The American Journal of Human Genetics, Volume *93*

Supplemental Data

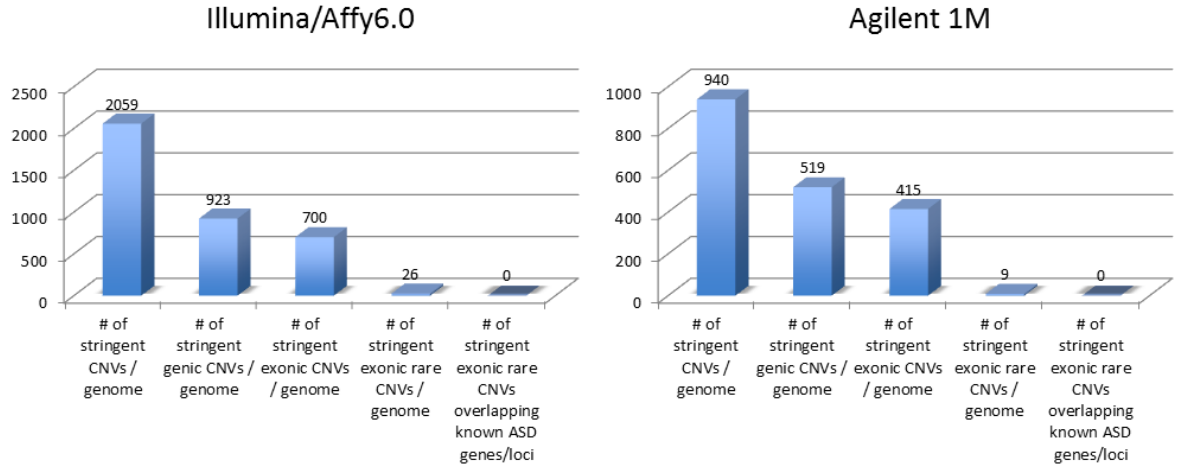
Detection of Clinically Relevant Genetic Variants in Autism Spectrum Disorder by Whole-Genome Sequencing

Yong-hui Jiang,[1,18] Ryan K.C. Yuen,[2,18] Xin Jin,[3,4,5]* Mingbang Wang,[3,18] Nong Chen,[3] Xueli Wu,[3] Jia Ju,[3] Junpu Mei,[3] Yujian Shi,[3] Mingze He,[3] Guangbiao Wang,[3] Jieqin Liang,[3] Zhe Wang,[3] Dandan Cao,[3] Melissa T. Carter,[6] Christina Chrysler,[7] Irene E. Drmic,[7] Jennifer L. Howe,[2] Lynette Lau,[2] Christian R. Marshall,[2,8] Daniele Merico,[2] Thomas Nalpathamkalam,[2] Bhooma Thiruvahindrapuram,[2] Ann Thompson,[7] Mohammed Uddin,[2] Susan Walker,[2] Jun Luo,[9] Evdokia Anagnostou,[10] Lonnie Zwaigenbaum,[11] Robert H. Ring,[12] Jian Wang,[3] Clara Lajonchere,[12] Jun Wang,[3,13,14,15,16] Andy Shih,[12] Peter Szatmari,[7] Huanming Yang,[3] Geraldine Dawson,[12,17] Yingrui Li,[3,]* and Stephen W. Scherer[2,8,]**

Figure S1

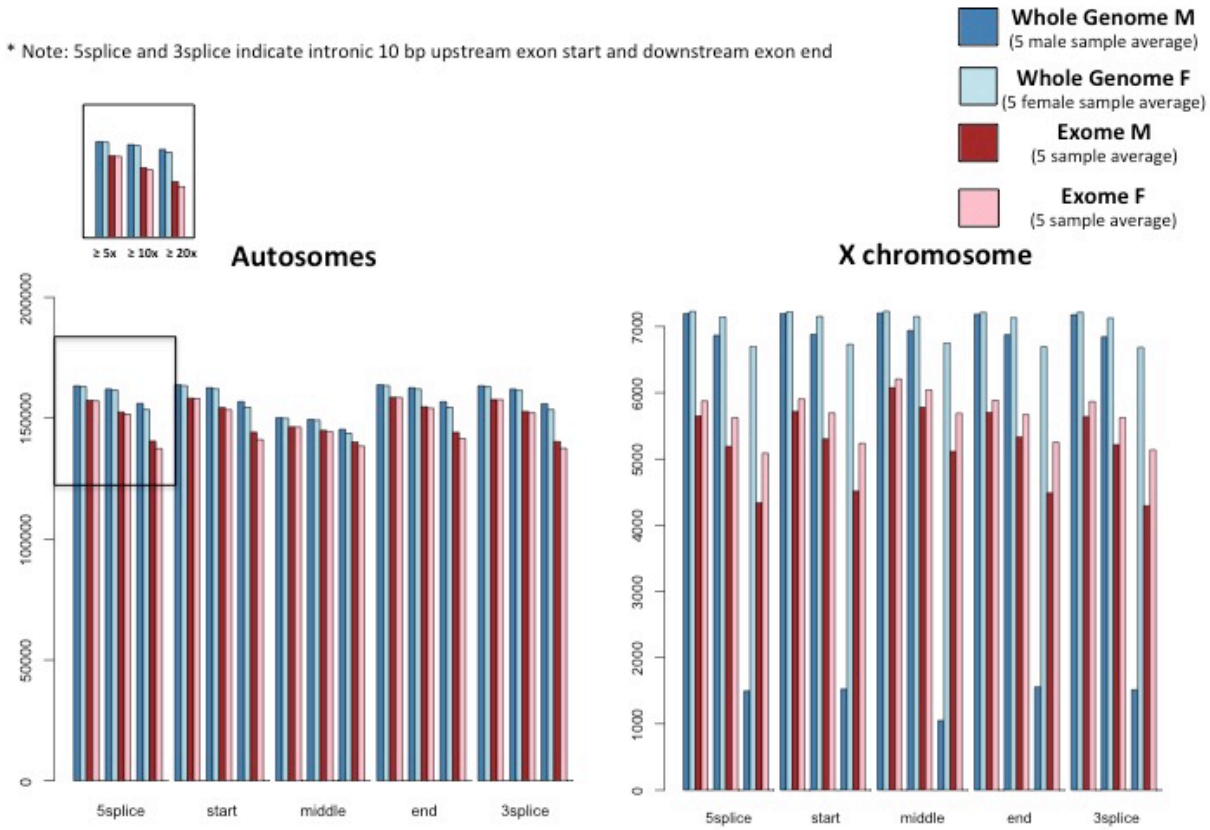
# of samples	Illumina1M - 1; Affy6.0 - 31	Agilent 1M - 26
Mean / Median Size (kb)	67 / 19.8	102 / 29
% Gain / Loss	27 / 71	42 / 58

	total	average/meadian per genome	total	average/meadian per genome
# of stringent CNVs / genome	2059	66 /66	940	36 / 36
# of stringent genic CNVs / genome	923	29 / 29	519	20 / 20
# of stringent exonic CNVs / genome	700	22 / 22	415	16 / 16
# of stringent exonic rare CNVs / genome	26	< 1	9	< 1
# of stringent exonic rare CNVs overlapping known ASD genes/loci	0	0	0	0



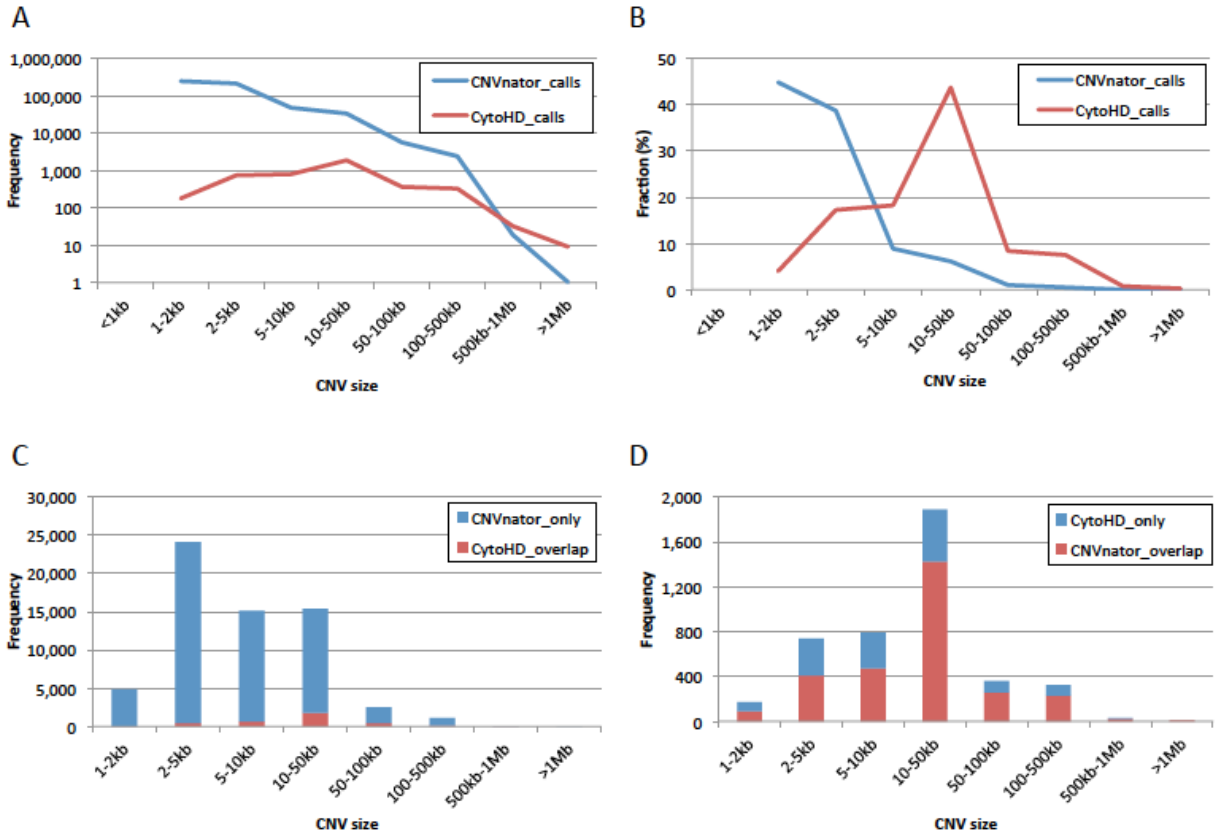
Copy number variation distribution in 32 samples. None of the sample contains exonic rare CNVs overlapping known ASD genes/loci.

Figure S2



Sequencing coverage in exonic regions with subdivision into different genomic locations.

Figure S3



Comparison of CNV calling performance between WGS and microarray. A) Size distribution of CNVs called by CNVnator (WGS) and by CytoScan HD (microarray). Total number of CNVs called by CNVnator is >100 times more than that by CytoHD. B) Size distribution of CNVs called by CNVnator and by CytoScan HD presented in fraction. CNV calling by CytoScan is more sensitive at size range 10-50kb, while calling by CNVnator is more sensitive at size range 1-2kb. C) Number of CNVs called by CytoHD overlap with that by CNVnator in regions where CytoHD has sufficient probe coverage (>5 probes). D) Number of CNVs called by CNVnator overlap with that by CytoHD in regions where stringent calls were made in CytoHD (see Materials and Methods).

