

1 **Both rare and common genetic variants contribute to autism in the Faroe**

2 **Islands**

3

4 **Short title: The genetic architecture of autism in the Faroe Islands**

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36 **Abstract**

37 **The number of genes associated with autism is increasing, but few studies have been**
38 **performed on epidemiological cohorts and in isolated populations. Here, we investigated**
39 **357 individuals from the Faroe Islands including 36 individuals with autism, 136 of their**
40 **relatives and 185 non-autism controls. Data from SNP array and whole exome**
41 **sequencing revealed that individuals with autism compared to controls had a higher**
42 **burden of copy-number variants ($p < 0.05$), higher inbreeding status ($p < 0.005$) and**
43 **higher load of homozygous deleterious variants ($p < 0.01$). Our analysis supports the role**
44 **of several genes/loci associated with autism (e.g. *NRXN1*, *ADNP*, 22q11 deletion) and**
45 **identified new truncating (e.g. *GRIK2*, *ROBO1*, *NINL* and *IMMP2L*) or recessive**
46 **deleterious variants (e.g. *KIRELL3* and *CNTNAP2*) affecting autism-risk genes. It also**
47 **revealed three genes involved in synaptic plasticity, *RIMS4*, *KALRN* and *PLA2G4A*,**
48 **carrying *de novo* deleterious variants in individuals with autism without intellectual**
49 **disability. In summary, our analysis provides a better understanding of the genetic**
50 **architecture of autism in isolated populations by highlighting the role of both common**
51 **and rare gene variants and pointing at new autism-risk genes. It also indicates that more**
52 **knowledge about how multiple genetic hits affect neuronal function will be necessary to**
53 **fully understand the genetic architecture of autism.**

54 **Author summary**

55

56 **Autism is characterized by problems in social communication and stereotyped behavior.**

57 **To improve our understanding of the genetic architecture of autism, we studied a group**

58 **of children and adolescents from the Faroe Islands, an isolated population living on**

59 **archipelago located in the North Atlantic Ocean, halfway between Norway, Iceland and**

60 **Scotland since the 9th century. The genetic profile of this small but genetically**

61 **homogenous epidemiological cohort of the Faroe individuals revealed that both rare**

62 **and common genetic variants contribute to the susceptibility to autism. Our analysis**

63 **supports the role of several genes previously associated with autism and points at new**

64 **candidate genes involved in the neuronal connectivity of the brain. In summary, our**

65 **analysis provides a comprehensive framework to analyze the complex heterogeneity of**

66 **autism in order to improve the diagnostic, the care and the integration of individuals**

67 **with autism.**

68

69 **Introduction**

70 Autism spectrum conditions (ASCs; henceforth 'autism') are diagnosed in 1-2% of the
71 population worldwide and are characterized by atypical social communication and the
72 presence of restricted interests, stereotyped and repetitive behaviors. Individuals with autism
73 can also suffer from psychiatric and medical conditions including intellectual disability (ID),
74 epilepsy, motor control difficulties, attention-deficit hyperactivity disorder (ADHD), tics,
75 anxiety, sleep disorders, depression or gastrointestinal problems (1). The genetic
76 susceptibility to autism can vary from one individual to another. In some cases, a single *de*
77 *novo* variant can be detected. On the contrary, in some cases, the genetic architecture is more
78 complex and involved thousands of common genetic variants, each one with low impact but
79 collectively increasing the susceptibility to autism (2). Most of our knowledge on the genetics
80 of autism comes from studies on unrelated individuals with autism who do not share a recent
81 common ancestor. Several studies investigated families with autism from countries where
82 consanguinity is high (3,4), but the genetic architecture of autism in isolated populations
83 remains largely unknown.

84 The Faroe Islands is an archipelago located in the North Atlantic Ocean, halfway
85 between Norway, Iceland and Scotland (Fig 1A). The population (approximately 49,000
86 inhabitants) was founded in the 9th century by a small number of emigrants from Norway. The
87 population remained at a small size for centuries until they experienced a rapid expansion in
88 the 1800s. Previous genetic studies indicated that individuals from Scotland, Norway,
89 Sweden, Ireland, Iceland and British Isles have significantly contributed to the current gene
90 pool of the Faroese population (5,6).

91 We previously showed that the prevalence of autism in the Faroe Islands (0.94% of the
92 population (7–9)) was similar to many other western countries. In this study, we ascertained
93 the genetic profile of 357 individuals including an epidemiological cohort of 36 individuals

94 with autism born between 1985 and 1994 (Fig 1 and S1 Fig), their relatives (n=136) and a
95 group of 185 controls. We first investigated the known causes of autism and then identified
96 new candidate genes. We also ascertained the impact of inbreeding and the load of deleterious
97 homozygous variants on the risk of autism. Finally, both rare and common genetic variants
98 were used to stratify individuals with autism and to compare their genetic and clinical
99 profiles.

100

101 **Results**

102 **The genetic diversity in the Faroe Islands**

103 A total of 67 children and adolescents with autism were detected in a total population study of
104 individuals aged 8-17 years living in the Faroe Islands and born between 1985 and 1994
105 (8,10). Thirty-six of the 67 individuals (54% of the total group) signed (or had parents who
106 signed) informed consent forms and were included in the genetic study. Participants and non-
107 participants in the genetic study were similar in terms of gender and cognitive abilities (Fig
108 1B). In addition, we collected DNA from 136 of their relatives and from 185 "non-autism
109 controls. The genetic profile included a high-density Illumina SNP array interrogating > 4.3
110 millions of single nucleotide polymorphisms (SNPs) and a whole exome sequencing (WES)
111 to discover new variants. Using identical-by-state (IBS) genomic distance (see materials and
112 methods), we first compared the population structure of individuals from the Faroe Islands
113 with worldwide populations (Fig 1C). All individuals were clustered in the Faroese
114 population (with the exception of seven controls, but still with European genetic background).
115 Using admixture, we showed that individuals from the Faroe Islands have their genome in
116 majority constituted from "European component" (S2 Fig). As expected from the
117 demographic history, individuals from the Faroese population displayed a higher degree of
118 inbreeding compared with others world populations (Fig 1D).

119

120 **Contribution of *de novo* variants**

121 We ascertained the burden of *de novo* variants since they are key players in the genetics of
122 autism (11,12). The *de novo* variants were identified for 31 independent families including 28
123 individuals with autism and 45 siblings for whom DNA of both parents was available (see
124 Clinical notes in S1 Appendix for the pedigrees). The combined analysis of genotyping and
125 WES data revealed the presence of *de novo* chromosomal abnormalities and exonic copy-

126 number variants (CNVs) in 3 out of 26 individuals with autism (11.5%) and 1 out of 43
127 siblings (2.3%). One female PN400129 had a trisomy of chromosome 21 and was diagnosed
128 with autism, ID and Down syndrome (S3 Fig and S1 Table). One female PN400533 with
129 atypical autism without ID carried a *de novo* 2.9 Mb deletion on chromosome 22q11 causing
130 DiGeorge/velocardial syndrome. A male PN400115 with atypical autism without ID carried a
131 *de novo* 425.5 kb deletion removing the six first exons of the *NRXN1α*. We also found a 91.4
132 kb deletion removing all exons of *ADNP* in a male with autism and ID (PN400125). The
133 deletion was not found in the mother and was most likely *de novo*, but father's DNA was not
134 available and none of the SNPs within the deletion were informative to confirm the *de novo*
135 status of the deletion. The *de novo* CNV observed in the sibling (PN400170) was a
136 duplication of 782 kb affecting 5 genes (*CNPY1*, *DPP6*, *EN2*, *HTR5A*, *INSIG1* and *PAXIP1*).

137 Using the WES data, we detected the presence of *de novo* single nucleotide variants
138 (SNVs) and small insertions/deletions (indels)(S2 Table). Overall, the rate of *de novo* exonic
139 SNV/indels was similar to other studies (13) and was not different in individuals with autism
140 (0.93) and their siblings (0.96). The variants were considered as probably deleterious when
141 they were likely gene disruptive (LGD, for example stop gain or frame shift variant) or
142 missense events with a combined annotation dependent depletion (CADD) score > 30
143 (MIS30) (14). There was also no significant increase in the rate of *de novo* deleterious
144 variants in individuals with autism compared to their siblings and no significant enrichment in
145 genes associated with autism (SFARI genes (15)) or expressed in the brain (Brain genes, see
146 subjects and methods for gene selection). Nevertheless, several deleterious variants were
147 identified in known genes for autism (*MECP2*) or compelling candidate genes (*RIMS4*,
148 *KALRN*, *PLA2G4A*) (S4 Fig). Clinical details on the individuals with autism carrying those
149 variants are available in the S1 Appendix.

150

151 **Contribution of rare CNVs and SNVs/indels variants**

152 The overall burden of exonic CNVs was higher in individuals with autism compared to
153 controls for both deletions ($p = 0.02$) or duplication ($p = 0.006$) (Fig 2A and S3 Table). The
154 burden of deletions was also higher for autism-associated genes listed in the SFARI database
155 ($p = 0.01$), for genes intolerant to loss-of-function variant (pLI > 0.9) (16) ($p = 0.01$) or genes
156 expressed in the brain ($p = 0.02$). For duplications, only genes expressed in the brain were
157 more frequently duplicated in individuals with autism compared to controls ($p = 0.005$).
158 These differences however do not survive corrections for multiple tests and we had no
159 significant difference between individuals with autism and their siblings. Among the SFARI
160 genes affected by the CNVs, we identified a 58 kb maternal inherited deletion including the
161 *IMMP2L*, a 2 Mb paternal inherited duplication on the pseudo-autosomal region 1 including
162 *SHOX* and *ASMT*, and a 39 kb maternal inherited duplication of *TBLIXR1* (S3 Table).

163 Our analysis was restricted to CNVs affecting exons, but a large 357 kb duplication
164 within intron 5 of the *NLGNI* gene and covering a long *NLGNI* antisense noncoding RNA
165 was paternally inherited in a female (PN400102) with autism and no ID. There was no such
166 rare intronic CNVs in the SFARI genes in siblings and controls.

167 We then run gene-wise association tests from rare SNV/indels (MAF < 5%). None of
168 associations were gene wide significant, but *KIF1B*, *FOXD3*, *RNF181* and *SDPR* were among
169 the top genes ($p < 0.001$) detected by both the CMC-EMMAX and SKAT-O analyses (Fig
170 2B, S5 Fig and S4-S5 Tables). For *FOXD3*, *RNF181*, and *SDPR* the association was mainly
171 driven by one missense variant, whereas for *KIF1B*, several variants contributed to the
172 association. Single variant tests were performed (S6 Table) and among the hits with
173 $p < 0.009$, two variants affect genes previously associated with neurodevelopmental disorders
174 (NDD). A variant p.R276W in *NINL*, a gene previously associated with autism (17) was more
175 frequent in individuals with autism (P400121 was homozygous) compared to controls

176 ($p = 0.0005$). This Ninein-like protein is known to associate with motor complex and interact
177 with the ciliopathy-associated proteins lebercilin, USH2A and CC2D2A. A *CHAMP1* variant
178 (p.A586S) was more frequent in individuals with autism compared with controls ($p < 0.003$)
179 and it was previously shown that *de novo* variants in *CHAMP1* cause ID with severe speech
180 impairment (18).

181 Finally, we found truncating variants affecting several SFARI genes (*e.g.* *PRODH*,
182 *ERBB4*, *GRIK2*, *ROBO1*, *RBMS3*, and *IMMP2L*; Fig 2B and S7 Table), but the number of
183 individuals with autism carrying these variants was too low to detect a significant association.

