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Exome sequencing in sporadic autism spectrum disorders identifies severe *de novo* mutations

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Abstract

Evidence for the etiology of autism spectrum disorders (ASD) has consistently pointed to a strong genetic component complicated by substantial locus heterogeneity^{1,2}. We sequenced the exomes of 20 sporadic cases of ASD and their parents, reasoning that these families would be enriched for *de novo* mutations of major effect. We identified 21 *de novo* mutations, of which 11 were protein-altering. Protein-altering mutations were significantly enriched for changes at highly conserved residues. We identified potentially causative *de novo* events in 4/20 probands, particularly among more severely affected individuals, in *FOXPI*, *GRIN2B*, *SCN1A*, and *LAMC3*. In the *FOXPI* mutation carrier, we also observed a rare inherited *CNTNAP2* mutation and provide functional support for a multihit model for disease risk³. Our results demonstrate that trio-based exome sequencing is a powerful approach for identifying novel candidate genes for ASD and suggest that *de novo* mutations may contribute substantially to the genetic risk for ASD.

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Author Contributions E.E.E., J.S., and B.J.O. designed the study and drafted the manuscript. E.E.E. and J.S. supervised the study. R.B. analyzed the clinical information and contributed to the manuscript. S.E.F. and P.D. designed cell-based functional experiments, analyzed data, interpreted results, and contributed to the manuscript. S.G., C.B., and L.V. generated and analyzed array CGH data. C.L. performed Illumina GAIIX sequencing. B.J.O. and E.K. developed analysis pipeline and analyzed sequence data. A.P.M. and S.B.N. designed and optimized capture protocol. B.J.O., L.V., A.P.M., and S.B.N. constructed exome libraries. B.J.O., L.V., A.P.M., and J.J.S. performed mutation validation and haplotype characterization. B.J.O. and J.J.S. performed the evaluation of 12817 lymphoblast cell lines. P.D. performed functional experiments. M.J.R. and D.A.N. performed sequencing of control samples.

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ASD are characterized by pervasive impairment in language and communication, social reciprocity, and having restricted interests or stereotyped behaviors¹. Several new candidate loci for ASD have recently been identified using genome-wide approaches that discover individually rare events of major effect². A number of genetic syndromes with features of the ASD phenotype, collectively referred to as syndromic autism, have also been described⁴. Despite this progress, the genetic basis for the vast majority of ASD cases remains unknown. Several observations support the hypothesis that the genetic basis for ASD in sporadic cases may differ from that of families with multiple affected individuals, with the former more likely to result from *de novo* mutation events rather than inherited variants^{1,5-7}. In this study, we sequenced the protein-coding regions of the genome (the exome)⁸ to test the hypothesis that *de novo* protein-altering mutations substantially contribute to the genetic basis of sporadic ASD. In contrast with array-based analysis of large *de novo* copy number variants (CNVs), this approach has greater potential to implicate single genes in ASD.

We selected 20 trios with idiopathic ASD, each consistent with sporadic ASD based on clinical evaluations (Supplementary Table 1), pedigree structure, familial phenotypic evaluation, family history, and/or elevated parental age. Each family was initially screened by array comparative genomic hybridization (CGH) using a customized microarray⁹. We identified no large (>250 kbp) *de novo* CNVs but did identify a maternally inherited deletion (~350 kbp) at 15q11.2 in one family (Supplementary Fig. 1). This deletion has been associated with increased risk for epilepsy¹⁰ and schizophrenia^{11,12} but has not been considered as causal for autism.

