SUPPLEMENTARY INFORMATION

Supplementary Table 1: Rates of PZMs across ASC datasets, with the median depth and mode allele fraction of the *de novos* (Group A) in each dataset. The asterisk (*) denotes the datasets where the median allele fraction was calculated instead of the mode allele fraction.

Dataset	Trios	Median depth	Mode allele fraction	All *de novos* (Group A)	Candidate PZMs (Group B)	High-confidence PZMs (Group C)
BCH	251	92	0.50	215	46 (21.4%)	26 (12.1%)
Broad_v8	643	75	0.50	586	113 (19.3%)	51 (8.7%)
Broad_v11	282	76	0.47	323	101 (31.3%)	56 (17.3%)
Finnish	51	93	0.50	57	16 (28.1%)	7 (12.3%)
Frankfurt	353	72	0.50	337	59 (17.5%)	19 (5.6%)
MSSM	11	45	0.40*	6	1 (16.7%)	1 (16.7%)
Sanger	67	75	0.40	48	2 (4.2%)	1 (2.1%)
UPenn	35	88	0.45	26	3 (11.5%)	2 (7.7%)
VU	37	70	0.50	31	9 (29.0%)	6 (19.4%)
SSC_Probands	2299	67	0.50	1832	436 (23.8%)	169 (9.2%)
ASC_Siblings	161	67	0.50	126	40 (31.7%)	17 (13.5%)
SSC_Siblings	1757	62	0.50	1259	287 (22.8%)	113 (9.0%)
Total	**5947**			**4846**	**1113 (23.0%)**	**468 (9.7%)**

Supplementary Table 2: Information of all probands and unaffected siblings in the study.

This list can be found in an attached Excel file.

Supplementary Table 3: List of all *de novo* mutations found in the probands and unaffected siblings.

This list can be found in an attached Excel file.

Supplementary Table 4: List of all *de novo* mutations that were validated using the different resequencing approaches.

This list can be found in an attached Excel file.

Supplementary Table 5: Quantitative RT-PCR results for assaying copy number variants.

This list can be found in an attached Excel file.

Supplementary Table 6: There is a strand bias towards the anti-sense (- strand) for PZMs in Groups B and C compared to the variants found only in Group A. The odds ratios and 2-tailed Fisher's Exact Test P-values are calculated with respect to the number of *de novo* variants found only in Group A.

	- strand	**+ strand**	**Odds ratio with 95% confidence intervals**	**P-value**
Group A	2438 (50.3%)	2408 (49.7%)		
Group B	600 (53.9%)	513 (46.1%)	1.21 [1.05, 1.38]	6.3×10^{-3}
Group C	263 (56.2%)	205 (43.8%)	1.30 [1.07, 1.58]	7.5×10^{-3}

Supplementary Table 7: There is an excess of A-C and T-G mutations (in yellow) among the post-zygotic mutations in Groups B and C.

Group A:

Mutant / Reference	A	C	G	T
A	-	145 (3.0%)	516 (10.6%)	98 (2.0%)
C	214 (4.4%)	-	240 (5.0%)	1181 (24.4%)
G	1229 (25.4%)	221 (4.6%)	-	216 (4.5%)
T	97 (2.0%)	518 (10.7%)	171 (3.5%)	-

Group B:

Mutant / Reference	A	C	G	T
A	-	54 (4.9%)	98 (8.8%)	22 (2.0%)
C	52 (4.7%)	-	58 (5.2%)	249 (22.4%)
G	273 (24.5%)	66 (5.9%)	-	47 (4.2%)
T	27 (2.4%)	104 (9.3%)	63 (5.7%)	-

Group C:

Mutant / Reference	A	C	G	T
A	-	29 (6.2%)	34 (7.3%)	10 (2.1%)
C	23 (4.9%)	-	20 (4.3%)	102 (21.8%)
G	110 (23.5%)	27 (5.8%)	-	24 (5.1%)
T	13 (2.8%)	42 (9.0%)	34 (7.3%)	-

Supplementary Table 8: The excess of A-C mutations (in blue) among the post-zygotic mutations in Groups B and C is predominantly found on the sense (+) strand, whereas the excess of T-G mutations (in pink) is predominantly found on the anti-sense (-) strand.

