**Table e-2:** In silico analysis of the *NDUFS4* mutation identified in the proband.

1. **Occurrence of the *NDUFS4* variant c.369C>A in exome, genome and clinical databases (update 2017-08-21).**

|  |  |  |
| --- | --- | --- |
| Databases | **Results** | **Link** |
| Exac | Unknown variant | <http://exac.broadinstitute.org/gene/ENSG00000164258> |
| Kaviar | Unknown variant | <http://db.systemsbiology.net/kaviar/cgi-pub/Kaviar.pl> |
| 1000 genomes | Unknown variant | <http://phase3browser.1000genomes.org/Homo_sapiens/Gene/Variation_Gene/Table?db=core;g=ENSG00000164258;r=5:52856463-52979168> |
| EVS | Unknown variant | <http://evs.gs.washington.edu/EVS/> |
| VARSOME | Unknown variant | <https://varsome.com/variant/hg19/chr5%3A52954399%3A1%3AA> |
| GnomAD | Unknown variant | <http://gnomad.broadinstitute.org/variant/5-52954399-C-A> |
| MARRVEL | Unknown variant | http://marrvel.org/search/pair/NDUFS4/5:52954399%20C%3EA |
| CG46 | Unknown variant | http://wannovar.wglab.org/index.php |
| Decipher | Unknown variant | https://decipher.sanger.ac.uk/browser#q/chr5:52954399/location/5:52954351-52954413 |

1. **Prediction tools used to evaluate the pathogenicity of the *NDUFS4* variant c.369C>A**

|  |  |  |
| --- | --- | --- |
| Prediction Tools | **Interpretation** | **Link** |
| CADD | deleterious | <http://cadd.gs.washington.edu/home> |
| LRT | deleterious | <http://wannovar.wglab.org/index.php> |
| Mutation assessor | possibly damaging | <http://mutationassessor.org/r3/> |
| Mutation taster | disease causing | <http://www.mutationtaster.org/> |
| Panther | probably damaging | <http://pantherdb.org/tools/csnpScoreForm.jsp> |
| PhD-SNP | disease causing | <http://snps.biofold.org/phd-snp/phd-snp.html> |
| Polyphen-2 (v2.2.2r398) | damaging | <http://genetics.bwh.harvard.edu/pph2/> |
| Provean | deleterious | <http://provean.jcvi.org/seq_submit.php> |
| Sift | damaging | <http://sift.jcvi.org/www/SIFT_enst_submit.html> |
| SNAP2 | deleterious | <https://rostlab.org/services/snap/> |
| UMDpredictor | pathogenic | <http://umd-predictor.eu/checkident.php> |

1. **Classification of the *NDUFS4* gene variant c.369C>A according to ACMG standards and guidelines (Richards, Aziz et al. 2015)**

|  |  |  |
| --- | --- | --- |
| **ACMG criteria** | | **Evidence for the NDUFS4 variant c.369C>A** |
| **PS3** | **Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product.** | Complex I enzyme deficiency in muscle and fibroblast cells.  Complex I assembly defect on BN-Page. |
| **PM2** | **Absent from controls in ESP, EXAC or 1000 genome project.** | Cf. Table e-2A |
| **PP3** | **Multiples lines of computational evidence support a deleterious effect on the gene or the gene product.** | Cf. Table e-2B |
| **PP4** | **Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology.** | Consanguinity and homozygous mutation  Mild dystonia phenotype and complex I deficiency with a homozygous variant in the *NDUFS4* gene previously associated with complex I deficiency (OMIM : 252010) |
| **Conclusion: According to the rules for combining criteria detailed in the “ACMG standards and guidelines”, the *NDUFS4* gene c.369A>G (p.Asn123Lys) is classified as likely pathogenic.** | | |

Richards, S., N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W. W. Grody, M. Hegde, E. Lyon, E. Spector, K. Voelkerding, H. L. Rehm and A. L. Q. A. Committee (2015). "Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology." Genet Med **17**(5): 405-424.