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"Exploring the Genetic Architecture of Tourette Syndrome in Children and Adolescents: Integrated Evidence from Copy Number Variants and Exome Sequencing"

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Abstract

Despite the extensive research on Tourette Syndrome (TS), critical gaps remain in the understanding of its pathophysiology. The marked clinical heterogeneity, the frequent occurrence of comorbid conditions and the complex genetic architecture, further contribute to making the overall landscape even less clear. Over the past decades, growing scientific interest and significant investment in addressing these complex challenges have fostered an increasing emphasis on early diagnosis and integrated management approaches. Analyzing the correlation between genetic findings and the detailed clinical phenotypes of TS patients may contribute to a better understanding of its heterogeneity and inform future precision medicine strategies. Advances in next-generation sequencing, especially whole-exome sequencing (WES), have greatly improved our understanding of the TS genetic architecture, allowing the detection of both *de novo* and inherited rare coding variants that affect crucial neurodevelopmental pathways. Furthermore, growing evidence highlights rare high-impact variants, including CNVs, as key determinants of TS susceptibility. In line with these premises, during my PhD I conducted two complementary studies aimed at clarifying the contribution of rare genetic variants to TS in pediatric cohorts. The first study systematically examined CNVs in clinically characterized children with TS using array-comparative genomic hybridization (array-CGH). This investigation revealed rare genomic imbalances in several patients, some overlapping with *loci* previously associated with neurodevelopmental disorders, thereby providing novel insights into the role of structural variation in the clinical heterogeneity of TS. The manuscript of this research project has been published in February 2023, with the title “Copy Number Variations in Children with Tourette Syndrome: Systematic Investigation in a Clinical Setting”, co-authored by Saia, F., Prato, A., Saccuzzo, L., Madia, F., Barone, R., Fichera, M., & Rizzo, R., on the scientific journal titled “Genes”, as part of the section “Molecular Genetics and Genomics” .

Building on these findings, the second study presented in this thesis, employed trio-based whole-exome sequencing (WES) to investigate the role of rare coding variants, both *de novo* and inherited.

Unlike the first study, which focused on structural genomic alterations, this approach enabled a systematic exploration of coding variants with potential functional effects, thus expanding the search for novel risk genes and refining genotype–phenotype correlations. The manuscript of this second research project has not yet been published. These studies are part of a broader scientific trajectory developed during my doctoral training, which has included studies on both clinical and biological aspects of TS and related neurodevelopmental disorders. For instance, I contributed to the characterization of sensory *phenomena* in children with TS and ASD (Prato et al., 2024), to the follow-up of functional tic-like behaviours emerging during the COVID-19 pandemic (Prato et al., 2023), and to the investigation of long-COVID symptoms in pediatric TS cohorts (Prato et al., 2023). Moreover, I co-authored case reports and descriptive studies on rare genomic syndromes, such as 17q21.31 microduplication (Saia et al., 2023) and Zhu-Tokita-Takenouchi-Kim syndrome with novel SON variants (Pavone et al., 2022), further consolidating my expertise in the interface between clinical neuropsychiatry and human genetics. Finally, I participated in international collaborations such as the ENIGMA-TS project, aimed at advancing neuroimaging genetics research through large-scale meta-analysis (Paschou et al., 2022). Taken together, these experiences allowed me to develop a multidisciplinary approach combining detailed clinical phenotyping with advanced genomic methodologies, ultimately converging on the present thesis project, where my recent focus has primarily been on the genetic architecture of Tourette Syndrome.

Summary

1. Introduction

Tourette Syndrome is a childhood-onset neurodevelopmental disorder characterized by the occurrence of both motor and vocal tics persisting for more than one year (American Psychiatric Association, 2013). Its prevalence is estimated at 0.3–1% worldwide, with a male-to-female ratio of approximately 3–4:1 (Robertson, 2008; Robertson et al., 2017), and a typical onset in childhood, predominantly at 5–6 years of age (Cath DC et al., 2011).

The clinical presentation is highly heterogeneous: while some individuals manifest mild symptoms with limited functional impact, others develop more severe and persistent tics that interfere substantially with daily life.

Comorbid conditions are very common, particularly obsessive–compulsive disorder (OCD) and attention-deficit/hyperactivity disorder (ADHD), but also anxiety, depression, and learning difficulties, all of which contribute to the complexity of clinical management (Cavanna & Rickards, 2013; Robertson, 2015). Increasingly, TS is understood as part of the broader spectrum of neurodevelopmental disorders, where overlapping genetic and neurobiological mechanisms contribute to diverse and often co-occurring clinical phenotypes (Gorman et al., 2021).

From a genetic perspective, TS is highly heritable, with twin and family studies estimating heritability above 70% (Davis et al., 2013), and its genetic architecture is complex and multifactorial.

Genome-wide association studies (GWAS) confirm the role of polygenic inheritance but explain only part of the risk (Scharf et al., 2013; Huang et al., 2017). They have highlighted the contribution of common variants with small individual effects (Yu et al., 2019), whereas rare high-impact variants, including copy number variants (CNVs) and *de novo* single nucleotide variants (SNVs), have also been implicated (Willsey et al., 2017; Wang et al., 2018).

These exomic variants include loss-of-function (LoF) and deleterious missense mutations in genes that are highly constrained and intolerant to variation. Several whole-exome sequencing (WES) studies in simplex trios have reported damaging *de novo* variants in approximately 10% of TS cases,

with high-confidence risk genes such as *CELSR3*, *WWC1*, *NIPBL*, *FN1*, and *ASH1L* emerging as relevant contributors (Wang et al., 2018; Deciphering Developmental Disorders Study, 2017). Beyond these core genes, recent research has highlighted the potential contribution of rare variants in candidate genes such as *PNKD* and *BRPF1*, which play key roles in presynaptic regulation and chromatin-mediated transcriptional control, respectively (Sun et al., 2018; Yan et al., 2017). Variants in these genes may disrupt synaptic vesicle dynamics or epigenetic modulation, both of which are essential for maintaining the functional integrity of cortico-striatal-thalamo-cortical circuits implicated in tic generation. Moreover, WES meta-analyses have reported an enrichment of X-linked and autosomal rare variants, helping to explain the sex bias observed in TS and its frequent comorbidity with ASD and ADHD (Wang et al., 2018; Nature Genetics, 2023).

Nevertheless, many of these rare variants converge on biological pathways underscoring a shared substrate with other neurodevelopmental disorders including ASD and intellectual disability (Deciphering Developmental Disorders Study, 2017; Sun et al., 2018): this convergence supports the view of TS as a disorder embedded within a neurodevelopmental *continuum* rather than an isolated clinical entity.

A growing number of variants of uncertain significance (VOUS), mutations for which the pathogenic potential remains unconfirmed, are being recognized as crucial to the expanding landscape of TS genetics. Despite their classification uncertainty, many VOUS occur in genes with plausible neurobiological roles, such as those involved in ion channel activity (e.g., *CACNA1D*, *SLC6A1*), GABAergic inhibition, chromatin remodeling, and neuronal migration (e.g., *RELN*, *ZMYM2*, *KMT2C*), (Joly-Amado & Kulkarni, 2023; Lossi, 2019; Vallianatos & Iwase, 2015; Li et al., 2023).Importantly, some of these VOUS may act as susceptibility loci or modifiers within a broader oligogenic or polygenic context, interacting with other rare or common variants to influence phenotypic expression and disease severity. (Willsey et al., 2017; Wang et al., 2018; Deciphering Developmental Disorders Study, 2017). Therefore, careful interpretation of VOUS, especially those

of *de novo* origin or affecting functionally constrained regions, is essential for refining the genotype-phenotype correlations in TS (Faundes et al., 2018).

In the next research projects, we investigated whether the occurrence of potentially pathogenic variants was associated with recurrent phenotypic features in two separate, clinically well-characterized pediatric TS cohorts. Specifically, we examined the relationship between the presence of such variants and dysmorphic traits, epilepsy, brain MRI anomalies, comorbid conditions, family history, and tic severity.

By systematically analyzing the clinical and molecular profiles of affected individuals, we aimed to identify potential genetic signatures underlying distinct clinical subtypes of TS, thereby advancing insights into its diversity and supporting the development of precision medicine approaches.