Figure S4

Statistics for ASD genes not well covered
(d < 5x: insufficient coverage, 5x ≤ d < 10: low coverage)

CDS overlap ≥ 25%	WGS d < 5x	WES d < 5x	WGS 5x ≤ d < 10x	WES 5x ≤ d < 10x	WES Not targeted
ASD gene N# at least *one* exon not well covered	12	11	18	21	11
ASD gene N# at least *two* exons not well covered	2	2	3	4	3
ASD gene exon N# not well covered	14	25	21	27	30

CDS overlap = 100%	WGS d < 5x	WES d < 5x	WGS 5x ≤ d < 10x	WES 5x ≤ d < 10x	WES Not targeted
ASD gene N# at least *one* exon not well covered	3	7	6	15	11
ASD gene N# at least *two* exons not well covered	1	2	3	4	3
ASD gene exon N# not well covered	4	20	9	20	30

* Autosomes, X, Y
* All subjects (5 WGS-M, 5 WGS-F, 5 WES-M, 5 WES-F)
* ASD genes total: 119 (AGP list)
* ASD exons total: 2035
* ASD exons total, CDS overlap = 100%: 1727

Coverage of WGS and (whole exome sequencing) WES in known ASD gene list. Listed are the numbers of genes that had at least one exon not covered by the platform. We reported the coverage statistics for known ASD genes, based on the number of exons with insufficient (d < 5x) or low coverage (5x ≤ d < 10x), or not on the exome capture target (Figure S3).

Table S1. Summary of number of variants detected in all samples

Sample ID	Sequencing depth	Coverage of genome(%)	Total SNPs	Total INDELs
2-0704-01	39.2	99.8	3,185,306	811,254
2-0704-02	36.7	99.8	3,170,877	807,224
2-0704-03	35.2	99.8	3,164,829	824,025
2-1116-01	36.5	99.8	3,233,019	816,870
2-1116-02	39.2	99.9	3,266,462	827,034
2-1116-03	42.1	99.9	3,241,117	841,398
2-1182-01	36.1	99.9	3,220,029	798,601
2-1182-02	39.3	99.9	3,177,106	794,421
2-1182-03	39.4	99.9	3,199,160	816,021
2-1186-01	34.8	99.8	3,231,424	796,610
2-1186-02	46.0	99.9	3,215,304	811,282
2-1186-03	44.1	99.8	3,205,158	823,067
2-1239-01	36.5	99.8	3,210,421	796,820
2-1239-02	38.7	99.8	3,215,488	804,988
2-1239-03	38.4	99.8	3,221,124	821,915
2-1244-01	42.5	99.8	3,204,198	808,489
2-1244-02	38.3	99.8	3,182,264	797,538
2-1244-03	43.0	99.8	3,171,956	816,921
2-1266-01	37.3	99.8	3,229,060	801,066
2-1266-02	35.5	99.8	3,173,747	787,001
2-1266-03	39.4	99.8	3,224,993	821,124
2-1268-01	41.5	99.8	3,197,338	811,544
2-1268-02	43.4	99.8	3,197,033	810,883
2-1268-03	40.1	99.8	3,187,370	824,590
2-1269-01	39.9	99.8	3,178,207	800,913
2-1269-02	42.1	99.8	3,156,867	796,563
2-1269-03	39.5	99.8	3,148,740	812,439
2-1272-01	43.3	99.8	3,230,825	808,617
2-1272-02	39.8	99.8	3,199,100	799,398
2-1272-03	36.7	99.8	3,196,376	813,334
2-1276-01	35.6	99.8	3,189,611	806,420
2-1276-02	41.3	99.8	3,153,014	805,522
2-1276-03	38.0	99.8	3,207,424	832,309
2-1280-01	40.8	99.9	3,303,969	834,195
2-1280-02	41.1	99.8	3,270,404	826,792
2-1280-03	39.0	99.8	3,317,472	855,153
2-1291-01	26.1	99.8	3,212,103	792,590
2-1291-02	39.8	99.8	3,178,590	794,986
2-1291-03	44.9	99.8	3,188,245	813,041

Sample ID	Sequencing depth	Coverage of genome(%)	Total SNPs	Total INDELs
2-1295-01	37.8	99.8	3,266,952	793,867
2-1295-02	38.6	99.8	3,209,822	788,055
2-1295-03	34.0	99.8	3,238,805	791,829
2-1303-01	36.8	99.8	3,206,628	790,300
2-1303-02	37.0	99.8	3,187,798	781,632
2-1303-03	37.0	99.8	3,190,293	801,778
2-1305-01	34.7	99.8	3,254,739	793,140
2-1305-02	34.3	99.8	3,285,790	779,863
2-1305-03	39.5	99.8	3,302,698	817,107
2-1314-01	43.3	99.9	3,262,059	825,726
2-1314-02	42.6	99.8	3,199,067	808,578
2-1314-03	42.5	99.8	3,303,886	850,776
2-1325-01	35.6	99.8	3,251,986	787,124
2-1325-02	38.9	99.8	3,196,285	778,569
2-1325-03	37.7	99.8	3,222,418	801,211
2-1329-01	36.5	99.8	3,232,169	803,678
2-1329-02	32.9	99.8	3,218,677	793,568
2-1329-03	35.0	99.8	3,192,710	810,297
2-1330-01	37.5	99.8	3,189,790	791,981
2-1330-02	38.5	99.8	3,199,170	789,259
2-1330-03	40.3	99.8	3,177,628	803,498
2-1334-01	34.2	99.8	3,242,908	785,564
2-1334-02	34.1	99.8	3,219,078	784,236
2-1334-03	40.6	99.8	3,201,641	805,279
2-1337-01	39.7	99.8	3,183,547	813,369
2-1337-02	38.5	99.8	3,205,460	811,795
2-1337-03	36.1	99.8	3,202,101	830,779
2-1342-01	36.9	99.8	3,197,399	794,754
2-1342-02	38.3	99.8	3,204,267	796,247
2-1342-03	37.8	99.9	3,219,052	818,424
2-1352-01	35.9	99.8	3,260,340	801,579
2-1352-02	37.8	99.8	3,183,155	787,276
2-1352-03	37.3	99.8	3,218,739	809,995
2-1353-01	37.4	99.8	3,216,606	793,050
2-1353-02	44.5	99.8	3,200,287	796,362
2-1353-03	36.9	99.8	3,189,371	802,718
2-1360-01	36.8	99.8	3,169,886	788,171
2-1360-02	42.5	99.8	3,140,750	787,847
2-1360-03	34.3	99.8	3,124,812	796,198
2-1363-01	36.4	99.8	3,221,195	783,155

Sample ID	Sequencing depth	Coverage of genome(%)	Total SNPs	Total INDELs
2-1363-02	38.7	99.8	3,195,709	777,179
2-1363-03	37.8	99.8	3,183,254	795,188
2-1368-01	42.2	99.8	3,257,014	828,150
2-1368-02	37.1	99.8	3,136,446	798,958
2-1368-03	36.7	99.8	3,258,165	839,574
2-1369-01	38.1	99.8	3,212,820	795,253
2-1369-02	35.9	99.8	3,163,723	787,114
2-1369-03	35.4	99.8	3,169,142	805,978
2-1370-01	34.4	99.8	3,218,321	790,796
2-1370-02	39.3	99.8	3,208,498	785,190
2-1370-03	35.5	99.9	3,201,081	804,470
2-1375-01	36.1	99.8	3,276,890	798,938
2-1375-02	37.4	99.8	3,173,223	778,049
2-1375-03	38.3	99.8	3,247,270	814,844
2-1379-01	39.7	99.8	3,233,335	801,286
2-1379-02	41.8	99.8	3,202,157	798,073
2-1379-03	44.5	99.8	3,200,016	814,284
Average	**38.4**	**99.8**	**3,210,237**	**804,635**

Table S2. Summary of number of *de novo* SNVs detected by filter method (DD-1) and Random Forest method (RF-2)

FamilyID	DD-1	RF-2	Overlap-Rate	exonic DD-1	exonic RF-2
2-0704	398	48	91.67%	2	1
2-1116	360	52	88.46%	1	0
2-1182	407	76	88.16%	1	1
2-1186	355	61	83.61%	1	1
2-1239	392	74	83.78%	1	2
2-1244	356	56	92.86%	0	0
2-1266	439	63	93.65%	1	2
2-1268	401	71	90.14%	1	0
2-1269	418	52	82.69%	0	0
2-1272	457	68	79.41%	3	3
2-1276	393	66	81.82%	1	1
2-1280	540	71	87.32%	1	2
2-1291	313	80	80.00%	1	1
2-1295	490	50	88.00%	3	2
2-1303	373	40	80.00%	1	1
2-1305	399	82	82.93%	3	1
2-1314	370	66	86.36%	1	1
2-1325	442	53	81.13%	0	0
2-1329	419	59	86.44%	2	2
2-1330	412	74	87.84%	0	0
2-1334	331	54	81.48%	0	1
2-1337	428	87	89.66%	0	0
2-1342	372	77	85.71%	1	1
2-1352	394	75	81.33%	3	3
2-1353	427	67	83.58%	1	0
2-1360	435	67	82.09%	2	1
2-1363	364	63	85.71%	3	4
2-1368	494	78	79.49%	1	2
2-1369	363	42	95.24%	1	1
2-1370	455	70	82.86%	3	3
2-1375	368	89	80.90%	0	0
2-1379	389	66	87.88%	1	1
Sum	12954	2097	-	40	38
Average	404.81	65.53	85.38%	1.25	1.19
Validation rate				80%	95%

Table S3. Summary of number of *de novo* indels detected by filter

FamilyID	No. of *de novo* Indels	No. after filtering simple repeat or dbSNP135	No. of in coding after filtering simple repeat or dbSNP135
2-0704	16	8	0
2-1116	13	3	0
2-1182	20	4	0
2-1186	15	5	0
2-1239	16	7	0
2-1244	17	6	0
2-1266	11	4	0
2-1268	14	2	0
2-1269	10	6	0
2-1272	14	8	0
2-1276	20	4	0
2-1280	17	8	0
2-1291	13	4	0
2-1295	12	3	0
2-1303	5	1	0
2-1305	17	5	0
2-1314	15	4	0
2-1325	25	7	0
2-1329	14	6	0
2-1330	16	8	0
2-1334	22	7	0
2-1337	11	5	1
2-1342	10	4	0
2-1352	9	5	0
2-1353	7	4	0
2-1360	9	2	0
2-1363	17	5	0
2-1368	16	8	0
2-1369	21	7	0
2-1370	15	6	1
2-1375	11	5	0
2-1379	10	3	0
Sum	458	164	2
Average	14.31	5.13	0.06
Validation rate			100%

Table S4. Summary of rare variants in probands

Sample ID	All nonsyn SNVs (missense, nonsense, splicing)	rare nonsyn SNVs (not in dbSNP/1000G)	rare splicing	rare damaging missense (predicted as damaging by SIFT and PolyPhen-2)	rare nonsense	All indels	rare indel (not in dbSNP/1000G)	rare frameshift
2-0704-03	8,785	305	75	45	7	453	342	132
2-1116-03	8,935	232	71	37	5	458	328	120
2-1182-03	9,142	221	82	38	7	447	336	140
2-1186-03	8,984	177	75	22	3	462	337	134
2-1239-03	8,980	260	73	41	6	433	314	127
2-1244-03	8,860	194	77	32	5	433	317	111
2-1266-03	8,828	203	69	35	9	435	330	122
2-1268-03	8,817	193	79	23	8	457	336	127
2-1269-03	8,790	195	74	35	4	422	309	115
2-1272-03	8,926	231	80	39	9	439	325	129
2-1276-03	8,936	228	77	25	6	473	349	123
2-1280-03	9,178	315	85	58	12	451	329	128
2-1291-03	8,965	207	74	32	8	418	309	118
2-1295-03	8,969	259	72	49	7	412	310	112
2-1303-03	8,898	166	66	24	6	428	310	111
2-1305-03	9,312	441	72	81	6	437	316	119
2-1314-03	9,365	296	87	36	8	485	364	141
2-1325-03	8,954	199	73	41	5	427	304	121
2-1329-03	8,796	173	62	26	6	446	329	169
2-1330-03	8,958	184	72	28	3	456	345	127
2-1334-03	8,932	212	79	43	4	442	329	124
2-1337-03	8,981	214	65	38	4	454	343	131
2-1342-03	8,891	237	81	47	6	418	305	132
2-1352-03	8,970	162	63	30	5	433	314	113
2-1353-03	9,023	194	73	41	3	433	317	127
2-1360-03	8,780	192	76	34	4	446	332	143
2-1363-03	8,919	188	75	38	2	426	309	119
2-1368-03	9,233	340	75	85	9	444	326	120
2-1369-03	8,995	192	74	38	8	450	339	122
2-1370-03	9,080	205	78	41	6	468	351	140
2-1375-03	9,087	349	73	73	10	445	324	109
2-1379-03	9,155	232	80	37	11	463	356	134
Average	**8,982**	**231**	**75**	**40**	**6**	**444**	**328**	**126**