Similar to Vissers and colleagues¹³, who reported exome sequencing on 10 parent-child trios with sporadic cases of moderate to severe intellectual disability (ID), we performed exome sequencing on each of the 60 individuals separately, by subjecting whole-blood derived genomic DNA to in-solution hybrid capture and Illumina sequencing (**Methods**). We obtained sufficient coverage to call variants for ~90% of the primary target (26.4 Mb) (Table 1). Genotype concordance with SNP microarray data was high (99.7%) (Supplementary Table 2) and on average 96% of proband variant sites were also called in both parents (Supplementary Table 3). Given the expected rarity of true *de novo* events in the targeted exome (<1/trio) (Supplementary Table 4)¹⁴, we reasoned that most apparently *de novo* variants would result from undercalling in parents or systematic false positive calls in the proband. We therefore filtered variants previously observed in dbSNP, 1000 Genomes Pilot Project data¹⁵, and 1490 other exomes sequenced at the University of Washington (Supplementary Fig. 2). We performed Sanger sequencing on the remaining *de novo* candidates (<5/trio), validating 18 events within coding sequence and three additional events mapping to 3' untranslated regions (Table 2). A list of predicted variant sites within these genes from the 1000 Genomes Pilot Project data¹⁵ is provided for comparison (Supplementary Table 5).

We observed subtle differences with respect to mutation rate and characteristics when compared to Vissers and colleagues¹³ (Supplementary Note). The overall protein-coding *de novo* rate (0.9 events/trio) was slightly higher than expected¹⁴ (0.59 events/trio), suggesting that we are identifying the majority of *de novo* events in these trios (Supplementary Table 4). The transition to transversion ratio was highly skewed (18:2), with eight transitions

mapping to hypermutable CpG dinucleotides¹⁴. The proportion of synonymous events was higher than expected based on a neutral model and may reflect selection against embryonic lethal nonsynonymous variants. We successfully determined the parent of origin for seven events, six of which occurred on the paternal haplotype (Table 2). Notably, the eight probands with two or more validated *de novo* events corresponded to families with higher parental age (Mann–Whitney U, Combined Age, One-Sided $P < 0.004$).

Eleven of the 18 coding *de novo* events are predicted to alter protein function. Each of these mutations occurred at a different gene, precluding a statistical assessment for any specific locus despite their deleterious nature (e.g. PolyPhen-2¹⁶). We assessed whether proband *de novo* mutations were enriched in the aggregate for disruptive events by considering two independent quantitative measures: the nature of the amino-acid replacement (Grantham matrix score¹⁷) and the degree of nucleotide-level evolutionary conservation (Genomic Evolutionary Rate Profiling (GERP)^{18,19}) (Fig. 1a,b). For comparison, we sequenced 20 exomes from unrelated ethnically matched controls (HapMap) and applied the same filters to identify coding-sequence mutations that were common or private to each of the samples. These control DNA were isolated from immortalized lymphoblasts; however, the counts of private variants in the cases and controls were highly similar suggesting that the contribution of novel somatic events is likely minimal (Supplementary Fig. 3).

We determined by simulation the expected mean GERP and Grantham distributions for 10 randomly selected common or private control single nucleotide variants (SNVs) (**Methods**). When we compared the observed means of the 10 *de novo* protein-altering ASD proband variants to the distribution of common control SNVs (Fig. 1a), they corresponded to more highly conserved (GERP: $p < 0.001$) and disruptive amino acid mutations (Grantham: $p = 0.015$). If we limited the analysis to the private control SNVs, which serve as a proxy for evolutionarily young mutation events (Fig. 1b), we again found the *de novo* events were at the right tail of these distributions. Only the mean GERP score, however, remained significant (GERP: $p = 0.02$, Grantham: $p = 0.115$). In total, these results suggest that these *de novo* mutation sites are subjected to stronger selection and likely to have functional impact.

We identified a subset of trios (4/20) with disruptive *de novo* mutations that are potentially causative, including genes previously associated with autism, ID, and epilepsy (Table 2 and Supplementary Note). We examined the available clinical data for each of these four families and found they were among the most severely affected individuals in our study based on intelligence quotient (IQ) measures and on calibrated severity score²⁰ (CSS), which is largely independent from IQ and focuses specifically on autistic features with a score of 10 being most severe (Fig. 1c,d). For example, in proband 12681 we identified a single-base substitution (IVS9-2A>G, CCDS8662.1) at the canonical 3' splice site of exon 10 in *Glutamate receptor, ionotropic, N-methyl D-aspartate 2B (GRIN2B)* (Supplementary Fig. 4a,b). She is severely affected (CSS 9), with evidence of early onset, possible regression, and comorbid for mild ID. Expression and association studies have suggested that glutamatergic neurotransmission may play a role in ASD⁴. Recently, Endeley and colleagues²¹ described *GRIN2A* and *GRIN2B* as sites of recurrent *de novo* mutations in individuals with mild to moderate ID and/or epilepsy suggesting variable expressivity. Our data suggest that *de novo* mutations in *GRIN2B* may also lead to an ASD presentation.