Group A + strand (2408 mutations):

Mutant / Reference	A	C	G	T
A	-	84 (3.5%)	290 (12.0%)	68 (2.8%)
C	103 (4.3%)	-	133 (5.5%)	588 (24.4%)
G	643 (26.7%)	105 (4.4%)	-	112 (4.7%)
T	39 (1.6%)	171 (7.1%)	72 (3.0%)	-

Group B + strand (513 mutations):

Mutant / Reference	A	C	G	T
A	-	31 (6.0%)	47 (9.2%)	18 (3.5%)
C	19 (3.7%)	-	26 (5.1%)	109 (21.2%)
G	138 (26.9%)	30 (5.8%)	-	23 (4.5%)
T	8 (1.6%)	40 (7.8%)	24 (4.7%)	-

Group C + strand (205 mutations):

Mutant / Reference	A	C	G	T
A	-	19 (9.3%)	16 (7.8%)	8 (3.9%)
C	9 (4.4%)	-	7 (3.4%)	37 (18.0%)
G	53 (25.9%)	14 (6.8%)	-	12 (5.9%)
T	3 (1.5%)	16 (7.8%)	11 (5.4%)	-

Group A - strand (2438 mutations):

Mutant / Reference	A	C	G	T
A	-	61 (2.5%)	226 (9.3%)	30 (1.2%)
C	111 (4.6%)	-	107 (4.4%)	593 (24.3%)
G	586 (24.0%)	116 (4.8%)	-	104 (4.3%)
T	58 (2.4%)	347 (14.2%)	99 (4.1%)	-

Group B - strand (600 mutations):

Mutant / Reference	A	C	G	T
A	-	23 (3.8%)	51 (8.5%)	4 (0.7%)
C	33 (5.5%)	-	32 (5.3%)	140 (23.3%)
G	135 (22.5%)	36 (6.0%)	-	24 (4.0%)
T	19 (3.2%)	64 (10.7%)	39 (6.5%)	-

Group C - strand (263 mutations):

Mutant / Reference	A	C	G	T
A	-	10 (3.8%)	18 (6.8%)	2 (0.8%)
C	14 (5.3%)	-	13 (4.9%)	65 (24.7%)
G	57 (21.7%)	13 (4.9%)	-	12 (4.6%)
T	10 (3.8%)	26 (9.9%)	23 (8.7%)	-

Supplementary Table 9: Association of PZMs in Groups B and C with late replication timing, but not early replication timing. The odds ratios and 2-tailed Fisher's Exact Test P-values are calculated with respect to the number of *de novo* variants found only in Group A.

	Number of variants	Early replication ($Z \geq 1$)	Odds ratio with 95% confidence intervals	P-value
Group A	4846	2691 (55.5%)		
Group B	1113	597 (53.6%)	0.91 [0.79,1.04]	0.15
Group C	468	246 (52.6%)	0.88 [0.72, 1.07]	0.19

	Number of variants	Late replication ($Z \leq -1$)	Odds ratio with 95% confidence intervals	P-value
Group A	4846	183 (3.8%)		
Group B	1113	47 (4.2%)	1.17 [0.81, 1.66]	0.37
Group C	468	23 (4.9%)	1.36 [0.83, 2.14]	0.20

Supplementary Table 10: Number of *de novo* germline and mosaic mutations on the paternal or maternal haplotypes.

	Paternal	Maternal	Ratio (Paternal/Maternal)	Binomial P
Group A	1321	781	1.69	1.50×10^{-32}
Group B	285	179	1.59	4.92×10^{-7}
Group C	90	78	1.15	0.2

Supplementary Table 11: Distribution of functional categories between probands and siblings.