Table S5. Annotations of ASD clinically relevant variants

Family ID	Chr	Coordinate (hg19)	Gene	Accession	Coding_Change	Protein_Change	Effect[a]	Proband-Gtype	State[b]	SIFT[c]	Polyphen-2[d]	Prediction[e]	MutationTaser[f]	PANTHER (subPSEC)	LRT	GERP	PHYLOP
2-1342	1	16376358	CLCNKB	NM_001165945	c.C408G	p.I136M	M	CG	D	T(0.14)	B(0.014)	B	PP(0.999)	-4.39652	0.4905	-6.31	0.059562
2-1272	1	113235478	MOV10	NM_001130079	c.G1067A	p.R356Q	M	GA	D	D(0.01)	PD(0.581)	PD	DC(0.999)	-4.05166	0.99993	3.35	0.994954
2-1329	1	159846398	CCDC19	NM_012337	c.G1300A	p.A434T	M	CT	D	T(0.41)	B(0.003)	B	PP(0.999)	-0.7287	0.492197	-3.74	0.029105
2-1363	1	201687561	NAV1	NM_020443	c.C904A	p.R302S	M	CA	D	D(0)	D(0.956)	D	PP(0.691)	-2.86073	0.999994	3.66	0.978374
2-1370	10	24802273	KIAA1217	NM_019590	c.T2087C	p.L696P	M	TC	D	D(0.03)	D(0.999)	D	DC(0.998)	-2.24912	0.999941	5.46	0.998258
2-1370	10	75294480	USP54	NM_152586	c.11194dup	p.S399Efs	F	1bp ins	D	-	-	D	DC(1)	-	-	-	-
2-1329	10	81702258	SFTPD	NM_003019	c.C319T	p.P107S	M	GA	D	-	PD(0.731)	PD	PP(0.946)	-1.60762	0.760274	4.11	0.998875
2-1295	10	105768025	SLK	NM_014720	c.C2695T	p.R899C	M	CT	D	D(0.01)	D(1)	D	DC(0.999)	-8.11095	1	5.59	0.999222
2-1276	11	12316171	MICALCL	NM_032867	c.1193_1194insA	p.S399Lfs	F	1bp ins	D	-	-	D	DC(1)	-	-	-	-
2-1305	11	34107924	CAPRIN1	NM_005898	c.C1195T	p.Q399X	N	CT	D	-	-	D	DC(1)	-	1	5.57	0.999197
2-1360	11	64852255	ZFPL1	NM_006782	c.G85A	p.V29I	M	GA	D	T(0.11)	D(0.957)	U	DC(0.999)	-1.98159	0.999941	3.51	0.982783
2-1370	11	92600180	FAT3	NM_001008781	c.G11932A	p.V3978M	M	GA	D	D(0)	B(0.419)	U	DC(0.959)	-	-	-	-
2-1244	12	2775890	CACNA1C	NM_001129842	c.G4565A	p.R1522Q	M	GA	I	D(0)	D(0.988)	D	DC(0.999)	-5.30958	-	-	-
2-1266	12	65269243	TBC1D30	NM_015279	c.C1961T	p.A654V	M	CT	D	T(1)	B(0)	B	-	-	-	-	-
2-1314	12	123213933	HCAR1	NM_032554	c.C954T	p.L318L	S	GA	D	-	-	-	PP(0.729)	-	-	-	-
2-1291	14	105354151	KIAA0284	NM_015005	c.G3365A	p.R1122H	M	GA	D	D(0.02)	D(0.992)	D	PP(0.931)	-5.64741	-	-	-
2-1368	15	49426143	COPS2	NM_001143887	c.C899T	p.P300L	M	GA	D	D(0)	D(0.987)	D	DC(0.999)	-3.73227	1	5.58	0.999759
2-1239	17	1411432	INPP5K	NM_001135642	c.G415A	p.G139S	M	CT	D	T(0.92)	B(0.001)	B	PP(0.999)	-4.30698	0.999756	0.575	0.078338
2-0704	17	43111636	DCAKD	NM_001128631	c.C235A	p.R79R	S	GT	D	-	-	-	PP(0.729)	-	-	-	-
2-1276	2	25466800	DNMT3A	NM_175629	c.C1903T	p.R635W	M	GA	D	D(0.01)	D(0.958)	D	DC(0.999)	-7.01189	1	4.03	0.990943
2-1329	2	39249972	SOS1	NM_005633	c.G1597A	p.E533K	M	CT	I	D(0.01)	D(0.977)	D	DC(0.999)	-3.39319	1	5.57	0.999202
2-1116	2	50724817	NRXN1	NM_001135659	c.C2653T	p.H885Y	M	GA	I	D(0.01)	D(0.983)	D	PP(0.923)	-5.58385	-	-	-
2-1116	2	97215971	ARID5A	NM_212481	c.G359T	p.G120V	M	GT	D	D(0.05)	D(0.982)	D	DC(0.945)	-3.6209	0.999918	3.95	0.998955
2-1379	2	153575211	ARL6IP6	NM_152522	c.C73G	p.R25G	M	CG	D	T(0.32)	PD(0.878)	U	PP(0.991)	-	0.342823	-3.5	0.089324
2-1291	2	166152415	SCN2A	NM_001040143	c.C82T	p.R28C	M	CT	I	D(0.04)	D(0.972)	D	DC(0.991)	-7.05459	0.054772	5.2	0.998825
2-1337	2	166170551	SCN2A	NM_001040143	c.1317_1348del	p.440_450del	F	32bp del	D	-	-	D	-	-	-	-	-
2-1334	2	200137173	SATB2	NM_015265	c.C1963T	p.P655S	M	GA	I	D(0.03)	D(0.999)	D	DC(0.985)	-2.17544	1	5.45	0.999714
2-1352	20	17923824	SNX5	NM_014426	c.A1094G	p.K365R	M	TC	D	T(0.13)	B(0.04)	B	DC(0.999)	-4.35885	1	5.3	0.997996
2-1269	20	62055529	KCNQ2	NM_172109	c.1247+1G>A	-	SS	CT	I	-	-	D	DC(0.999)	-	-	-	-
2-1363	22	18387403	MICAL3	NM_001122731	c.A467G	p.H156R	M	TC	D	D(0)	D(1)	D	PP(0.999)	-	-	-	-
2-1272	3	47957453	MAP4	NM_001134364	c.A1864G	p.M622V	M	TC	D	T(0.67)	B(0.004)	B	PP(0.999)	-2.03529	0.580192	-2.24	0.076731
2-1368	3	48621941	COL7A1	NM_000094	c.C4096T	p.R1366W	M	GA	D	-	unknown(0)	unknown	PP(0.995)	-7.16169	0.702023	2.91	0.986511
2-1239	3	49064457	IMPDH2	NM_000884	c.G555C	p.L185F	M	CG	D	D(0)	D(0.978)	D	DC(0.999)	-4.57509	1	3.72	0.945302
2-1370	3	120628487	STXBP5L	NM_014980	c.G62A	p.G21D	M	GA	D	T(0.12)	B(0.084)	B	PP(0.654)	-1.65615	0.998397	4.95	0.999723
2-1182	5	422895	AHRR	NM_020731	c.C505G	p.Q169E	M	CG	D	T(0.08)	B(0.284)	B	DC(0.983)	-1.76868	1	4.49	0.997447
2-1239	5	156679654	ITK	NM_005546	c.G1829A	p.R610H	M	GA	D	T(0.32)	B(0)	B	PP(0.932)	-3.55725	0.937182	3.09	0.964439
2-1272	5	168175351	SLIT3	NM_003062	c.G2226T	p.G742G	S	CA	D	-	-	-	PP(0.648)	-	-	-	-
2-1369	6	152771947	SYNE1	NM_033071	c.C3229T	p.P1077S	M	GA	D	-	B(0.264)	possibly B	PP(0.745)	-3.86778	0.999809	5.73	0.9998
2-1186	6	153075412	VIP	NM_003381	c.T219A	p.Y73X	N	TA	D	-	-	D	DC(0.999)	-	0.997782	1.55	0.893102
2-1295	7	127670456	LRRC4	NM_022143	c.C238G	p.L80V	M	GC	D	D(0)	D(0.996)	D	DC(0.957)	-5.611	0.99999	4.11	0.999151
2-1303	7	133842813	LRGUK	NM_144648	c.A696G	p.G232G	S	AG	D	-	-	-	PP(0.729)	-	-	-	-
2-1352	8	10583692	SOX7	NM_031439	c.G723A	p.P241P	S	CT	D	-	-	-	PP(0.729)	-	-	-	-
2-1272	8	61742924	CHD7	NM_017780	c.G3566A	p.R1189H	M	GA	I	D(0)	D(1)	D	DC(0.990)	-7.94252	-	-	-
2-1363	8	104439424	DCAF13	NM_015420	c.T1024G	p.C342G	M	TG	D	T(0.38)	B(0.042)	B	DC(0.832)	-1.9283	0.999985	5.51	0.998337
2-1363	9	133942350	LAMC3	NM_006059	c.G2351A	p.R784Q	M	GA	D	T(0.06)	B(0.159)	B	PP(0.982)	-4.37859	0.983004	3.33	0.98462
2-1352	9	140710435	EHMT1	NM_024757	c.C3295G	p.P1099A	M	CG	I	D(0)	D(0.991)	D	DC(0.992)	-4.71053	1	4.95	0.998448
2-1280	X	3248358	MXRA5	NM_015419	c.A410G	p.N137S	M	TC	D	D(0)	D(0.956)	D	DC(0.513)	-	0.999553	3.33	0.986939
2-1303	X	8522080	KAL1	NM_000216	c.C1267T	p.R423X	N	AA	D	-	-	D	DC(0.999)	-	0.999999	-4.56	0.21329
2-1330	X	10104728	WWC3	NM_015691	c.G2819A	p.R940Q	M	AA	D	D(0)	D(1)	D	DC(0.999)	-4.76344	0.999999	5.4	0.999687
2-1266	X	30327222	NR0B1	NM_000475	c.G259T	p.A87S	M	CA	D	D(0)	D(0.999)	D	PP(0.930)	-	0.996977	3.72	0.998193
2-1330	X	32364157	DMD	NM_004009	c.G5477T	p.R1826I	M	AA	I	D(0)	D(0.933)	D	DC(0.772)	-4.80584	0.999436	5.4	0.999041
2-1353	X	64719083	ZC3H12B	NM_001010888	c.G953A	p.R318Q	M	AA	I	D(0.01)	D(0.973)	D	DC(0.998)	-5.98218	-	-	-
2-1352	X	70386983	NLGN3	NM_001166660	c.G916A	p.V306M	M	AA	I	T(0.08)	B(0.052)	B	PP(0.999)	-4.54339	0.999973	4.79	0.999658
2-1375	X	129150017	BCORL1	NM_021946	c.G3269C	p.R1090P	M	CC	I	D(0)	D(0.909)	D	PP(0.722)	-4.62477	0.994831	3.62	0.999269

Family ID	Chr	Coordinate (hg19)	Gene	Accession	Coding_Change	Protein_Change	Effect[a]	Proband-Gtype	State[b]	SIFT[c]	Polyphen-2[d]	Prediction[e]	MutationTaser[f]	PANTHER (subPSEC)	LRT	GERP	PHYLOP
2-1353	X	135795456	ARHGEF6	NM_004840	c.G806T	p.R269I	M	AA	I	D(0)	D(0.905)	D	DC(0.999)	-4.78686	1	4.7	0.985039
2-1269	X	148069012	AFF2	NM_001170628	c.G2662A	p.V888I	M	AA	I	D(0)	D(0.957)	D	-	-3.52349	0.999999	5.52	0.999736

