Supplementary Material – Additional file 1

Twin Research and Human Genetics

Copy Number Variation Analysis of 100 Twin Pairs Enriched for Neurodevelopmental Disorders

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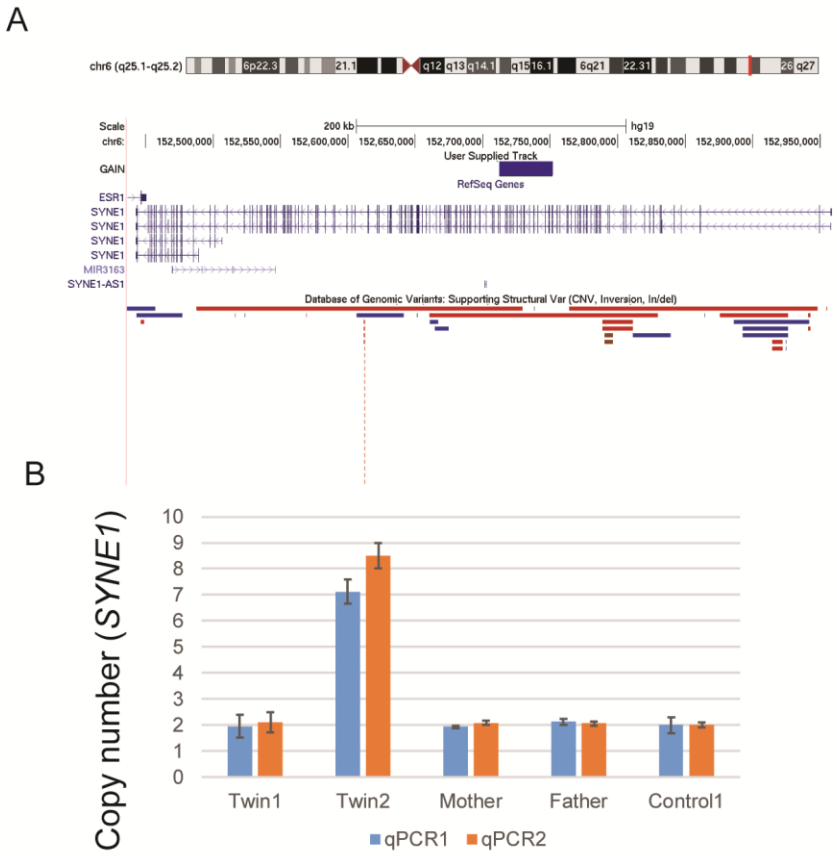
Supplementary Figure



Supplementary Figure S1. Visualization and validation of 39 kb gain on chr6q25.2 (GRCh37/hg19, chr6:152,712,302-152,751,762) present in a male twin (twin 2) with autism spectrum disorder and intellectual disability from dizygotic discordant twin pair 31. A) Visualization of the chromosome region affected by the gain using UCSC genome browser. CNVs present in the region are shown from the Database of Genomic Variants tract (blue boxes represent gains and red boxes represent losses present in control samples). B) Quantitative PCR (qPCR) validation of the gain the family and unrelated male control. The qPCR was performed twice in quadruplicates. Probe targeting *TERT* was used as a reference.

Supplementary Tables

Supplementary Table S1. Predicted pre- and postzygotic *de novo* copy number variants in the analyzed 18 families and 55 MZ twins from the sample.

Prediction	Family	twin nr	Zygosity	Primary concordance	chr	region	start	end	CNV	Size	Genes	qPCR validation, inheritance
prezygotic *de novo (DZ pair)*	61	3	DZ	Discordant ASD/Discordant NDD	7		100721337	100732844	Loss	11,508	TRIM56	Not validated, False positive in microarray
prezygotic *de novo*	12	1, 2	MZ	Discordant NDD (ADHD)	8		68277883	68352280	Gain	74,398	CPA6	Validated, found in both twins, maternal
postzygotic *de novo*	40	1	MZ	Discordant ASD/Discordant NDD	15		31350121	31376365	Gain	26,245	MIR211;TRPM1	Validated, found in both twins
postzygotic *de novo*	40	2	MZ	Discordant ASD/Discordant NDD	2		186617835	186673830	Gain	55,996	FSIP2	Not validated, false positive
postzygotic *de novo*	40	2	MZ	Discordant ASD/Discordant NDD	6		49825048	49937323	Gain	112,276	DEFB114;CRISP1;DEFB113;DEFB133	Not validated, false positive
postzygotic *de novo*	50	1	MZ	Concordant ASD/Concordant NDD	13		21742287	21780383	Loss	38,097	SKA3;MRPL57	Not validated, false positive
postzygotic *de novo*	64	1	MZ	Discordant NDD (ADHD)	9		140395219	140417248	Gain	22,030	PNPLA7	Validated in both twins

Supplementary Table S2. Copy number variant concordance of the different variant calling algorithms and the stringent CNVs used for the study.

Algorithm	Number of pairs with 100% CNV concordance	Percentage of pairs with 100% CNV concordance	Mean % of CNV concordance within the twin pairs	Mean number of shared CNVs	Mean number of CNVs in Twin 1	Mean number of CNVs in Twin 2
PennCNV	2	3.6%	30.5	3.9	22.2	22.5
QuantiSNP	4	7.3%	37.8	4.1	13	14.2
iPattern	13	23.6%	65.3	4.8	6.3	7
iPsychCNV*	0	0	13.6	6.4	41.3	34.3
Stringent CNVs	**16**	**29.1%**	**64.2**	**2.2**	**3.1**	**2.9**
Stringent CNVs (≥100 Kbp)	**24**	**43.6%**	**73.35**	**1.6**	**1.9**	**2.06**

*iPsychCNV did not call any CNVs in 15 MZ pairs.

Supplementary Table S3. All rare stringent copy number variants in the study samples after quality control.

See Additional file 2

Supplementary Table S4. The number of rare copy number variants overlapping genes implicated in the abnormality of nervous system in the Human Phenotype Ontology (HPO-NS) and genes earlier implicated in neurodevelopmental disorders (see methods in the main article). Within-pair comparison for the rates within the dizygotic (DZ) discordant pairs were done using McNemar's pair test.

	Discordant ASD		**Within-pair comparison**	**Discordant NDD**		**Within-pair comparison**
	Proband (N=13)	**Co-twin (N=13)**		**Proband (N=15)**	**Co-twin (N=15)**	
Number of rare CNVs overlapping genes in HPO-NS	2	2	p-value=1 OR=1, 95% CI 0.01-78.49	1	2	p-value=1 OR=0, 95% CI 0-39
Number of rare CNVs overlapping genes earlier implicated in NDDs	3	1	p-value=0.5 OR=Inf, 95% CI 0.18-Inf	2	1	p-value=1 OR=Inf, 95% CI 0.02-Inf