Title: A Novel Nonsense Mutation at the NSUN2 Gene Causes Dubowitz Syndrome in Two Members of a North-West Iranian Family

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To appear in: **Basic and Clinical Neuroscience**

Received date: 2023/08/15

Revised date: 2024/10/19

Accepted date: 2024/11/11

Please cite this article as:

Akhavan, H., Ahmadabadi, F., Hosseini-Asl, S.E., Hosseini-Asl, S.S. (In Press). A Novel Nonsense Mutation at the NSUN2 Gene Causes Dubowitz Syndrome in Two Members of a North-West Iranian Family. Basic and Clinical Neuroscience. Just Accepted publication Jul. 10, 2024. Doi: http://dx.doi.org/10.32598/bcn.2024.5782.2

DOI: http://dx.doi.org/10.32598/bcn.2024.5782.2

Abstract

Background: Growth retardation, distinctive facial dysmorphism, and intellectual disability are hallmark features of Dubowitz syndrome. Pathogenic variants at the NSUN2 and LIG4 genes are related to this syndrome. Case presentation: A male patient, 8 years old, with a clinical diagnosis of Dubowitz syndrome, was referred to the Homa Medical Genetic Laboratory. WES analysis determined a novel nonsense mutation NSUN2(NM_01755.6):c.346C>T(p.Gln116Ter) has been detected as a homozygous genotype. Sanger-based PCR-Sequencing confirmed the finding. Segregation analysis revealed heterozygosity in his unaffected parents and sister, as well as homozygosity for the mutant allele in his mother's uncle with the same phenotype. Conclusions: About 32 loss-of-function variants were reported to be responsible for Dubowitz syndrome. Being a nonsense variant leading to a truncated protein, not found in the genomic database, and finally, this variant should be categorized as pathogenic based on PVS1, PP4, PP1, and PM2, according to the segregation analysis results.

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Background

In 1965, Dubowitz documented four cases of malformations, which included growth retardation, short stature, and intellectual disability (1). Dubowitz syndrome is an autosomal recessive intellectual and developmental genetic disorder characterized by growth and/or mental retardation, facial asymmetry, hyperactivity, short stature, microcephaly, eczema, and learning problems. The pathogenic variants of the NSUN2 and LIG4 genes are known to be responsible for this disorder (2, 3). The NSUN2 gene, also known as NOP2/Sun RNA Methyltransferase 2, is located in the 5p15.31 region and is expressed in brain tissue (4). This enzyme methylated the tRNA precursors' cytosine to 5-methylcytosine (5). Catalyzing the C(5)-methylation of some mRNAs helps stabilize them by preventing mRNA decay (6). In this report a novel pathogenic variant of the NSUN2 gene has been detected in an affected Iranian child with Dubowitz syndrome symptoms. The Ardabil University of Medical Sciences has granted approval for ethical and regulatory issues related to the collection of human specimens for research purposes (Approval ID: IR.ARUMS.MEDICINE.REC.1401.142).

Case presentation

The Homa Medical Genetics Laboratory in Ardabil, Iran received a referral for an 8-year-old boy with mental retardation. Clinical examination indicated intellectual disability, hyperactivity, learning difficulties, a small face, hypertelorism, and short stature. The observed clinical symptoms were suggestive of Dubowitz syndrome. He was the result of a consanguineous marriage. His parents and sister were healthy, but his mother's uncle also had the same disease. The affected relative's parents were also consanguineous (see Figure 1).

Methods

Whole exome sequencing (WES) followed by Sanger-based PCR-Sequencing was used for the genetic analysis. After genomic DNA isolation and quantification by a filter-based methodology, Using the Agilent SureSelect Human All Exon V7 kit (Agilent Technologies, CA, USA), sequence libraries were created and then fed into the Novaseq 6000 Illumina Sequencer. A Unix-based operating system was utilized to conduct data quality control, analysis, and interpretation on the G9 generation of HP servers. The average read depth was 100X, and more than 98% of the targeted genomic sequence had a depth of 20X or greater. In addition to the two genes

responsible for Dubowitz syndrome, two panels associated with intellectual disability (containing 563 genes), and short stature (153 genes) were selected for analysis of the WES results (Table 1).