184

185 **Contribution of recessive variants**

186 Since inbreeding increases the risk for individuals to carry homozygous deleterious variants,
187 we first compared the inbreeding status of the individuals with autism, their relatives and
188 controls. Patients ($p < 0.005$) as well as their siblings ($p < 0.01$) had a higher inbreeding
189 coefficient compared with controls (Fig 3A). More interestingly, we found that individuals
190 with autism were carrying more deleterious homozygous variants (LGD, MIS30, gnomAD
191 MAF < 1%) than controls ($p < 0.0005$; Fig 3B and S8 Table). Genes carrying deleterious
192 homozygous variants in affected individuals were significantly enriched in the combined
193 gene-set lists (SFARI + pLI > 0.9 + Brain genes) compared to controls ($p < 0.05$; Fig 3C).

194 In one consanguineous family, we found a *KIRREL3* homozygous damaging missense
195 variant (p.R562L) affecting a conserved residue in the cytoplasmic domain of this synaptic
196 adhesion molecule (19) listed in SFARI and associated with NDD (20,21) (22) (Fig 3D).
197 Interestingly, the female PN400528 with autism and a normal IQ was also homozygous for
198 another deleterious variant (p.N687K) affecting *TECTA*, a SFARI gene associated with
199 autism (21) and deafness (23).

200 In another family, the male PN400579 was homozygous for two variants affecting
201 *CNTNAP2* and *PEX6* (Fig 3E). Recessive *CNTNAP2* variants are associated with Pitt-
202 Hopkins like syndrome 1 and cortical dysplasia-focal epilepsy syndrome (MIM #610042).
203 The *CNTNAP2* p.E680K variant affects a highly conserved amino acid within the fibrinogen
204 domain of the protein. Recessive *PEX6* variants are associated with Heimler syndrome 2 a
205 recessive peroxisome disorder characterized by sensorineural hearing loss, amelogenesis
206 imperfecta and nail abnormalities, with or without visual defects (MIM #616617). The
207 homozygous variant p.R601Q carried by the male with autism can be considered pathogenic
208 since it was previously detected in several independent patients diagnosed with Heimler
209 syndrome 2 (24). Details on the clinical profiles of the families are available in S1 Appendix.
210

211 **Contribution of common variants**

212 In order to complete the genetic profile of all the individuals, we investigated the contribution
213 of the common variants (MAF > 5%) using three complementary approaches: (i) genome
214 wide association studies (GWAS) in cases and controls using three models (allelic, recessive
215 and dominant), (ii) a burden/collapsing test that aggregates the variants located in a gene, (iii)
216 a calculation of the autism polygenic risk score.

217 The results of the GWAS are presented in S6-S7 figs and a list of the top 45 loci that
218 display p-values under 10^{-5} are shown in Fig 2B and S9 Table. Two of these loci were located
219 within or nearby genes previously associated with NDD. *TNIK* on chromosome 3q26
220 (rs1492859; $p = 5.59 \times 10^{-7}$) encodes a key synaptic partner of *DISC1*, a gene associated with
221 psychiatric disorders (25). *TMEFF2* on chromosome 2q32.3 (rs6737056; $p = 3.47 \times 10^{-7}$) is
222 highly expressed in the brain and is known to modulate the AKT/ERK signaling pathways
223 that are supposedly perturbed in autism (26). *TMEFF2* is also a target of *CHD8*, a chromatin
224 remodeling gene associated with autism (27,28). Using the gene-based analysis implemented

225 in MAGMA (29), only one gene *WHAMM* on chromosome 15q25.1 had a $p < 10^{-5}$ (S10-S11
226 Tables). *WHAMM* is expressed in the brain and acts as a regulator of membrane dynamics that
227 functions at the interface of the microtubule and actin cytoskeletons (30).

228 Finally, we ascertained the autism polygenic risk score (PRS-autism) for each
229 individual (Fig 4). The PRS-autism was calculated using PRSice-2 from a previous GWAS
230 using over 16,000 individuals with autism (31) who do not overlap with this sample. We
231 found a significantly higher PRS-autism in individuals with autism compared to controls
232 ($p < 0.01$; Fig 4A). Remarkably, in the autism group, the PRS-autism was significantly higher
233 in individuals without ID compared to those with ID ($p < 0.05$, Fig 4B).

234

235 **Genetic stratification of autism in the Faroe Islands**

236 In order to stratify individuals with autism, we used the number of rare deleterious variants in
237 SFARI genes (including CNVs) and the PRS-autism estimated from the common variants
238 (Fig 5). Hierarchical clustering found four clusters. The first one comprised seven individuals
239 with high PRS-autism and high burden of deleterious variants in SFARI genes. In this cluster,
240 none of the individuals had ID. The second "cluster" included only one individual who was
241 diagnosed with autism and Down syndrome with a low PRS-autism and high burden of
242 SFARI deleterious variants. In the third cluster, fourteen individuals had low PRS-autism and
243 low burden of SFARI genes deleterious variants. In this cluster 50% of the individuals had
244 ID. In the fourth cluster, fourteen individuals had higher PRS-autism, but low burden of
245 SFARI deleterious variants. In this cluster, 29% of the individuals had ID and it includes the
246 three individuals with autism with epilepsy from the cohort.

247

248 **Discussion**

249 In this study, we investigated a group of individuals with autism that has two distinctive
250 features. First, the group is representative of a general population cohort of all young people
251 living in the Faroe Islands at one point in time, meaning that it was not biased for
252 inclusion/exclusion criteria used for research studies. Secondly, the Faroese population has a
253 more homogeneous genetic background compared to most other populations.

254

255 **Autism-risk genes in the Faroe Islands**

256 We found a subset of individuals carrying strongly deleterious variants (some of which
257 appeared *de novo*) affecting single gene or chromosomal regions. The chromosomal
258 abnormalities included on case each of trisomy 21 and 22q11 deletion (causing Down and
259 DiGeorge/velocardial syndromes, respectively). This was not surprising to find such known
260 genetic disorders in an epidemiologic cohort since the prevalence of autism in individuals
261 diagnosed with these syndromes is higher than in the general population (16-37% for Down
262 syndrome (32), and 23-50% for DiGeorge/velocardial syndrome deletion (33,34)). We also
263 revealed new variants in known autism-risk genes (*ADPNP*, *NRXN1*, *NINL*, *MECP2*) and
264 points at new compelling candidate genes such as *KALRN*, *PLA2G4A* and *RIMS4*.

265 The *KALRN* gene encodes for a guanine nucleotide exchange factor (GEF) with strong
266 homology to *TRIO*, a gene previously associated with autism (35). *KALRN* is expressed in
267 neuronal tissue during embryonic development (36) and has been associated with
268 schizophrenia risk through association analysis and postmortem analyses of individuals with
269 autism' cortical *KALRN* mRNA and protein levels (37)(38). It is also a binding partner of the
270 Huntingtin and a regulator of structural and functional plasticity at dendritic spines. The *de*
271 *novo* variant (p.N2024D; CADD=26.7) was never observed in the general population affects a
272 key amino acid of the GEF domain conserved through evolution and present in *Drosophila*

273 *melanogaster* and *Caenorhabditis elegans*. The male individual (PN400117) carrying this *de*
274 *novo* *KALRN* variant has no ID.

275 *PLA2G4A* gene encodes the cytosolic phospholipase A2 α that catalyzes the hydrolysis
276 of membrane phospholipids to produce arachidonic acid. Mice lacking *Pla2g4a* display
277 abnormalities in neuronal maturation (narrow synaptic cleft) (39) and long-term potentiation
278 (LTP) (40). The *de novo* variant (p.R485C; CADD=35) has never been observed in the
279 general population and is predicted as a deleterious variant falling in the catalytic domain of
280 the protein. The female PN400102 carrying this *de novo* variant has no ID.

281 *RIMS4* codes for a presynaptic proteins that plays a key role for dendritic and axonal
282 morphogenesis (41). *RIMS1* and *RIMS3* have already been associated with autism (42–44) .
283 The individual (PN400137) carrying the *de novo* *RIMS4* stop variant (p.Y204*; CADD=38)
284 has a normal IQ (Performance IQ=108, Verbal IQ=116). The variant is predicted to truncate
285 the last quarter of the protein and was never observed in the general population. Interestingly,
286 RIM proteins interact with voltage-dependent Ca(2+) channels (VDCCs) and suppress their
287 activity at the presynaptic active zone to regulate neurotransmitter release. Knockdown of
288 gamma-RIMs (*RIMS3* and *RIMS4*) attenuated glutamate release to a lesser extent than that of
289 alpha-RIMs (*RIMS1* and *RIMS2*). As a consequence, competition between alpha- and gamma-
290 RIMs seems to be essential for modulating the release of glutamate at the synapse (45). We
291 can therefore hypothesize that the *de novo* *RIMS4* truncating stop variant perturbs the fine-
292 tuning of glutamatergic release at the synapse and contributes to autism.

293

294 **High inbreeding slightly increases the risk of autism, but no evidence for a founder**
295 **effect for autism in the Faroe Islands.**

296 In genetic isolates, it is frequent to observe an increase frequency of diseases due to the
297 presence of deleterious variants that were present in the genomes of the small group of

298 migrants who settled the population. In the Faroe Islands, this “founder effect” was
299 documented for several genetic diseases such as Bardet-Biedl syndrome (46), cystic fibrosis
300 (47), 3-Methylcrotonyl-CoA carboxylase deficiency (48), glycogen storage disease type IIIA
301 (49) and retinitis pigmentosa (50). We confirmed that inbreeding in the Faroe Island is higher
302 than expected compared with other populations. The median of inbreeding is
303 $F = 0.015 \pm 0.001$ in the control sample and is similar to the one reported by Binzer et al.
304 (2014) in their study on multiple sclerosis in the Faroe Islands ($F = 0.018$) (51). This level of
305 inbreeding corresponds approximately to children from parents with a second-cousin
306 relationship ($F = 0.016$). We also observed that individuals with autism from the Faroe
307 Islands have significantly higher level of inbreeding and burden of recessive deleterious
308 variants compared to their geographically matched controls. The homozygous deleterious
309 variants carried by individuals with autism were enriched in genes included in our list of
310 genes of interest (*e. g.* high intolerance for loss of function variants, expressed in the brain
311 and previously associated with autism). However, one should note that the increased
312 probability for having autism due to inbreeding in the Faroe Islands is relatively small
313 ($F_{\text{autism}} = 0.0189$; $F_{\text{controls}} = 0.0148$; $OR = 1.28$; $p = 0.0047$).

314 In contrast to other genetic conditions, we could not detect a founder effect for autism
315 in the Faroe Islands. Moreover, the loci identified in our study do not overlap with those
316 detected in a previous genetic microsatellite association study in the Faroese population
317 pointing at regions on 2q, 3p, 6q, 15q, 16p, and 18q (52). We also found no overlap between
318 the variants identified in our study and those found in Faroese individuals with autism
319 diagnosed with panic (53) or bipolar (54) disorders. This absence of a founder effect is also in
320 agreement with the epidemiological observation that the prevalence of autism in the Faroese
321 population is not higher compared to more outbred populations.