2. Array-CGH Study

2.1 Materials and Methods

2.1.1 Participants

This study was conducted at the Child and Adolescent Neurology and Psychiatry of the Medical and Experimental Department, Catania University. A total of 93 patients with a clinical diagnosis of TS according to DSM-5 criteria, have been enrolled for the study between April 2021 and April 2022. We excluded patients with a primary psychiatric disorder different from TS, and/or presence of known etiology such as genetic or inherited metabolic diseases. Each participant was clinically assessed for tics and associated comorbidities. Investigations were carried out as part of the routine clinical care of the patients in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Prior to enrolment, written informed consent was obtained from all

participants' parent or legal guardian. The study was approved by the local Ethics Committee at our institution.

2.1.2 Procedures

Medical history was obtained from participant's parents with a focus on developmental delay, seizures, neuropsychiatric disorders, and positive family history for neurodevelopmental disorder and metabolic diseases. All participants underwent physical and neurological examination. Fasting blood samples and urine samples were collected for routine blood analyses and to rule out inherited metabolic diseases. The occurrence of epileptic seizures and isolated electroencephalogram (EEG) anomalies were evaluated. Moreover, brain MRI using a 1.5 T MRI scanner, were analyzed to detect morphologically visible signs of altered brain development.

2.1.3 Clinical assessment

All participants underwent a full neuropsychiatric assessment. According to participants' ages, the Wechsler Intelligence Scale for Children (WISC-IV) was administered to evaluate the intelligence quotient (IQ) of patients (Wechsler, 2012). Symptoms of TS were evaluated using the Yale Global Tic Severity Rating Scale (YGTSS) (Leckman et al., 1989), a clinician-rated scale used to assess the motor and phonic tic severity considering the number, frequency, duration, intensity, and complexity of tics.

2.1.4 Genetic analysis

Array-CGH analyses were performed by using the Human Genome array-CGH 8x60K Microarray (Agilent Technologies, Palo Alto, CA), with an average probe spacing of around 55 Kb. The arrays were performed using Agilent Reference DNAs, analyzed with the Agilent Microarray Scanner Feature Extraction Software version 11.5, and Agilent Genomic Workbench 7.0.4.0 software using the ADM-2 algorithm. Genomic positions of the rearrangements refer to the public UCSC database

GRCh37/hg19. Inheritance of non-polymorphic CNVs was assessed either by Multiplex Ligation-dependent Probe Amplification or Real Time-PCR assays.

2.1.5 Array CGH data analysis and CNVs classification

"Causative" deletions/duplications, "non-causative" or potentially benign familiar variants were established based on the scientific literature and on the careful consultation of public and private databases such as the database of genomic variants (http://dgv.tcag.ca/dgv/app/home), the database of human CNVs hosted by IRCCS Oasi Maria SS of Troina, (http://gvarianti.homelinux.net/gvariantib37/index.php), the Decipher database (https://decipher.sanger.ac.uk/), the database of human genomic structural variation (https://www.ncbi.nlm.nih.gov/dbvar), and the OMIM catalogue (http://www.ncbi.nlm.nih.gov/omim). In the present study we considered pathogenic or "causative variants" (C-CNVs) all the CNVs described as associated with TS in the OMIM database. Further, we included in the C-CNV potentially causative CNVs having a more uncertain role in the disease, but sporadically associated with TS in the literature or affecting genes known to be linked to TS or other neuropsychiatric conditions. The "non-causative" CNVs (NC-CNVs) included variants of unknown significance (VOUS) which have never been described in literature (unknown CNVs) or never reported in association with TS as well as VOUS-likely benign or benign (Kearney et al., 2011). When a C-CNV was found, the study of the parents was requested to ascertain its origin and decide the strategy for family management and future genetic counselling. There were cases where the origin of the imbalances was not possible to determine, either because of unwillingness of both parents to be evaluated, in situations of adoption or in mono-parental subjects in which the available progenitor was not a carrier of the proband's CNVs.

2.1.6 Statistical analysis

Data were analyzed using SPSS software (SPSS, Inc., Chicago, IL, USA, IBM, Somers, NY, USA). Children with C-CNV and NC-CNV and children W-CNV were compared to verify the effects of presence/absence of CNVs on dysmorphic features, comorbidities, ID, YGTSS scores, as well as on the rate of epilepsy, EEG anomalies and brain MRI anomalies. Clinical characteristics of participants were summarized by randomized group using mean (SD) for continuous data or count (%) for categorical data. Students' t-tests were used to compare the neuropsychological scores and characteristics between groups. A p-value < 0.05 was considered to indicate statistical significance.

2.2 Results

2.2.1 Genetic analysis

Out of 93 children with TS, 20 (16 males, 4 females) tested positive for CNVs (21.5 %) and 73 (55 males, 18 females) had no CNVs (78.5%). Eleven patients (9 males and 2 females) had C-CNVs (11.8%) and nine (7 males and 2 females) had NC-CNVs (9.7 %). A total number of 20 imbalances were detected (13 duplications and 7 deletions) (Table 1). All children showed a single CNV. Five deletions located on chromosomes 22q11.21, 6q26, 15q11.2 (2 deletions), 16p13.3, and six duplications on chromosomes 2p16.1, 22q11.21, 4p14, 7q11.23, 17q12, 5q33.3-q34 were classified as pathogenic/causative CNVs (55% of all the CNVs) (Table 2). Nine CNVs (2 deletions and 7 duplications) were considered as non-causative CNVs (45.0% of all the CNVs). Among 12 imbalances studied for the parental origin, 4 were paternally inherited, 3 were maternally inherited and 5 arose *de novo*.

Table 1. Demographic and genetic characteristics of TS patients

	Patients (*n*)	Age (years)	M: F Ratio	Deletions (*n*)	Duplications (*n*)
All	93	12.1± 3.1	71:22	/	/
Patients without CNVs	73	11.9± 3.2	55:18	/	/
Patients with CNVs	20	12.4± 2.8	16:4	7	13
C-CNVs	11	12.3± 2.8	9:2	5	6
NC-CNVs	9	12.7± 3.0	7:2	2	7

2.2.2 Participants' characteristics

In this study, we enrolled a total of 93 subjects aged 6-18 years (mean age = 12.1 ± 3.1; male (M)/female (F) = 71:22; male =76.3%). All patients were affected by TS. Somatic and neuropsychiatric features of patients with TS are displayed in table 2.

The mean age of tic onset was 6.6 ± 2.9. Of the 93 patients affected by TS, 42 subjects (45.2%) had a family history of TS, OCD, or other neuropsychiatric disorders. Among the individuals diagnosed with TS, the most common comorbid neuropsychiatric disorder was OCD (33.3%, n= 31). Forty-nine patients (52.7%) presented "pure-TS" phenotype. Mean full scale IQ off all participants was 85.5 (± 19.4 SD). Nineteen patients (20.4%) had ID. Furthermore, the rate of patients with ID was significantly different among children with C-CNVs, NC-CNVs and W-CNVs (p= 0.025). Mean score for YGTSS was 20.4 (± 9.2 SD). Results on YGTSS score showed no significant differences over the groups (p= 0.4). No significant differences were also computed among groups about the rate of patients with comorbidities (p= 0.1) (Table 2).

2.2.3 Physical measures

Thirty-four of 93 children with TS (37.6%) had some degree of dysmorphisms with significant differences observed among the three groups. The number of children with dysmorphism was higher in the group with C-CNV (63.6%) compared to NC-CNVs (44.4%) and to W-CNVs (31.5%) (p= 0.1) (Table 2).

2.2.4 Epilepsy and EEG anomalies

Only seven out of 93 patients (7.5%) had epilepsy that required medical therapy, and/or isolated EEG anomalies (focal spikes or sharp waves, slow waves). Significant differences were found among groups for the occurrence of epilepsy or isolated EEG anomalies (p=0.026) (Table 2).

2.2.5 MRI studies

MRI studies were analyzed to detect morphologically visible signs of altered brain development, areas with abnormal white matter signal as well as atrophic changes. MRI anomalies were observed in 13 of 93 study patients (14.0%). The proportion of patients with MRI anomalies was 36.4% in the C-CNV group, 0% and 12.3% in the NC-CNV and W-CNV groups respectively with significant differences (p = 0.045) (Table 2).

