Supplementary Methods

Whole exome sequencing and identification of *ACTL6A* variants

The research WES study and transfer of clinical exome data were approved by the human subject ethics committees at Baylor College of Medicine and Radboud University Medical Center. The families provided informed consent prior to participation. Subject 1 had research WES, which was performed with capture reagents developed at the Baylor College of Medicine Human Genome Sequencing Center (BCM-HGSC), as previously described (Campeau, et al., 2012). Briefly, exome was captured on Nimblegen's Baylor VCRome library (Roche NimbleGen) and sequencing was performed on the Illumina HiSeq 2000 platform (Illumina). Sequence reads were aligned to the hg19 iteration of the reference human genome. Variant annotation and analysis was performed as previously described (Campeau, et al., 2012). Subject 2 had clinical WES at Baylor Genetics. Library construction, exome capture using VCRome version 2.1 (Roche NimbleGen, Madison WI) (Bainbridge, et al., 2011), HiSeq next-generation sequencing, and data processing were performed as previously described (Yang, et al., 2013). Subject 3 had clinical WES at Nijmegen as previously described (Lelieveld, et al., 2016). Since the clinical analysis did not reveal a pathogenic variant in any known disease-causing gene, the families consented for transfer of data to a research study. GeneMatcher tool (https://genematcher.org/) (Sobreira, et al., 2015) assisted in the recruitment of subject 3. The pathogenicity of candidate variants was predicted based on conservation, and comparison to available databases, including the Exome Aggregation Consortium database (ExAC, Cambridge, MA, http://exac.broadinstitute.org/) (Lek, et al., 2016) and the 1000 Genomes database (http://browser.1000genomes.org/) (Auton, et al., 2015). WES findings were validated by PCR and Sanger sequencing. The primers were designed with the primer3 design software (http://bioinfo.ut.ee/primer3/), and will be provided upon request.

RNA study in Subject 3 lymphoblasts

RNA was isolated using Trizol reagent (Direct-zol, Zymo Research). The Superscript III RT system (Invitrogen) was used to synthesize cDNA from total RNA according to the manufacturer's protocol. RT-PCR was performed using gene-specific primers to amplify the full length of the *ACTL6A* transcript. RT-PCR products were purified (QIAquick PCR purification kit, Qiagen) and cloned into pGEM T Easy vector (Promega). DNA was isolated from bacterial colonies (QIAprep Spin Mini Prep kit, Qiagen) and submitted to Genewiz (South Plainfield, NJ) for Sanger sequencing.

Co-immunoprecipitation of ACTL6A and BRG1 in Subject 3 lymphoblasts

EBV-transformed Lymphoblastoid cell pellets were lysed in RIPA buffer (50mM Tris pH 7.5, 150 mM NaCl, 0.5% NP-40 and 0.05% SDS), containing protease and phosphatase inhibitors (GenDEPOT). Whole cell lysates were incubated overnight with Dynabeads Protein G (ThermoFisher Scientific), and ACTL6A antibody (Abcam). Normal rabbit IgG served as control (Santa Cruz Biotechnology). Immunoprecipitated products were washed in PBS, re-suspended in SDS-PAGE loading buffer and separated on 4-15% SDS-PAGE protein gel (Bio-Rad). Western blots were labeled with either BRG1, ACTL6A antibodies (Abcam) or GAPDH antibody (Sigma-Aldrich). Intensity of signal was quantified using ImageJ program (https://imagej.nih.gov/ij/index.html).

Cell cycle analysis in Subject 3 lymphoblasts

EBV-transformed Lymphoblastoid cell pellets were fixed in 70% Ethanol and incubated with Propidium Iodide/Rnase staining solution (FxCycle™, ThermoFisher Scientific). Cell cycle was

analyzed using Attune flow cytometer at the Baylor College of Medicine Cytometry and Cell Sorting Core.

References

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