322

323 **Perspectives**

324 Our study confirms that both rare and common genetic variants contribute to the susceptibility
325 to autism. Indeed, although, we identified previously known genetic causes for autism and
326 pointed at new compelling candidate genes, we also showed a contribution of the common
327 variants illustrated by the higher level of PRS-autism in individuals with autism (especially
328 those with no ID) compared to controls. To date, in the literature, very few genes are
329 identified in individuals diagnosed with autism, but with intact general intelligence. Based on
330 the genes previously reported (*NLGN3*, *NLGN4X*, duplication of *SHANK3*) (55,56) and the
331 genes found in this study (*RIMS4*, *KALRN*, *PLA2G4A*), it seems that the proteins involved in
332 autism without ID converge to different part of the post- and pre-synapse rather than to
333 pathways such as gene regulation and chromatin remodeling, but this has to be confirmed on
334 larger cohorts. Indeed, the main limitation of our study is the small number of individuals
335 with autism. Several LGD variants affecting autism-risk genes such as *GRIK2* (57) or *ASMT*
336 (58) were found exclusively or more frequently in individual with autism compared to
337 controls, but we lack a replication samples to confirm the contribution of these variants in the
338 susceptibility to autism in the Faroe Islands.

339 In summary, this study improves our knowledge on the genetic architecture of autism
340 in epidemiological cohorts and in genetic isolates by showing that the contribution of both
341 rare and common gene variants to autism can be detected in small, but genetically
342 homogeneous populations. It also provides new compelling candidate genes and reveals that
343 high inbreeding and high load of homozygous deleterious variants can be a risk factor for
344 autism. Such combined analysis investigating both rare and common gene variants might
345 represent a useful framework to investigate, from groups to individuals, the complex genetic
346 architecture of autism.

347

348 **Materials and Methods**

349 **Ethics statement**

350 This study was approved by the IRB of the “Institut Pasteur” of Paris (IRB00006966 Institut
351 Pasteur, approval 2010-003).

352

353 **Patients**

354 All individuals with autism in this study were recruited from an epidemiological cohort
355 through a two-phase screening and diagnostic process targeting all children born in the 10-
356 year period from 1985 through 1994 and living in the Faroe Islands in 2002 (7-16 years,
357 n=7,689 children) and 2009 (15-24 years, n= 7,128 children) (8)-(10). The total number of
358 children diagnosed with autism was 67 which corresponds to an autism prevalence of 0.94%.
359 Among the individuals with autism, 23% were diagnosed with childhood autism, 56% with
360 Asperger syndrome and 21% with atypical autism. There were 49 males (73.1%) and 18
361 females (26.9%). DNA was available for 36 individuals with autism including 11 diagnosed
362 with childhood autism (31%), 17 with Asperger syndrome (47%), and 8 with atypical autism
363 (22%). There were 28 males (78%) and 8 females (22%). The non-autism controls were
364 recruited by issuing an invitation with information on the study to all pupils at the high school
365 level in winter 2008-2009. The schools invited are in Eysturoy, Suduroy and Torshavn. The
366 age of the invited was from 14-24 years. For those under 18 years a letter was sent to the
367 parents that could sign the consent for their children.

368

369 **Screening and diagnosis**

370 In 2002, screening included the use of the Autism Spectrum Screening Questionnaire (ASSQ)
371 (59). Screen-positive children were thoroughly examined via Diagnostic Interview for Social
372 and Communication Disorder (DISCO-10 in 2002 and DISCO-11 in 2009) (60) of one or both

373 parents and the Wechsler Intelligence Scale for Children – 3^r edition (WISC) or Wechsler
374 Adult Intelligence Scale – Revised (WAIS). Whenever overall and verbal abilities allowed it
375 possible, children were also interviewed in an unstructured/semistructured manner about
376 interests and skills patterns, peer relations, family relationships and about formal general
377 information knowledge. The following diagnostic criteria used when making clinical
378 diagnoses were (a) ICD-10 criteria for childhood autism/autistic disorder; (b) Gillberg criteria
379 for Asperger syndrome; (c) ICD-10 criteria for atypical autism with the added requirement
380 that a case thus diagnosed could not meet full criteria for childhood autism or Asperger
381 syndrome; and (d) ICD-10 criteria for disintegrative disorder.

382 The majority of children in the atypical autism and Asperger syndrome groups had
383 been tested with the WISC-R. Those with childhood autism had usually been tested on other
384 tests. In those intellectually low-functioning individuals for whom no test was available, IQ
385 was estimated on the basis of the Vineland developmental portion that is part of the DISCO-
386 interview.

387

388 **Genotyping**

389 The cohort available for the genotyping is shown in Fig 1 and S1 Fig. It includes 36
390 individuals with autism, 208 controls, 132 close relatives of the individuals with autism (61
391 siblings and 68 parents) and 10 close relatives from the controls. DNA was extracted from
392 blood leukocytes. The genotyping was performed at the “Centre National de Recherche en
393 Génomique Humaine (CNRGH)” using the Infinium IlluminaOmni5-4 BeadChip (> 4.3
394 millions of markers) from Illumina. Sample quality controls such as Sex check (based on the
395 X chromosome homozygosity rate), Mendel errors (transmission errors within full trios) and
396 Identity By State (IBS, see section below) were performed using PLINK 1.90 (61).

397

398 **Population genetic structure**

399 Genome-wide pairwise IBS calculations and Multidimensional scaling (mds) analysis on
400 genome-wide IBS pairwise distances matrix was calculated using PLINK 1.90. IBS values
401 have been calculated for 376 individuals from Faroe Islands and 1,184 individuals from
402 HapMap phase 3 project with the following calculation: $1 - (0.5 * IBS1 + IBS2)/N$; N is the
403 number of tested markers; IBS1 and IBS2 are the number of markers for which one pair of
404 individuals share either 1 or 2 identical allele(s), respectively. Out of the 376 individuals, 32
405 individuals were removed from further analyses, including 7 ancestry outliers (all controls), 9
406 siblings of controls, one swap and 15 control individuals involved in pairs with IBS score
407 superior to 0.9.

408 For the estimation of the inbreeding status, SNPs with genotyping call rate < 95%,
409 minor allele frequency < 0.05, strong linkage disequilibrium $r > 0.5$ or failing Hardy
410 Weinberg equilibrium test ($p < 10^{-6}$) were filtered out of the Faroe SNP genotyping dataset.
411 All homozygosity analyses were performed with Plink 1.09 on autosomes including
412 identification of Runs Of Homozygosity (ROH) and Inbreeding coefficients calculation. For
413 ROH detection, a threshold of 50 consecutive homozygous SNPs with a minimum density of
414 1 SNP / 5,000 kb and no minimum length 50 SNPs was used following Howrigan et al.'s
415 guidelines (62). No heterozygous markers were allowed in the 50 SNPs-window. In this analysis,
416 the maximum gap between two consecutive SNPs within a run was set to 5,000 kb. Inbreeding
417 coefficients were calculated by estimating the proportion of the autosomal genome that is in
418 ROH. This method was proposed by McQuillan and al (2008)(63) and has been showed to be
419 the most reliable, especially with small sample size(64). Faroe inbreeding coefficients were
420 compared to inbreeding coefficient of HapMap phase 3 project populations.

421

422 **Genome-wide association study (GWAS)**

423 Prior association analyses, SNPs with genotyping call rate < 90%, minor allele
424 frequency < 0.05 or failing Hardy Weinberg equilibrium test ($p < 10^{-6}$) were filtered out of the
425 Faroe SNP genotyping dataset. The global genome wide genotyping call rate of all the
426 individuals was superior to 90%. A total of 1,690,491 variants and 212 independent
427 individuals (including 36 cases and 176 controls) passed filters and QC. Allelic, recessive and
428 dominant GWAS were performed with Plink 1.09 using Chi-squared statistics. Manhattan and
429 Quantile-Quantile (Q-Q) plots were generated using R. Gene and gene-set (including SFARI,
430 pLI > 0.9 and Brain gene lists) analyses were performed with MAGMA v1.06 (29) using
431 principal components regression and linear regression model, respectively.

432

433 **Polygenic risk score (PRS) for autism**

434 The computation of the PRS was performed with the tool PRSice2 (65) on the Faroes SNP
435 array data using as a reference the PGC GWAS summary statistics (31). SNPs were not
436 imputed since we used high density arrays (over 4 millions SNPs). For our dataset, PRSice2
437 with default parameters defined a p-value threshold of 0.197 which gives us a R^2 (squared
438 correlation coefficient) of 0.04.

439

440 **Whole-Exome Sequencing (WES)**

441 Blood leukocytes DNA from 286 individuals was enriched for exonic sequences through
442 hybridization SureSelect Human All Exon V5 (Agilent) by the CNRGGH. For 67 individuals
443 for whom the available quantity of DNA was low, they used a low-input protocol using only
444 200 ng of DNA compared to 3 μ g for the normal protocol. The captured DNA was sequenced
445 using a HiSeq 2000 instrument (Illumina). Coverage/depth statistics have been accessed as
446 quality control criteria. We required that more than 90% of each exome have 10X coverage

447 and more than 80% have 20X coverage. Short read sequences were then aligned to hg19 with
448 BWA v0.7.8, duplicate reads were removed with PicardTools MarkDuplicates. Reads with a
449 global quality under 30 and a mapping quality under 20 were excluded from the analysis.
450 Variants were predicted using FreeBayes (66) and GATK (67) with a minimum of 10 reads
451 covering the position. VEP (using RefSeq and Ensembl 91) was used to annotate the variants.
452 We used the GEMINI (68) framework that automatically integrates the VCF file into a
453 database for exploring genetic variant for disease and population genetics. Genetic variants
454 were analyzed using GRAVITY, a Cytoscape (69) plugin designed in the lab specifically for
455 interpreting WES results using Protein-Protein Interaction networks
456 (<http://gravity.pasteur.fr/>). Gravity uses a user-friendly interface and makes easier prioritizing
457 variants according to damage prediction, mode of inheritance, gene categories and variant
458 frequency in databases. It allows filtering variants with many parameters, such as quality
459 parameters (DQ, MQ, GQ), allelic fraction, frequency of the variant in the cohort or in
460 databases, damage prediction scores (CADD, SIFT, Polyphen2) and many more. Since WES
461 does not detect the *FMRI* amplification, 33 individuals with autism were tested for Fragile-X
462 syndrome using the AmplideX™ *FMRI* PCR kit from Theradiag. There were no individuals
463 with “pre-mutation” or “full-mutation” of CGG repeats in the 5' UTR region of the fragile X
464 mental retardation-1 (*FMRI*) gene.

465

466 **Copy-number variants (CNVs)**

467 CNVs were identified from both SNP genotyping and WES data. Quality controls were the
468 following: call rate > 0.99, standard deviation of the Log R ratio < 0.35, standard deviation of
469 the B allele frequency < 0.08 and absolute value of the wave factor < 0.05. CNVs were
470 detected by both PennCNV(70) and QuantiSNP(71) algorithm using the following
471 filters: ≥ 3 consecutive probes, CNV size > 1kb and CNV detection confidence score ≥ 15 .

472 CNV detections from PennCNV and QuantiSNP were merged using CNVision(17). CNVs
473 with CNVision confidence score < 30 , CNV size < 50 kb, overlap $> 50\%$ with segmental
474 duplication or known large assembly gaps (greater than 150 kb) or copy number = 2 in
475 pseudo autosomal regions (PARS) in males were filtered out. CNV annotations were
476 performed using ANNOVAR (72) and CNV frequencies in Faroese and in database of
477 genomic variant cohorts (DGV, <http://dgv.tcag.ca/dgv/app/home>) were assessed using in
478 house python scripts based on reciprocal overlap $\geq 80\%$. We also detected CNVs from the
479 WES sequencing data using the XHMM software(73). CNVs with QSOME score < 90 ,
480 number of targets < 5 , or overlap $> 50\%$ with segmental duplication or known large assembly
481 gaps (greater than 150 kb) were filtered out. CNV annotations were performed using
482 ANNOVAR (72) and CNV frequencies in Faroese were assessed using in house python
483 scripts based on reciprocal overlap $\geq 80\%$. *De novo* and inherited CNVs were validated by
484 visual inspection using SnipPeep (<http://snippeep.sourceforge.net/>).