Proband 12499 has a missense variant (p.P1894L, CCDS33316.1) predicted to be functionally deleterious and at a highly conserved position in *Sodium channel, voltage-gated, type I, alpha subunit (SCN1A)* (Supplementary Fig. 4c). He is severely affected (CSS 8) with evidence of early onset, possible regression, language delay, a diagnosis of epilepsy and mild ID. *SCN1A* was previously associated with epilepsy and suggested as an ASD candidate^{22,23}, although limited screening has been conducted in idiopathic ASD. Hundreds of disease-associated mutations have been described in epilepsy and typically patients with *de novo* events show more severe phenotypes²⁴. The proband also carries the maternally inherited 15q11.2 deletion increasing the risk for epilepsy¹⁰.

Proband 11666 has a missense variant (p.D399G, CCDS6938.1) predicted to be functionally deleterious and at a highly conserved position within the second laminin-type epidermal growth factor-like domain of *Laminin, gamma 3 (LAMC3)* (Supplementary Fig. 4d). He is severely affected (CSS 10) with evidence of early onset and moderate ID. *LAMC3* is not known to be involved in neuronal development; however, human microarray data have shown expression in many areas of the cortex and limbic system²⁵. Additional study is warranted since laminins have structural similarities to the neuexin and contactin-associated families of proteins, both of which have been associated with ASD².

The fourth example of a potentially causative mutation is a single-base insertion in *Forkhead box P1 (FOXP1)*, introducing a frameshift and premature stop codon (p.A339SfsX4, CCDS2914.1) in proband 12817 (Fig. 1e). He is severely affected (CSS 8) with evidence for regression, language delay, and comorbidity for moderate ID and nonfebrile seizures. Recently, rare occurrences of large *de novo* deletions and a nonsense variant disrupting *FOXP1* were reported in individuals with mild to moderate ID and language defects, with or without ASD features^{26,27}. *FOXP1* encodes a member of the forkhead-box family of transcription factors and is closely related to *FOXP2*, a gene implicated in rare monogenic forms of speech and language disorder^{28–31}. Functional evidence of heterodimer formation and overlapping neural expression patterns suggests that *FOXP1* and *FOXP2* can co-regulate gene expression in the brain^{32,33}. We assessed relative levels of the mutant transcript in proband derived lymphoblasts finding strong evidence for nonsense-mediated decay (NMD) (Supplementary Fig. 5a). HEK293T cell-based functional assays further demonstrated that, if translated, the protein would be truncated and mislocalized from the nucleus to the cytoplasm—similar to results obtained with *FOXP2* mutations³¹ (Supplementary Fig. 5b,c).

Remarkably, in addition to the *FOXP1* mutation, proband 12817 also carried an inherited missense variant (p.H275A, CCDS5889.1) in *Contactin associated protein-like 2 (CNTNAP2)* predicted to be functionally deleterious and at a highly conserved position. This variant is likely to be extremely rare or private as it was not observed in 942 previously sequenced controls³⁴ or in 1490 other exomes. *CNTNAP2* is directly downregulated by *FOXP2*³⁵ and has been independently associated with ASD and specific language impairment^{34–37}. In HEK293T cells, we found that wild-type *FOXP1* significantly reduced expression of *CNTNAP2* (p=0.0005), while the truncated protein was associated with a three-fold expression increase (p=0.0056) (Supplementary Note, Fig. 5d). Overall, we hypothesize that *FOXP1* haploinsufficiency (due to NMD), combined with dysfunction of

FOXP1 mutant proteins that escape this process, may yield overexpression of CNTNAP2 proteins, amplifying any deleterious effects of p.H275A in the proband.

