Supplementary Table 1. Presumably causal variants of unknown significance

ID	Gender	Age	Phenotype	NGS results	Arguments for the potential causative role	EAGLE gene score	SFARI gene score	Segregation analysis
3375	m	5 y	ASD, DD	*DSCAM* NM_001389.5 c.4670C>T (p.Ala1557Val) rs374349326	EAGLE score >12; CADD >20	13.5	1	maternal
3438	f	7 y 4 mos	ASD, DD	*SHANK2* NM_012309.5 c.1229C>T (p.Thr410Met) rs532499001	EAGLE score >12; CADD >20	18.55	1	maternal
				AUTS2 NM_015570.4 c.2116G>C (p.Ala706Pro)	EAGLE score >12; CADD >20	35.5	1	maternal
3745	m	3 y	ASD, DD	*SCN1A* NM_001165963.4 c.5632G>A (p.Glu1878Lys) rs148703212	CADD >30	-	1s	nd
3746	m	2 y	ASD	*CHD2* NM_001271.4 c.4814A>C (p.Lys1605Thr) rs780701076	EAGLE score >12; CADD >20	25	1s	maternal
				DEAF1 NM_021008.4 c.1141G>A (p.Val381Met) rs753669832	EAGLE score >12; CADD >20	30.9	1s	paternal
				EHMT1 NM_024757.5 c.786G>C (p.Gln262His)	EAGLE score >12; CADD >20	13.5	1s	maternal
3747	f	9 y	ASD, DD	*YY1* c.233_241del NM_003403.5 (p.His78_His80del) rs753530895	LGD	-	1s	nd

				CHD2 NM_001271.4 c.4688A>G (p.Glu1563Gly) rs774715709	EAGLE score >12; CADD >20	25	1s	nd
3808	f	11 y	ASD, DD epilepsy; micrognatia, broad forehead, oligodontia, low-set ears	*GRIN2B* NM_000834.5 c.4078G>A (p.Gly1360Arg)	EAGLE score >12; CADD >20	29.65	1	not detected in healthy mother, fathers' sample unavailable
3851	m	6 y	ASD, DD	*PCDH19* NM_001184880.2 c.1552C>G (p.Leu518Val) rs1197941611 hemizygous	Rare variants observed in 2 more ASD families; CADD >20	-	1s	maternal
3897	m	6 y	ASD, DD	*PCDH19* NM_001184880.2 c.1552C>G (p.Leu518Val) rs1197941611 hemizygous	Rare variants observed in 2 more ASD families; CADD >20	-	1s	maternal
4028	m	3 y 6 mos	ASD, DD	*ARID1B* NM_001374828.1 c.4062C>G (p.Ser1354Arg) rs369035728	EAGLE score >12; CADD >20	34.75	1s	paternal
4070	m	7 y 1 mo	ASD, mild ID, speech dyspraxia; CHD (left heart hypoplasia, VSD), facial hypotonia	*FOXP1* NM_001349338.3 c.319A>G (p.Ile107Val)	EAGLE score >12; CADD >20	60.45	1s	nd
				ATRX NM_000489.6 c.169_171del (p.Met57del) hemizygous	LGD	9.25	1	nd
4117	m	7 y 6 mos	ASD, DD	*AUTS2* NM_015570.4 c.1673C>T (p.Thr558Met) rs552275264	EAGLE score >12; CADD >20	35.5	1	nd