Table 2: Somatic and neuropsychiatric features in patients with TS according to CNVs

	C-CNVs (11)	NC-CNVs (9)	W-CNVs (73)	P-value
Dysmorphic features	7 (63.6%)	4 (44.4%)	23 (31.5%)	**0.1**
Epilepsy/EEG anomalies	3 (27.3%)	0 (0%)	4 (5.5%)	**0.026**
Brain MRI anomalies	4 (36.4%)	0 (0%)	9 (12.3%)	**0.045**
Familiarity for neuropsychiatric disorders	7 (63.6%)	7 (77.8%)	28 (38.4%)	**0.03**
Presence of Comorbidities	8 (72.7%)	5 (55.6%)	29 (39.7%)	0.1
Intellectual disability	5 (45.5%)	4 (44.4%)	10 (13.7%)	**0.01**
IQ	75.7± 26.1	74.4± 23.2	88.3± 17.0	**0.025**
YGTSS	17.0 ± 7.5	22.0 ± 11.7	20.7 ± 9.1	0.4

2.3 Discussion

In this study, we aimed to identify whether the occurrence of C-CNVs is associated with recurrent clinical features in the TS cohort. We systematically studied for array-CGH a cohort of patients with a diagnosis of primary TS recruited prior to the study period and considered patients with C-CNV compared to patients with NC-CNV or W-CNV. Twenty of ninety-three patients (21.5 %) tested positive for CNVs. Eleven children (11.8%) had pathogenic CNV (C-CNV) of which five had a *de novo* aberration. Clinical and molecular characteristics of patients with C-CNV are reported in Table 3 (proband 1-20).

Table 3. Clinical and molecular characteristics associated with CNVs in patients with TS.

Proband	CNVs	Type of CNVs	Genes involved	Tourette	Comorbidities	Dysmorphism
1	**arr[GRCh37]2p16.1-p15(60,294,104-62,030,285)x3 dn**	Causative	BCL11A, CASK, USP 24 and PEX13	Onset: 7. YGTSS:12.	Mild intellectual disability. OCD. Headache. Behavior disorder.	Small forehead, thick eyebrows with horizontal orientation, low implantation ears, hypoplasia of the tragus, short and flat nasal filter, ogival palate. Adipose tissue abundantly represented. 5 inch finger like, clinodactyly
2	**arr[GRCh37]22q11.21(18,641,409-21,440,514)x3 dn**	Causative	About 76 genes; (MIM:# 608363)	Onset: 7. YGTSS:12.	ADHD.	Broad forehead, thick eyebrows, epicanth, horizontal eyelid rhymes, low implantation large ears, tragus hypoplasia, flat filter, protrudent columella, ogival palate, thin upper lip, V-finger clinodactyl, finger pads
3	**arr[GRCh37]9q21.33(88,234,157-88,408,246)x1**	Non causative	AGTPBP1	Onset: 12. YGTSS:15.	Mild intellectual disability. OCD.	Small eyes, small forehead, hypolorism, low implantation ears, short filter, clinodactylly V finger, finger-like thumb, syndactyl II, III and IV finger. Accentuated arch
4	**arr[GRCh37]16q22.1-q22.2(69,833,053-70,883,845)x3 pat**	Non causative	WWP2, CLEC18A, PDPR, CLEC18C, EXOSC6, AARS1 (#OMIM 601065), DDX19B, DDX19A, ST3GAL2, FUK, COG4 (#OMIM 606976), SF3B3, IL34, MTSS1L	Onset:5. YGTSS:45.	Headache. Anxiety disorder.	Flat filter. Small nose. Tapered hands, thin fingers. Pectus excavatum. Sindattilia II-III toes
5	**arr[GRCh37]4p14(37,684,725-40,379,119)x3 dn**	Causative	30 genes including TLR1, WDR19, RFC1, LIAS, UGDH and RHOH	Onset:10. YGTSS:14.	Speech disorder.	No dysmorphism
6	**arr[GRCh37]12q13.12(49,488,325-49,811,367)x3 mat**	Non causative	DHH, LMBR1L, TUBA1A, TUBA1B, TUBA1C, PRPH, TROAP, C1QL4, DNAJC22, SPATS2	Onset:6. YGTSS:13.	Mild intellectual disability. OCD. Behavior disorder. Microcephaly.	Coffee-milk stains of varying sizes spread throughout the body. Broad forehead, prominent frontal drafts
7	**arr[GRCh37]Xp22.31(6,552,712-8,115,153)x2**	Non causative	HDHD1, STS, VCX, PNPLA4 (X)	Onset:7. YGTSS:15.	No	Large eyes with low implantation, sparse eyebrows, horizontal eyelid rhymes, hypolorism. Short filter, ogival palate,

						clinodactylly V finger, mild hypotonia, Sindattilia II and III toe bilaterally, finger-like thumbs.
8	**arr[GRCh37]22q11.21(20,754,422-21,440,514)x1 pat**	Causative	ZNF74, SCARF2(MIM: 613619), KLHL22, MED15, PI4KA(MIM: 600286), SERPIND1(MIM:142360), SNAP29(MIM:604202), CRKL, AIFM3, LZTR1(MIM:600574), THAP7, P2RX6, SLC7A4	Onset:2. YGTSS:34.	OCD. Behavior disorder. Speech disorder.	Large eyes with low implantation, sparse eyebrows, horizontal eyelid rhymes, hypolorism. Short filter, ogival palate, clinodactylly V finger, mild hypotonia, Sindattilia II and III toe bilaterally, finger-like thumbs.
9	**arr[GRCh37]7q11.23(72,645,480-74,197,434)x3 dn**	Causative	33 genes	Onset:6. YGTSS:20.	Mild intellectual disability. OCD. ADHD. ODD. Anxiety disorder.	Turrecephalia, high forehead, epicanth, hypolorism, horizontal eyelid rhymes, pointed columella, microretrograzia, thin lips, mentonyl dimple, ogival palate, clinodactyly of the V. finger, winged scapulae.
10	**arr[GRCh37]10q22.3(78,651,963-79,327,368)x3 pat**	Non causative	KCNMA1 (#MIM 600150)	Onset:11. YGTSS:9.	Mild intellectual disability. OCD.	No dysmorphism
11	**arr[GRCh37]6q22.31(123,539,625_124,280,442)x3**	Non causative	TRDN (#OMIM 603283)	Onset:4. YGTSS:20.	Anxiety disorder.	No dysmorphism
12	**arr[GRCh37]17q12(34,817,422-36,168,104)x3 pat**	Causative	20 genes: HNF1B (MIM: 189907),ACACA (MIM:200350), PIGW(MIM:610275) , ZNHIT3(MIM:604500)	Onset:13. YGTSS:10.	Epilepsy.	Small forehead. Sinofrisi. Hypolorism. Poorly shaped and low-implanted ears. Horizontal eyelid rhymes. Prominent columella. Short filter. Epitrochlear dimples. Pointed palate. Thumb pretends like
13	**arr[GRCh37]Xq28(148,798,762-149,572,551)x2**	Non causative	MAGEA11, HSFX1, MAGEA9B, MAGEA9, MAGEA8, CXorf40B, MAMLD1 (#OMIM 300120)	Onset:6. YGTSS:25.	Severe intellectual disabilities. Behavior disorder.	No dysmorphism
14	**arr[GRCh37]6q26(161,878,327-162,683,777)x1 mat**	Causative	PARK2 (#OMIM 602544)	Onset:3 YGTSS:19.	OCD. Specific Learning Disorder.	No dysmorphism
15	**arr[GRCh37]15q11.2(22,784,523-23,179.948)x1**	Causative	TUBGCP5, CYFIP1, NIPA2, NIPA1 (MIM:608145)	Onset:2. YGTSS:11.	OCD.	Mild turryphaly, sparse eyebrows, poorly structured ears, mild bilateral gynecomastia and inverted

						nipples. Pterygian neck.
16	**arr[GRCh37]5q33.3-q34(158,829,101-161,658,146)x3**	Causative	ADRA1B, TTC1, PWWP2A, FABP6, CCNJL, C1QTNF2, C5orf54, SLU7, ATP10B, PTTG1, GABRB2 (#OMIM 600232), GABRA6, GABRA1 (#OMIM 137160) , GABRG2 (#OMIM 137164)	Onset:6. YGTSS:10.	Mild intellectual disability. ODD. Behavior disorder.	No dysmorphism
17	**arr[GRCh37] 16p12.2(21,599,687-21,739,885)x1 mat**	Non causative	OTOA (MIM:607038)	Onset:7. YGTSS:36.	Mild intellectual disability. Behavior disorder. Microcephaly.	No dysmorphism
18	**arr[GRCh37] 16p13.3 (6,880,891-7,008,524)x1 pat**	Causative	RBFOX1	Onset:7. YGTSS:25.	Severe intellectual disabilities. Disorder of the autistic spectrum.	Flat front on the right, low implantation ears, horizontal eyelid rhymes. Right eyelid ptosis. Excavated chest. Clinodattilia V finger.
19	**arr[GRCh37]15q11.2(22,784,523-23,179.948)x1**	Causative	TUBGCP5, CYFIP1, NIPA2, NIPA1 (MIM:608145)	Onset:5. YGTSS:20.	Mild intellectual disability. ODD.	No dysmorphism.
20	**arr[GRCh37]4q26(119,461,777-119,830,456)x3**	Non causative	METTL14, SEC24D (#OMIM 607186), SYNPO2	Onset:3. YGTSS:20.	No	No dysmorphism