Among the ~110 (85 SNVs, 25 indels) novel inherited protein-altering variants in each proband, we identified several rare inherited variants in genes overlapping the SFARI Gene³⁸, a curated database of potential ASD candidate loci, but no excessive burden in cases relative to controls (Supplementary Table 6). While the numbers from our pilot study are few, we do observe two cases with a significant *de novo* event and a potential inherited risk variant (12817p1:FOXP1/CNTNAP2 and 12499.p1: SCN1A/15q11.2 deletion) highlighting that in some sporadic families a multihit model may be playing a role³ (Supplementary Table 7). In the future, this hypothesis could be further explored by comparing burden in a much larger number of affected/unaffected sibling pairs.

The probands with the four potentially causative *de novo* events met strict criteria for a diagnosis of autistic disorder (Supplementary Note). Our finding of *de novo* events in genes that have also been disrupted in children with ID without ASD, ID with ASD features, and epilepsy provides further evidence that these genetic pathways may lead to a spectrum of neurodevelopmental outcomes depending on the genetic and environmental context^{2,4}. Recent data suggest that CNVs may also blur these lines with diverse conditions all showing association to the same loci^{2,4}. Distinguishing primary from secondary effects will require a better understanding of the underlying biology and identification of interacting genetic and environmental factors within the phenotypic context of the family. The identification of *de novo* events along with disruptive inherited mutations underlying “sporadic” ASD has the potential to fundamentally transform our understanding of the genetic basis of ASD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Bailey A, et al. Autism as a strongly genetic disorder: evidence from a British twin study. *Psychol Med*. 1995; 25:63–77. [PubMed: 7792363]
2. O’Roak BJ, State MW. Autism genetics: strategies, challenges, and opportunities. *Autism Research*. 2008; 1:4–17. [PubMed: 19360646]
3. Girirajan S, et al. A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. *Nat Genet*. 2010; 42:203–9. [PubMed: 20154674]