[a]Predicted effect of mutation by ANNOVAR. F: frameshift, M: missense, N: nonsense, S: synonymous, SS: splice site

[b]Inheritance state of the mutation. D: de novo, I: inherited

[c]Predicted functional effect by SIFT. D: damaging, T: tolerated

[d]Predicted functional effect by Polyphen-2. B: benign, D: probably deleterious, PD: possibly deleterious

[e]Concluded prediction based on SIFT and Polyphen-2. B: benign, PB: possibly benign (benign based on one tool), PD: possibly deleterious (deleterious based on one tool), D: deleterious, U: undetermined (when SIFT and Polyphen-2 gave opposite prediction)

[f]Predicted functional effect by MutationTaser. DC: disease cause, PP: Polymorphism

Table S5. Annotations of ASD clinically relevant variants (cont'd)

Family ID	Chr	Coordinate (hg19)	Gene	Accession	Coding_Change	Protein Change	Exome captured	dbSNP	ESP6500(E) (%) /1000G(G) (%) /HGMD(H)	Exome papers	Known ASD gene	OMIM	Known inheritance	MGI (behavior /nervous system)	Genotype
2-1342	1	16376358	CLCNKB	NM_001165945	c.C408G	p.I136M	TRUE	-	-	-	FALSE	Bartter syndrome	recessive	-	-
2-1272	1	113235478	MOV10	NM_001130079	c.G1067A	p.R356Q	TRUE	-	-	-	FALSE	-	-	-	-
2-1329	1	159846398	CCDC19	NM_012337	c.G1300A	p.A434T	TRUE	-	0.0077(E)	-	FALSE	-	-	-	-
2-1363	1	201687561	NAV1	NM_020443	c.C904A	p.R302S	FALSE	rs146415554	0.0077(E)	-	FALSE	-	-	-	-
2-1370	10	24802273	KIAA1217	NM_019590	c.T2087C	p.L696P	TRUE	-	-	-	FALSE	-	-	-	-
2-1370	10	75294480	USP54	NM_152586	c.11194dup	p.S399Efs	TRUE	-	-	-	FALSE	-	-	-	-
2-1329	10	81702258	SFTPD	NM_003019	c.C319T	p.P107S	TRUE	-	-	-	FALSE	-	-	behavior/ nervous system	hom
2-1295	10	105768025	SLK	NM_014720	c.C2695T	p.R899C	TRUE	-	-	-	FALSE	-	-	-	-
2-1276	11	12316171	MICALCL	NM_032867	c.1193_1194insA	p.S399Lfs	TRUE	-	-	Neale: Q581E	FALSE	-	-	-	-
2-1305	11	34107924	CAPRIN1	NM_005898	c.C1195T	p.Q399X	TRUE	-	-	-	FALSE	-	-	behavior/ nervous system	hom
2-1360	11	64852255	ZFPL1	NM_006782	c.G85A	p.V29I	TRUE	-	-	-	FALSE	-	-	-	-
2-1370	11	92600180	FAT3	NM_001008781	c.G11932A	p.V3978M	TRUE	-	-	-	FALSE	-	-	nervous system	hom
2-1244	12	2775890	CACNA1C	NM_001129842	c.G4565A	p.R1522Q	TRUE	-	-	-	TRUE	Brugada syndrome/Timothy syndrome	dominant	behavior	het
2-1266	12	65269243	TBC1D30	NM_015279	c.C1961T	p.A654V	TRUE	-	0.001(G)	-	FALSE	-	-	-	-
2-1314	12	123213933	HCAR1	NM_032554	c.C954T	p.L318L	TRUE	-	-	-	FALSE	-	-	-	-
2-1291	14	105354151	KIAA0284	NM_015005	c.G3365A	p.R1122H	TRUE	-	0.0083(E)	-	FALSE	-	-	-	-
2-1368	15	49426143	COPS2	NM_001143887	c.C899T	p.P300L	TRUE	-	-	-	FALSE	-	-	-	-
2-1239	17	1411432	INPP5K	NM_001135642	c.G415A	p.G139S	TRUE	rs61733755	0.3537(E)	-	FALSE	-	-	-	-
2-0704	17	43111636	DCAKD	NM_001128631	c.C235A	p.R79R	TRUE	-	-	-	FALSE	-	-	-	-
2-1276	2	25466800	DNMT3A	NM_175629	c.C1903T	p.R635W	TRUE	rs144689354	0.0079(E)	Sanders: V665L	FALSE	-	-	nervous system	condition
2-1329	2	39249972	SOS1	NM_005633	c.G1597A	p.E533K	TRUE	-	-	-	FALSE	Fibromatosis, gingival/Noonan syndrome	dominant	nervous system	het/hom
2-1116	2	50724817	NRXN1	NM_001135659	c.C2653T	p.H885Y	TRUE	-	0.0994(E)	Lossifov: Y587X	TRUE	Pitt-Hopkins-like syndrome/Schizophrenia, susceptibility to	dominant /recessive	behavior/ nervous system	hom
2-1116	2	97215971	ARID5A	NM_212481	c.G359T	p.G120V	TRUE	-	-	-	FALSE	-	-	-	-
2-1379	2	153575211	ARL6IP6	NM_152522	c.C73G	p.R25G	TRUE	-	-	-	FALSE	-	-	-	-
2-1291	2	166152415	SCN2A	NM_001040143	c.C82T	p.R28C	TRUE	-	0.0384(E)	Lossifov: T1420M; Sanders: C959X, G1013X	TRUE	Epileptic encephalopathy, early infantile/Seizures, benign familial infantile	dominant	behavior/ nervous system	hom
2-1337	2	166170551	SCN2A	NM_001040143	c.1317_1348del	p.440_450del	TRUE	-	-	Lossifov: T1420M; Sanders: C959X, G1013X	TRUE	Epileptic encephalopathy, early infantile/Seizures, benign familial infantile	dominant	behavior/ nervous system	hom
2-1334	2	200137173	SATB2	NM_015265	c.C1963T	p.P655S	TRUE	-	-	-	TRUE	Cleft palate and mental retardation	dominant /recessive	-	-

Family ID	Chr	Coordinate (hg19)	Gene	Accession	Coding_Change	Protein Change	Exome captured	dbSNP	ESP6500(E) (%) /1000G(G) (%) /HGMD(H)	Exome papers	Known ASD gene	OMIM	Known inheritance	MGI (behavior /nervous system)	Genotype
2-1352	20	17923824	SNX5	NM_014426	c.A1094G	p.K365R	FALSE	-	-	Lossifov: A236V	FALSE	-	-	-	-
2-1269	20	62055529	KCNQ2	NM_172109	c.1247+1G>A	-	FALSE	-	-	-	FALSE	Epileptic encephalopathy, early infantile/Myokymia/Seizures, benign neonatal	dominant	behavior/ nervous system	het
2-1363	22	18387403	MICAL3	NM_001122731	c.A467G	p.H156R	FALSE	-	-	-	FALSE	-	-	-	-
2-1272	3	47957453	MAP4	NM_001134364	c.A1864G	p.M622V	TRUE	-	-	O'Roak: D122W	FALSE	-	-	-	-
2-1368	3	48621941	COL7A1	NM_000094	c.C4096T	p.R1366W	TRUE	rs147089666	0.0308(E)	-	FALSE	EBD inversa/EBD, Bart type/EBD, localisata variant/Epidermolysis bullosa dystrophica, AD/Epidermolysis bullosa dystrophica, AR/Epidermolysis bullosa pruriginosa/Epidermolysis bullosa, pretibial/Toenail dystrophy, isolated/Transient bullous of the newborn	dominant/recessive	-	-
2-1239	3	49064457	IMPDH2	NM_000884	c.G555C	p.L185F	TRUE	-	-	-	FALSE	IMPDH2 enzyme activity, variation in	dominant	-	-
2-1370	3	120628487	STXBP5L	NM_014980	c.G62A	p.G21D	TRUE	-	-	-	FALSE	-	-	-	-
2-1182	5	422895	AHRR	NM_020731	c.C505G	p.Q169E	TRUE	-	-	-	FALSE	-	-	-	-
2-1239	5	156679654	ITK	NM_005546	c.G1829A	p.R610H	TRUE	rs138438785	0.0154(E)	-	FALSE	Lymphoproliferative syndrome, EBV-associated, autosomal	dominant	-	-
2-1272	5	168175351	SLIT3	NM_003062	c.G2226T	p.G742G	TRUE	-	-	-	FALSE	-	-	-	-
2-1369	6	152771947	SYNE1	NM_033071	c.C3229T	p.P1077S	TRUE	-	-	-	FALSE	Emery-Dreifuss muscular dystrophy/Spinocerebellar ataxia	dominant /recessive	behavior/ nervous system	hom
2-1186	6	153075412	VIP	NM_003381	c.T219A	p.Y73X	FALSE	-	-	-	FALSE	-	-	behavior	het
2-1295	7	127670456	LRRC4	NM_022143	c.C238G	p.L80V	TRUE	-	-	-	FALSE	-	-	behavior	hom
2-1303	7	133842813	LRGUK	NM_144648	c.A696G	p.G232G	TRUE	-	-	-	FALSE	-	-	-	-
2-1352	8	10583692	SOX7	NM_031439	c.G723A	p.P241P	TRUE	-	-	-	FALSE	-	-	-	-
2-1272	8	61742924	CHD7	NM_017780	c.G3566A	p.R1189H	TRUE	-	-	O'Roak: G996S	TRUE	CHARGE syndrome/Hypogonadotropic hypogonadism with or without anosmia/Scoliosis, idiopathic	dominant	behavior/ nervous system	het
2-1363	8	104439424	DCAF13	NM_015420	c.T1024G	p.C342G	FALSE	-	-	-	FALSE	-	-	-	-
2-1363	9	133942350	LAMC3	NM_006059	c.G2351A	p.R784Q	TRUE	-	0.0231(E)	-	FALSE	Cortical malformations, occipital	recessive	nervous system	hom
2-1352	9	140710435	EHMT1	NM_024757	c.C3295G	p.P1099A	TRUE	-	-	-	TRUE	Kleefstra syndrome	dominant /recessive	behavior/ nervous system	het
2-1280	X	3248358	MXRA5	NM_015419	c.A410G	p.N137S	TRUE	-	-	-	FALSE	-	-	-	-
2-1303	X	8522080	KAL1	NM_000216	c.C1267T	p.R423X	TRUE	-	R423X(H)	-	FALSE	Hypogonadotropic hypogonadism with or without anosmia (Kallmann syndrome 1)	X-linked	-	-
2-1330	X	10104728	WWC3	NM_015691	c.G2819A	p.R940Q	TRUE	-	-	-	FALSE	-	-	-	-
2-1266	X	30327222	NR0B1	NM_000475	c.G259T	p.A87S	TRUE	-	-	-	FALSE	46XY sex reversal 2, dosage-sensitive/Adrenal	X-linked	-	-