Among children with C-CNV, one male subject (proband 1) has a *de novo* duplication of the short arm of chromosome 2 (2p16.1p15 60,294,104-62,030,285) of about 1.73 Mb encompassing many genes including *BCL11A, PAPOLG, REL, PUS10, PEX13, KIAA1841, AHSA2, USP34, XPO1, C2orf74* (Mimouni-Bloch et al., 2015, Félix et al., 2010). The boy, affected by TS in comorbid with OCD and mild intellectual disability, presented also dysmorphic traits, like clinodactyly of the 5th finger and bilateral 2nd-3rd toes syndactyly. This patient was already reported as a case-report in 2018 (Pavone et al., 2018). A similar rearrangement was reported in a patient with a *de novo* interstitial microduplication involving 2p16.1-p15 (60,150,427-61,816,209) (Mimouni-Bloch et al., 2015). Proband 1 and the patient reported by Mimouni-Bloch et al. (2015) present with some similar findings, consisting of mild cognitive delay, behavioral anomalies with noticeable OCD features, hypotonia and motor dyspraxia, and some facial dysmorphisms (puffy eyelid, broad philtrum). Interestingly, this region is also associated with the 2p15p16.1 microdeletion syndrome characterized

by a complex phenotype including neurodevelopmental delay, facial dysmorphisms, and autistic behavior (Levy et al 2017), suggesting that this chromosomal segment may harbor dosage sensitive genes linked to neuropsychiatric symptoms.

Proband 2, a male affected by TS associated with hypotonia, learning disabilities and ADHD, showed a *de novo* microduplication of chromosome 22q11.21, a region involved in the recurrent 22q11.2 deletion and duplication syndromes, including the DiGeorge syndrome (DGS; 188400) and velocardiofacial syndrome (VCFS; 192430. While the 22q11.2 deletion syndrome is associated with a high risk of neuropsychiatric disorders, including intellectual disability, schizophrenia, learning disabilities, attention deficit hyperactivity disorder, autism spectrum disorder, anxiety disorders, seizures and epilepsy, and early-onset Parkinson's disease (Zinkstok et al. 2019), the 22q11.2 microduplication syndrome shows a highly variable phenotype, ranging from asymptomatic to severe, characterized by intellectual disability, ADHD, and dysmorphic features. Furthermore, significant cardiac heart defects were reported on patients with 22q11.2 deletion and duplication syndromes (Portnoi, 2009; Wentzel et al., 2008). Accordingly, our patient also presented an hypomobility of interatrial septum and mild dysmorphic features. Remarkably, Clark et al. (2009) identified a 22q11.2 microduplication in a patient with TS and Klippel-Feil anomaly.

Proband 5, a female subject affected by TS, speech disorder and EEG abnormalities, has a partial duplication of the short arm of chromosome 4p14 of about 2.7 Mb, encompassing about 30 genes including *TLR1* (MIM:601194), *WDR19* (MIM:608151), *RFC1* (MIM:102579), *LIAS* (MIM:607031), *UGDH* (MIM:603370), *RHOH* (MIM:602037). Among them, *RCF1* gene is associated with cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (MIM:614575) (Thieme et al., 2022), while the *UGDH* gene was reported in patients affected by developmental and epileptic encephalopathy (Hengel et al., 2020). The duplication was also identified in our probands' father who showed intellectual disability, speech impairment and dysmorphic features. Considering the large size of duplication, and the presence of the same duplication and psychopathologies in her affected father, we classified this variation as causative.

Proband 8, a male child, presents a partial deletion of the long arm of chromosome 22 (22q11.21) of about 686 Kb, inherited by his father. The proband presented TS with comorbid OCD, speech disorder and minor dysmorphic traits. Furthermore, his father was affected by TS and OCD. The 22q11.21 deletion encompasses several genes including *SCARF2* (MIM: 613619), *PI4KA* (MIM: 600286), *SERPIND1* (MIM:142360), *SNAP29* (MIM:604202), *LZTR1* (MIM:600574). Germline loss-of-function mutations in *LZTR1* predispose to Noonan syndrome (MIM:605275) and an inherited disorder of multiple schwannomas (Burnside, 2015; Piotrowski et al. 2014). The same CNV was already reported in a patient affected by TS and OCD in a large genome-wide investigation of rare CNVs in OCD and TS (McGrath et al., 2014).

Proband 9, a male with TS in comorbid with speech delay, mild intellectual disability, OCD, ADHD, ODD, anxiety disorder and dysmorphic features, has a *de novo* partial duplication of the long arm of chromosome 7 (7q11.23) of about 1.6 Mb, that involves about 33 genes (Van der Aa et al. 2009; Dentici et al., 2020). The chromosome 7q11.23 duplication syndrome is a multisystem developmental disorder with variable manifestations, most commonly speech delay, intellectual disability, and mild craniofacial anomalies, including thin lips, broad forehead, abnormal columella, deep set eyes, such us in our proband (Van der Aa et al. 2009; Dentici et al., 2020).

Proband 12 is a boy affected by TS, intellectual disability, epilepsy, and mild dysmorphic features carrying 1.3 Mb paternally inherited duplication of chromosome 17q12, inherited from his father. The duplicated 17q12 region encompasses about 20 genes, including *HNF1B* (MIM: 189907), *ACACA* (MIM:200350), *PIGW*(MIM:610275), *ZNHIT3*(MIM:604500).

The 17q12 microduplication syndrome is associated with an increased risk for neurodevelopmental disorders, including developmental delay, ID (mild to severe), ASD, psychotic disorder, anxiety, and bipolar disorder (Mefford et al., 2007; Milone et al., 2021). In addition, patients with 17q12 duplication display a wide phenotypic spectrum due to reduced penetrance and variable expressivity (Mefford et al., 2007; Milone et al., 2021).

The proband 14, a male affected by TS, OCD, learning disorder and dysmorphic traits like turricephaly and joint hyperlaxity, has a 805 Kb deletion of chromosome 6q26 inherited by his mother. A similar CNV was already reported in a patient affected by TS and OCD in a large genome-wide investigation of rare CNVs in OCD and TS (McGrath et al., 2014). This deletion encompasses the *PARK2* gene (OMIM:602544), a neurodevelopmental gene originally discovered as one of the causes of early-onset Parkinson disease (PD) (Yin et al., 2016). More recently, this gene has been associated with schizophrenia, ASD, and attention-deficit/hyperactivity disorder (ADHD) (Yin et al. 2016). In disorder-specific analyses, the neurodevelopmental deletion burden was larger in OCD than in TS. Most frequent neurodevelopmental CNVs have been identified at 16p13.11 and 22q11 regions (McGrath et al. 2014).

Both proband 15, a boy affected by TS, OCD, behavioral problems, and mild dysmorphic features, and proband 19, a girl affected by TS, mild intellectual disability, and ODD, present the recurrent 15q11.2 deletion of about 395 Kb, which includes *TUBGCP5, CYFIP1, NIPA1* and *NIPA2* genes. Three of these genes are widely expressed in the central nervous system (*CYFIP1, NIPA1, NIPA2*), while *TUBGCP5* gene is specifically expressed in the subthalamic nuclei (Butler, 2017). Neuropsychiatric disorders and mild dysmorphic features with incomplete penetrance and variable expressivity are associated with genomic imbalances of the 15q11.2 BP1–BP2 region (Cox & Butler, 2015).