4. Abrahams BS, Geschwind DH. Advances in autism genetics: on the threshold of a new neurobiology. *Nat Rev Genet.* 2008; 9:341–55. [PubMed: 18414403]
5. Sebat J, et al. Strong association of de novo copy number mutations with autism. *Science.* 2007; 316:445–9. [PubMed: 17363630]
6. Marshall CR, et al. Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet.* 2008; 82:477–88. [PubMed: 18252227]
7. Durkin MS, et al. Advanced parental age and the risk of autism spectrum disorder. *Am J Epidemiol.* 2008; 168:1268–76. [PubMed: 18945690]
8. Ng SB, et al. Targeted capture and massively parallel sequencing of 12 human exomes. *Nature.* 2009; 461:272–6. [PubMed: 19684571]
9. Bailey JA, et al. Recent segmental duplications in the human genome. *Science.* 2002; 297:1003–7. [PubMed: 12169732]
10. de Kovel CG, et al. Recurrent microdeletions at 15q11.2 and 16p13.11 predispose to idiopathic generalized epilepsies. *Brain.* 2009; 133:23–32. [PubMed: 19843651]
11. Stefansson H, et al. Large recurrent microdeletions associated with schizophrenia. *Nature.* 2008; 455:232–6. [PubMed: 18668039]
12. Kirov G, et al. Support for the involvement of large cnvs in the pathogenesis of schizophrenia. *Hum Mol Genet.* 2009
13. Vissers LE, et al. A de novo paradigm for mental retardation. *Nat Genet.* 2010
14. Lynch M. Rate, molecular spectrum, and consequences of human mutation. *Proc Natl Acad Sci U S A.* 2010; 107:961–8. [PubMed: 20080596]
15. Durbin RM, et al. A map of human genome variation from population-scale sequencing. *Nature.* 2010; 467:1061–73. [PubMed: 20981092]
16. Adzhubei IA, et al. A method and server for predicting damaging missense mutations. *Nat Methods.* 2010; 7:248–9. [PubMed: 20354512]
17. Grantham R. Amino acid difference formula to help explain protein evolution. *Science.* 1974; 185:862–4. [PubMed: 4843792]
18. Cooper GM, et al. Distribution and intensity of constraint in mammalian genomic sequence. *Genome Res.* 2005; 15:901–13. [PubMed: 15965027]
19. Cooper GM, et al. Single-nucleotide evolutionary constraint scores highlight disease-causing mutations. *Nat Methods.* 2010; 7:250–1. [PubMed: 20354513]
20. Gotham K, Pickles A, Lord C. Standardizing ADOS scores for a measure of severity in autism spectrum disorders. *J Autism Dev Disord.* 2009; 39:693–705. [PubMed: 19082876]
21. Endele S, et al. Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. *Nat Genet.* 2010
22. Claes L, et al. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *Am J Hum Genet.* 2001; 68:1327–32. [PubMed: 11359211]
23. Weiss LA, et al. Sodium channels SCN1A, SCN2A and SCN3A in familial autism. *Mol Psychiatry.* 2003; 8:186–94. [PubMed: 12610651]
24. Mulley JC, et al. SCN1A mutations and epilepsy. *Hum Mutat.* 2005; 25:535–42. [PubMed: 15880351]
25. Lein ES, et al. Genome-wide atlas of gene expression in the adult mouse brain. *Nature.* 2007; 445:168–76. [PubMed: 17151600]
26. Hamdan FF, et al. De Novo Mutations in FOXP1 in Cases with Intellectual Disability, Autism, and Language Impairment. *Am J Hum Genet.* 2010; 87:671–8. [PubMed: 20950788]
27. Horn D, et al. Identification of FOXP1 deletions in three unrelated patients with mental retardation and significant speech and language deficits. *Hum Mutat.* 2010; 31:E1851–60. [PubMed: 20848658]
28. Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP. A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature.* 2001; 413:519–23. [PubMed: 11586359]
29. Feuk L, et al. Absence of a paternally inherited FOXP2 gene in developmental verbal dyspraxia. *Am J Hum Genet.* 2006; 79:965–72. [PubMed: 17033973]

