012). Mutations in SLITRK5 are associated with obsessive compulsive disorder (Song et al., 2017). Another TS/ASD proband carries a deleterious missense mutation in *HRH2*, encoding the H2 histamine receptor. The histamine pathway has been implicated in TS, with the identification of a TS family carrying a nonsense mutation in histidine decarboxylase (*HDC),* which converts histidine to histamine (Alexander et al., 2016; Rapanelli and Pittenger, 2016). *URB1* acts downstream of mTOR, which regulates the actin cytoskeleton and is implicated in ASD (Yeung et al., 2017). TS candidate genes in this pathway include the mTORC2 subunit *RICTOR* and the TOR pathway inhibitor *TBC1D7* (Tsetsos et al., 2016). *De novo* mutations in *URB1* and *RICTOR* have now been found in two different studies. Five genes (*COL6A6*, *GIGYF2*, *NOTCH1*, *OFD1*, *RP1L1*) in which *de novo* LGD mutations have occurred in TS/ASD probands and that are in enriched pathways could be considered higher priority candidate genes. Interestingly, *GIGYF2* is also a candidate gene for Parkinson Disease

A



B



(caption on next page)

Fig. 2. Genes containing *de novo* variants in TS/ASD probands identified in pathways overlap with previously identified TS pathways. (A) ClueGO functionally grouped networks with Gene Ontology (GO) terms shown as same colored nodes connected based on Cohen's kappa score (≤ 3). The node size represents the statistical significance of the term enrichment. Nodes with multiple colors represent genes that participate in several GO networks. Arrows represent regulatory interactions generated from predictive databases. GO terms with highest significance were highlighted for simplicity. All GO terms identified are listed in Supplementary Fig. 1 and are color coded. (B) Node pie charts display over-represented biological pathways (GO terms) generated by ClueGO. For each term, the percentage of genes is proportionally represented in the pie chart. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

while *NOTCH1* is mutated in some children with aortic stenosis and *OFD1* mutations are associated with ciliopathies. There was no apparent enrichment for other categories of genes commonly mutated in autism cohorts, such as those encoding proteins important for transcription, splicing and post-transcriptional events, cell cycle regulation and chromatin remodeling (Krishnan et al., 2016). However, the use of Gene Ontology to detect enrichment for certain pathways and processes is limited by the fact that not all genes are properly annotated in the Gene Ontology Consortium. This may result in an underrepresentation of pathways and processes in which TS candidate genes participate.

Other studies have mined data from the SSC to unravel the clinical and genetic basis for the dual diagnoses of ASD and obesity (Cortes and Wevrick, 2018) and ASD and poor motor skills (Buja et al., 2018). Studies that use the SSC to identify genes for dual diagnosis phenotypes, including this one, may overemphasize certain genes that were originally put forward as candidate genes because of studies that used the SSC. Nonetheless, one common conclusion from these studies, ours included, is that expanding the number of dual diagnosis children with ASD and other medical findings from whom clinical and genetic data have been collected will be key to understanding how genes control intersecting developmental biological pathways, in turn leading to better therapeutics for these conditions.

Contributorship statement

RW planned the study and takes responsibility for the overall content. KVC and RW performed the data analysis and wrote the manuscript.

Conflicts of interest

The authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2019.01.023.

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