Proband 16, a male subject affected by TS, mild intellectual disability, ODD and MRI abnormalities, has a duplication of about 2.8 Mb of chromosome 5q33.3-q34 involving several genes including *GABRB2* (MIM:600232), *GABRA1*(MIM:137160), *GABRG2* (MIM:137164). These genes are associated with developmental and epileptic encephalopathy (Johannesen et al., 2016; Shen et al., 2017; Yang et al., 2020). In this case, we could not ascertain the inheritance of the rearrangement, because of lack of both parents' sample. To date, this imbalance is not clearly associated with a typical clinical phenotype. However, its role as a contributing cause of disease cannot be excluded.

Then last proband with C-CNV, a male child with TS, severe intellectual disability, ASD and mild dysmorphisms features, carried an interstitial deletion of chromosome of 116p13.3 inherited by his father. Loss of the 16p13.3 locus partially includes the RBFOX1, a gene encoding an RNA binding protein that has consistently been associated with aggressive and antisocial behavior (Vaht et al., 2020). In addition, a recent study suggests a significant role of RBFOX1 gene in ASD susceptibility (Bacchelli et al., 2020).

Several studies have investigated the presence and importance of causative CNVs in TS. Approximately 1% of patients with TS carry one known or potentially pathogenic CNV. Overall, chromosomal structural variations and extensive CNV increases, which are very important in many common and rare clinical diseases, also have a significant impact on the genetic structure of TS (Lin et al., 2022).

In the present series, we found about 28% of patients with C-CNV with seizures and isolated epileptiform EEG changes while 37% with C-CNV patients showed qualitative anomalies of clinical MRI. Data on the prevalence of epilepsy and EEG abnormalities in TS are very heterogeneous. Patients with Tourette syndrome have a higher rate of epilepsy, and are also sometimes misdiagnosed with seizures. Only few studies have reported an association between Tourette syndrome and epilepsy (Diamond, et al., 2006; Doherty & Sloan, 2010; Rizzo, et al., 2010). Rizzo et al. (2010) reported a case series of eight patients with Tourette syndrome presenting with comorbidid ADHD and epilepsy. Most of these patients developed epilepsy before the onset of Tourette syndrome, which was easily controlled by medications (Rizzo et al., 2010). Wong et al. (2016), showed that the incidence rate of epilepsy in the Tourette syndrome group was 4.0%, whereas that in the control group was 0.2% ($P < 0.001$). On the other hand, 37% patients with C-CNV showed anomalies of clinical MRI. It has been suggested that TS arises from neurobiological abnormalities, of which imaging studies have revealed many relevant clues (Albin & Mink, 2006). It has been demonstrated that TS patients in structural imaging studies can present smaller corpus-callosum volumes and thinner sensorimotor cortices, like

our probands #5, #16 and #18. Furthermore, some of these regions with grey matter abnormalities may be associated with cortico-striato-thalamo-cortical (CSTC) circuits in TS.

As to neurobehavioral features, patients with TS harboring a C-CNV had more severe comorbid conditions including ADHD, OCD and ODD than individuals with NC-CNV or W-CNV. Indeed, most of the clinical variants founded in our cohort are just associated with other comorbidities (e.g., ADHD, conduct disorder) rather than TS in literature studies, probably because the diagnosis of TS is often underestimated respect on other neurological or psychiatric disorders.

The results of this study also showed that rare deletions and duplications highlighting relevant genes for neurodevelopment had a statistically higher occurrence in children with tics and additional comorbidities. In our cohort we determined an incidence of CNV-C of about 12%. Our result is higher in respect of another previous study conducted by Sundaram et al (2010) on a cohort of 111 TS patients, that showed an incidence of about 9% of significant CNVs. Moreover, most of the CNVs identified in our TS patients, such as the 22q11.21 microduplication, the 17q12 duplication, and the 15q11.2 deletion, show incomplete penetrance and variable expressivity and are associated with different clinical presentations in the literature.

3. Exome-based study

3.1 Materials and Methods

3.1.1 Participants

This study was conducted at the Child and Adolescent Neurology and Psychiatry of the Medical and Experimental Department, Catania University. A total of 80 patients (average age 12.84, M: F 70:10), with a clinical diagnosis of TS according to DSM-5 criteria, have been enrolled for the study between April 2023 and April 2025.

We excluded patients with a primary psychiatric disorder different from TS, and/or presence of known etiology such as genetic or inherited metabolic diseases. Each participant was clinically assessed for tics and associated comorbidities. Investigations were carried out as part of the routine clinical care of the patients in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Prior to enrolment, written informed consent was obtained from all participants' parent or legal guardian. The study was approved by the local Ethics Committee at our institution.

3.1.2 Procedures

Medical history was obtained from participant's parents with a focus on developmental delay, seizures, neuropsychiatric disorders, and positive family history for neurodevelopmental disorder and metabolic diseases. All participants underwent physical and neurological examination. Fasting blood samples and urine samples were collected for routine blood analyses and to rule out inherited metabolic diseases. The occurrence of epileptic seizures and isolated electroencephalogram (EEG) anomalies were evaluated. Moreover, brain magnetic resonance imaging (MRI) using a 1.5 T MRI scanner, was examined to detect morphologically visible signs of altered brain development and atrophic alterations.

3.1.3 Clinical assessment

All participants underwent a full neuropsychiatric assessment for TS and related comorbidities. According to participants' ages, the Wechsler Intelligence Scale for Children (WISC-IV) was administered to evaluate the intelligence quotient (IQ) of patients (Wechsler, 2012). Symptoms of TS were evaluated using the Yale Global Tic Severity Rating Scale (YGTSS) (Leckman et al., 1989), a clinician-rated scale used to assess the motor and phonic tic severity considering the number, frequency, duration, intensity, and complexity of tics.

3.1.4 Genetic analysis

A multigene (exome-based) analysis was performed on DNA extracted from peripheral blood samples of the probands and the parents using the standard phenol extraction protocol. Library preparation for sequencing analysis was carried out using the SureSelect All Exon v6 kit (Agilent Technologies, Santa Clara, CA, USA), following the manufacturer's instructions, with a focus on Neurodevelopmental Disorders (including both SNVs and CNVs) and Extrapyramidal Movement Disorders (SNVs and CNVs).

Sequencing was conducted by using NGS technology with 100 bp paired-end reads and HiSeq 2500 platform (Illumina).

Bioinformatic analysis included sequence alignment to the human reference genome GRCh37/hg19 using the BWA algorithm, removal of non-specific alignments, variant calling through GATK**,** samtools**,** and bcftools, and quality control of target gene coverage and read depth within the requested gene panel. The clinical exome analysis examined more than 1,250 primary genes and over 1,100 candidate genes associated with neurodevelopmental disorders. Variant validation was performed using Sanger sequencing.

Variant interpretation was conducted according to the 2015 ACMG (American College of Medical Genetics and Genomics) guidelines. Identified variants were annotated using population databases such as GnomAD v.4.1.0, Exome Aggregation Consortium (ExAC), 1000 genome database, Exome Variant Server, and clinical genetic databases (i.e. OMIM, ClinVar, HGMD, GWAS). Each variant was subjected to in silico analysis to predict its impact on protein structure and/or function, as well as its evolutionary conservation, using tools such as PolyPhen-2, SIFT, CADD, MutationTaster, MutationAssessor, and PhyloP.

3.1.5 Exome data analysis and variants classification

"Potentially Causative" variants (PC-V), "Non-causative Variants" (NC-V) or "VOUS variants" (V-V), were established based on the scientific literature and on the careful consultation of public and private databases such as the database of genomic variants (http://dgv.tcag.ca/dgv/app/home), the Decipher database (https://decipher.sanger.ac.uk/), the database of human genomic structural variation (https://www.ncbi.nlm.nih.gov/dbvar), and the OMIM catalogue (http://www.ncbi.nlm.nih.gov/omim). In the present study we considered potentially pathogenic all the variants described as associated with TS in the OMIM database. Further, we included in the VOUS variants, those having a more uncertain role in the disease, but sporadically associated with TS in the literature or affecting genes known to be linked to TS or other neuropsychiatric conditions. The "non-causative" variants have never been described in literature or never reported in association with TS. When a VOUS or potentially causative variants were found, the study of the parents was requested to ascertain its origin and decide the strategy for family management and future genetic counselling. There were cases where the origin of the imbalances was not possible to determine, either because of unwillingness of both parents to be evaluated, in situations of adoption or in mono-parental subjects in which the available progenitor was not a carrier of the proband's variant.