30. MacDermot KD, et al. Identification of FOXP2 truncation as a novel cause of developmental speech and language deficits. *Am J Hum Genet.* 2005; 76:1074–80. [PubMed: 15877281]
31. Vernes SC, et al. Functional genetic analysis of mutations implicated in a human speech and language disorder. *Hum Mol Genet.* 2006; 15:3154–67. [PubMed: 16984964]
32. Li S, Weidenfeld J, Morrisey EE. Transcriptional and DNA binding activity of the Foxp1/2/4 family is modulated by heterotypic and homotypic protein interactions. *Mol Cell Biol.* 2004; 24:809–22. [PubMed: 14701752]
33. Teramitsu I, Kudo LC, London SE, Geschwind DH, White SA. Parallel FoxP1 and FoxP2 expression in songbird and human brain predicts functional interaction. *J Neurosci.* 2004; 24:3152–63. [PubMed: 15056695]
34. Bakkaloglu B, et al. Molecular cytogenetic analysis and resequencing of contactin associated protein-like 2 in autism spectrum disorders. *Am J Hum Genet.* 2008; 82:165–73. [PubMed: 18179895]
35. Vernes SC, et al. A functional genetic link between distinct developmental language disorders. *N Engl J Med.* 2008; 359:2337–45. [PubMed: 18987363]
36. Arking DE, et al. A Common Genetic Variant in the Neurexin Superfamily Member CNTNAP2 Increases Familial Risk of Autism. *Am J Hum Genet.* 2008; 82:160–4. [PubMed: 18179894]
37. Alarcon M, et al. Linkage, Association, and Gene-Expression Analyses Identify CNTNAP2 as an Autism-Susceptibility Gene. *Am J Hum Genet.* 2008; 82:150–159. [PubMed: 18179893]
38. Banerjee-Basu S, Packer A. SFARI Gene: an evolving database for the autism research community. *Dis Model Mech.* 2010; 3:133–5. [PubMed: 20212079]
39. Fischbach GD, Lord C. The Simons Simplex Collection: a resource for identification of autism genetic risk factors. *Neuron.* 2010; 68:192–5. [PubMed: 20955926]
40. Hurley RS, Losh M, Parlier M, Reznick JS, Piven J. The broad autism phenotype questionnaire. *J Autism Dev Disord.* 2007; 37:1679–90. [PubMed: 17146701]
41. Constantino JN, Todd RD. Intergenerational transmission of subthreshold autistic traits in the general population. *Biol Psychiatry.* 2005; 57:655–60. [PubMed: 15780853]
42. Selzer RR, et al. Analysis of chromosome breakpoints in neuroblastoma at sub-kilobase resolution using fine-tiling oligonucleotide array CGH. *Genes Chromosomes Cancer.* 2005; 44:305–19. [PubMed: 16075461]
43. Itsara A, et al. Population analysis of large copy number variants and hotspots of human genetic disease. *Am J Hum Genet.* 2009; 84:148–61. [PubMed: 19166990]
44. Igartua C, et al. Targeted enrichment of specific regions in the human genome by array hybridization. *Curr Protoc Hum Genet.* 2010; Chapter 18(Unit 18):3. [PubMed: 20582915]
45. Ng SB, et al. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nat Genet.* 2010; 42:790–3. [PubMed: 20711175]
46. Roach JC, et al. Analysis of genetic inheritance in a family quartet by whole-genome sequencing. *Science.* 2010; 328:636–9. [PubMed: 20220176]
47. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics.* 2009; 25:1754–60. [PubMed: 19451168]
48. Li H, et al. The Sequence Alignment/Map format and SAM tools. *Bioinformatics.* 2009; 25:2078–9. [PubMed: 19505943]
49. Bailey JA, Yavor AM, Massa HF, Trask BJ, Eichler EE. Segmental duplications: organization and impact within the current human genome project assembly. *Genome Res.* 2001; 11:1005–17. [PubMed: 11381028]
50. Sudmant PH, et al. Diversity of human copy number variation and multicopy genes. *Science.* 2010; 330:641–6. [PubMed: 21030649]
51. Hach F, et al. mrsFAST: a cache-oblivious algorithm for short-read mapping. *Nat Methods.* 2010; 7:576–7. [PubMed: 20676076]
52. Andres AM, et al. Balancing selection maintains a form of ERAP2 that undergoes nonsense-mediated decay and affects antigen presentation. *PLoS Genet.* 2010; 6:e1001157. [PubMed: 20976248]

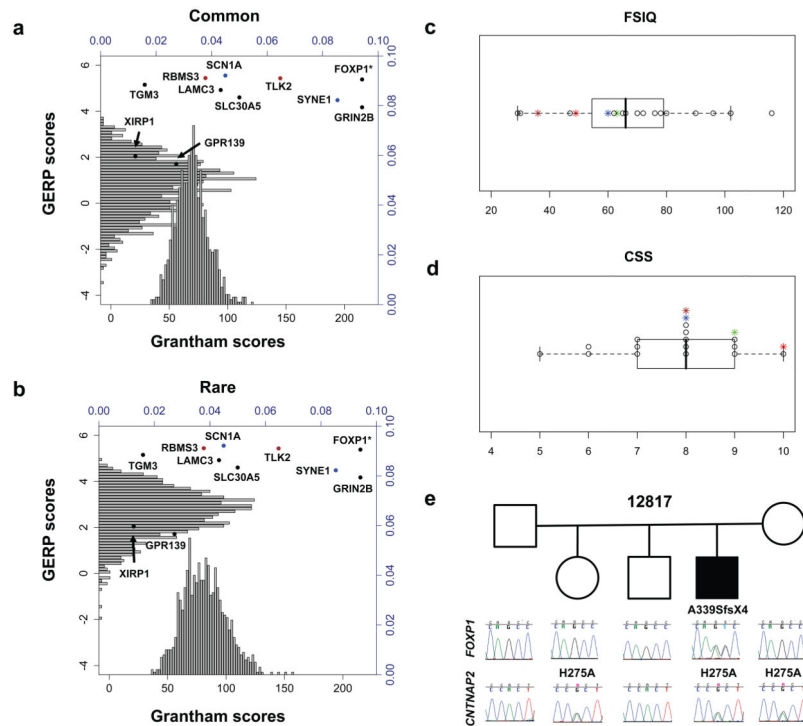


Figure 1.

