

**Supplemental Figure 1. Minor allele frequencies (MAF) plotted against the CADD score of missense and Loss of function (LoF) variants in POLR3F.** All missense and LoF variants in POLR3F from the GnomAD database were queried for their CADD score. Subsequently, the MAF for each variant were plotted against the CADD score, with each point representing one variant, colored according to the annotation for the variant as indicated.The variant observed in the twins is highlighted in bold with the consequence of their mutation: p.R50W. CADD, common annotation dependent depletion; MAF, minor allele frequency.

Rare alleles with a MAF<0.01 in ExAC and GnomAD, and with a CADD score higher than predicted Mutational Significance Cut-off for POLR3F (13) of 99% confidence were considered. Of these, five are homozygous missense variants with a CADD score between 16 and 24, i.e. above the MSC cutoff. One of these variants is reported mostly in the latino population, and the others in the “other” and South asian population. The MAF for these variants are ~5.5 x 10-3 (the variant observed in the latino population), whereas the others are lower than 8.5 x 10-5. The high frequency variant is present only within the Ashkenazi Jewish population. Altogether, the global frequency of stop gained mutations is < 10-4. Further population genetic analyses, based on study of POLR3F variations from the GnomAD database, demonstrate a very low frequency of non-synonymous and loss-of-function mutations with a CADD score >12, and a global minor allele frequency (MAF) < 10-4 for nonsense mutations. Moreover, when performing natural selection assessment based on mouse and human gene homologous comparison, the POLR3F gene appears to be under purifying selection as reflected by a dN/dS score of 0.0172 (below the threshold of 1). Finally, the gene damage index is 61.74 with a selective pressure neutrality index of 0.1264, predicting moderate purifying selection (Figure 1A and supplementary Figure 1).