nature medicine

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41591-019-0711-0

In the format provided by the authors and unedited.

Autism risk in offspring can be assessed through quantification of male sperm mosaicism

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Supplementary Information

Clinical Notes

Likely Pathogenic Variants in F01-F20

All of the variants were prioritized using published approaches (Brandler et al., 2018; Brandler et al., 2016; Iossifov et al., 2014), AMCG guidelines (Kleinberger et al., 2016; Richards et al., 2015), and previously published literature where possible. All dSNVs fulfilled ACMG-classification of pathogenic or likely pathogenic. All dSV would be classified as being of unknown significance – likely pathogenic (Kearney et al., 2011).

F01: *CACNG2*, 22q12.3 Del
Has been previously reported in intellectual disability (Hamdan et al., 2011); many members of the calcium channel family have been implicated in ASD; high likelihood of impact; reported by us previously (Brandler et al., 2016).

F02: *GIGYF1*, stop-gain
Listed as score 3 gene in SFARI GENE (suggestive evidence).

F03: GTF2I, stop-gain
Listed as score 4 gene in SFARI GENE (minimal evidence).

F04: SLC35A2, start-loss
SLC35A3 has been implicated in ASD; note that we were unable to design a ddPCR assay for this variant and did not include it in our analyses.

F05: *TSC2*, missense/splice site mutation
Involved in various disorders; listed as score 3S in SFARI GENE (suggestive evidence, syndromic).

F06: 1p36.32 Dup
Large effect size duplication; complex rearrangement of this locus was connected to developmental phenotypes with variable expressivity (Tonk et al., 2005).

F07: *SON*, stop-gain
Strongly implicated in the autosomal dominant ZTTK syndrome (Zhu et al., 2015), for which intellectual disability has been described.

F08: *DCHS1*, missense
Homozygous mutations cause Van Maldergem syndrome 1 which includes autistic features (Cappello et al., 2013); heterozygous variants have been described in autistic females (Butler et al., 2015).

F09: *GRIN2A*, splice site mutation
Multiple reports of this specific mutation in a range of neurodevelopmental disorders (Carvill et al., 2013; Lemke et al., 2013); listed as score 4 in SFARI GENE (minimal evidence).

F10: *BCL11A*, missense
Heterozygous mutations have been reported in Dias-Logan Syndrome (Dias et al., 2016), for which intellectual disability has been described; listed as score 2S in SFARI GENE (strong candidate, syndromic).

F11: *SCN1A*, missense
Implicated in various epileptic disorders with intellectual disabilities (Mulley et al., 2005); listed as score 3S in SFARI GENE (suggestive evidence, syndromic).

F12: *PAK1*, missense
Novel candidate; expression in the developing brain; mutations in the gene of its binding partner PAK3 cause intellectual disability, some of these variants impair the interaction between the two proteins.

F13: *NR2F1*, missense
Implicated in Bosch-Boonstra-Schaaf optic atrophy syndrome (Bosch et al., 2014), for which intellectual disability is reported; patient has ocular findings that are consistent with this syndrome; listed as score 4S in SFARI GENE (minimal evidence, syndromic).

F14: *CTCF*, stop-gain
Implicated in intellectual disability (Gregor et al., 2013); listed as score 3 in SFARI GENE (suggestive evidence).

F15: *DYRK1A*, stop-gain
Implicated in intellectual disability (van Bon et al., 2011); listed as score 1S in SFARI GENE (high confidence, syndromic).

F16: *SCN2A*, missense
Implicated in epileptic disorders with intellectual disabilities (Berkovic et al., 2004); listed as score 1 in SFARI GENE (high confidence).

F17: *DIP2C*, frameshift
Listed as score 2 in SFARI GENE (strong candidate).

F18: 7q11.23 Dup and 16p13.11 Del
The chromosome 7 duplication is in the Williams syndrome locus (MIM194050); likely to confer risk; the chromosome 16 deletion is in a risk region that contains *NDE1 (Alkuraya et al., 2011)* and 16p13.11 microdeletion is a cause of syndromic developmental delay (Bartsch et al., 2006).

F19: 15q13.1-q13.3 Del

Deletions of this locus have been implicated in intellectual disability with variable expressivity (Sharp et al., 2008).