Evaluation of *de novo* mutations by simulation, proband severity, and family 12817. **a,b** We compared the mean Grantham (black x-axis) and GERP scores (black y-axis) of the 10 proband *de novo* protein-changing substitutions to 20 HapMap control samples by building a distribution of the mean values of 10 randomly selected common or private variants over 1000 trials. Splice-site and nonsense events were given a maximum Grantham score (215) and indels were not included in the simulation. Histograms show the relative frequency (blue axes) of each distribution. Points show the proband variants, with variants from the same individual highlighted (blue=13708.p1, red=12499.p1). Proband mean values, GERP: 4.349 and Grantham: 104.3. **FOXP1* not included in proband mean values. **a**, Control common variants (GERP: $p < 0.001$, Grantham: $p = 0.015$). **b**, Control rare variants (GERP: $p = 0.026$, Grantham: $p = 0.098$). **c,d** We evaluated the disease severity of the mutation carriers 12817.p1-*FOXP1* (brown), 12681.p1-*GRIN2B* (green), 12499-*SCN1A* (blue) and 11666.p1-*LAMC3* (red). **c**, Box and whisker plot of Full Scale Intelligence Quotient (FSIQ) values. **d**, Box and whisker plot of Calibrated Severity Scores (CSS) based on the Autism Diagnostic Observation Schedule (ADOS). Data were available for 19/20 probands; CSS were estimated for two probands based on ADOS module 4 data. **e**, Pedigree for 12817 showing chromatogram traces surrounding *FOXP1* (top) and *CNTNAP2* (bottom) mutation events. Proband carries a *de novo* single-base (+A relative to mRNA) frameshifting mutation p.A339SfsX4 in *FOXP1* and an inherited missense variant p.H275A in *CNTNAP2*.

Table 1

Summary of the exome sequencing results from of 20 sporadic ASD probands

Family SSC/SAGE ID#	Proband Sex	Fa Age months	Mo Age months	Mo Age months	Trio Bases [‡]	% target +/- 2 bp	Coding SNV (- dbSNP/1kG)	Rare Disruptive SNV [□]	Coding Indels (- dbSNP/1kG)	Rare Indels	Protein Coding De Novo Events
11048	M	358	358	358	23,901,726	88.19	14,095 (752)	131	74 (44)	27	0
11307	M	421	407	407	23,549,536	86.89	13,509 (583)	75	64 (40)	19	0
11580	M	443	305	305	23,823,712	87.90	13,912 (642)	89	62 (36)	24	1
11666	M	398	370	370	24,179,474	89.21	14,306 (622)	77	59 (40)	25	1
12325	M	363	313	313	24,088,772	88.88	13,866 (629)	79	65 (43)	24	1
12499	M	425	372	372	25,217,651	93.04	14,479 (634)	86	80 (47)	21	3
12575	M	351	317	317	24,259,870	89.51	14,568 (679)	78	80 (55)	26	0
12647	M	541	413	413	24,669,129	91.02	14,144 (830)	78	68 (42)	22	1
12680	M	502	471	471	24,437,989	90.16	14,124 (642)	69	70 (42)	24	2
12681	F	399	375	375	24,723,806	91.22	14,750 (691)	93	68 (39)	20	2
12817	M	485	430	430	24,520,475	90.47	14,364 (656)	83	72 (38)	24	2
12974	M	366	365	365	24,235,164	89.42	13,990 (555)	52	54 (37)	23	0
13095	M	337	322	322	24,460,239	90.25	14,605 (645)	66	89 (54)	29	0
13253	M	436	427	427	24,070,345	88.81	13,775 (610)	96	41 (25)	16	2
13284*	M	300	302	302	24,911,060	91.91	17,806 (639)	111	151 (79)	53	1
13466	M	353	385	385	24,676,574	91.05	14,023 (591)	72	58 (39)	23	0
13683	M	470	402	402	24,139,439	89.06	14,419 (725)	73	72 (49)	22	0
13708	M	397	382	382	23,933,169	88.30	13,997 (686)	77	78 (41)	26	2
13970	M	313	234	234	24,465,009	90.26	14,293 (626)	84	89 (58)	31	0
SAGE4022	F	271	283	283	24,130,743	89.03	14,538 (713)	141	86 (56)	29	0
AVG	18M:2F	397	362	362	24,319,694	89.73	14,378 (658)	86	74 (45)	25	0.9