Family ID	Chr	Coordinate (hg19)	Gene	Accession	Coding_Change	Protein Change	Exome captured	dbSNP	ESP6500(E) (%) /1000G(G) (%) /HGMD(H)	Exome papers	Known ASD gene	OMIM	Known inheritance	MGI (behavior /nervous system)	Genotype
												hypoplasia, congenital, with hypogonadotropic hypogonadism			
2-1330	X	32364157	DMD	NM_004009	c.G5477T	p.R1826I	TRUE	-	0.0189(E)	-	TRUE	Becker muscular dystrophy/Cardiomyopathy, dilated, 3B/Duchenne muscular dystrophy	X-linked	behavior/ nervous system	hemi
2-1353	X	64719083	ZC3H12B	NM_001010888	c.G953A	p.R318Q	TRUE	-	-	O'Roak: R67H	FALSE	-	-	-	-
2-1352	X	70386983	NLGN3	NM_001166660	c.G916A	p.V306M	TRUE	-	-	-	TRUE	Asperger syndrome susceptibility/Autism susceptibility	X-linked	behavior/ nervous system	hemi
2-1375	X	129150017	BCORL1	NM_021946	c.G3269C	p.R1090P	TRUE	-	-	Sanders: C797Y	FALSE	-	-	-	-
2-1353	X	135795456	ARHGEF6	NM_004840	c.G806T	p.R269I	TRUE	-	-	-	TRUE	Mental retardation	X-linked	behavior/ nervous system	hemi
2-1269	X	148069012	AFF2	NM_001170628	c.G2662A	p.V888I	TRUE	-	-	-	TRUE	Mental retardation	X-linked	behavior/ nervous system	hemi

[a]Predicted effect of mutation by ANNOVAR. F: frameshift, M: missense, N: nonsense, S: synonymous, SS: splice site

[b]Inheritance state of the mutation. D: de novo, I: inherited

[c]Predicted functional effect by SIFT. D: damaging, T: tolerated

[d]Predicted functional effect by Polyphen-2. B: benign, D: probably deleterious, PD: possibly deleterious

[e]Concluded prediction based on SIFT and Polyphen-2. B: benign, PB: possibly benign (benign based on one tool), PD: possibly deleterious (deleterious based on one tool), D: deleterious, U: undetermined (when SIFT and Polyphen-2 gave opposite prediction)

[f]Predicted functional effect by MutationTaser. DC: disease cause, PP: Polymorphism

Table S6. Clinical description of individuals carrying rare or *de novo* variant

Family/ ID	Variant	Relation to Proband/ Diagnosis	Clinical Details
Family A (2-1186)			
-01 (F)		Mother	**Language**: PPVT-IV (SS=93); **Other Medical Info**: hypothyroidism, allergies
-02 (M)		Father	**Language**: PPVT-IV (SS=99)
-03 (M)	*VIP* Y73*	Proband: **Clinical dx**: Asperger; **ADI** & **ADOS dx**: Autism	**IQ**: WPPSI-III: verbal IQ=115, performance IQ=95, full scale IQ=102; Leiter-R Brief: IQ=102; Merrill-Palmer: developmental idex SS=99 (47 %ile), cognitive SS=111 (77 %ile); **Language:** OWLS: listening comprehension SS=102, oral expression SS=111, oral composite SS=107; PPVT-IV: SS=104; PLS-4: auditory comprehension SS=135, expressive communication SS=126; **Adaptive Behavior**: VABS-II: communication SS=89, daily living SS=87, socialization SS=77, motor SS=81, adaptive behavior composite SS=80; **Other Medical Info**: iron deficient
-04 (M)	*VIP* Y73*	Full sibling	**Language**: PPVT-IV: SS=131; **Other Medical Info**: trouble with bowels; speech delay; clumsy as a preschooler and would not put hands down to stop the fall; **Microarray analysis**: arr 3q25.32q25.3(159,815,284-160,267,487)x1 maternal inherited
Family B (2-1305)			
-01 (F)		Mother	**IQ**: WASI-I: verbal IQ=115, performance IQ=110, full scale IQ=115; **Language**: PPVT-IV: SS=123
-02 (M)		Father	**IQ**: WASI-I: verbal IQ=111, performance IQ=124, full scale IQ=119; **Language**: PPVT-IV: SS=115
-03 (M)	*CAPRIN1* Q399*	Proband**: Clinical dx**: Asperger; **ADI** & **ADOS dx**: Autism	**IQ**: WISC-IV: verbal comprehension composite=121, perceptual reasoning composite=129, working memory composite=104, processing speed composite=94, full scale IQ=119; Leiter-R Brief: IQ=98; **Language**: OWLS: listening comprehension SS=93, oral expression SS=84, oral composite SS=87; PPVT-IV: SS=135; **Adaptive Behavior**: VABS-II: communication SS=88, daily living SS=76, socialization SS=64, adaptive behavior composite SS=74; **Other Medical Info**: OCD suspected; had fetal distress and had trouble breathing, was cyanotic and needed oxygen; had some gastrointestinal issues.
Family C (2-1337)			
-01 (F)		Mother	**Language**: PPVT-IV: SS=105; **Other Medical Info**: seasonal allergies, hypothyroidism
-02 (M)		Father	**Language**: PPVT-IV: SS=98; **Other Medical Info**: suspected ADHD

Family/ ID	Variant	Relation to Proband/ Diagnosis	Clinical Details
-03 (F)	*SCN2A* 440_450del	Proband: **Clinical dx**: Autism; **ADI** & **ADOS dx**: Autism	**IQ**: Leiter-R Brief: IQ=too low functioning to score; **Adaptive Behavior**: VABS-II: communication SS=47, daily living SS=69, socialization SS=66, motor SS=64, adaptive behavior composite SS=59; **Other Medical Info**: reactive asthma when sick
Family D (2-1370)			
-01 (F)		Mother	**Language**: PPVT-IV: SS=113; **Other Medical Info**: allergies
-02 (M)		Father	**Language**: PPVT-IV: SS=107
-03 (M)	*USP54* S399Efs (*KIAA1217* L696P; *FAT3* V3978M; *STXBP5L* p.G21D)	Proband: **Clinical dx**: Asperger; **ADI** & **ADOS dx**: Autism	**IQ**: WISC-IV: verbal comprehension composite=96, perceptual reasoning composite=111; Leiter-R Brief: IQ=87; **Language**: OWLS: listening comprehension SS=101, oral expression SS=104, oral composite SS=102; PPVT-IV: SS=123; **Adaptive Behavior**: VABS-II: communication SS=92, daily living SS=76, socialization SS=66, adaptive behavior composite SS=76; **Other Medical Info**: allergies
Family E (2-1303)			
-01 (F)		Mother	**Language**: PPVT-IV: SS=109; **Other Medical Info**: underactive thyroid
-02 (M)		Father	**Language**: PPVT-IV: SS=107; **Other Medical Info**: high blood pressure
-03 (M)	*KAL1* R423* (*LRGUK* G232G)	Proband: **Clinical dx:** Atypical/PDD-NOS/ASD; **ADI** & **ADOS dx**: Autism	**IQ**: WASI-I: verbal IQ=78, performance IQ=96, full scale IQ=86; **Language**: OWLS: listening comprehension SS=72, oral expression SS=74, oral composite SS=71; PPVT-IV: SS=78; **Adaptive Behavior**: VABS-II: communication SS=68, daily living SS=72, socialization SS=60, adaptive behavior composite SS=65; **Other Medical Info**: Kallmann syndrome; has only one kidney; born with micro-penis, mirror movements; Type 1 diabetes
Family F (2-1276)			
-01 (F)		Mother	**Language**: PPVT-IV: SS=120; CELF-4: core language SS=124, %ile rank=95, expressive language SS=120, %ile rank=91; **Other Medical Info**: seasonal allergies, asthma
-02 (M)		Father	**Other Medical Info**: recovered from Hepatitis C
-03 (F)	*MICALCL* S399Lfs (*DNMT3A* R635W)	Proband: **Clinical dx**: Atypical/PDD-NOS/ASD; **ADI** & **ADOS dx**: Autism	**IQ**: Leiter-R Brief: IQ=38; Stanford-Binet: Abbreviated IQ SS=47; **Language**: PPVT-IV: SS=20; **Adaptive Behavior**: VABS-II: communication SS=43, daily living SS=40, socialization SS=40, motor SS=56, adaptive behavior composite SS=39; **Other info**: has 4 other relatives with ASD (cousins)

Family/ ID	Variant	Relation to Proband/ Diagnosis	Clinical Details
Family G (2-1269)			
-01 (F)	*AFF2* V888I	Mother	**Language**: PPVT-IV: SS=103; **Other Medical Info**: asthma, hypertension; self-reported dyslexic
-02 (M)	*KCNQ2* c.1247+1G>A	Father: **Clinical dx**: Asperger	**Languag**e: PPVT-IV: SS=90; **Other Medical Info**: depression; had seizures as an infant
-03 (M)	*AFF2* V888I; *KCNQ2* c.1247+1G>A	Proband: **Clinical dx**: Autism; **ADI** & **ADOS dx**: Autism	**IQ**: Leiter-R Brief: IQ=117; **Language**: OWLS: listening comprehension SS=79, oral expression SS=59, oral composite SS=67; PPVT-IV: SS=82; **Adaptive Behavior**: VABS-II: communication SS=78, daily living SS=75, socialization SS=74, motor SS=72, adaptive behavior composite SS=71; **Other Medical Conditions**: at 3 days developed seizures, meningitis suspected but father also had seizures as an infant, was on phenobarbitol for 1 month; **Other info**: paternal half uncle has male child with ASD; paternal half aunt has female child with severe autism
-04 (F)	*KCNQ2* c.1247+1G>A	Full sibling	**Other Medical Inf**o: skull x-ray requested in 2012 because head circumference is smaller than expected
-13 (F)	*AFF2* V888I	Maternal Grandmother	
-15 (F)	*KCNQ2* c.1247+1G>A	Paternal Grandmother	
Family H (2-1353)			
-01 (F)	*ARHGEF6* R269I; *ZC3H12B* R318Q	Mother	**Language**: PPVT-IV: SS=115; **Other Medical Info**: allergies to bees and aspirin
-02 (M)		Father	**Language**: PPVT-IV: SS=88 (ESL); **Other Medical Info**: allergies
-03 (M)	*ARHGEF6* R269I; *ZC3H12B* R318Q	Proband: **Clinical dx**: Atypical/PDD-NOS/ASD; **ADI** & **ADOS dx**: Autism	**IQ**: Leiter-R Brief: IQ=70; **Adaptive Behavior**: VABS-II: communication SS=64, daily living SS=69, socialization SS=59, adaptive behavior composite SS=64;
-04 (F)	*ARHGEF6* R269I; *ZC3H12B* R318Q	full sibling of proband	**Language**: PPVT-IV: SS=99

Family/ ID	Variant	Relation to Proband/ Diagnosis	Clinical Details
Family I (2-1330)			
-01 (F)	*DMD* R1826I	Mother	**Language**: PPVT-IV: SS=100
-02 (M)		Father	**Language**: PPVT-IV: SS=109; **Other Medical info**: recovered from brain tumour 3 years ago, kidney stones
-03 (M)	*DMD* R1826I (*WWC3* R940Q)	Proband: **Clinical dx**: Asperger; **ADI** & **ADOS dx**: Autism	**IQ**: WASI-I: verbal IQ=95, performance IQ=93, full scale IQ=93; **Language**: OWLS: listening comprehension SS=86, oral expression SS=94, oral composite SS=89; PPVT-IV: SS=91; **Adaptive Behavior**: VABS-II: communication SS=96, daily living SS=85, socialization SS=89, adaptive behavior composite SS=88
-05 (F)	*DMD* R1826I	Maternal Grandmother	
Family J (2-1375)			
-01 (F)	*BCORL1* R1090P	Mother	**Language**: PPVT-IV: SS=100
-02 (M)		Father	**Language**: PPVT-IV: SS=118
-03 (M)	*BCORL1* R1090P	Proband: **Clinical dx**: Atypical/PDD-NOS/ASD; **ADI** & **ADOS dx**: Autism	**IQ**: WASI-I: verbal IQ=125, performance IQ=112, full scale IQ=121; **Languag**e: OWLS: listening comprehension SS=111, oral expression SS=102, oral composite SS=107; PPVT-IV: SS=119; **Adaptive Behavior**: VABS-II: communication SS=79, daily living SS=87, socialization SS=71, adaptive behavior composite SS=77
-05 (F)	*BCORL1* R1090P	Full Sibling	
Family K (2-1272)			
-01 (F)		Mother	**Language**: PPVT-IV: SS=106; **Other Medical Info**: lactose intolerant
-02 (M)	*CHD7* R1189H	Father	**Language**: PPVT-IV: SS=115
-03 (M)	*CHD7* R1189H (*MOV10* R356Q; *MAP4* M622V; *SLIT3* G742G)	Proband: **Clinical dx**: Asperger; **ADI** & **ADOS dx**: Autism	**IQ**: WPPSI-III: verbal IQ=118, performance IQ=108, full scale IQ=113; **Language**: OWLS: listening comprehension SS=103, oral expression SS=104, oral composite SS=103; PPVT-IV: SS=100; **Adaptive Behavior**: VABS-II: communication SS=100, daily living SS=93, socialization SS=70, motor SS=94, adaptive behavior composite SS=87; **Other Medical Info**: recurrent ear infections, tonsils and adenoids removed, had sleep apnea; eats and sleeps well but still low energy