485

486 **Gene-set lists and prioritization of variants**

487 Three gene-set lists were used : (i) “SFARI genes” (n=990) that includes genes implicated in
488 autism (15) (Simons Foundation Autism Research Initiative gene database -
489 <https://gene.sfari.org/>); (ii) “pLI > 0.9 genes” that includes genes with strong probability of
490 being loss-of function intolerant (n=3,230) (74); (iii) “Brain genes” that includes genes
491 specifically or strongly expressed (above 1 Standard Deviation) in fetal or adult human brain
492 using data from Su et al (n=3,591)(75).

493 A combination of approaches was used to prioritize the genes and to estimate the
494 deleterious effect of a variant. We prioritized genes using gene sets (SFARI genes, pLI ≥ 0.9
495 and Brain genes, previously defined). We prioritized Likely Gene Disruptive (LGD) variants
496 (stopgains, splice site variants, frameshift indels) over missense variants or synonymous

497 variants. Additionally, we used the CADD score (14) (a CADD ≥ 30 means that the variants
498 belong to the 0.1% most deleterious variants) to assess the deleterious effect missense
499 variants. Minor allele frequency (MAF) was estimated in the general population from the
500 gnomAD database(74). In order to filter out common variants that was not listed in gnomAD,
501 we also excluded variants that were present in more than 15% of our Faroese control cohort.
502 For the detection of deleterious homozygous variants, we kept only LGD and MIS30 with
503 MAF $< 1\%$.

504

505 **Burden analysis**

506 Rare variant association studies (MAF $<5\%$) were performed using EPACTS v3.2.6
507 (<https://genome.sph.umich.edu/wiki/EPACTS>). Prior association analysis, variants identified
508 by WES were filtered using VCFtools (http://vcftools.sourceforge.net/man_latest.html) with
509 the following metrics: minimum genotyping quality ≥ 30 , min depth of coverage ≥ 10 ,
510 maximum of missing data ≤ 10 , no InDel (small insertion or deletion), only bi-allelic sites and
511 no site failing Hardy Weinberg equilibrium test ($p < 10^{-6}$). The annotation of the variants was
512 done using EPACTS and the variants included in the Gene-wise association analyses were
513 non-synonymous, essential splice site, normal splice site, start loss, stop loss and stop gain
514 variants. Logistic Score Test (“b.score” in S1 Table) was used to test single variant
515 association ($n_{\text{Cases}}=36$; $n_{\text{Controls}}=107$; $n_{\text{Variants}}=155,284$). For Gene-wise tests, we used two
516 approaches (including $n_{\text{Cases}}=36$; $n_{\text{Controls}}=107$ and $n_{\text{groups}}= 15,005$): (i) collapsing burden test
517 using EMMAX (Efficient Mixed Model Association eXpedited (76), “CMC-EMMAX” in S1
518 Table) and (ii) Optimal SNP-set sequence Kernel Association Test (“SKAT-O” in S1 Table).
519 The advantage of the CMC-EMMAX is that this test is accounting for population structure
520 and high relatedness between individual (based on kinship matrix). The advantage of SKAT is

521 that this test is particularly powerful in the presence of protective and deleterious variants and

522 null variants. For both Gene-wise tests, a $10^{-6} \leq \text{MAF} \leq 0.05$ was used.

523

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528

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748

749

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763

764 **Figure Captions**

765 **Fig 1. Genetic background of the Faroese population.** A. Geographic localization of the
766 Faroe Islands (R software and “maps” and “mapdata” packages were used to draw the map).
767 B. Demographic comparison of the epidemiological and genetic cohorts of the Faroe Islands.
768 The epidemiological and the genetic cohort are composed of 67 and 36 individuals with
769 autism, respectively. C. Multidimensional scaling plots (MDS) of genome-wide identity by
770 state (IBS) pairwise distances between human populations. Each dot represents an individual
771 and the distance between two dots corresponds to genetic distance based on genome-wide
772 pairwise IBS calculations. D. Degree of inbreeding across HapMap 3 and Faroese
773 populations. The inbreeding coefficients of the Faroe non-autism control individuals (n=176)
774 were compared to the HapMap3 populations. Faroe individuals displayed a higher degree of
775 inbreeding compared with others human populations (Median F: $F_{\text{FAROES}} = 0.015$;
776 $F_{\text{ASW}} = 0.0014$; $F_{\text{CEU}} = 0.0071$; $F_{\text{CHD}} = 0.0081$; $F_{\text{CHB}} = 0.0083$; $F_{\text{GIH}} = 0.011$; $F_{\text{JPT}} = 0.0093$;
777 $F_{\text{LWK}} = 0.0045$; $F_{\text{MXL}} = 0.0095$; $F_{\text{MKK}} = 0.0026$; $F_{\text{TSI}} = 0.0066$; $F_{\text{YRI}} = 0.0028$; Paired samples
778 Wilcoxon test: $W_{\text{ASW}} = 12.56$, $p_{\text{ASW}} < 0.0001$; $W_{\text{CEU}} = 12.27$, $p_{\text{CEU}} < 0.0001$; $W_{\text{CHD}} = 7.14$,
779 $p_{\text{CHD}} < 0.0001$; $W_{\text{CHB}} = 7.11$, $p_{\text{CHB}} < 0.0001$; $W_{\text{GIH}} = -3.06$, $p_{\text{GIH}} < 0.002$; $W_{\text{JPT}} = -5.83$,
780 $p_{\text{JPT}} < 0.0001$; $W_{\text{LWK}} = -10.86$, $p_{\text{LWK}} < 0.0001$; $W_{\text{MXL}} = -5.66$, $p_{\text{MXL}} < 0.0001$; $W_{\text{MKK}} = -14.52$,
781 $p_{\text{MKK}} < 0.0001$; $W_{\text{TSI}} = -8.64$, $p_{\text{TSI}} < 0.0001$; $W_{\text{YRI}} = -14.41$, $p_{\text{YRI}} < 0.0001$; p are nominal p -
782 values, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$). N, number; ID, intellectual disability; ASW,
783 African ancestry in Southwest USA (n=83); CEU, Utah residents with Northern and Western
784 European ancestry from the CEPH collection (n=165); CHB, Han Chinese in Beijing, China
785 (n=84); CHD, Chinese in Metropolitan Denver, Colorado (n=85); GIH, Gujarati Indians in
786 Houston, Texas (n=88); JPT, Japanese in Tokyo, Japan (n=86); LWK, Luhya in Webuye,
787 Kenya (n=90); MXL, Mexican ancestry in Los Angeles (n=77), California; MKK, Maasai in

788 Kinyawa, Kenya (n=171); TSI, Toscani in Italia (n=88); YRI, Yoruba in Ibadan, Nigeria
789 (n=167).

790

791 **Fig 2. Rare and common variants in Faroese individuals with autism.** A. Copy-number
792 variant (CNV) analysis among gene-set lists within Faroe individuals. The number of exonic
793 CNV carriers altering any genes or gene-set lists (SFARI genes, pLI > 0.9 genes and Brain
794 genes, see Materials and Methods section) were compared between individuals with autism,
795 siblings and controls (Fisher's exact test: $n_{\text{autism}} = 36$, $n_{\text{controls}} = 107$, $p_{\text{CNV_loss_All_genes}} = 0.02$,
796 $\text{OR}_{\text{CNV_loss_All_genes}} = 2.79$; $p_{\text{CNV_loss_SFARI}} = 0.014$, $\text{OR}_{\text{CNV_loss_All_SFARI}} = 13.25$;
797 $p_{\text{CNV_loss_pLI>0.9}} = 0.014$, $\text{OR}_{\text{CNV_loss_pLI>0.9}} = 13.25$; $p_{\text{CNV_loss_Brain}} = 0.024$,
798 $\text{OR}_{\text{CNV_loss_All_Brain}} = 5.59$; $p_{\text{CNV_gain_All_genes}} = 0.0056$, $\text{OR}_{\text{CNV_gain_All_genes}} = 3.28$;
799 $p_{\text{CNV_gain_SFARI}} = 0.067$, $\text{OR}_{\text{CNV_gain_All_SFARI}} = 4.33$; $p_{\text{CNV_gain_pLI>0.9}} = 0.11$,
800 $\text{OR}_{\text{CNV_gain_pLI>0.9}} = 2.47$; $p_{\text{CNV_gain_Brain}} = 0.0049$, $\text{OR}_{\text{CNV_gain_All_Brain}} = 4.76$; p are nominal p -
801 values). B. Heatmap combining signals obtained from rare and common variant association
802 tests and rare deleterious variants altering SFARI genes throughout Faroese individuals with
803 autism. "Burden of non-synonymous" includes results from SKAT-O and CMC-EMMAX
804 obtained from WES data (see Materials and Methods section and S5 fig; $p < 10^{-3}$). "Common
805 variants" are the top hits of the genome wide association study (GWAS) for both allelic and
806 recessive model obtained from genome-wide genotyping data ($p < 10^{-6}$). "Rare LGD SFARI
807 variants" are rare likely gene disrupting (LGD) variants altering SFARI genes identified by
808 (MAF < 1% in gnomAD). "The controls freq." column indicates the proportion of non-ASD
809 Faroese controls carrying the corresponding variant. P-Values are nominal. ID, intellectual
810 disability.

811

812 **Fig 3. Genetic recessive mutations in Faroese individuals with autism.** A. Distribution of
813 the inbreeding coefficient in Faroese individuals (Mann Whitney U-test: $n_{\text{autisms}} = 36$,

814 $n_{\text{controls}} = 176$, $n_{\text{siblings}} = 30$; $U_{\text{controls.vs.autisms}} = 2,297$, $p_{\text{controls.vs.autisms}} = 0.0047$;
815 $U_{\text{controls.vs.siblings}} = 1,953$, $p_{\text{controls.vs.siblings}} = 0.011$; * indicates the one withstanding multiple
816 testing). B. Number of rare LGD+MIS30 homozygous mutations carried per individual (Mann
817 Whitney U-test: $n_{\text{autisms}} = 36$, $n_{\text{controls}} = 107$, $n_{\text{siblings}} = 30$; $U_{\text{controls.vs.autisms}} = 1,321$,
818 $p_{\text{controls.vs.autisms}} = 0.00049$; $U_{\text{controls.vs.siblings}} = 1,293$, $p_{\text{controls.vs.siblings}} = 0.025$; * indicates the one
819 withstanding multiple testing). C. Venn diagram of the genes carrying the variants from B.
820 Genes names are in bold and annotated when they are part of our gene-set lists (SFARI genes,
821 pLI > 0.9 genes and Brain genes, see subject and methods section). The plot on the right shows
822 the proportion of individuals in each category carrying at least one mutated gene in our gene-
823 sets lists (Fisher's exact test: $p_{\text{controls.vs.autisms}} = 0.03$; $p_{\text{controls.vs.siblings}} = 0.03$). D. and E. are
824 describing two specific families carrying multiple variants. "0" and "1" refer to wildtype or
825 mutated allele, respectively. The localizations of the variants are indicated along the proteins
826 and alignments throughout species showed the strong conservation of the altered amino acids.
827 LGD, likely gene disruptive; MIS30, missense variants with CADD score ≥ 30 ; IgD,
828 immunoglobulin domain; TIL, Trypsin Inhibitor-like; FA5/8C, Coagulation factor 5/8 type C
829 domain; LamG, Laminin G domain; EGF, epidermal growth factor like domain; Fibr.,
830 Fibrinogen, alpha/beta/gamma chain, C-terminal globular domain; AAA: ATPases associated
831 domains.