The candidate variant identified through WES has been confirmed by Sanger sequencing using specific primers 5'- GCTCAAACAGTGTGGATTGCTT-3' and 5'- TCGCATGAAAACTTGCGTAGC-3' (Product size: 470 bp; Tm=60°C). The primers were designed by Primer3 software for amplifying the exon 3 of the NSUN2 gene. In order to accurately validate the results, an ABI 3130xl Genetic Analyzer was used to perform Sanger sequencing. Co-segregation analysis was applied to verify the zygosity status of the mutation in the participants.

Outcome

Among the variants, only the likely pathogenic variant NSUN2(NM_017755.6):c.346C>T(p.Gln116Ter) has been considered. After confirming that the proband was homozygous for the mutant allele, segregation analysis was conducted in both his normal and affected relatives. The participants were including the parents, an unaffected sister, as well as his mother's uncle with the same symptoms of abnormality (Figure 1). The heterozygosity of the normal individuals and homozygosity of the uncle for the mutant allele was determined (Figure 2).

Conclusions

Some previous investigations have reported an association between pathogenic variants in the NSUN2 gene and Dubowitz syndrome (8-10). Recently, a 7-year-old girl from Zahedan, Iran, who exhibits a moderate intellectual disability, ptosis, an elongated face, and short stature, has been reported to be homozygous for the mutation c.593T>G in the NSUN2 gene (11). In addition, the cause of Dubowitz syndrome was identified as nonsense mutations (c.679C>T and c.1114C>T) and a splicing mutation in three Iranian families (8).

The variant c.346C>T at the NSUN2 gene has not been previously reported for its pathogenicity. Because of creating a premature translation stop signal in the NSUN2 gene (767 amino acids in the normal product are replaced by 116), it is expected to result in a disrupted protein product. The disease is known to be caused by mutations that result in loss-of-function (LOF) at the

NSUN2 gene (12). There were about 32 pathogenic variants at the NSUN2 gene (13). Computational-based in silico analysis containing BayesDel_addAF, BayesDel_noAF, EIGEN, and EIGEN PC predicts this variant as deleterious. Also, its CADD score (44) is above the score of 20, so, it could be mentioned as a deleterious variant. This variant has been introduced as likely pathogenic by Varsome and Franklin with the criteria PVS1, and PM2 (14, 15). In accordance with the guidelines of the American College of Medical Genetics and Genomics (ACMG), with the criteria PVS1 for being a nonsense mutation, PP4 for detecting as homozygous genotype in two members of a family with the clinical diagnosis of Dubowitz syndrome, PP1 for not detecting in the unaffected family members, and finally PM2 for not reporting in the genomic database, we classify this variant as pathogenic.

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Table 1. The list of the genes responsible for Dubowitz syndrome, Intellectual disability, and Short stature (based on the Fulgent (7))