Family/ ID	Variant	Relation to Proband/ Diagnosis	Clinical Details
Family L (2-1291)			
-01 (F)	*SCN2A* R28C	Mother	**Language**: PPVT-IV: SS=101; **Other Info**: procrastinates, worries
-02 (M)		Father	**Language**: PPVT-IV: SS=118; **Other Medical Info**: severe arthritis came on in his early teens, some learning difficulties
-03 (M)	*SCN2A* R28C (*KIAA0284* R1122H)	Proband: **Clinical dx**: Asperger; **ADI** & **ADOS dx**: Autism	**IQ**: WISC-IV: verbal comprehension composite=98, perceptual reasoning composite=107, working memory composite=97, processing speed composite=75, full scale IQ=93; **Language**: OWLS: listening comprehension SS=87, oral expression SS=92, oral composite SS=88; PPVT-IV: SS=111; **Adaptive Behavior**: VABS-II: communication SS=79, daily living SS=78, socialization SS=69, adaptive behavior composite SS=74
-04 (F)	*SCN2A* R28C	Full Sibling	**Other Medical Info**: born with urinary reflux -microplasty both ureters at age 6;; poor at reading social cues, relates better to adults, mother questions ASD
-05 (F)	*SCN2A* R28C	Full Sibling	**Language**: PPVT-IV: SS=111; **Other Medical Info**: heart murmur; **Other Info**: procrastinator, time management hard
-06 (F)	*SCN2A* R28C	Maternal Grandmother	
Family M (2-1116)			
-01 (F)		Mother	**Language**: PPVT-IV: SS=98; EVT-2: SS=115; **Other Medical Info**: allergies, eczema, alopecia (no body hair)
-02 (M)	*NRXN1* H885Y	Father	**Language**: PPVT-IV: SS=103; EVT-2: SS=112
-03 (M)	*NRXN1* H885Y (*ARID5A* G120V)	Proband: **Clinical dx**: Autism; **ADI** & **ADOS dx**: Autism	**IQ**: Leiter-R Brief: IQ=89; **Language**: OWLS: listening comprehension SS=61, oral expression SS=58, oral composite SS=57; **Adaptive Behavior**: VABS-II: communication SS=67, daily living SS=66, socialization SS=61, adaptive behavior composite SS=64; **Other Medical Info**: eczema; one incident of no body hair - triggered by stress
-04 (M)	*NRXN1* H885Y	Full Sibling	**Language**: OWLS: listening comprehension SS=117, oral expression SS=110, oral composite SS=114; **Other Medical Info**: eczema; infant asthma, learning disability- Central Auditory Processing Disorder (CAPD)
Family N (2-1244)			
-01 (F)		Mother	**Language**: PPVT-IV: SS=123; **Other Medical Info**: epilepsy
-02 (M)	*CACNA1C* R1522Q	Father	**Language**: PPVT-IV: SS=115; **Other Medical Info**: Wolff-Parkinson-White syndrome

Family/ ID	Variant	Relation to Proband/ Diagnosis	Clinical Details
-03 (M)	*CACNA1C* R1522Q	Proband: **Clinical dx**: Atypical/PDD-NOS/ASD; **ADI** & **ADOS dx**: Autism	**IQ**: WISC-IV: verbal comprehension composite=102, perceptual reasoning composite=107, working memory composite=111, processing speed composite=85, full scale IQ=101; **Language**: OWLS: listening comprehension SS=66, oral expression SS=93, oral composite SS=78; PPVT-IV: SS=113; **Adaptive Behavior**: VABS-II: communication SS=88, daily living SS=103, socialization SS=91, adaptive behavior composite SS=92; **Other Medical Info**: ADHD, migraines, peanut allergy
-04 (M)	*CACNA1C* R1522Q	Full Sibling	**Language**: PPVT-IV: SS=111; **Other Medical Info**: milk allergy
Family O (2-1352)			
-01 (F)	*NLGN3* V306M; *EHMT1* P1099A	Mother	**Language**: PPVT-IV: SS=93; **Other Medical Info**: allergy-induced asthma
-02 (M)		Father	**Langage**: PPVT-IV: SS=100; **Other Medical Info**: mitrovalve prolapse
-03 (M)	*NLGN3* V306M; *EHMT1* P1099A (*SNX5* K365R; *SOX7* P241P)	Proband: **Clinical dx**: Asperger; **ADI** & **ADOS dx**: Autism	**IQ**: WASI-I: verbal IQ=119, performance IQ=114, full scale IQ=119; **Language**: OWLS: listening comprehension SS=97, oral expression SS=105, oral composite SS=101; PPVT-IV: SS=105; **Adaptive Behavior**: VABS-II: communication SS=104, daily living SS=97, socialization SS=76, adaptive behavior composite SS=90
-04 (F)	*NLGN3* V306M	Full Sibling	**Language**: PPVT-IV: SS=114
-05 (F)	*EHMT1* P1099A	Maternal Grandmother	
Family P (2-1334)			
-01 (F)		Mother	**Language**: PPVT-IV: SS=97
-02 (M)	*SATB2* P655S	Father	**Language**: PPVT-IV: SS=98
-03 (M)	*SATB2* P655S	Proband: **Clinical dx**: Autism; **ADI** & **ADOS dx**: Autism	**IQ**: Leiter-R Brief: IQ=62; **Adaptive Behavior**: VABS-II: communication SS=61, daily living SS=64, socialization SS=63, motor SS=61, adaptive behavior composite SS=59;
-06 (M)	*SATB2* P655S	Paternal Grandfather	
2-1342			
-01 (F)		Mother: **Clinical dx**: Asperger	**IQ**: WISC-R: verbal IQ=124, performance IQ=131, full scale IQ=131; Language: PPVT-IV: SS=123; **Other Medical Info**: depression
-02 (M)		Father: **Clinical dx**:	**Language**: PPVT-IV: SS=107; **Other Medical Information**: diabetes (could be a metabolic

Family/ ID	Variant	Relation to Proband/ Diagnosis	Clinical Details
		Asperger	syndrome), takes insulin injection
-03 (F)	*CLCNKB* I136M	Proband: **Clinical dx**: Asperger; **ADI** & **ADOS dx**: Autism	**IQ**: WASI-I: verbal IQ=114, performance IQ=128, full scale IQ=124; **Language**: OWLS: listening comprehension SS=96, oral expression SS=86, oral composite SS=90; PPVT-IV: SS=129; **Adaptive Behavior**: VABS-II: communication SS=94, daily living SS=76, socialization SS=64, adaptive behavior composite SS=76
2-1182			
-01 (F)		Mother	
-02 (M)		Father	
-03 (M)	*AHRR* Q169E	Proband: **Clinical dx**: Autism; **ADI** & **ADOS dx**: Autism	**IQ**: Leiter-R Brief: IQ=105; **Language**: OWLS: listening comprehension SS=85, oral expression SS=101, oral composite SS=92; **Adaptive Behavior**: VABS-II: communication SS=97, daily living SS=85, socialization SS=85, motor SS=94, adaptive behavior composite SS=88; **Other Info**: maternal aunt has male child with ASD
2-1379			
-01 (F)		Mother	**Language**: PPVT-IV: SS=113
-02 (M)		Father	**Language**: PPVT-IV: SS=95
-03 (M)	*ARL6IP6* C73G	Proband: **ADI** & **ADOS dx**: Autism	**IQ/Language**: Mullen: visual reception T-score=20, fine motor T-score=<20, receptive language T-score=<20, expressive language T-score=24, early learning composite SS=50; **Adaptive Behavior**: VABS-II: communication SS=69, daily living SS=77, socialization SS=72, motor SS=67, adaptive behavior composite SS=68
2-1266			
-01 (F)		Mother	**Language**: PPVT-IV: SS=100; **Other Medical Info**: underactive thyroid, ulcers
-02 (M)		Father	**Language**: PPVT-IV: SS=120; **Other Medical Inf**o: hereditary tremor (father and brothers also have same condition)
-03 (F)	*TBC1D30* A654V; *NR0B1* A87S	Proband**: Clinical dx**: Asperger; **ADI** & **ADOS dx**: Autism	**IQ**: WASI-I: verbal IQ=93, performance IQ=103, full scale IQ=98; **Language**: OWLS: listening comprehension SS=81, oral expression SS=86, oral composite SS=82; PPVT-IV: SS=94; **Adaptive Behavior**: VABS-II: communication SS=81, daily living SS=83, socialization SS=73, adaptive behavior composite SS=77
2-1314			
-01 (F)		Mother	**Language**: PPVT-IV: SS=125
-02 (M)		Father	**Language**: PPVT-IV: SS=123

Family/ ID	Variant	Relation to Proband/ Diagnosis	Clinical Details
-03 (M)	*HCAR1* L318L	Proband: **Clinical dx**: Autism; **ADI** & **ADOS dx**: Autism	**IQ**: Leiter-R Brief: IQ=68; **Language**: OWLS: listening comprehension SS=45, oral expression SS=50, oral composite SS=45; PPVT-IV: SS=64; **Adaptive Behavior**: VABS-II: communication SS=79, daily living SS=81, socialization SS=75, motor SS=72, adaptive behavior composite SS=73
2-1268			
-01 (F)		Mother	**Other Medical Info**: depression, learning disability, auditory processing problems
-02 (M)		Father	**Other Medical Info**: depression, speech delay
-03 (M)		Proband: **Clinical dx**: Atypical/PDD-NOS/ASD; **ADI** & **ADOS dx**: Autism	**IQ/Language**: Mullen: visual reception T-score=20, fine motor T-score=20, receptive language T-score=<20, expressive language T-score=<20, early learning composite SS=49; **Adaptive Behavior**: VABS-II: communication SS=65, daily living SS=85, socialization SS=72, motor SS=72, adaptive behavior composite SS=70
2-1239			
-01 (F)		Mother	**Language**: PPVT-IV: SS=99; **Other Medical Info**: hypoglycemic
-02 (M)		Father	
-03 (F)	*INPP5K* G139S; *IMPDH2* L185F	Proband: **Clinical dx**: Autism; **ADI** & **ADOS dx**: Autism	**IQ**: Leiter-R Brief: IQ=36; **Language**: OWLS: listening comprehension SS=40, oral expression SS=40, oral composite SS=40; PPVT-IV: SS=20; **Adaptive Behavior**: VABS-II: communication SS=47, daily living SS=56, socialization SS=43, adaptive behavior composite SS=48
2-1280			
-01 (F)		Mother	**Language**: PPVT-IV: SS= 73 (ESL)
-02 (M)		Father	**Language**: PPVT-IV: SS=76 (ESL)
-03 (F)	*MXRA5* N137S	Proband: **Clinical dx**: Atypical/PDD-NOS/ASD; **ADI dx**: Autism; **ADOS dx**: Autism Spectrum	**IQ**: WASI-I: verbal IQ=110, performance IQ=97, full scale IQ=105; **Language**: OWLS: listening comprehension SS=94, oral expression SS=85, oral composite SS=88; PPVT-IV: SS=101; **Adaptive Behavior**: VABS-II: communication SS=79, daily living SS=75, socialization SS=68, motor SS=88, adaptive behavior composite SS=74
2-1368			
-01 (F)		Mother	**Language**: PPVT-IV: SS=107
-02 (M)		Father	**Language**: PPVT-IV: SS=99