F20: 10q21.3-q22.1 Dup
Duplications of an overlapping region have been implicated in cases with several syndromic features and intellectual disability (Aalfs et al., 1995; Alesi et al., 2017).

Supplementary Table 1. ACMG Classification of Variants.

GENE	EFFECT	EVIDENCE
GIGYF1	Likely pathogenic (II)	PS2, PM2, PP3, PM4
GTF2I	Likely pathogenic (II)	PS2, PM4, PM2, PP3
TSC2	Likely pathogenic (II)	PS2, PM4, PP3, PM2
SON	Likely pathogenic (II)	PS2, PM2, PM4, PP3
DCHS1	Likely pathogenic (II)	PS2, PM2, PP3
GRIN2A	Pathogenic (Ia)	PS1, PS2, PS3, PM2, PM4, PVS1
BCL11A	Likely pathogenic (II)	PS2, PM2, PP2
SCN1A	Likely pathogenic (II)	PS2, PM2, PP3
PAK1	Likely pathogenic (II)	PS2, PM2, PP3
NR2F1	Likely pathogenic (II)	PS2, PM2, PP3
CTCF	Likely pathogenic (II)	PS2, PM2, PM4, PP3
DYRK1A	Pathogenic (Ia)	PVS1, PS2, PM2, PM4, PP3
SCN2A	Likely pathogenic (II)	PS2, PM2, PP3
DIP2C	Likely pathogenic (II)	PS2, PM2, PM4, PP3

ACMG-classification of the 14 dSNVs (Kleinberger et al., 2016; Richards et al., 2015).

Supplementary Table 2. F09 Clinical Information.

Patient	*F01:II-1*	*F01:II-1*	*F01:II-1*
Sex	Male	Female	Male
Age at last examination	28 years	24 years	19 years
Age at seizure onset	12 years	5 years	5 years
Seizure Type			
Tonic-clonic	Y	Y	Y
Complex partial	N	Y	Y
Partial with secondary generalizations	N	Y	Y
Status epilepticus	N	Y	N
Major seizure type	Tonic-clonic	Partial with secondary generalizations	Tonic-clonic
Lifetime seizure total	2	Many	Many
Anti-epileptic medications (AEMs)	Depakote	Dilantin, Tegretol, Trileptal, Topomax, Lamictal	Depakote, Lamictal, Keppra, Prednisone, Topomax
AGE at AEM usage	12-14 years	5 years to present	6 years to present
Age at most recent seizure	12 years	16 years	13 years
ASD symptoms (pr)/ clinical diagnosis	Absent (0/7)/No	Absent (0/7)/No	Present (4/7)/Yes
ADHD symptoms (pr)/ clinical diagnosis	Present (7/17)/Yes	Present (4/17)/No	Present (6/17)/No

Clinical information for the affected children in F09. pr: parental report; symptoms were assessed *post hoc* through a conversation with the parents using DSM-5 as a reference (American Psychiatric Association, 2013).

Additional Information

Primer3 settings ddPCR

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PRIMER_MAX_TEMPLATE_MISPRIMING=12.00
PRIMER_PAIR_MAX_MISPRIMING=24.00
PRIMER_PAIR_MAX_TEMPLATE_MISPRIMING=24.00
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PRIMER_PRODUCT_OPT_TM=
PRIMER_PRODUCT_MAX_TM=
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PRIMER_PRODUCT_SIZE_RANGE=40-100 100-150 150-200
PRIMER_GC_CLAMP=0
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PRIMER_MIN_SIZE=13
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SCRIPT_DETECTION_USE_PRODUCT_SIZE=0
SCRIPT_DETECTION_PRODUCT_MIN_SIZE=100
SCRIPT_DETECTION_PRODUCT_OPT_SIZE=200
SCRIPT_DETECTION_PRODUCT_MAX_SIZE=1000
SERVER_PARAMETER_FILE=Default
PRIMER_NAME_ACRONYM_LEFT=F
PRIMER_NAME_ACRONYM_INTERNAL_OLIGO=IN
PRIMER_NAME_ACRONYM_RIGHT=R
PRIMER_NAME_ACRONYM_SPACER=_

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