3.1.6 Statistical analysis

Children with PC-Variants, VOUS-Variants and NC-Variants were statistically compared to verify the effect of presence/absence of variants on dysmorphic features, comorbidities, ID, YGTSS scores, as well as on the rate of epilepsy, EEG anomalies and brain MRI anomalies. Clinical characteristics of participants were summarized by randomized group using mean (SD) for continuous data or count and percentage (%) for categorical data. Pairwise comparisons were performed using one-way ANOVA or the Kruskal–Wallis test for continuous variables, according to data distribution, and the

chi-square test (χ^2) for categorical variables. The Kolmogorov-Smirnov test was applied to assess data distribution and guide the choice between parametric and non-parametric methods. A p-value < 0.05 was considered to indicate statistical significance. When the χ^2 test or ANOVA/Kruskal-Wallis test showed significant results, post hoc pairwise comparisons were conducted, with a Bonferroni correction applied to control for potential inflation of Type I error. After this adjustment, the significance threshold (alpha) was set at 0.017. Data were analyzed using GraphPad Prism version 10.0.0 (GraphPad Software, San Diego, CA, USA).

3.2 Results

3.2.1 Genetic Findings

Among the 80 children with TS, 11 patients (8 males and 3 females) were classified as having PC-Vs (13.75%), 29 patients (25 males and 4 females) as having V-Vs (36.25%), and 40 patients (37 males and 3 females) as carrying NC-Vs (50%) (Table 4).

Table 4. Demographic and genetic characteristics of TS patients. Abbreviations: TS, Tourette Syndrome; PC-CNVs, potentially causative variants; V-V, VOUS variants; NC-CNVs, non-causative variants; F, female; M, male; *n*, number; SD, standard deviation.

	Patients (*n*)	**Age (Years) (mean ± SD)**	**M: F Ratio**	**Percentage/all**
All	80	12.84 ± 4.65	70:10	100 %
Patients with PC-Vs	11	14.45 ± 5.32	8:3	13.75 %
Patients with V-Vs	29	12.03 ± 4	25:4	36.25 %
Patients with NC-Vs	40	12.98 ± 4.88	37:3	50 %

Eleven variants were classified as pathogenic/causative CNVs, six of which were identified as *de novo*. The clinical and genetic features associated with PC-Vs are presented in Table 5.

Table 5. Clinical and molecular characteristics associated with PC-CNVs in patients with TS.

Patient ID	Gene involved	Type of Variant	Clinical features
Patient_1	***KMT2C***	*De novo* frameshift, c.10400dup p.(Pro3468ThrfsTer27)	Low IQ (72), dysmorphic features, ADHD
Patient_2	***PNKD***	Heterozygous deletion, c.76_103del p.(Ala26IlefsTer38)	MRI anomalies, EEG anomalies, OCD, anxiety
Patient_3	***BRPF1***	*De novo* missense, c.1545C>G p.(Asp515Glu)	ADHD
Patient_4	***PNKD***	heterozygous missense, c.1126C>T	No major findings
Patient_5	***SMARCA2***	*De novo* missense, c.1279C>T p.(Arg427Cys)	Dysmorphic features, specific learning disorder
Patient_6	***CACNA1D*** ***RERE***	Heterozygous missense, c.3439G>A p.(Glu1147Lys) in *CACNA1D* gene; Heterozygous in-frame deletion, c.3925_3954del p.(Ile1309_Glu1318del) in *RERE* gene	Developmental delay, epilepsy, ADHD, Intellectual disability, dysmorphic features, behavioral problems
Patient_7	***MAPK8IP3***	*De novo* missense, c.3299G>T p.(Arg1100Leu)	Language disorder, motor tics, learning difficulties
Patient_8	***SLC20A2***	Heterozygous missense, c.541C>T p.(Arg181Trp)	MRI anomalies, dysmorphic features, OCD
Patient_9	***PTEN***	*De novo* nonsense, c.1003C>T p.(Arg335Ter)	Low IQ (66), MRI anomalies, dysmorphic features, ADHD
Patient_10	***ANKRD17***	*De novo* variants	Low IQ (75), dysmorphic features, motor/vocal tics within a broader neurodevelopmental syndrome
Patient_11	***SLC6A1***	*De novo* missense, c.1195C>G p.(Cys399Arg)	Epilepsy, anxiety, OCD, ADHD

3.2.2 Participants' Characteristics

In this study, we recruited 80 patients (mean age = 12.84 ± 4.65; male/female = 70/10) with a clinical diagnosis of TS, as determined by the Yale Global Tic Severity Rating Scale (YGTSS). Table 6 reports the results of χ^2 tests and ANOVA/Kruskal–Wallis analyses for the clinical and neuropsychiatric

features of TS participants, categorized by variant status. Significant pairwise differences are illustrated in Figure 1.

Table 6. Somatic and neuropsychiatric features in patients with TS according to variant classification. Abbreviations: PC-CNVs, potentially causative variants; V-V, VOUS variants; NC-CNVs, non-causative variants; Y.G.T.S.S., Yale Global Tic Severity Scale; IQ, intelligence quotient; OCD, Obsessive-Compulsive Disorder; ODD, Oppositional Defiant Disorder; EEG, electroencephalography; MRI, Magnetic Resonance Imaging. Superscript "a" indicates results obtained with the Chi-square (χ^2) test; superscript "b" indicates results obtained with one-way analysis of variance (ANOVA); superscript "c" indicates results obtained with Kruskall-Wallis test. Data are presented as means ± standard deviations or as counts and percentages. Statistically significant values ($p < 0.05$) are indicated in bold.

	PC-Vs (11)	**V-Vs (29)**	**NC-Vs (40)**	***p-value***
Y.G.T.S.S.	28 ± 8.21	24.17 ± 7.7	18.45 ± 6.98	**0.001**[c]
Brain MRI anomalies	3 (27.27%)	10 (34.5%)	9 (22.5%)	0.546[a]
EEG anomalies	1 (9.09%)	6 (20.7 %)	3 (7.5 %)	0.245[a]
Dysmorphic features	6 (54.55%)	18 (62.1%)	18 (45%)	0.371[a]
IQ	67.55 ± 31.02	90.79 ± 23.08	86.36 ± 20.25	**0.020**[b]
OCD	2 (18.18%)	11 (37.9%)	17 (42.5%)	0.336[a]
ADHD	3 (27.27%)	6 (20.7%)	6 (15%)	0.617[a]
ODD	0	2 (6.9%)	2 (5%)	0.671[a]
Headache	0	0	2 (5%)	0.359[a]
Anxiety	2 (18.18)	7 (24.1%)	8 (20%)	0.885[a]
Presence of comorbidities	3 (27.27%)	17 (58.6 %)	21 (52.5%)	0.203[a]
Family history of tics	3 (27.27%)	8 (27.6%)	22 (55%)	**0.044**[a]
Parental mutations	5 (45.45%)	21 (72.4%)	22 (55%)	0.197[a]

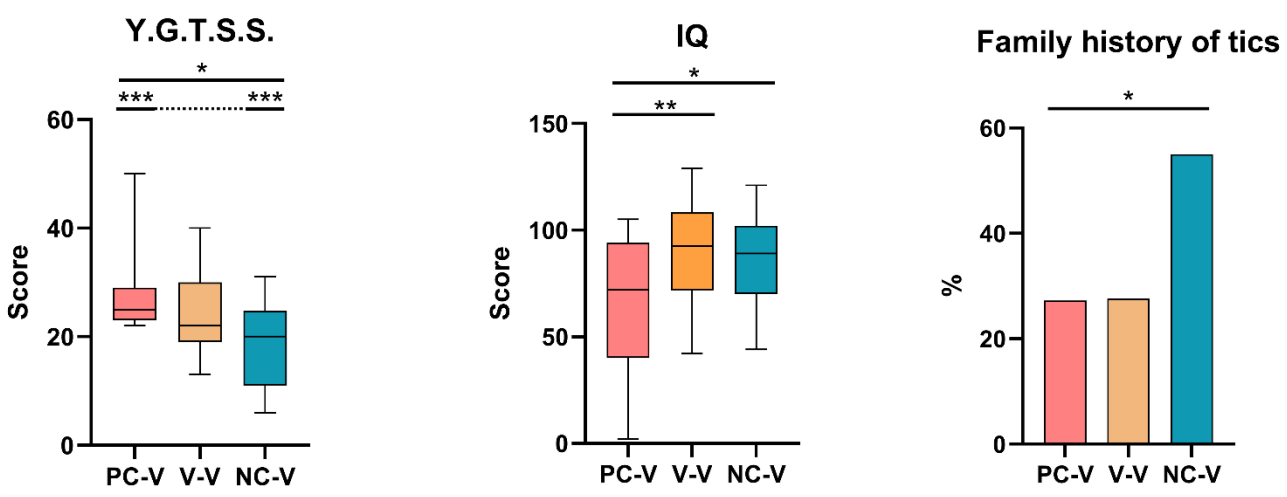

Figure 1. Clinical variables with statistically significant differences among TS children, according to variant classification. Paired post hoc comparisons with Bonferroni adjustment were used. * $p < 0.05$; ** $p < 0.017$; *** $p < 0.008$.