Family/ ID	Variant	Relation to Proband/ Diagnosis	Clinical Details
-03 (F)	*COPS2* P300L; *COL7A1* R1366W	Proband: **Clinical dx**: Autism; **ADI** & **ADOS dx**: Autism	**IQ**: WASI-I: verbal IQ=102, performance IQ=112, full scale IQ=108; **Language**: OWLS: listening comprehension SS=80, oral expression SS=96, oral composite SS=87; PPVT-IV: SS=89; **Adaptive Behavior**: VABS-II: communication SS=74, daily living SS=71, socialization SS=59, adaptive behavior composite SS=66; Other Medical Info: stomach issues; **Other info**: GFCF diet
2-0704			
-01 (F)		Mother	
-02 (M)		Father	
-03 (M)	*DCAKD* C235A	Proband: **ADI** & **ADOS dx**: Autism	**IQ**: Leiter-R Brief: IQ=40; **Language**: OWLS: listening comprehension SS=40, oral expression SS=40, oral composite SS=40; **Adaptive Behavior**: VABS 1984: communication SS=19, daily living SS=19, socialization SS=21, adaptive behavior composite SS=19; **Other Info**: Father's cousin has male child with ASD
2-1295			
-01 (F)		Mother	**IQ**: WASI-I: verbal IQ=104, performance IQ=114, full scale IQ=109; **Language**: PPVT-IV: SS=92 (ESL)
-02 (M)		Father	**Language**: PPVT-IV: SS=85 (ESL); **Other Info**: parents are 2nd cousins; has twin brothers both dx with Schizophrenia
-03 (M)	*SLK* R899C; *LRRC4* L80V	Proband: **Clinical dx**: Autism; **ADI** & **ADOS dx**: Autism	**IQ:** WPPSI-III: verbal IQ=76, performance IQ=93, full scale IQ=82; **Language**: OWLS: listening comprehension SS=75, oral expression SS=82, oral composite SS=77; PPVT-IV: SS=85; **Adaptive Behavior**: VABS-II: communication SS=87, daily living SS=97, socialization SS=83, motor SS=88, adaptive behavior composite SS=86
2-1325			
-01 (F)		Mother	**Language**: PPVT-IV: SS=129; **Other Medical Info**: Crohn's Disease; exercised induced asthma
-02 (M)		Father	**Language**: PPVT-IV: SS=128
-03 (M)		Proband: **Clinical dx**: Autism; **ADI** & **ADOS dx**: Autism	**IQ**: WPPSI-III: verbal IQ=117, performance IQ=129, full scale IQ=130; **Language**: PLS-4: auditory comprehension SS=129, expressive communication SS=136, total language SS=135; PPVT-IV: SS=130; **Adaptive Behavior**: VABS-II: communication SS=106, daily living SS=111, socialization SS=88, motor SS=104, adaptive behavior composite SS=102
2-1329			
-01 (F)	*SOS1* E533K	Mother	**Language**: PPVT-IV: SS=122; **Other Medical Info**: fibromyalgia, chemical sensitivities, migraines, interstitial bladder disease

Family/ ID	Variant	Relation to Proband/ Diagnosis	Clinical Details
-02 (M)		Father	**Language**: PPVT-IV: SS=120; **Other Medical Info**: depression, anxiety, OCD, takes prozac, dyslexic
-03 (M)	*SOS1* E533K; *CCDC19* A434T; *SFTPD* P107S	Proband: **Clinical dx**: Autism; **ADI** & **ADOS dx**: Autism	**IQ**: WASI-I: verbal IQ=55, performance IQ=84, full scale IQ=69; **Language**: OWLS: listening comprehension SS=50, oral expression SS=40, oral composite SS=43; PPVT-IV: SS=57; **Adaptive Behavior**: VABS-II: communication SS=61, daily living SS=65, socialization SS=61, adaptive behavior composite SS=61; **Other Medical Info**: exercise-induced asthma, allergies; eczema; occassionaly takes prozac for OCD traits; **Other Info**: GFCF diet
2-1360			
-01 (F)		Mother	**Language**: PPVT-IV: SS=99; **Other Medical Info**: age 18 had accident - blind in one eye and deaf in one ear, acquired brain injury, functioning fine; allergic to sulfa
-02 (M)		Father	**Language**: PPVT-IV: SS=90; **Other Medical Info**: born with right hand amputation and umbilical cord wrapped around arm; hay fever, skin allergies
-03 (M)	*ZFPL1* V29I	Proband: **Clinical dx**: Atypical/PDD-NOS/ASD; **ADI** & **ADOS dx**: Autism	**IQ**: WASI-I: verbal IQ=89, performance IQ=94, full scale IQ=90; **Language**: OWLS: listening comprehension SS=99, oral expression SS=77, oral composite SS=87; PPVT-IV: SS=99; **Adaptive Behavior**: VABS-II: communication SS=69, daily living SS=71, socialization SS=66, adaptive behavior composite SS=67
2-1363			
-01 (F)		Mother	**IQ**: WASI-I: verbal IQ=98, performance IQ=116, full scale IQ=107; **Language**: PPVT-IV: SS=95
-02 (M)		Father	**Language**: PPVT-IV: SS=108; **Other Medical Info**: may have depression
-03 (M)	*NAV1* R302S; *MICAL3* H156R; *DCAF13* C342G; *LAMC3* R784Q	Proband: **Clinical dx**: Atypical/PDD-NOS/ASD; **ADI** & **ADOS dx**: Autism	**IQ**: WASI-I: verbal IQ=109, performance IQ=103, full scale IQ=107; **Language**: OWLS: listening comprehension SS=102, oral expression SS=88, oral composite SS=94; PPVT-IV: SS=111; **Adaptive Behavior**: VABS-II: communication SS=100, daily living SS=87, socialization SS=75, motor=94, adaptive behavior composite SS=86; **Other Medical Info**: emergency C-section, birth wt 11 lbs, 4.1 oz, unable to breath normally at birth, required O2, dehydrated and had malnutrition for 3 days after birth,occassionally would turn blue, jaundice
2-1369			
-01 (F)		Mother	**Language**: PPVT-IV: SS=106; **Other Medical Info**: OCD (lose does medication), used to have high blood pressure
-02 (M)		Father	**Language**: PPVT-IV: SS=115; **Other Medical Info**: medication for cholesterol

Family/ ID	Variant	Relation to Proband/ Diagnosis	Clinical Details
-03 (M)	*SYNE1* P1077S	Proband: **Clinical dx**: Asperger; **ADI dx**: Autism; **ADOS dx**: Autism Spectrum	**IQ**: WASI-I: verbal IQ=108, performance IQ=136, full scale IQ=124; **Language**: OWLS: listening comprehension SS=127, oral expression SS=105, oral composite SS=117; PPVT-IV: SS=121; **Adaptive Behavior**: VABS-II: communication SS=69, daily living SS=71, socialization SS=57, adaptive behavior composite SS=64; **Other Medical Info**: ADHD

F = Female; M = Male; dx = diagnosis

All test scores are presented in percentiles using the following classifications Above Average (75th->99th %), Average (73rd -25th %), Below Average (24th-2nd%), Mild Delay (2-0.1%) Moderate Delay (0.1%) and Severe Delay(<0.1%).

IQ was measured with an age-appropriate Weschler Scale (WPPSI, WISC, WASI) or Leiter-R Brief. Score are presented in percentiles for Full Scale IQ (FSIQ),Verbal IQ (VIQ), Nonverbal IQ (NVIQ).

Language was measures with the Oral and Written Language Scales (OWLS) percentiles scores are presented for Total Language(TL),Receptive Language (RL), Expressive Language (EL). Receptive Vocabulary (RV) was assessed using the Peabody Picture Vocabulary Test (PPVT-4th Edition).

Adaptive Behaviour was measured using the Vineland Adaptive Behaviour Scales- Second Edition (VABS-II). Percentiles are presented for Adaptive Behaviour Composite (ABC), communication (COM), Daily Living Skills (DLS), Socialization (SOC), and Motor (MOT;only for children under 7 years).

Table S7. Diagnostic data and management implication for families with ASD-relevant variants

ID	Variant	Diagnosis (age of dx)	Phenotype Comments	Family Information (if any)	Management Implications
Family A 2-1186-03 (male)	VIP p.Tyr73*, de novo	**Clinical dx**: Asperger (3.2 yrs); **ADI & ADOS dx**: Autism (3.2 yrs)	**IQ:** FSIQ Average 55%, VIQ Above Average 84%, NVIQ Average 37%, **Language**: TL Average 68%, RL Average 55%, EL Above Average 77%, RV: Average 61%; **Adaptive**: All Below Average, ABC 9%, COM 23%, DLS 19%, SOC 6%, MOT 10%; **Other**: Iron deficient	Mother (-01): hypothyroidism, allergies Sibling (-04, male): **Language**: RV Above Average 98%, trouble with bowels; speech delay; clumsy	Possible deleterious ASD mutation. Assessment of male sibling *VIP* Y73* mutation carrier for ASD. Genetic counselling of family, noting probable gonadal mosaicism. Prioritized for clinical assessment.
Family B 2-1305-03 (male)	CAPRIN1 p.Gln399*, de novo	**Clinical dx**: Asperger (6.2); **ADI & ADOS dx**: Autism (7.1 yrs)	**IQ:** All Above Average FSIQ 90%, VIQ=92%, NVIQ=97%; **Language**: TL Below Average 19%, RL Average 32%, EL Below Average 14%, RV Above Average 99%; **Adaptive:** ABC Below Average 4%, COM Below Average 21%, DLS Below Average 5%, SOC Mild Delay 1%; **Other**: OCD suspected; had fetal distress and had trouble breathing, was cyanotic and needed oxygen; Gastrointestinal issues		Possible deleterious ASD mutation. Detection *of de novo* mutation relevant to genetic counselling. Proband may be a candidate for mGluR5 drug trial. Prioritized for clinical assessment.
Family C 2-1337-03	SCN2A p.440_45	**Clinical dx**: Autism (2.4	**IQ**: NVIQ Mod Delay <0.1; **Adaptive**: ABC Mild Delay	Mother (-01): seasonal allergies, hypothyroidism	Possible deleterious ASD mutation. Detection of *de*

(female)	0 del, de novo	yrs); **ADI & ADOS dx**: Autism (4.0 yrs)	0.3%, COM: Mod Delay <0.1%, DLS Mild Delay 2%, SOC Mild Delay 1%, MOT Mild Delay 1%; **Other**: reactive asthma when sick	Father (-02): suspected ADHD Sibling (-04, female): **Clinical dx**: Atypical/PDD-NOS/ASD; **ADI & ADOS dx**: ASD (7.9 yrs); See Supplemental data for details	*novo* mutation relevant to genetic counselling noting female ASD sibling does not have the mutation. Assess mutation carrier for epilepsy. Prioritized for clinical assessment.
Family D 2-1370-03 (male)	USP54 . p.Ser399 Glufs*10, de novo	**Clinical dx**: Asperger (7.7 yrs); **ADI & ADOS dx:** Autism (11.3 yrs)	**IQ**: NVIQ Below Average 19%; **Language**: TL Average 55%, RL Average 53%, EL Average 61%, RV Above Average 94%; **Adaptive**: ABC Below Average 5% COM Average 30%, DLS Below Average 5%, SOC Mild Delay 1%; **Other:** allergies	Mother (-01): allergies	Candidate ASD mutation, which needs to monitored in medical literature. Proband also carries three other predicted benign *de novo* mutations to follow.
Family E 2-1303-03 (male)	KAL1 p.Arg423 *, de novo	**Clinical dx**: Atypical/PDD -NOS/ASD (3.8 yrs); **ADI & ADOS dx**: Autism (14.4 yrs)	**IQ**: FSIQ Below Average 18%, VIQ Below Average 7%, NVIQ Average 39%; **Language**: All Below Average, RL 3%, EL 4%, TL 3%, RV 7%; **Adaptive**: ABC Mild Delay 1%, COM Mild Delay 2%, DLS Below Average 3%, SOC Mild Delay 0.4%, **Other**: Kallmann syndrome; one kidney; micro-penis; mirror movements; Type 1 diabetes	Mother (-01): underactive thyroid Father (-02): high blood pressure	Proband has Kallmann syndrome and ASD. Potential hormone treatment. Prioritized for clinical assessment.
Family F 2-1276-03	MICALCL p.Ser399 Leufs*10,	**Clinical dx**: Atypical/PDD -NOS/ASD	**IQ**: NVIQ Severe Delay <0.1%; **Language**: RV Severe Delay <0.1%; **Adaptive**: ABC	Mother (-01): seasonal allergies, asthma	Candidate autosomal *MICALCL and DNMT3A*