Panel	Gene list
Dubowitz syndrome	NSUN2, LIG4 (2 genes)
Intellectual disability	ABCD1, ACAT1, ACOX1, ACSL4, ACTB, ACTG1, ACY1, ADAR, ADNP, ADSL, AFF2, AGTR2, AHDC1, AHI1, AIFM1, ALDH18A1, ALDH4A1, ALDH5A1, ALG1, ALG11, ALG12, ALG13, ALG2, ALG3, ALG6, ALX4, AMER1, ANK3, ANKRD11, AP1S1, AP1S2, AP3B1, AP4B1, AP4E1, AP4M1, AP4S1, AR, ARG1, ARHGEF6, ARHGEF9, ARID1A, ARID1B, ARX, ASAH1, ASPM, ASS1, ASXL1, ASXL3, ATL1, ATP10A, ATP13A2, ATP1A2, ATP6AP2, ATP7A, ATRX, AUH, AUTS2, AVPR1A, AVPR2, BBIP1, BBS1, BBS10, BBS12, BBS2, BBS4, BBS7, BBS9, BCL11A, BCOR, BCS1L, BDNF, BIN1, BRAF, BRSK2, BRWD3, BUB1B, CA2, CACNA1A, CACNA1C, CACNG2, CAMTA1, CANT1, CASK, CBS, CC2D1A, CC2D2A, CCDC22, CCDC88C, CDC42, CDH15, CDK13, CDK16, CDKL5, CDKN1C, CEP290, CEP41, CEP57, CHAMP1, CHD2, CHD7, CHD8, CHRNA4, CIC, CLCN4, CLIC2, CLN3, CLN5, CLN6, CLN8, CNKSR2, CNTNAP2, CNTNAP5, COG5, COG7, COG8, COL4A3BP, CP, CPA6, CPS1, CRADD, CRBN, CREBBP, CSNK2A1, CTC1, CTCF, CTNNB1, CTNND2, CTSA, CTSD, CTSF, CUL4B, CYB5R3, CYP27A1, D2HGDH, DARS2, DBT, DCX, DDX3X, DHCR24, DHCR7, DKC1, DLG3, DLGAP2, DMD, DNM1, DNMT3A, DOCK4, DPP10, DPP6, DPYD, DYNC1H1, DYRK1A, EBP, EFNB1, EFTUD2, EHMT1, EIF2S3, ELOVL4, EP300, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6, ERCC8, EZH2, FAAH2, FAM126A, FANCB, FANCG, FBN1, FBXO11, FGD1, FGF14, FGFR1, FGFR2, FGFR3, FKRP, FKTN, FLNA, FMR1, FOLR1, FOXG1, FOXP1, FOXP2, FRMPD4, FTO, FTSJ1, G6PC3, GABRB3, GABRG2, GALE, GAMT, GAN, GATAD2B, GBA, GBE1, GCK, GDI1, GFAP, GFM1, GHR, GK, GLI3, GLRA1, GLUL, GLYCTK, GM2A, GNAO1, GNAS, GNPAT, GNPTAB, GNPTG, GPC3, GRIA3, GRIK2, GRIN1, GRIN2A, GRIN2B, GRM1, GRN, GSPT2, GSS, GUSB, HAX1, HCCS, HCFC1, HDAC4, HDAC8, HECW2, HEPACAM, HEXB, HOXA1, HPD, HPRT1, HRAS, HSD17B10, HSPD1, HUWE1, IDS, IGBP1, IGF1, IGF1R, IL1RAPL1, IMMP2L, IMPA1, INSR, IQSEC2, IRX5, ITGA7, ITPR1, KAT6A, KAT6B, KATNAL2, KCNB1, KCNJ10, KCNJ11, KCNK9, KCNQ2, KCTD13, KCTD7, KDM5B, KDM5C, KDM6A, KIF11, KIF1A, KIF1BP, KIF21A, KIF5A, KIF7, KIRREL3, KLF8, KMT2A, KMT2D, KMT5B, KRAS, L1CAM, LAMA2, LAMC3, LAMP2, LARGE1, LAS1L, LHX3, LIG4, LMBRD1, LYST, MACF1, MAGEL2, MAGT1, MAN1B1, MAN2B1, MANBA, MAOA, MAPK8IP3, MAT1A, MBD5, MBTPS2, MCCC1, MCCC2, MCOLN1, MCPH1, MECP2, MED12, MED13L, MED17, MED23, MEF2C, MFSD8, MGAT2, MID1, MKKS, MMADHC, MOCS1, MOCS2, MRAP, MTFMT, MTHFR, MTOR, MTR, MYCN, MYO5A, MYT1L, NAA10, NAGA, NALCN, NBN, NDP, NDUFA1, NDUFAF5, NDUFS1, NEGR1, NEXMIF, NF1, NGF, NGLY1, NHEJ1, NHP2, NHS, NIPBL, NLGN3, NLGN4X, NPC1, NPC2, NPHP3, NR2F1, NRXN1, NSD1, NSDHL, NSUN2, NTNG1, OCRL, OFD1, OGT, OPHN1, ORC1, OTC, PACS1, PAFAH1B1, PAH, PAK3, PAX6, PCDH19, PCDH9, PCGF2, PCNT, PDE4D, PDHA1, PDHX, PDSS1, PEX7, PGK1, PHF6, PHF8, PHKA2, PHKG2, PIGA, PIGL, PIGO, PIGV, PIP5K1B, PLA2G6, PLP1, PMM2, PNKP, POGZ, POMGNT1, POMT1, POMT2, PON3, PORCN, POU1F1, PPOX, PPP1CB, PPP2R1A, PPP2R5D, PPT1, PQBP1, PRKAR1A, PRMT7, PRPS1, PRSS12, PTCHD1, PTEN, PTPN11, PURA, PYCR1, PYGL, RAB39B, RAI1, RAPSN, RBBP8, RBFOX1, RBM10, RELN, RIT1, RORA, RPGRIP1L, RPL10, RPS6KA3, SACS, SAMHD1, SATB2, SCN1A, SCN2A, SCN8A, SDCCAG8, SETBP1, SETD5, SGSH, SHANK2, SHANK3, SHOC2, SHROOM4, SIL1, SKI, SLC16A2, SLC20A2, SLC25A12, SLC25A13, SLC25A15, SLC2A1, SLC2A2, SLC35A2, SLC35C1, SLC46A1, SLC4A4, SLC5A5, SLC6A1, SLC6A4, SLC6A8, SLC7A7, SLC9A6, SLC9A9, SLX4, SMARCA2, SMARCA4, SMARCB1, SMC1A, SMG6, SMS, SNAP25, SNIP1, SNRPN, SOBP, SOS1, SOS2, SOX10, SOX2, SOX3, SOX5, SPAST, SPATA5, SPR, SPRED1, SPTAN1, SRD5A3, SRPX2, ST3GAL3, ST7, STAT5B, STK3, STRA6, STX11, STXBP1, SUCLG1, SYN1, SYNGAP1, SYP, SYT14, TAF1, TAF6, TBC1D24, TBCE, TBL1XR1, TBR1, TBX1, TCF4, TECR, TET3, TGIF1, TH, THOC2, THRB, TIMM8A, TINF2, TMCO1, TMEM165, TMEM216, TMEM231, TMEM67, TMEM70, TPH2, TPK1, TPP1, TRAPPC9, TRHR, TRIO, TRIP12, TSC1, TSC2, TSHR, TSPAN7, TTC37, TTC8, TUBA1A, TUBA8, TUBB2B, TUBB3, TUSC3, TWIST1, UBE2A, UBE3A, UNC80, UPB1, UPF3B, UROC1, USP9X, VLDLR, VPS13B, WAC, WDR13, WDR45, WDR62, WDR73, WDR81, XPNPEP3, ZBTB16, ZBTB24, ZC4H2, ZCCHC12, ZDHHC9, ZEB2, ZFP57, ZFYVE26, ZIC2, ZMYM3, ZNF41, ZNF507, ZNF674, ZNF711, ZNF804A, ZNF81, ZNHIT6 (563 genes)
Short stature	ACAN, ACTB, ACTG1, ALMS1, AMMECR1, ANKRD11, ARCN1, ARID1A, ARID1B, ATR, ATRIP, B3GAT3, BLM, BMP2, BRAF, BRF1, BTK, CBL, CCDC8, CDC45, CDC6, CDT1, CENPJ, CEP152, CEP63, COL10A1, COL11A1, COL11A2, COL1A1, COL27A1, COL2A1, COL9A1, COL9A2, COL9A3, COMP, CREBBP, CRIPT, CUL7, DHCR7, DNA2, DONSON, DVL1, EP300, ERCC6, ERCC8, EVC, EVC2, FANCA, FANCC, FANCG, FBN1, FGD1, FGFR3, FN1, GH1, GHR, GHRHR, GHSR, GLI2, GLI3, GNAS, HDAC8, HESX1, HRAS, HSPG2, IDUA, IGF1, IGF1R, IGF2, IGFALS, IHH, INSR, KDM6A, KMT2D, KRAS, LARP7, LFNG, LHX3, LHX4, LIG4, LMNA, LZTR1, MAP2K1, MAP2K2, MATN3, MRAS, NBN, NF1, NIPBL, NOTCH2, NPPC, NRAS, NSMCE2, OBSL1, ORC1, ORC4, ORC6, OSGEP, OTX2, PCNT, PDE4D, PIK3R1, PISD, PLK4, POC1A, POP1, POU1F1, PPP1CB, PPP3CA, PRKAR1A, PRMT7, PROP1, PTH1R, PTPN11, PUF60, RAD21, RAF1, RALA, RASA2, RBBP8, RIT1, RNU4ATAC, ROR2, RPS6KA3, RRAS, RTTN, SGMS2, SHOC2, SHOX, SMARCA2, SMARCA4, SMARCAL1, SMARCB1, SMARCE1, SMC1A, SMC3, SOS1, SOS2, SOX11, SOX2, SOX3, SOX9, SPRED1, SRCAP, STAT5B, TALDO1, TBX2, TBX3, TOP3A, TRIM37, TRMT10A, WNT5A, XRCC4 (153 genes)

