RAPID COMMUNICATION

A homozygous nonsense mutation in *DNAJC30* causes Leber's hereditary optic neuropathy with Leigh-like phenotypes

Nuclear encoded genes can cause early-onset mitochondria-related disorders such as Leigh or Leigh-like syndrome. Defects in *DNAJC30* have been implicated in mitochondria-related diseases such as Leber's hereditary optic neuropathy (LHON) and Williams syndrome (WS). However, the role of *DNAJC30* in disease progression concerning mitochondrial dysfunction has yet to be fully understood. Here we report a 12-year-old boy with acute dystonia onset at age 10. Brain magnetic resonance imaging (MRI) showed bilateral basal ganglion and thalamic hyperintensities concerning putaminal necrosis. He then developed bilateral optic atrophy and rapid progressive bilateral visual loss. Exome sequencing analysis of his peripheral blood sample revealed a homozygous nonsense germline variant, c.24G>A (p.W8X) in the *DNAJC30* gene, proven to result in a complete loss of DNAJC30 protein expression in cells. Remarkably, the same germline variant was then identified by whole genome sequencing in another unrelated patient, a 17-year-old male. He also showed acute bilateral optic atrophy, subacute central vision loss, and abnormal brain MRI. *In vitro* functional analysis further confirmed that *DNAJC30* deletion inhibited cell growth and induced mitochondrial disorders, evidenced by decreased oxygen consumption rate (OCR), reduced mitochondrial membrane potential ($\Delta\Psi$m), and increased cellular and mitochondrial reactive oxygen species (ROS), presumably due to decreased complex I enzyme activity. Collectively, a homozygous nonsense germline variant in *DNAJC30* (c.24G>A) was identified to cause mitochondria-related disorders. This rare *DNAJC30* pathogenic variant will be useful in their diagnosis/prognosis and highlights the significance of the roles of DNAJC30 in the maintenance of normal mitochondrial function and brain development.

A 12-year-old male of Guatemalan ancestry exhibited acute dystonia onset at age 10. At the initial evaluation of acute dystonia, the parent reported a change in his gait—he appeared to be limping while walking. He was also noted to have intermittent shaking of his arms, increased clumsiness, and a change in his handwriting. Brain MRI showed focal areas of low attenuation in the posterior putamen bilaterally, concerning putaminal necrosis. He then developed bilateral optic atrophy and rapidly progressive visual loss. In our examination, his speech had a mild slur and was slow and hypotonic while his hearing was grossly intact to voice. Laboratory studies showed elevated anti-strptolysin O titer (409; normal rang: < 99) and lactic acid level (30.2; normal range: 6.3—18.9). A panel testing concerning neuro-metabolic disorders was unable to reach a diagnosis. Developmentally, he was reported normal. He was born at full term to healthy, nonconsanguineous parents with no known contributing family history [see Supplementary Data (Individual 1) for detailed developmental history].

Trio exome sequencing and analysis of the patient and parents' peripheral blood samples revealed a homozygous nonsense germline variant, c.24G>A (p.W8X), in the *DNAJC30* gene (reference sequence: NM_032317.3) in the 12-year-old patient, while both parents were heterozygous carriers (Fig. S1). This variant occurs at the N-terminal of DNAJC30 and is predicted to result in a truncated protein of only 8 amino acids in length, likely leading to complete loss of DNAJC30 function. So far, this nonsense variant has not been reported either as a benign or disease-causing variant in human individuals. This particular sequence change has only been detected in Latino/Admixed American population and observed in 9 out of the total 243978 alleles without homozygotes in the gnomAD database (https://gnomad.broadinstitute.org/).