832

833 **Fig 4. Distribution of the polygenic risk score for autism in Faroese individuals. A.**

834 Distribution of the polygenic risk score for autism (PRS-autism) of controls, autisms and
835 siblings (Mann Whitney U-test: $n_{\text{autisms}} = 36$, $n_{\text{controls}} = 107$, $n_{\text{siblings}} = 53$;

836 $U_{\text{controls.vs.autisms}} = 2,344$, $p_{\text{controls.vs.autisms}} = 0.0070$; * indicates the one withstanding multiple

837 testing). B. Distribution of the PRS-autism for the cases without intellectual disability (ID)

838 and the cases with ID (Mann Whitney U-test: $n_{\text{autisms-with-ID}} = 12$, $n_{\text{autisms-without-ID}} = 24$; $U_{\text{ID.vs.no-}}$

839 ID = 86, $p_{ID.vs.no-ID} = 0.027$; * indicates the one withstanding multiple testing). The PRS was
840 calculated using PRSice-2 (see Materials and Methods section).

841

842 **Fig 5. Stratification of autism in Faroese individuals.** On the left, the stratification was built
843 using hierarchical clustering on the number of genes carrying rare deleterious variants altering
844 SFARI genes (MIS30, LGD or CNV) and on the polygenic risk score for autism (PRS-autism).
845 The other columns were not used for the clustering. The genetic profile contains variants with
846 a predicted impact on the condition of the individual with autism, the one in bold are most likely
847 causatives. The clinical profile gives a subset of relevant information for each individual with
848 autism. ID, intellectual disability; M, male; F, female; del, deletion; dup, duplication.

849

850

851 **Supporting information**

852 **S1 Fig 1. Pipeline of the study.** QC, quality control; SD LRR, standard deviation of the Log
853 R ratio; SD BAF, standard deviation of the B allele frequency; |WF|, absolute value of the
854 wave factor; BWA, Burrows-Wheeler Aligner; GATK, Genome Analysis Toolkit; VEP,
855 Variant Effect Predictor; GQ, genotyping quality; Htz_R, heterozygosity ratio; CNV, copy
856 number variant; SNP/V, single nucleotide polymorphism/variation; WES, whole exome
857 sequencing; Hmz, homozygote; Htz, heterozygote; pLI, probability of being loss-of function
858 intolerant.

859

860 **S2 Fig. Population stratification of the Faroe Islands.** Combination of the ethnic genetic
861 background for each individuals of the Faroe and the individuals from HapMap3 using
862 admixture. Legend of HapMap 3 population: Africa (ASW, African ancestry in Southwest
863 USA (n=83); LWK, Luhya in Webuye, Kenya (n=90); MKK, Maasai in Kinyawa, Kenya
864 (n=171); YRI, Yoruba in Ibadan, Nigeria (n=167)); Asia (CHB, Han Chinese in Beijing,
865 China (n=84); CHD, Chinese in Metropolitan Denver, Colorado (n=85); JPT, Japanese in
866 Tokyo, Japan (n=86)); TSI, Toscani in Italia (n=88); CEU, Utah residents with Northern and
867 Western European ancestry from the CEPH collection (n=165); GIH, Gujarati Indians in
868 Houston, Texas (n=88); MXL, Mexican ancestry in Los Angeles (n=77), California.

869

870 **S3 Fig. CNVs altering genes involved in neurodevelopmental disorders.** A. *De novo*
871 Trisomy 21 in patient with autism, ID and Down syndrome. B. *De novo* deletion of 425.5 kb
872 removing the six first exons of the *NRXN1α* in individual with autism. C. *De novo* 2.9 Mb
873 deletion on chromosome 22q11 in individual with autism and DiGeorge/velocardial
874 syndrome. D. A 91.4 kb deletion removing all exons of *ADNP* in a male with autism and ID.
875 Each dot shows Log R Ratio (LRR; in red) and B allele frequency (BAF; in green). The copy

876 number (CN) is indicated with a blue line. Patients with ID and patients without ID are
877 represented in black and grey, respectively. ID, Intellectual disability.

878

879 **S4 Fig. The *de novo* SNVs in individuals with autism.** Sanger sequencing was performed to
880 validate *de novo* SNVs altering *MECP2*, *KIF17* (A), *PLA2G4A* (B), *RIMS4* (C) and *KALRN*
881 (D). Sanger chromatograms are shown for each trios. Individuals with ID and without ID are
882 represented in black and grey, respectively. The position of the SNVs is indicated on the
883 protein and the amino acid alignment of the region throughout several species (Human,
884 Rhesus, Mouse, Dog, Zebrafish) shows the high conservation of the altered amino acids; ID,
885 Intellectual disability.

886

887 **S5 Fig. Gene-wise association study using the whole exome sequencing data.** To test for
888 Gene-wise association, a collapsing burden test using EMMAX “CMC-EMMAX” (A) and
889 optimal SNP-set sequence Kernel Association Test “SKAT-O” (B) were used. The dashed
890 line indicates $p\text{-value} < 10^{-3}$. EMMAX, Efficient Mixed Model Association eXpedited.

891

892 **S6 Fig. Results from the Genome Wide Association Study (GWAS) using different**
893 **models (allelic, Recessive or Dominant).** QQ plots and Manhattan plots for allelic, recessive
894 and dominant GWAS are represented in panel A and B, respectively. QQ, quantile-quantile.

895

896 **S7 Fig. Locus zoom of the top hits detected by the GWAS.** LocusZoom for regional
897 visualization of the top hits isolated from the allelic and recessive GWAS was used.

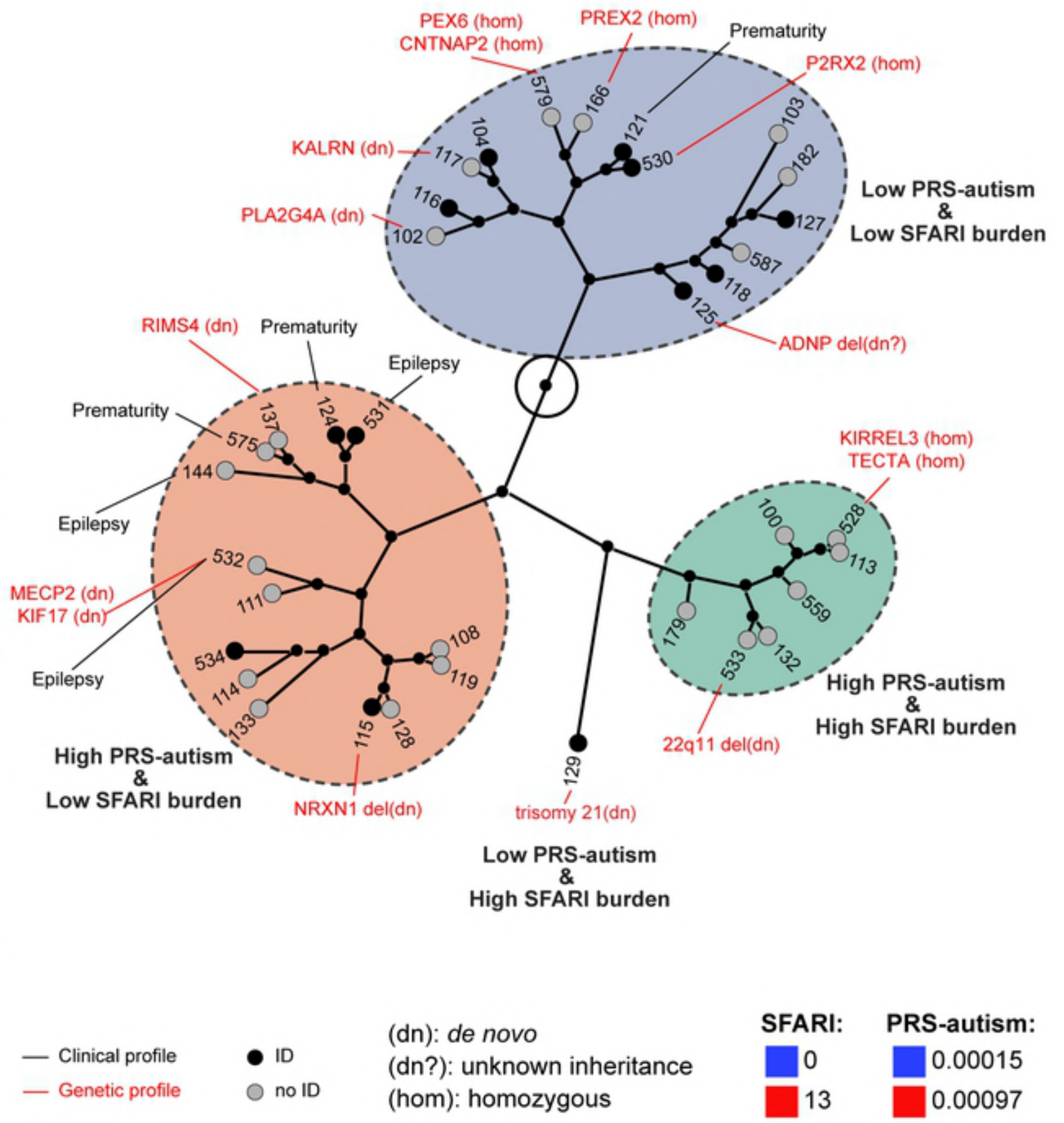
898

899 **S1 Appendix. Clinical notes**

900

- 901 **S1 Table: *de novo* CNVs**
- 902 **S2 Table: *de novo* SNVs**
- 903 **S3 Table: CNVs within SFARI, pLI > 0.9 an Brain genes**
- 904 **S4 Table: CMC-emmax gene-wise association test from WES**
- 905 **S5 Table: SKAT-O gene-wise association test from WES**
- 906 **S6 Table: b.score single variant association test from WES**
- 907 **S7 Table: rare LGD or MIS30 SNV within SFARI, pLI and Brain genes (< 1%**
- 908 **gnomAD)**
- 909 **S8 Table: recessive SNVs**
- 910 **S9 Table: GWAS from SNP genotyping data**
- 911 **S10 Table: MAGMA Gene-based association study from SNP genotyping data**
- 912 **S11 Table: MAGMA Gene-set-based association study from SNP genotyping data**

