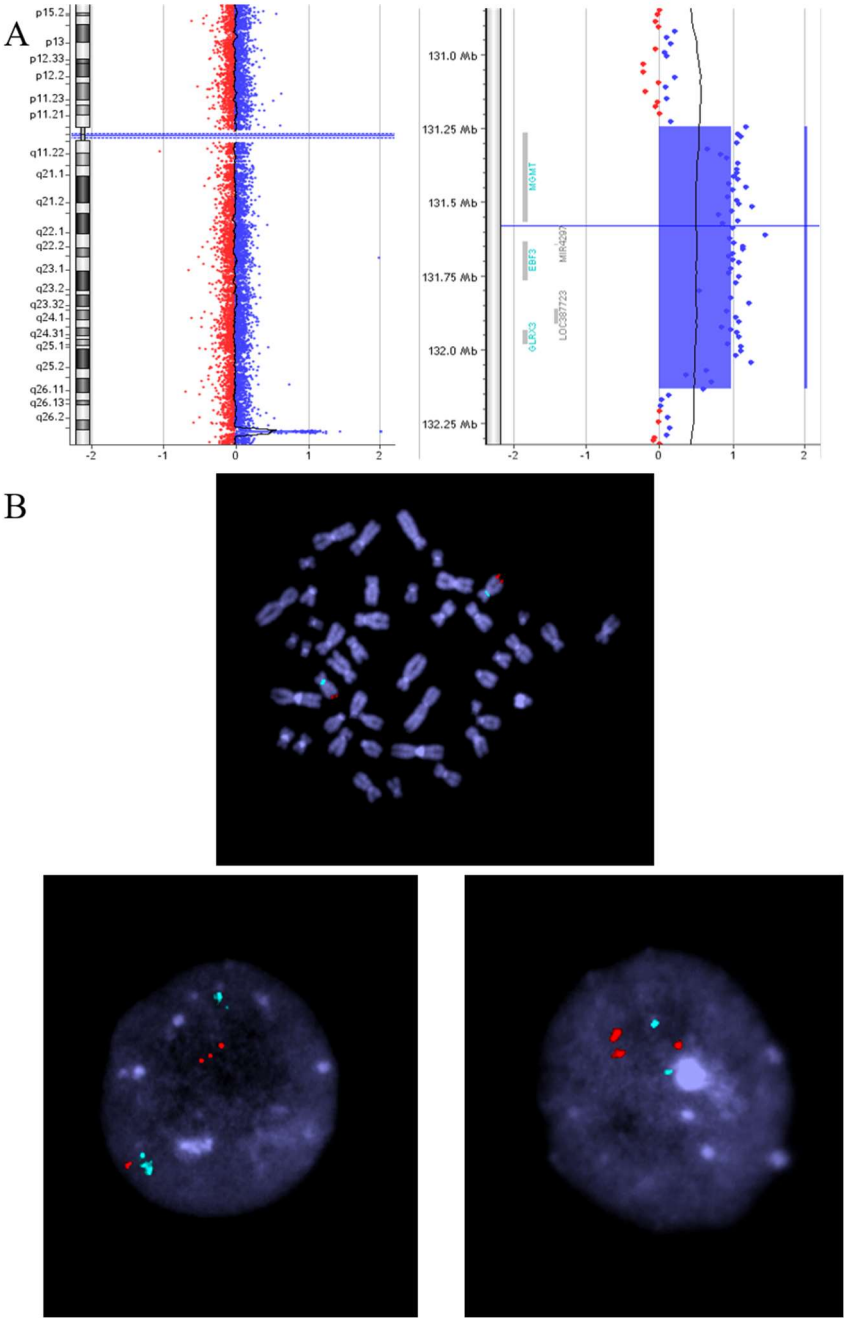
Supplementary Methods

Supplementary Figure 1: P11 duplication/triplication mosaicism identification and analysis



A) Array comparative genomic hybridization (array-CGH) was performed using the Agilent Human Genome CGH Microarray Kit 180K (Agilent Technologies Inc., Santa Clara, CA, USA). Data analysis was performed using Agilent Cytogenomics version 4.0.3.12. Array-CGH indicated a duplication or possible triplication: NC_000010.10:g.(131225843_131243689)_(132131959_132151948)dup/[3]. All nucleotide positions refer to the Human Genome Feb 2009 Assembly (GRCh37/hg19).

B) Fluorescence *in situ* hybridization (FISH) analysis of patient lymphocytes revealed a tandem duplication/triplication mosaicism. 60/100 of the cells had a triplication and 40/100 of the cells a duplication.

Red signal: BAC FISH probe RP11-343L20 (10q26.3, 200 kb, Empire Genomics, Buffalo, NY, USA)

Aqua signal: control probe CEP 10 that recognizes the chromosome 10 centromere area (Vysis Abbott, Abbott Park, IL, USA).

Somatic mosaicism in the mother of P8

Using droplet digital polymerase chain reaction (ddPCR) the mother of P8 was confirmed to be a mosaic carrier of the NM_001005463.2:c.1183C>T variant.

Targeted wild-type and mutation probes for the *EBF3* variant were designed and pre-validated by Bio-Rad (www.biorad.com, Bio-Rad, Hercules, CA, USA) and 2 µl of the extracted DNA was used for each duplicate reaction. The QX200 Droplet Generator partitioned the samples (20 µl into 20,000 droplets) for PCR amplification. Following amplification using a thermal cycler, droplets from each sample were analyzed individually on the QX200 Droplet Reader, where PCR-positive and PCR-negative droplets were counted to provide absolute quantification of the target DNA in digital form. The results were analyzed with the QuantaSoft Analysis Pro Software (v.1.0, Bio-Rad, Hercules, CA, USA).

In the clinically unaffected mother of P8, the fractional abundance of the NM_001005463.2:c.1183C>T variant was 22%, 22% and 33% in leukocytes, buccal cells and urine cells, respectively, implying mosaicism for the variant. In P8, the fractional abundance of the NM_001005463.2:c.1183C>T variant was 50%, 50% and 51% in leukocytes, buccal cells and urine cells, respectively, in line with her being heterozygous for the variant.

Neuropsychological assessments used in evaluations

Age 2 years, The Bayley Scales of Infant Development, Third Edition

Age 3-6 years, The Wechsler Preschool and Primary Scale of Intelligence – Revised or Third Edition (edition unknown n=2)

Age 7-15 years, The Wechsler Intelligence Scale for Children – Revised, Third or Fourth Edition

Age 17-31 years, The Wechsler Adult Intelligence Scale – Third or Fourth Edition

Used assessment unknown: n = 1

Finnish norms and versions were used for all the scales.