All Probands:

	LoF	Missense	Silent	Total
Group A	265 (7.7%)	2386 (68.9%)	810 (23.4%)	3461
Group B	50 (6.4%)	521 (66.3%)	215 (27.4%)	786
Group C	16 (4.7%)	231 (68.3%)	91 (26.9%)	338

All Siblings:

	LoF	Missense	Silent	Total
Group A	61 (4.4%)	958 (69.2%)	366 (26.4%)	1385
Group B	15 (4.6%)	233 (71.3%)	79 (24.2%)	327
Group C	6 (4.6%)	95 (73.1%)	29 (22.3%)	130

SSC Probands:

	LoF	Missense	Silent	Total
Group A	148 (8.1%)	1261 (68.8%)	423 (23.1%)	1832
Group B	24 (5.5%)	299 (68.6%)	113 (25.9%)	436
Group C	6 (3.6%)	122 (72.2%)	41 (24.3%)	169

SSC Sibings:

	LoF	Missense	Silent	Total
Group A	57 (4.5%)	862 (68.5%)	340 (27.0%)	1259
Group B	14 (4.9%)	202 (70.4%)	71 (24.7%)	287
Group C	6 (5.3%)	79 (70.0%)	28 (24.8%)	113

Supplementary Table 12: Distribution of predicted deleterious missense mutations in probands and siblings. The P-values are calculated using a 1-tailed Fisher's Exact Test.

	Predicted deleterious missense mutations in probands	Total missense mutations in probands	Predicted deleterious missense mutations in siblings	Total missense mutations in siblings	P-value
Group A	872 (61.1%)	1428	317 (56.1%)	565	0.024
Group B	178 (59.9%)	297	67 (50.4%)	133	0.041
Group C	70 (55.1%)	127	27 (50%)	54	0.32

Supplementary Table 13: Gene-specific mutation rates for post-zygotic mutations estimated from the rare inherited variants.

This list can be found in an attached Excel file.

Supplementary Table 14: Recurrent non-synonymous PZMs in probands, with genes that are expressed in the brain in red text.

	Expected	Observed	Hypergeometric P
KLF16	0/84448	2/571	$<1\times10^{-6}$
MSANTD2	1/84448	2/571	$<1\times10^{-6}$
POLA2	2/84448	2/571	4.6×10^{-5}
SMARCA4	11/84448	3/572	4.9×10^{-5}
AZGP1	4/84448	2/571	2.7×10^{-4}
CNGB3	5/84448	2/571	4.5×10^{-4}
HNRNPU	5/84448	2/571	4.5×10^{-4}
SCN2A	5/84448	2/571	4.5×10^{-4}
EPPK1	58/84448	4/571	6.6×10^{-4}
CARD11	7/84448	2/571	9.4×10^{-4}
SYNJ1	8/84448	2/571	1.2×10^{-3}
MEGF11	10/84448	2/571	2.0×10^{-3}
SASH1	10/84448	2/571	2.0×10^{-3}
MYO5C	14/84448	2/571	3.9×10^{-3}
TECTA	14/84448	2/571	3.9×10^{-3}
MUC12	233/84448	6/571	5.3×10^{-3}
DYNC1H1	21/84448	2/571	8.8×10^{-3}
PRKDC	30/84448	2/571	0.018
USH2A	35/84448	2/571	0.023
RP11-1407O15.2	39/84448	2/571	0.029
DNAH17	40/84448	2/571	0.030
NBPF10	70/84448	2/571	0.082
NBPF1	77/84448	2/571	0.096
HRNR	115/84448	2/571	0.18
AHNAK2	221/84448	3/571	0.19
FLG	200/84448	2/571	0.39
MUC4	471/84448	4/571	0.39

Supplementary Table 15: Recurrent non-synonymous PZMs in siblings, with genes that are expressed in the brain in red text.

	Expected	Observed	Hypergeometric P
TRPS1	5/84448	2/248	8.5×10^{-5}
MYCBP2	26/84448	2/248	2.7×10^{-3}

Supplementary Note: List of Autism Sequencing Consortium members.

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