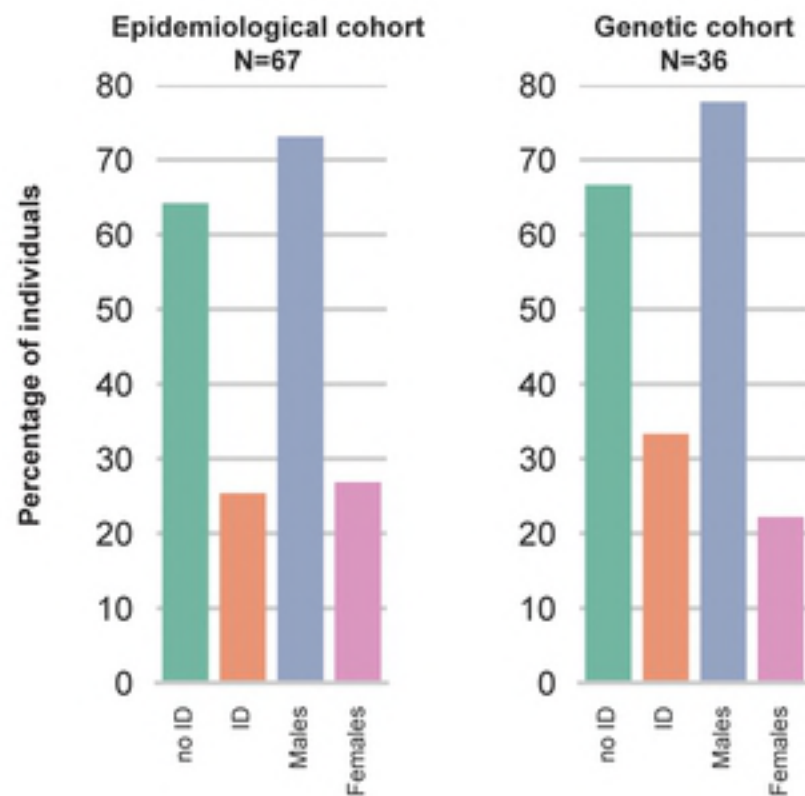
Patient	ID	Sex	PRS-autism	SFARI
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PN400528	●	F		
PN400113	●	M		
PN400559	●	M		
PN400132	●	M		
PN400533	●	F		
PN400179	●	M		
PN400129	●	M		
PN400108	●	M		
PN400119	●	F		
PN400128	●	M		
PN400115	●	M		
PN400133	●	M		
PN400114	●	M		
PN400534	●	F		
PN400111	●	M		
PN400532	●	F		
PN400144	●	M		
PN400575	●	F		
PN400137	●	M		
PN400124	●	M		
PN400531	●	F		
PN400102	●	M		
PN400116	●	M		
PN400117	●	M		
PN400104	●	M		
PN400579	●	M		
PN400166	●	M		
PN400121	●	F		
PN400530	●	M		
PN400103	●	M		
PN400182	●	M		
PN400127	●	M		
PN400587	●	M		
PN400118	●	M		
PN400125	●	M		



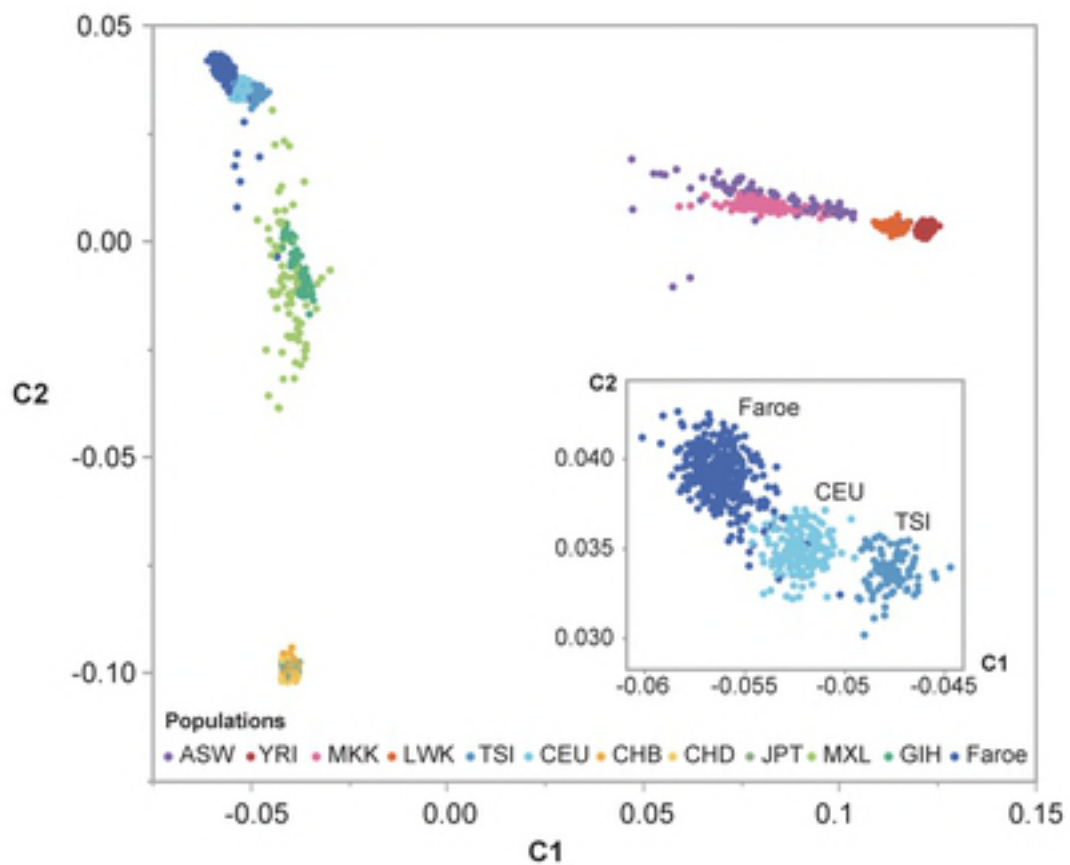
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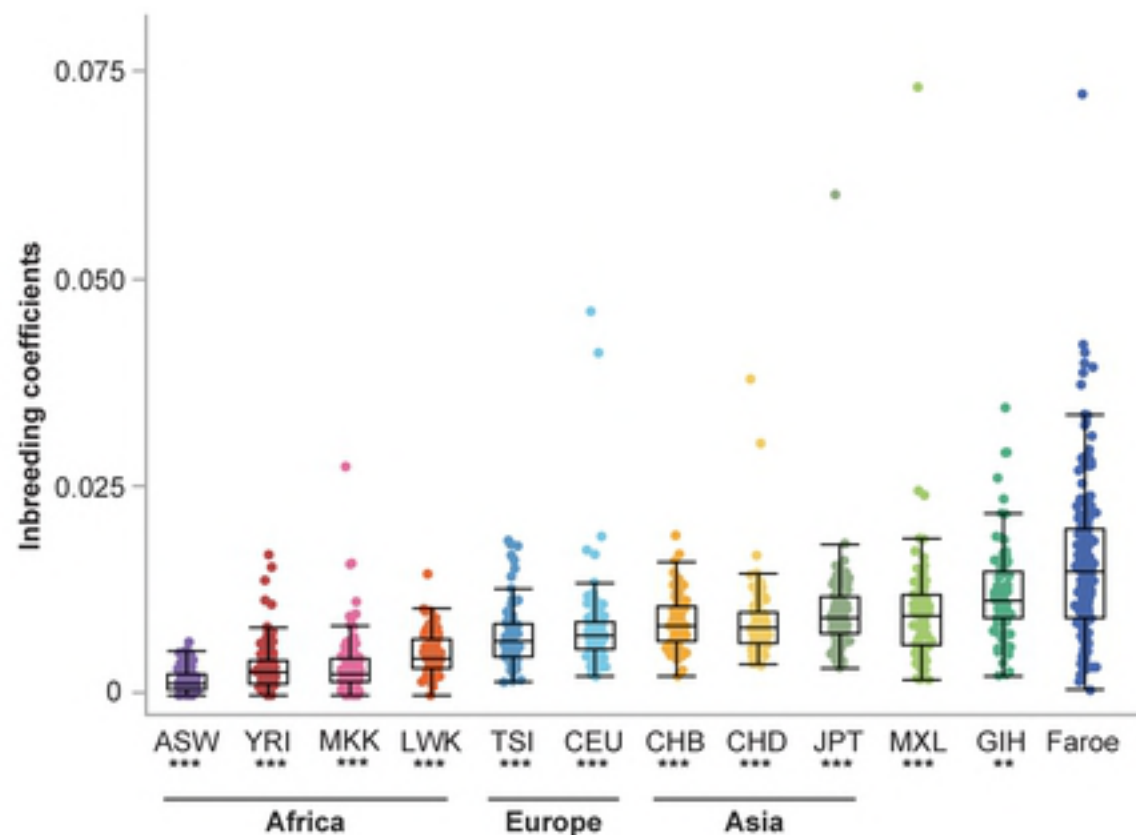
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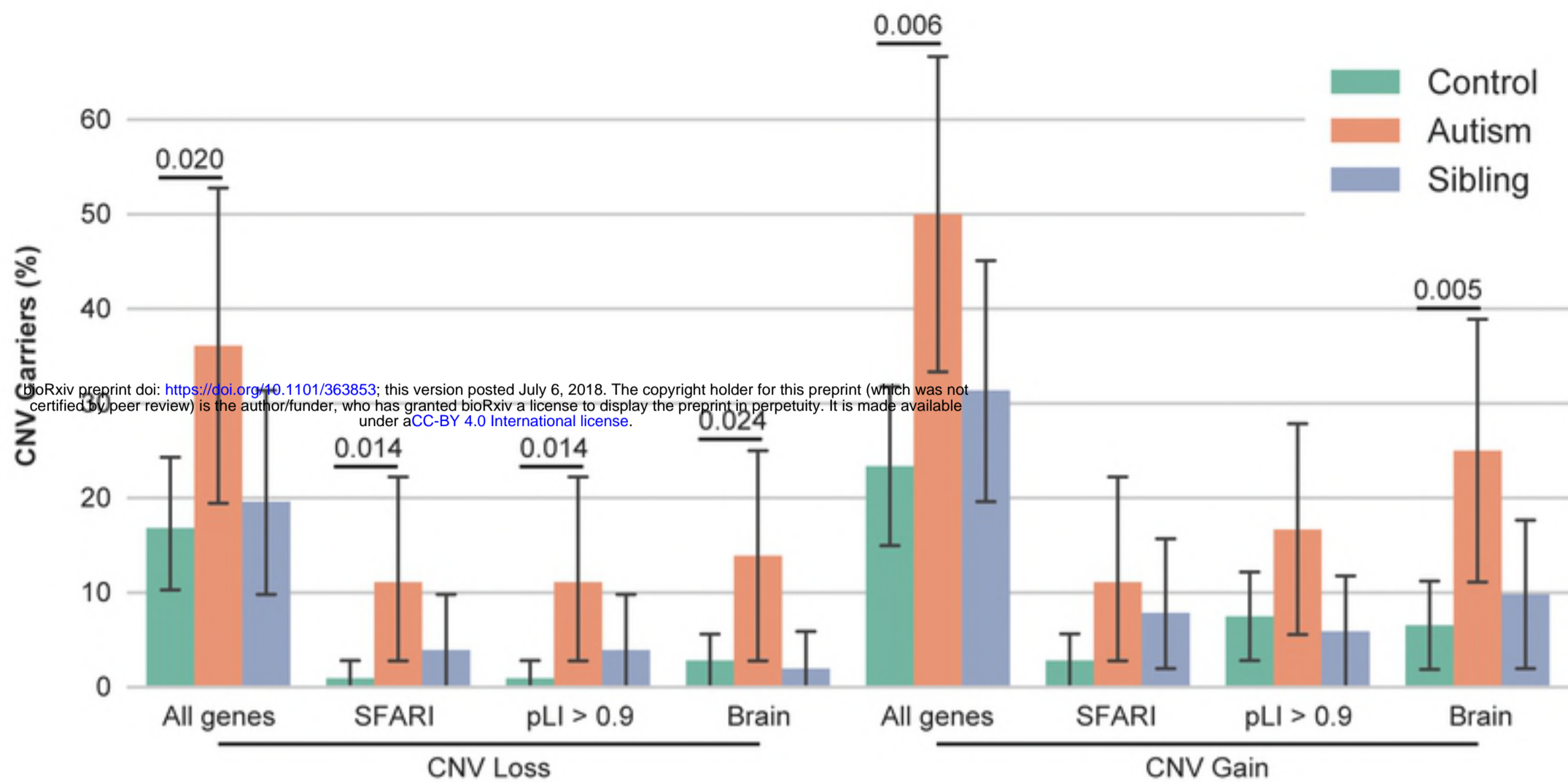