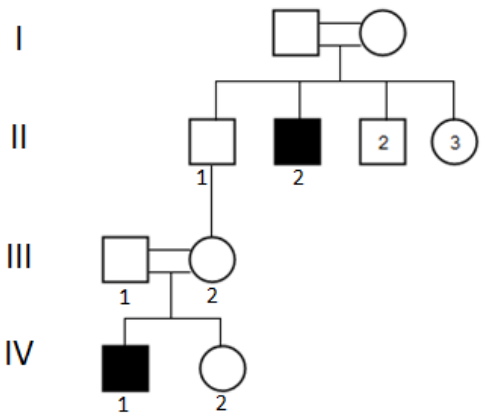

Figure 1. Pedigree of an Iranian family with two affected cases (II2, and IV1) with Dubowitz syndrome

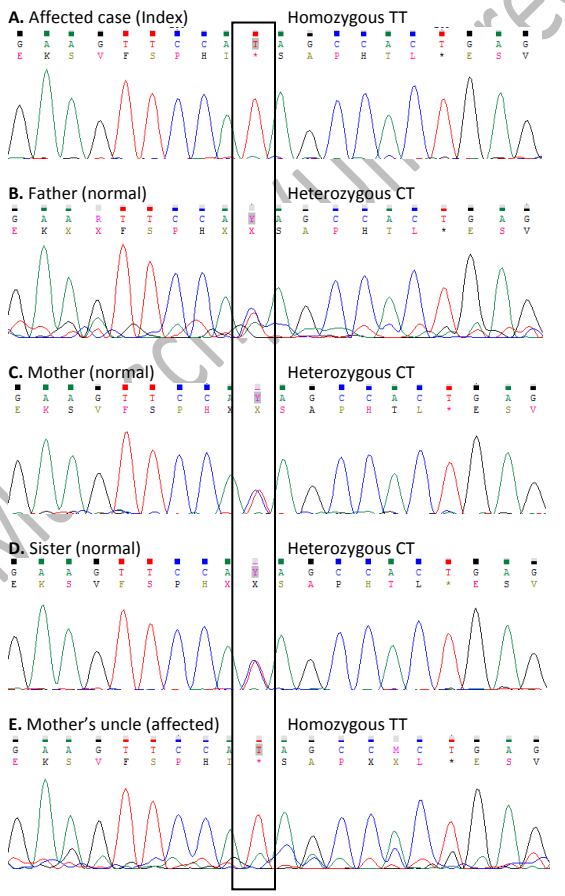

Figure 2. Genotypes of the affected and unaffected cases by Sanger-based PCR-Sequencing: Affected cases (II2, and IV1) have been detected to be homozygous for the mutant allele (T), and the unaffected relatives were heterozygous.