(female)	de novo; DNMT3A p.Arg635 Trp, de novo	(2.7 yrs); **ADI & ADOS dx**: Autism (13.9 yrs)	Severe Delay <0.1%, COM Mod Delay <0.1%, DLS Mod Delay <0.1%, SOC Mod Delay <0.1%, MOT Mild Delay 0.2%	Father (-02): recovered from Hepatitis C Extended family members with ASD	ASD mutations, which need to be followed in the medical literature and in the context of 4 related family members also with ASD.
Family G 2-1269-03 (male)	AFF2 p.ValV888 Ile, (maternal); KCNQ2 c.1247+1 G>A (paternal)	**Clinical dx**: Autism (3.3 yrs); **ADI & ADOS dx**: Autism (3.9 yrs)	**IQ**: NVIQ Above Average 87%; **Language**: TL Mild Delay 1%, RL Below Average 8%, EL Mild Delay 0.3%, RV: Below Average 12%; **Adaptive**: All Below Average, ABC 3%, COM 7%, DLS 5%, SOC 4%, MOT 3%; **Other**: at 3 days developed seizures; meningitis suspected, was on phenobarbitol for 1 month	Mother (-01): asthma, hypertension, self-reported dyslexia Father (-02): **Clinical dx**: Aspergers (40.0 yrs); **Language**: RV Average 25%; **Other:** depression, seizures as an infant Sibling (-04, female): small head circumference Extended paternal family members reported with ASD.	Possible deleterious ASD mutation in X-linked *AFF2* and autosomal *KCNQ2* mutation in known epilepsy gene. Genetic counselling for nuclear and extended family, in particular for the female sibling mutation carrier. Proband may be a candidate for mGluR5 drug trial. Prioritized for clinical assessment.
Family H 2-1353-03 (male)	ARHGEF6 p.Arg269I le, (maternal); ZC3H12B p.Arg318	**Clinical dx**: Atypical/PDD -NOS/ASD (2.7 yrs); **ADI & ADOS dx**: Autism (8.7 yrs)	**IQ**: NVIQ Below Average 2%; **Adaptive**: All Mild Delay ABC 1%, COM 1%, DLS 2%, SOC 0.3%	Mother (-01): allergies to bees and aspirin Father (-02): allergies	Possible deleterious mutation in known *ARHGEF6* ASD gene. Follow medical literature for maternal X-linked *ZC3H12B* mutation. Prioritized for clinical assessment.

	Gln, (maternal)				
Family I 2-1330-03 (male)	DMD p.Arg1826Ile, (maternal); WWC3 p.Arg940Gln de novo	**Clinical dx**: Asperger (3.7 yrs); **ADI & ADOS dx**: Autism (11.2 yrs)	**IQ**: All Average, FSIQ 32%, VIQ 37%, NVIQ 32%; **Language:** TL Below Average 23%, RL Below Average 18%, EL Average 34%, RV Average 27%; **Adaptive**: ABC Below Average 21%, COM Average 39%, DLS Below Average 16%, SOC Below Average 23%	Father (-02): recovered from brain tumour 3 years ago, kidney stones	Proband to be assessed for muscular dystrophy. Genetic counselling of family. Follow medical literature for maternal X-linked *WWC3* mutation. Prioritized for clinical assessment.
Family J 2-1375-03 (male)	BCORL1 p.Arg1090Pro, (maternal)	**Clinical dx**: Atypical/PDD-NOS/ASD (4.8 yrs); **ADI & ADOS dx**: Autism (10 yrs)	**IQ**: FSIQ Above Average 92%, VIQ Above Average 95%, NVIQ Below Average 79%; **Language**: TL Average 68%, RL Above Average 77%, EL Average 55%, RV Above Average 90%; **Adaptive**: All Below Average, ABC 6%, COM 8%, DLS 19%, SOC 3%		Genetic counselling of family. Follow medical literature for maternal X-linked *BCORL1* mutation.
Family K 2-1272-03 (male)	CHD7 p.Arg1189His, (paternal)	**Clinical dx**: Asperger (5.9 yrs); **ADI & ADOS dx**: Autism (6.4 yrs)	**IQ**: FSIQ Above Average 81%; VIQ Above Average 88%, NVIQ Below Average 70%; **Language**: All Average, TL 58%, RL 58%, EL 61%, RV 50%; **Adaptive**: ABC Below Average 19%, COM Average 50%, DLS Average 32%, SOC	Mother (-01): lactose intolerant	Assess proband and paternal mutation carrier for CHARGE syndrome. Prioritized for clinical assessment.

			Below Average 2%, MOT Average 34%; **Other**: recurrent ear infections, tonsils and adenoids removed, had sleep apnea; eats and sleeps well but no energy		
Family L 2-1291-03 (male)	SCN2A p.Arg28Cys, (maternal) KIAA0284 p.Arg1122His, de novo	**Clinical dx**: Asperger (6.4 yrs); **ADI & ADOS dx**: Autism (8.1 yrs)	**IQ**: All average, FSIQ 32%, VIQ 45%, NVIQ 68%; **Language**: TL Below Average 21%, RL Below Average 19%, EL Average 30%, RV Above Average 77%; **Adaptive**: All Below Average, ABC 4%, COM 8%, DLS 7%, SOC 2%; **Other:** Anxiety and OCD; idiopathic ventricular tachycardia in infancy; learning disability – hard time reading; low muscle tone; developmental coordination disorder	Mother (-01): procrastinate, worrier Father (-02): arthritis – from early teens and is quite severely affected; dyslexia Sibling (-04, female): poor at reading social cues, relates better to adults, concerns expressed about ASD Sibling (-05, female): heart murmur, but outgrew it; procrastinator, time management hard	Candidate deleterious *SCN2A* ASD mutation in male proband and two female sibling carriers. Genotype and phenotype (including gender) study (see Discussion). Assess mutation carriers for epilepsy. Follow medical literature for *KIAA0284*. Prioritized for clinical assessment.
Family M 2-1116-03 (male)	NRXN1 p.His885Tyr, (paternal); ARID5A	**Clinical dx**: Autism (4.8 yrs); **ADI & ADOS dx**: Autism (8.6 yrs)	**IQ**: NVIQ Below Average 23%; **Language**: All Mild Delay, TL 0.2%, RL 0.5%, EL 0.3%; **Adaptive**: All Mild Delay, ABC 1%, COM 1%, DLS 1%, SOC 0.5%; **Other**: eczema; one incident of no body hair	Mother (-01): allergies, eczema, alopecia Sibling (-04, male): eczema; infant asthma, learning disability- Central Auditory Processing Disorder (CAPD)	Candidate damaging inherited mutation in known ASD candidate *NRXN1* and ASD candidate *ARID5A.* Assess female sibling *NRXN1* mutation carrier for ASD. Prioritized for clinical

	p.Gly120 Val, de novo				assessment.
Family N 2-1244-03 (male)	CACNA1C p.Arg152 2Gln, (paternal)	**Clinical dx**: Atypical/PDD -NOS/ASD (4.7 yrs); **ADI & ADOS dx**: Autism (8.9 yrs)	**IQ**: All Average, FSIQ 53%, VIQ 55%, NVIQ 68%; **Language**: TL Below Average 7%, RL Mild Delay 1%, EL Average 32%, RV Above Average 81%; **Adaptive**: All Average, ABC 30%, COM 21%, DLS 58%, SOC 27%; **Other**: ADHD, migraines, peanut allergy	Mother (-01): epilepsy Father (-02): Wolff Parkinson-White syndrome Sibling (-04, male): milk allergy	Candidate damaging mutation in known ASD gene *CACNA1C.* Assess paternal and male sibling mutation carriers for ASD. Prioritized for clinical assessment.
Family O 2-1352-03 (male)	EHMT1 p.Pro109 9Ala, (maternal); NLGN3 p.Val306 Met, (maternal)	**Clinical dx**: Asperger (4.6); **ADI & ADOS dx**: Autism (7.3 yrs)	**IQ**: All Above Average, FSIQ 90%, VIQ 90%, NVIQ 82%; **Language**: All average, TL 53%, RL 42%, EL 63%, RV 63%; **Adaptive**: TL Average 25%, COM Average 61%, DLS Average 42%, SOC Below Average 5%	Mother (-01): allergy-induced asthma Father (-02): mitrovalve prolapse	Candidate damaging mutation in ASD candidate gene *EHMT1*. Rare predicted benign mutation in known ASD gene *NLGN3*. Prioritized for clinical assessment.
Family P 2-1334-03 (male)	SATB2 p.Pro655S er, rare inherited	**Clinical dx**: Autism (3.3 yrs); **ADI & ADOS dx**: Autism (4.9	**IQ**: NVIQ Mild Delay 1%; **Language**: RV Mild Delay 0.4%; **Adaptive**: All Mild Delay, ABC 0.3%, COM 0.5%,		Candidate damaging mutation in known ASD gene *SATB2*. Assess paternal carrier for ASD. Prioritized for clinical

	(paternal)	yrs)	DLS 1%, SOC 1%, MOT 0.5%		assessment.

Test scores are presented in percentiles (%) using the following classifications: Above Average (75^{th} -99^{th} %), Average (74^{th} -25th %), Below Average (24^{th}-2^{nd} %), Mild Delay (2^{nd}-0.1^{st}%) Moderate Delay (0.1%) and Severe Delay (<0.1%).

Intelligence (IQ) was measured with an age-appropriate Wechsler Scale (Wechsler Preschool and Primary Scale of Intelligence, WPPSI-III;Wechsler Intelligence Scale for Children, WISC-IV; Wechsler Abbreviated Scale of Intelligence, WASI) or Leiter-R Brief (Leiter-R only measures nonverbal IQ). Score are presented in percentiles for Full Scale IQ (FSIQ), Verbal IQ (VIQ), Nonverbal IQ (NVIQ).

Language was measured with the Oral and Written Language Scales (OWLS) and percentile scores are presented for Total Language (TL), Receptive Language (RL), Expressive Language (EL). Receptive Vocabulary (RV) was assessed using the Peabody Picture Vocabulary Test (PPVT-4th Edition).

Adaptive Behaviour was measured using the Vineland Adaptive Behaviour Scales- Second Edition (VABS-II) and percentiles are presented for Adaptive Behaviour Composite (ABC), Communication (COM), Daily Living Skills (DLS), Socialization (SOC), and Motor (MOT; only for children under 7 years).

Other abbreviations: ASD: autism spectrum disorder, ADHD: attention deficit hyperactivity disorder, ADI: autism diagnostic interview, ADOS: autism diagnostic observation schedule

All female X-linked variant carriers were tested for X-inactivation skewing. In family G (2-1269), maternal grandmother has skewed X-inactivation (>70% variant allele inactivated), while mother is not informative for the X-inactivation test. In family I (2-1330), both mother and maternal grandmother have skewed X-inactivation (>70% and >90% respectively, but their genotypes were not informative to determine the direction of skewing).