				TRIO NM_007118.4 c.1300G>A (p.Ala434Thr)	EAGLE score >12; CADD >20	-	1	nd
4123	m	8 y 9 mos	ASD, DD Tall forehead, broad nasal bridge, downslanted palpebral fissures, epicanthus	*EP300* NM_001429.4 c.3031G>A (p.Glu1011Lys) rs775368605	EAGLE score >12; CADD >20	23.6	1s	maternal
				PCDH19 NM_001184880.2 c.1651G>T (p.Val551Phe) hemizygous	Rare variants observed in 2 more ASD families; CADD >20	-	1s	maternal
4124	m	6 y 4 mos	ASD, DD	*AUTS2* NM_015570.4 c.3776G>A (p.Arg1259Gln) rs148604002	EAGLE score >12; CADD >20	35.5	1	nd
				GRIN2A NM_001134407.3 c.82_84del (p.Glu28del) rs767188122	LGD	3.6	1	nd
4125	m	9 y	ASD, DD Short philtrum, macrodontism, upper micrognatia	*NCKAP1* NM_013436.5 c.526C>G (p.Pro176Ala) rs746137721	EAGLE score >12; CADD >20	24.75	1	nd
4133	m	9 y	ASD, ID	*CHD8* NM_001170629.2 c.7357G>A (p.Gly2453Ser) rs1212438664	EAGLE score >12; CADD >20	97.56	1s	nd
				APBA2 NM_001353788.2 c.1417C>T (p.Arg473Cys) rs202208854	CADD>30	-	2	nd
4223	m	6 y 9 mos	ASD, DD	*EP300* NM_001429.4 c.1870A>G (p.Asn624Asp) rs771836492	EAGLE score >12; CADD >20	23.6	1s	paternal
4225	m	9 y	ASD, ID	*ASTN2* NM_001365068.1 c.2414A>T (p.Lys805Met)	CADD>30	2.5	2	nd

				ANKRD11 NM_013275.6 c.2212C>T (p.Arg738Cys) rs139523271	EAGLE score >12; CADD >20	22.6	1s	nd
4234	m	4 y 3 mos	ASD, DD	*NR4A2* NM_006186.4 c.1349G>A (p.Arg450Gln) rs776299489	CADD>30	11.5	1	nd
4266	m	4 y 4 mos	ASD, DD	*NBEA* NM_001385012.1 c.5699G>A (p.Arg1900His) rs890073712	CADD>30	-	1s	nd
4350	m	8 y 5 mos	ASD, ID	*ASTN2* NM_001365068.1 c.1652G>A (p.Arg551His) rs571117828	CADD>30	2.5	2	nd
4433	f	18 y	ASD, ID; epileptiform EEG abnormalities	*TRIP12* NM_001348323.3 c.734C>T (p.Ser245Phe) rs761102548	EAGLE score >12; CADD >20	27	1s	maternal
4448	m	5 y	ASD, DD	*PCDH19* NM_001184880.2 c.841G>A (p.Val281Ile) rs997198770 hemizygous	Rare variants observed in 2 more ASD families; CADD >20	-	1s	maternal
4449 monozygotic twin of 3851	m	5 y	ASD, DD	*PCDH19* NM_001184880.2 c.841G>A(p.Val281Ile) rs997198770 hemizygous	Rare variants observed in 2 more ASD families; CADD >20	-	1s	maternal
4544	m	5 y	ASD, DD	*MBD5* NM_001378120.1 c.83G>A (p.Arg28His) rs201699340	EAGLE score >12; CADD >20	46.6	1s	paternal
4605	m	12 y 8 mos	ASD, ID	*POGZ* NM_015100.4 c.2585G>A (p.Arg862His) rs778395899	EAGLE score >12; CADD >20	35.4	1s	nd

4606	m	6 y 1 mos	ASD, DD	*ARID1B* NM_001374828.1 c.3230C>T (p.Ser1077Leu) rs1267578477	EAGLE score >12; CADD >20	34.75	1s	nd
4646	m	6 y 11 mos	ASD, DD	*TRIP12* NM_001348323.3 c.1916G>A (p.Ser639Asn) rs776142962	EAGLE score >12; CADD >20	27	1s	nd

CHD – congenital heart defect; DD – developmental delay; ID – intellectual deficiency; LGD – likely gene damaging variant; LP - likely pathogenic; nd – no data; P - pathogenic; VSD - ventricular septum defect