C



D

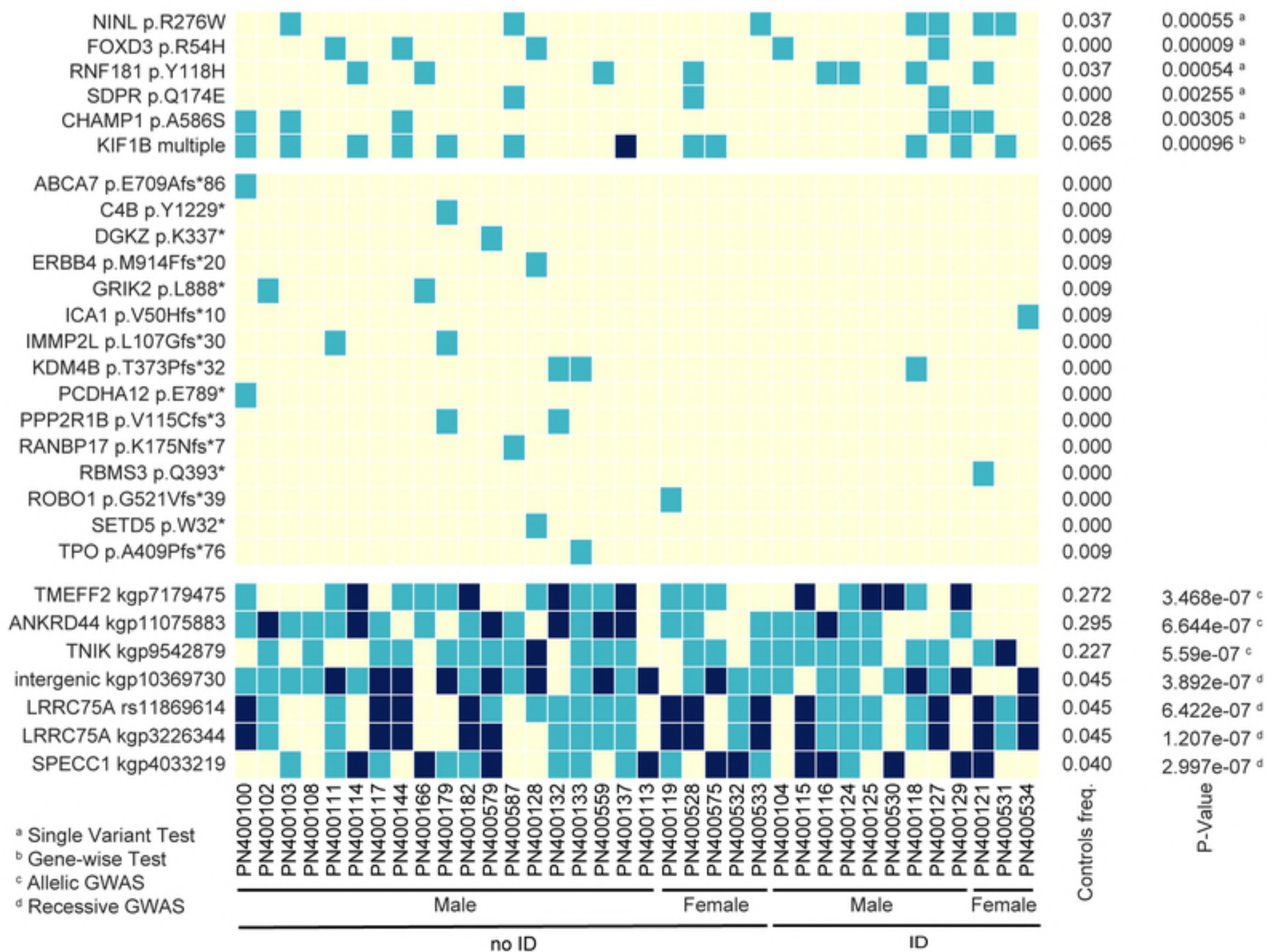


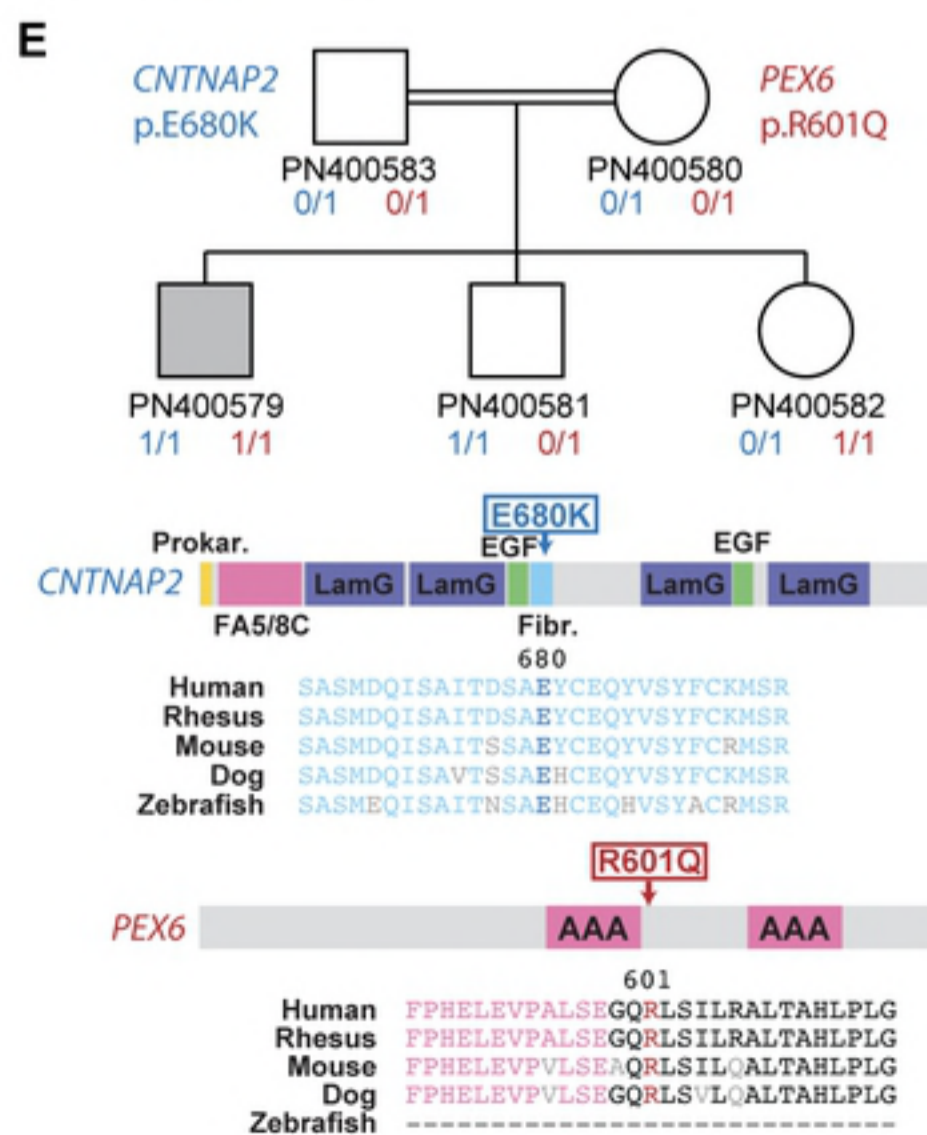
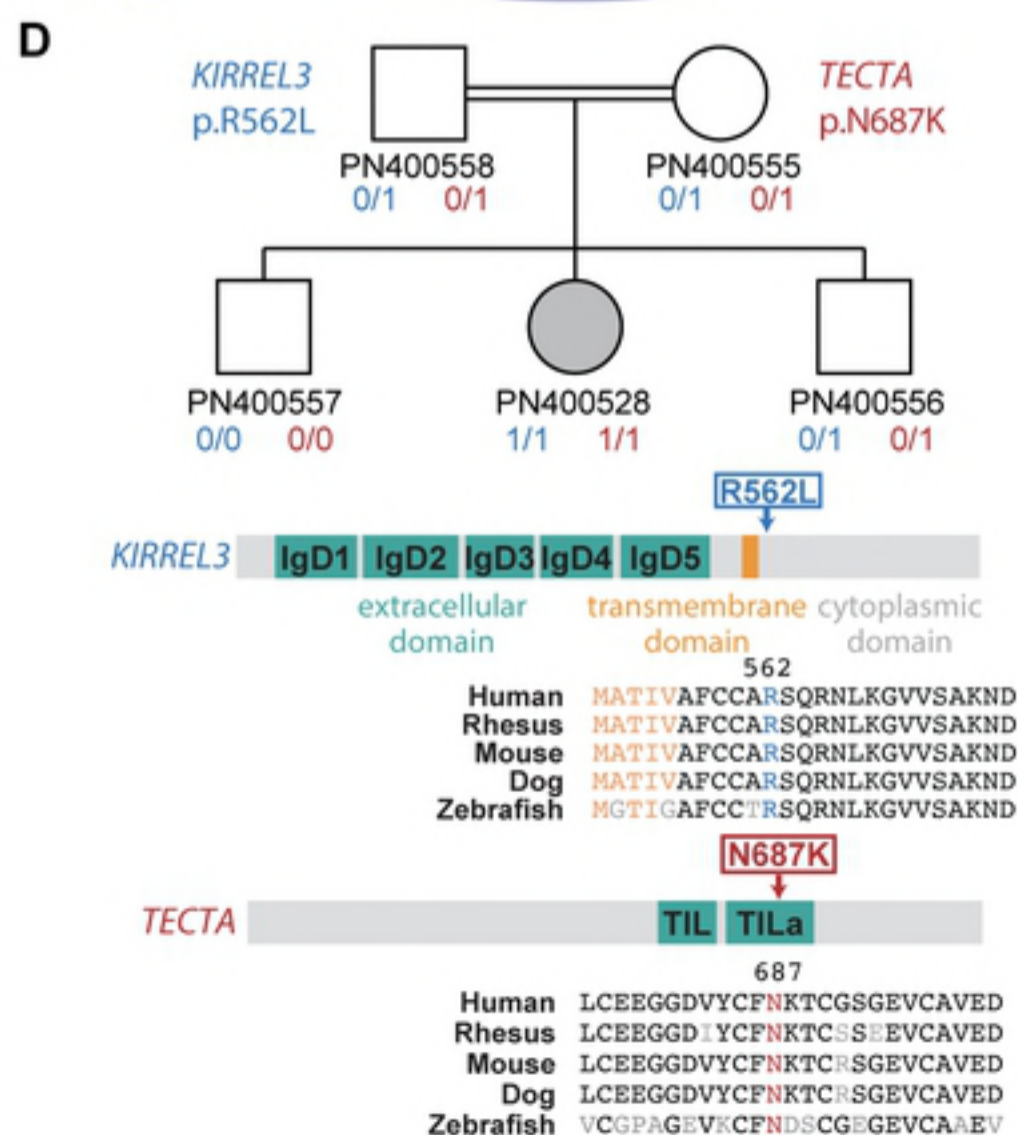
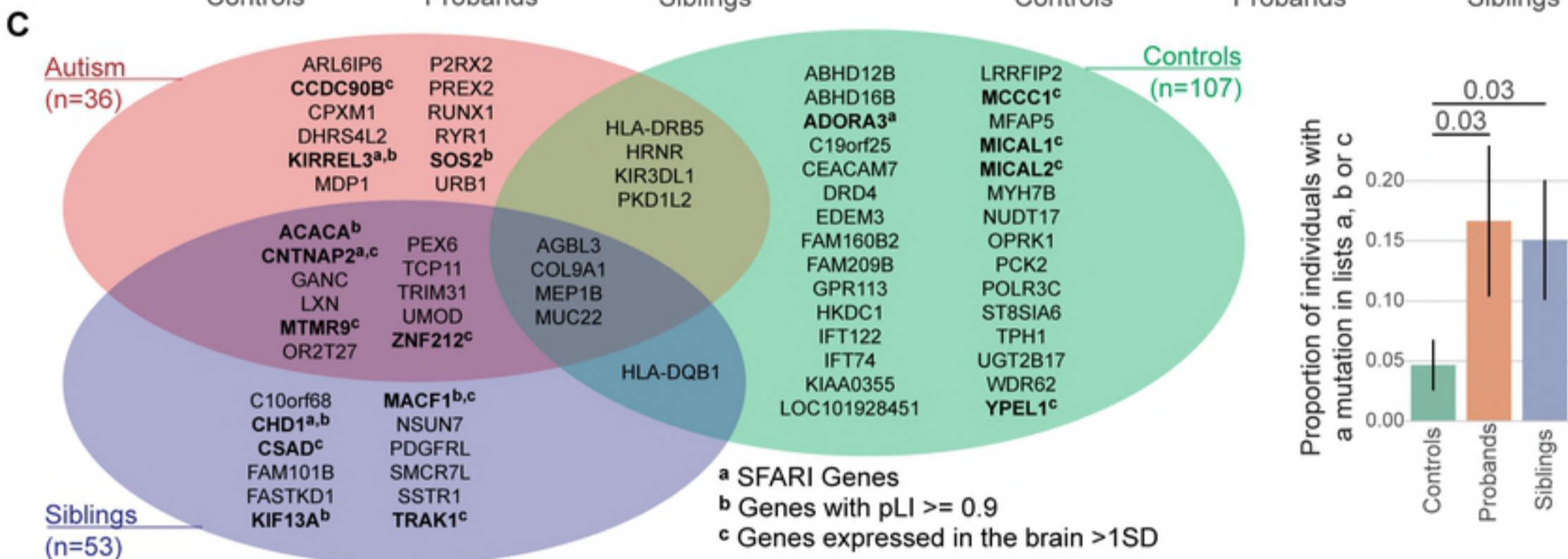