PC-CNVs, potentially causative variants; V-Vs, VOUS variants; NC-CNVs, non-causative variants.

3.2.3 Tic severity

The severity of tic symptoms, assessed with the Yale Global Tic Severity Scale (YGTSS), showed a progressive gradient: children with potentially causative variants (PC-Vs) exhibited the highest mean scores (28 ± 8.21), followed by those with variants of uncertain significance (VOUS, 24.17 ± 7.7), and finally the non-causative group (NC-Vs), which displayed the lowest scores (18.45 ± 6.98). The intergroup difference reached statistical significance ($p = 0.001$), indicating that the presence of potentially pathogenic variants is associated with greater clinical burden in terms of tic manifestation.

3.2.4 Cognitive evaluation

Mean IQ was markedly reduced in the PC-V group (67.55 ± 31.02), compared with both VOUS (90.79 ± 23.08) and NC-Vs (86.36 ± 20.25). The differences between groups were statistically significant ($p = 0.020$), suggesting that rare high-impact variants may contribute not only to tic severity but also to broader neurodevelopmental impairment.

3.2.5 EEG Anomalies

Electroencephalographic abnormalities were documented in a minority of patients, with frequencies ranging from 7.5% in NC-Vs to 20.7% in VOUS and 9.1% in PC-Vs. Despite these variations, the overall comparison did not reach statistical significance ($p = 0.245$), suggesting that EEG alterations are not systematically linked to variant type in this cohort.

3.2.6 MRI analysis

Structural brain anomalies identified on MRI were relatively common but comparably distributed across groups. They were present in 27.3% of PC-V patients, 34.5% of VOUS, and 22.5% of NC-Vs, without significant group differences ($p = 0.546$). These findings suggest that, while MRI alterations are not uncommon in TS, they do not appear to segregate according to the genetic classification adopted in this study.

3.2.7 Physical Measures

Dysmorphic features were observed with notable frequency across all subgroups, ranging from 45% in NC-Vs to 62.1% in VOUS carriers. However, these variations did not reach statistical significance ($p = 0.371$). This indicates that, although dysmorphisms are frequent in pediatric TS patients, they cannot be specifically attributed to one genetic subgroup.

3.2.8 Comorbidities

Psychiatric comorbidities were highly prevalent in the cohort, though their distribution did not significantly differ among genetic subgroups. Obsessive–compulsive disorder (OCD) was most

frequently reported in the NC-V group (42.5%), followed by VOUS (37.9%), and less commonly in PC-Vs (18.2%) (p = 0.336). Attention-deficit/hyperactivity disorder (ADHD) showed the opposite trend, being most frequent among PC-V carriers (27.3%), compared with 20.7% in VOUS and 15% in NC-Vs (p = 0.617). Other comorbidities such as oppositional defiant disorder (ODD), anxiety, and headache occurred at lower rates overall, without significant intergroup differences. The overall prevalence of psychiatric comorbidity was highest in the VOUS (58.6%) and NC-Vs (52.5%) subgroups, compared to 27.3% in the PC-V group (p = 0.203).

3.2.9 Family history of tics

A statistically significant difference emerged when analyzing the distribution of family history of tics across the three subgroups (p = 0.044). In the PC-V and VOUS groups, familial aggregation was relatively uncommon, being reported in only 27.3% and 27.6% of patients, respectively. In contrast, more than half of the NC-V subgroup (55%) had at least one family member with a history of tic disorders.

3.2 Discussion

By integrating genetic findings with detailed neuropsychiatric assessments, this study aimed to providing novel insights into the contribution of rare exome variants, including both *de novo* and inherited mutations, to the clinical heterogeneity of Tourette syndrome.

Our cohort of 80 children and adolescents with TS was characterized by a predominance of males (male-to-female ratio 7:1) and a mean age of 12.8 years, in line with epidemiological data showing the higher prevalence and earlier onset of tics in boys (Robertson, 2008; Scharf et al., 2015). Across the sample, tics ranged from mild to severe, and psychiatric comorbidities such as obsessive–compulsive disorder (OCD), attention-deficit/hyperactivity disorder (ADHD), anxiety, and oppositional defiant disorder (ODD) were frequent, reflecting the well-established clinical spectrum

of TS (Billnitzer & Jankovic, 2020). Dysmorphic traits, intellectual disability, and epilepsy were also observed in a relevant proportion of patients, especially those carrying potentially causative variants (PC-Vs). MRI anomalies were present in about a quarter of the cohort, and EEG abnormalities in around 10–20%, findings consistent with the variable but nonspecific neurological alterations described in the literature (Szejko et al., 2021).

Genetic subgrouping revealed clear clinical distinctions. Children with PC-Vs displayed the highest tic severity scores on the Yale Global Tic Severity Scale (YGTSS), along with significantly lower IQ values compared to those with VOUS or non-causative variants. In contrast, patients without candidate variants more often reported a positive family history of tics, supporting the hypothesis that inherited polygenic burden drives familial clustering, while *de novo* high-effect variants underlie more severe simplex and syndromic presentations. These demographic and clinical patterns reinforce the dual model of TS genetic architecture, in which rare variants act as strong risk factors for severe phenotypes, and common variants contribute to broader familial risk (Willsey et al., 2017; Wang et al., 2018; Zhao et al., 2020).

Building on these observations, the detailed analysis of individual PC-V cases provided further insight into how specific genes contribute to tic severity, cognitive outcome, and associated comorbidities (Table 5).

Patient 1, carrying a *de novo* truncating variant in *KMT2C*, presented with borderline intellectual functioning, dysmorphic features, ADHD, and Tourette syndrome. Pathogenic *KMT2C* variants have been associated with developmental delay and dysmorphism (Faundes et al., 2018; Satterstrom et al., 2020), but tics have not been systematically described, suggesting that our case expands the known phenotype. Patient 2, harboring a truncating variant in *PNKD*, showed severe tics with OCD and anxiety but preserved cognition, a pattern strikingly similar to the familial cases reported by Sun et al. (2018).

By contrast, Patient 4, with a missense variant in the same gene, displayed only moderate tics without comorbidities, exemplifying the uncertainty in interpreting non-truncating variants in synaptic genes.

Variants in chromatin regulators also emerged as relevant contributors. Patient 3, with a *de novo* *BRPF1* variant, presented with ADHD and tics but preserved cognition and no dysmorphism, diverging from the more syndromic presentations described in the literature (Yan et al., 2017). Patient 5 carried a *SMARCA2* missense variant, typically linked to Nicolaides–Baraitser syndrome with severe intellectual disability and seizures (Santen et al., 2013), but in our case associated with preserved IQ, a learning disorder, and moderate tics. These cases illustrate how chromatin-remodeling genes, while generally linked to syndromic neurodevelopmental disorders, may predispose to tic phenotypes in milder allelic presentations.

Some patients presented complex phenotypes involving multiple or pleiotropic gene effects. Patient 6 carried dual variants in *CACNA1D* and *RERE* and exhibited developmental delay, epilepsy, and tics. Both genes are individually implicated in neurodevelopmental disorders (Fregeau et al., 2016; Pinggera et al., 2017), and their co-occurrence suggests additive or oligogenic effects, as previously hypothesized in TS cohorts with multiple rare variants (Willsey et al., 2017).

