Supplemental Data of

**ARF1-related disorder: phenotypic and molecular spectrum.**

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Supplementary note 1:

During the compilation of ARF1 putative loss-of-function variant, we excluded four gnomAD individuals with presumed loss-of-function variants. Indeed, two individuals in gnomADv2.1.1 (rs755638275, available at https://gnomad.broadinstitute.org/variant/1-228285417-G-GACCTCCCCAAGCCATGAATGCGGCCAGATCACAGACAAGCTGGGGCTGCAC?dataset=gnomad_r2_1) and two individuals in gnomAD v3.1.1 (79 nucleotides deletion, c.148+2_149del, available at https://gnomad.broadinstitute.org/variant/1-228097260-TAGGTGAGGGGGGCGACAGGAGTGCTGGGCTGCGCTGGCAAGGATCAAAGCCTACCCTGCATCCCCGACACC-T?dataset=gnomad_r3) were identified with alleles predicted to be splice disruptive but could not be counted as germline loss-of-function variants with enough confidence after examination. We observed the following issues: long alternative alleles matching a processed pseudogene and poor read support. The two variants in gnomADv2.1.1 (rs755638275) had high strand bias (Phred-scaled p-value of Fisher’s exact test = 45.347) and a very low QD score (QD < 6), which failed to meet satisfying confidence for germline variants. Alternative alleles were compatible with a processed pseudogene (inserted/deleted nucleotides concordant with exon4/exon5 junction, intronic heterozygous SNV present in the supposedly heterozygous 79pb deletion). This issue has been submitted to gnomAD production team and resulted in the suppression of the read data of the gnomAD v3.1.1 individuals.

Supplementary note 2:

The in-vitro activation of Arf1Y35H in transfected cells previously reported was weaker compared to Arf1WT transfected cells but stronger compared to basal activation, which is compatible with the Arf1 overexpression in transfected cells compared to non-transfected cells.3
**Supp. Fig. 1**

A) Missense deleteriousness predictions from CADD (GRCh37-v1.6), REVEL, MISTIC, BayesDel (noAF), M-CAP and Varity_ER of benign ARF1 missense variants, and pathogenic variants of this cohort (eight substitutions, seven distinct missense variations, red). Vertical axis: deleterious prediction scores; horizontal axis: ARF1 residues.

*One missense variant in gnomADv3.1 (1-228097853-A-C (GRCh38); p.(Asp129Ala), available at: https://gnomad.broadinstitute.org/variant/1-228097853-A-C?dataset=gnomad_r3 has been excluded from this analysis because of highly suspicious quality metrics (QD=1.136) and poor read support for germline heterozygous substitution (allelic fraction of 29% based on 5/17 reads and 3/5 having a base quality <7). This issue has been reported to gnomAD Production Team.*

**The better discrimination superiority of MISTIC was not biased by the presence of three missense variants in HGMD* or ClinVar* (p.Y35H, p.R99H and p.K127E), since the positive training sets used for MISTIC did not include these variants or any other variants of our cohort.**
MISTIC discriminates pathogenic from benign variants with better accuracy than CADD, M-CAP, BayesDel, REVEL or Varity. Potential splicing alteration of the cohort variants were investigated with spliceAI, which predicted no impact.\textsuperscript{10}

B) RNA results for NM_001658.4(ARF1):c.384+1G>T. Above panel showing SpliceAI-visual predictions.\textsuperscript{11} Above: predictions for the wild-type sequence, middle: predictions for the c.384+1G>T variant, below: close-up of the predicted amino-acid sequence inserted after Gln128. The variant is highlighted in red. Below panel showing ARF1 cDNA PCR and Sanger analysis.
Supp. Fig. 2

Stabilization of the Lys127 sidechain by Asp93. The second phosphate of GDP is interacting with the Ala26-Asp27 backbone, in close contact with Arg99.
Structure of ARF1 (grey cartoon) in its inactive GDP-bound conformation (1r8s)\(^\text{12}\), with 3 mutated residues in yellow. Dashed blue lines: H-bonds; dashed yellow lines: ionic bonds; the red ball marked by a white asterisk represents H\(_2\)O molecule.
Sup. Fig. 3

GEF uses Phe51 hydrophobic ring to ‘pinch off’ ARF1 switch loop. This interaction is required for the GEF to push out the GDP during activation of ARF1. The Phe to Leu missense is likely to alter the strength of this interaction.

In blue and in grey: GEF, ARF1, respectively, according to the structure 1s9d (right) or to the modelled PheS1Leu by Swiss-Model using 1s9d as template (left). Grey dashed lines: hydrophobic interactions.

Supp. Fig. 4

Molecular hypothesis for recurrence of chr1(GRCh38):g.228097627G>A p.(Arg99His).

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<tr>
<th>Strand</th>
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<td>GCGTG</td>
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<td>GCATG</td>
</tr>
<tr>
<td>- (3’ &lt;= 5’)</td>
<td>GCAC</td>
<td>GCAC</td>
<td>GTAC</td>
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The nucleotidic context of the recurrent substitution is compatible with a spontaneous deamination of a methylated cytosine, known to be in high proportion among de novo substitutions. The methylated cytosine (“m”, in red: methyl radical), spontaneously deaminates in thymine (red “T”), followed by mismatch repair. NB: in this figure, only the incorrect mismatch repair causing the missense is shown (strand +, G>A), the correct mismatch repair (strand −, T>C) is not depicted.
Supp. Fig 5. Brain MRI images

A) Individual #1, 2 years, axial T1-weighted MRI section showing periventricular nodular heterotopia.
B) Individual #9, 12 years old. Sagittal T1-weighted section showing relative microcephaly, cerebral atrophy, partial hypoplasia of corpus callosum (especially posterior), and cerebellar vermis hypoplasia. Axial T2-weighted sections showing bilateral enlargement of parietal subarachnoid spaces.
C) Individual #11, 2 years old. Sagittal T1-weighted section showing thin aspect of the corpus callosum (more pronounced at the splenium), relative microcephaly and cerebellar vermis hypoplasia.
D) Individual #13, age unknown, T2-weighted sections showing PNH (red arrows).

Supplemental data references