Paternal and maternal ages at time of conception were estimated based on month-year birth information assuming a 9-month pregnancy

[‡]Number of bases covered at 8x and Q30 in all three individuals

* 13284 Included Additional RefSeq Targets

[□]Not observed in 1490 other exomes sequenced at the University of Washington

Simons Simplex Collection (SSC) or Study of Autism Genetics Exploration (SAGE) family number

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Table 2

Summary of confirmed *de novo* mutation events

SNV Proband	Type	Chromosome: Position	Gene Symbol	Variant	AA Change	GERP Score	Grantham Score	PolyPhen-2	CpG	Ts/Tv	Mut Origin
11580.pl	missense	chr20:2239665	TGM3	R	V144I	5.15	29	probably damaging	Y	Ts	Mo
11666.pl	missense	chr9:132904111	LAMC3*	R	D339G	4.92	94	probably damaging	N	Ts	Fa
12325.pl	3'UTR	chr12:55708658	MYO1A	R		2.23			N	Ts	
12325.pl	missense	chr16:19951169	GPR139	Y	S151G	1.71	56	benign	N	Ts	
12499.pl	missense	chr2:166556317	SCN1A*	R	P1894L	5.55	98	probably damaging	N	Ts	Fa
12499.pl	synonymous	chr3:38033207	PLCD1	K		-8.24			Y	Tv	
12499.pl	missense	chr6:152865504	SYNE1	Y	Y282C	4.48	194	probably damaging	N	Ts	
12575.pl	3'UTR	chr9:32619906	TAF1L	R		-1.02			Y	Ts	
12647.pl	3'UTR	chr16:223585994	DCTN5	Y		-0.989			N	Ts	
12647.pl	missense	chr5:68453390	SLC30A5	S	S561R	4.6	110	possibly damaging	N	Tv	
12680.pl	synonymous	chr2:101992478	IL1R2	Y		-1.53			N	Ts	
12680.pl	synonymous	chr5:132251451	AFF4	Y		-11.2			Y	Ts	Fa
12681.pl	3' splice	chr12:13614220	GRIN2B*	Y		4.17	215#		N	Ts	Fa
12681.pl	synonymous	chr7:142274902	EPHB6	Y		-3.14			Y	Ts	Fa
12817.pl	synonymous	chr2:143724639	ARHGAP15	R		3.51			N	Ts	
13253.pl	missense	chr3:39204494	XIRP1	Y	V483M	2.04	21	probably damaging	N	Ts	
13253.pl	synonymous	chr16:74121475	CHST5	Y		-3.22			Y	Ts	
13284.pl	synonymous	chr2:179145956	TTN	Y		0.328			Y	Ts	
13708.pl	missense	chr17:58033198	TLK2	Y	S595L	5.43	145	probably damaging	Y	Ts	
13708.pl	missense	chr3:30004687	RBMS3	Y	T383M	5.44	81	probably damaging	Y	Ts	
Indel											
12817.pl	frameshift	chr3:71132860	FOXP1*	+T	A339SfsX4	5.38 [‡]	215#		NA	NA	Fa

* Disruptive *de novo* mutations that are potentially causative

Maximum Grantham score given for splice and frameshifting variants

‡ Average GERP score for two sites flanking the insertion