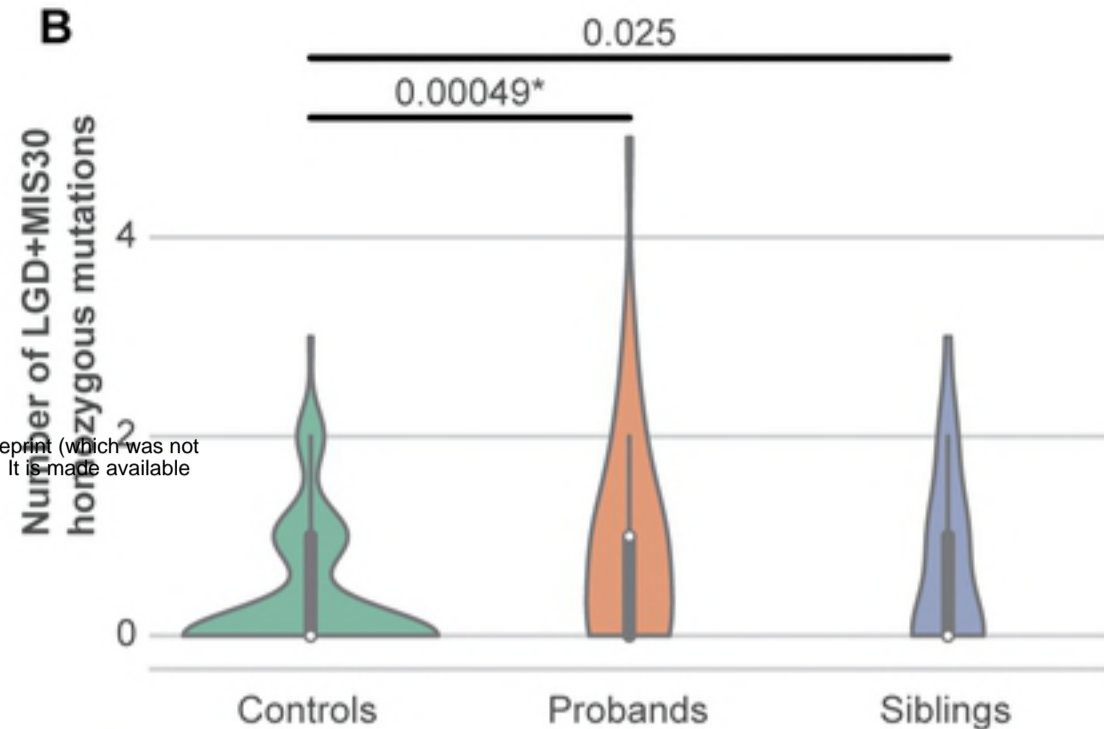
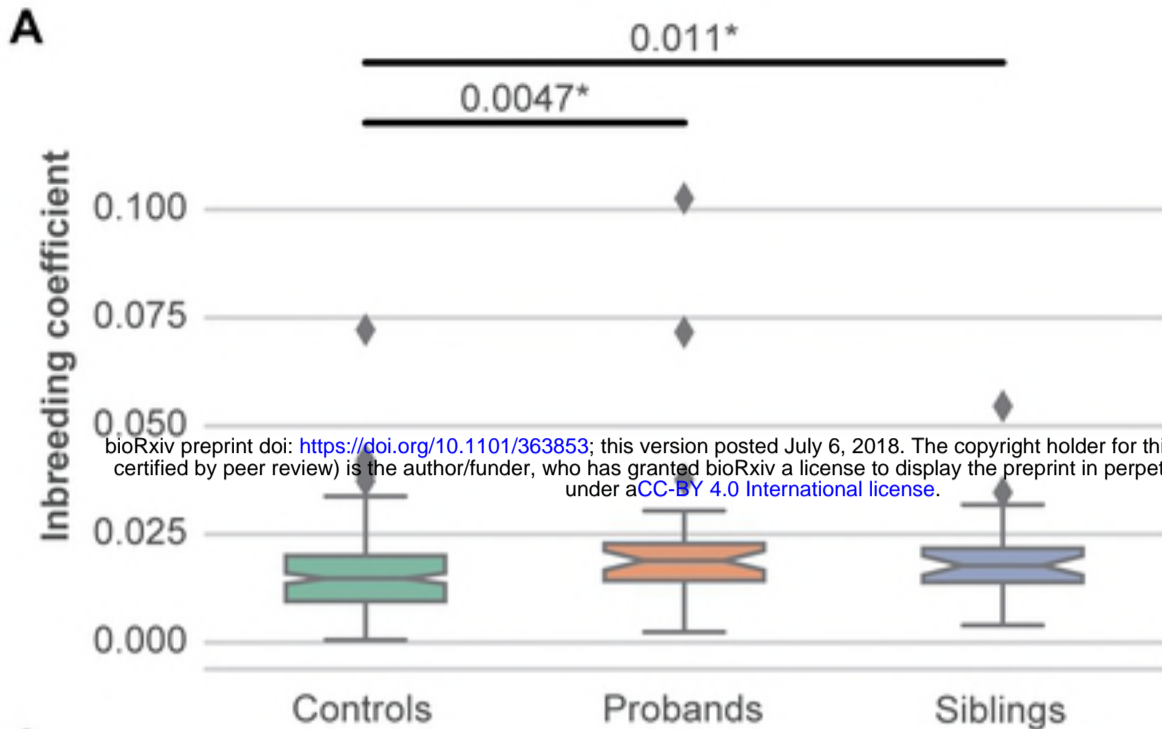
A**B**

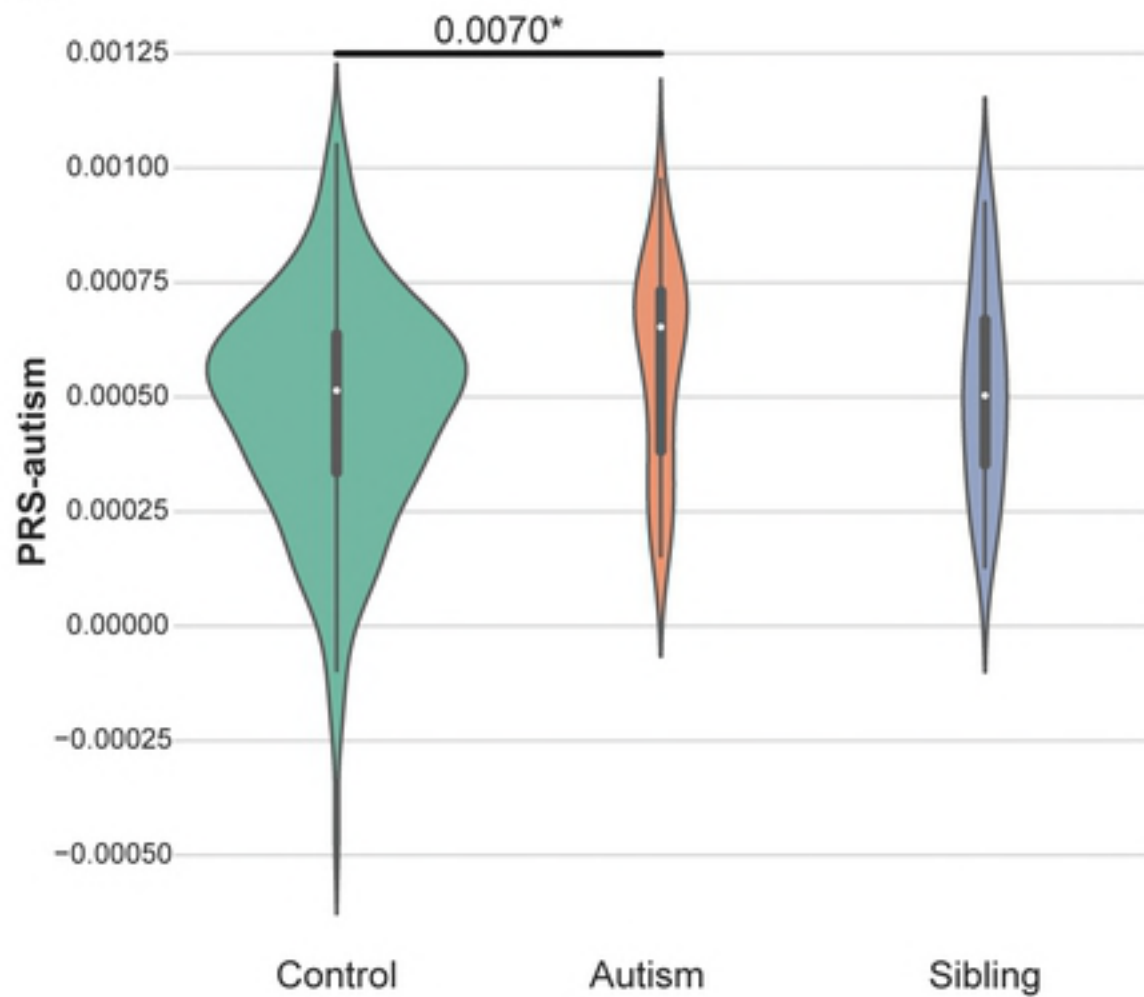
Burden of non-synonymous

Rare LGD SFARI variants

Common variants





A**B**