Similarly, Patient 7 carried a *de novo* variant in *MAPK8IP3*, a gene associated with intellectual disability and axonal transport dysfunction (Platzer et al., 2019). Although tics have not been reported in *MAPK8IP3*-related disorders, their presence in our case suggests that axonal transport defects may also predispose to movement disorders, extending the clinical spectrum of this condition.

Other findings highlighted interpretative challenges. Patient 8 harbored a heterozygous missense variant in *SLC20A2*, classically linked to adult-onset primary familial brain calcification (Wang et al., 2012). In this child, the absence of calcifications renders the variant more consistent with a VOUS, analogous to CNV findings in pediatric TS where variants in late-onset genes are difficult to classify. In contrast, Patient 9 carried a *de novo PTEN* nonsense mutation and exhibited macrocephaly, dysmorphism, intellectual disability, ADHD, and tics, a constellation highly consistent with PTEN hamartoma tumor syndrome (Yehia et al., 2020). Patient 10, with a *de novo ANKRD17* variant, showed dysmorphism, borderline cognition, and tics. While *ANKRD17* has been linked to intellectual

disability and behavioral disturbances (Soden et al., 2014), tics have not been reported, suggesting an expansion of the phenotypic spectrum.

Finally, Patient 11 carried a *de novo* missense variant in *SLC6A1* and presented with epilepsy, ADHD, OCD, anxiety, and tics. Pathogenic *SLC6A1* variants are well-recognized causes of epilepsy with developmental delay and psychiatric comorbidities (Johannesen et al., 2018). The strong phenotypic overlap with published cases supports impaired GABAergic inhibition as a convergent mechanism in Tourette syndrome (Willsey et al., 2017).

Taken together, these cases highlight recurring themes: truncating and *de novo* variants in constrained genes are associated with more severe and syndromic phenotypes, while inherited or missense variants remain more challenging to interpret but may still act as modifiers. The overlap with genes implicated in broader neurodevelopmental syndromes underscores the pleiotropy and shared vulnerability across psychiatric and neurodevelopmental disorders. Psychiatric comorbidities such as OCD, ADHD, and anxiety were frequent in all groups but did not significantly differ by variant type, suggesting they are more strongly influenced by polygenic and environmental factors (Billnitzer & Jankovic, 2020). Likewise, MRI and EEG anomalies did not differentiate between genetic subgroups, consistent with clinical guidelines that do not recommend routine imaging in typical TS (Szejko et al., 2021).

Our findings underscore the complementary contributions of rare high-effect variants and inherited polygenic burden in the pathogenesis of Tourette syndrome. Patients with potentially pathogenic variants tend to exhibit higher tic severity, lower cognitive scores, and syndromic features, while familial cases without such variants reflect polygenic inheritance. Comprehensive genomic testing is particularly warranted in patients with atypical or syndromic features, while future studies integrating rare and common variant analyses with longitudinal phenotyping will be critical to refine risk stratification and develop mechanism-driven interventions.

4. Integrated Conclusions and Unified Perspectives

The first study of this project aimed to systematically investigate the contribution of copy number variants (CNVs) to the genetic architecture of Tourette Syndrome in a pediatric cohort. Using array-CGH, we sought to detect recurrent or novel structural imbalances, assess their correlation with clinical phenotypes, and identify overlaps with loci previously associated with neurodevelopmental disorders. The second study, presented in this thesis, focused instead on rare exome variants identified through trio-based whole-exome sequencing (WES). The objective was to evaluate both inherited and *de novo* variants, clarify their potential role in the pathogenesis of TS, and refine genotype–phenotype correlations through systematic variant classification and pathway analysis. Taken together, the overarching goal of this research was to integrate complementary genomic approaches, structural (CNV) and sequence-level (WES), to obtain a more comprehensive understanding of the genetic basis of Tourette Syndrome. By combining these methods, we aimed not only to disentangle the relative contribution of rare high-impact variants but also to explore convergent biological pathways and to position TS within the broader neurodevelopmental spectrum. The diagnostic yield was consistent between CNV (11.8%) and WES (13.7%) cohorts, in line with previous reports highlighting the complementary utility of these approaches in neurodevelopmental disorders (Coe et al., 2014; Deciphering Developmental Disorders Study, 2017). *De novo* variants accounted for nearly half of pathogenic findings (45–55%), corroborating prior evidence of their enrichment in simplex and syndromic presentations (Willsey et al., 2017; Wang et al., 2018; Iossifov et al., 2014; Sanders et al., 2015). Inherited variants, including recurrent CNVs (22q11.21) and missense variants in chromatin remodelers, frequently showed incomplete penetrance, echoing the complex inheritance patterns already described in other neurodevelopmental conditions (McGrath et al., 2014; Zinkstok et al., 2019). Pathway-level convergence was observed in chromatin remodeling (KMT2C, BRPF1, SMARCA2), synaptic and GABAergic signaling (PNKD, SLC6A1, CACNA1D), and structural hotspots with pleiotropy (22q11.21, 15q11.2, 17q12, 6q26). These overlaps mirror findings in autism,

epilepsy, and intellectual disability (Satterstrom et al., 2020; de Rubeis et al., 2014; Shen et al., 2017), reinforcing the pleiotropy of rare high-effect variants (Vallianatos & Iwase, 2015) and supporting the interpretation of TS as part of a shared neurodevelopmental continuum (Mataix-Cols et al., 2015; Robertson, 2015). Recent GWAS and transcriptomic studies have provided additional insight. For example, a large-scale TWAS highlighted FLT3 expression in the dorsolateral prefrontal cortex and immune-related splicing alterations in TS (Liao et al., 2022). Pathway analyses have linked TS-associated variants to ligand-gated ion channel signaling and cell adhesion, reinforcing GABAergic involvement (Tsetsos et al., 2021). A Taiwanese GWAS identified a novel locus at DRAM1 (12q23.2), suggesting a role for autophagy and apoptosis (Lin et al., 2024). Meta-analyses also show TS polygenic risk is enriched in loss-of-function–intolerant genes and neurodevelopmental pathways (Strom et al., 2025). Of particular relevance, rare *de novo* variants affecting neuronal cell polarity have been implicated in TS, pointing to disrupted polarity as a mechanism for altered cortical wiring (Wang et al., 2018). This aligns with broader findings on the role of polarity proteins in cortical development and neurodevelopmental disease (Hakanen et al., 2019). Clinically, these findings underline the importance of genomic testing in TS, especially in children with syndromic or atypical features. Early identification of pathogenic variants enables refined diagnosis, prognosis, and tailored surveillance for comorbidities such as epilepsy, intellectual disability, and psychiatric disorders (Billnitzer & Jankovic, 2020; Robertson et al., 2017). However, the detection of inherited variants with incomplete penetrance necessitates nuanced counselling strategies (Leblond et al., 2012; Girirajan et al., 2013). Our study has limitations: the cohorts were recruited separately, parental samples were sometimes unavailable, and whole-genome sequencing was not performed, limiting detection of non-coding or complex rearrangements (Huang et al., 2017; Werling et al., 2018). Functional validation was beyond scope but remains crucial to establish pathogenicity (Kearney et al., 2011; Srivastava et al., 2019). Despite these caveats, the findings support a dual genetic model of TS, in which rare high-impact variants contribute to severe and sporadic cases, while polygenic inheritance accounts for familial clustering of milder forms (Willsey et al., 2017; Yu et al., 2019).

This duality resonates with recent insights from international consortia, including TIC Genetics and the PGC (Dietrich et al., 2022; Paschou et al., 2022). Looking ahead, future research should integrate polygenic risk scores with rare variant analyses to refine stratification, expand multicenter pediatric cohorts for statistical power, and adopt functional approaches—including transcriptomics, proteomics, iPSC-derived neuronal models, and brain organoids—to clarify how candidate variants impact neurodevelopment, synaptic function, and neuronal polarity. Ultimately, such strategies may pave the way toward precision medicine in Tourette Syndrome, offering better risk prediction, personalized clinical management, and eventually pathway-directed therapies targeting chromatin remodeling, GABAergic transmission, or cell polarity mechanisms (Pinggera et al., 2017; Yehia et al., 2020).

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