Very recently, we recorded another unrelated patient transferred to our hospital who was a 17-year-old male with the same homozygous nonsense *DNAJC30* variant (c.24G>A,

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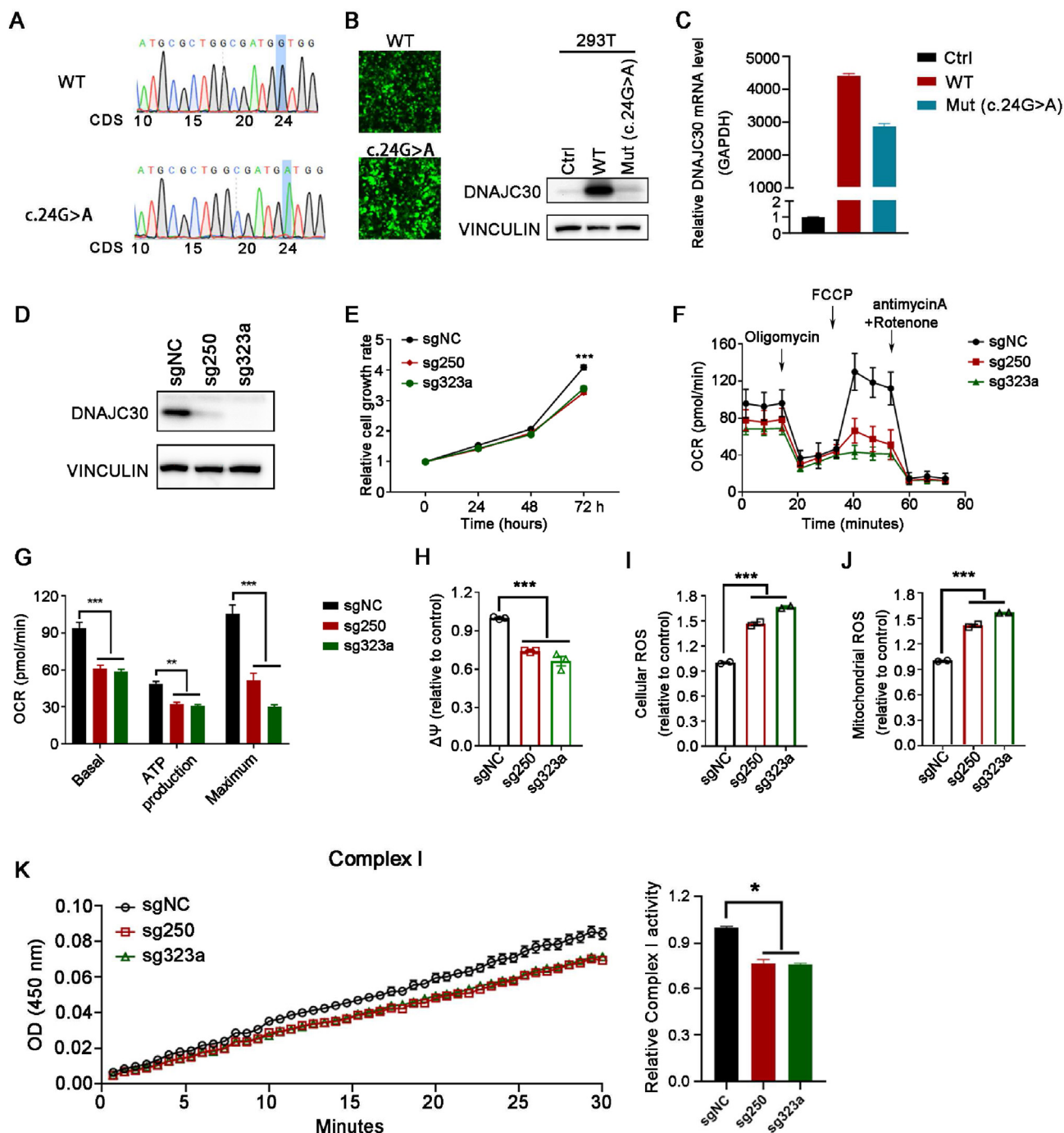
Figure 1 c.24G>A mutation blocks DNAJC30 protein expression in cells, and DNAJC30 deficiency inhibits cell growth and affects mitochondrial functions. **(A)** Sanger sequencing confirms the c.24G>A mutation in *DNAJC30* mutant expressing plasmids. **(B, C)** 293 T cells were transfected with empty vector (Ctrl), wild-type (WT) or mutant (Mut (c.24G>A)) DNAJC30 plasmids. Transfection efficiency was confirmed by GFP percentage (B, left); DNAJC30 protein and mRNA levels were detected by Western blot (B, right) and RT-qPCR (C). **(D)** CRISPR-cas9 expressing 293 T cells were transduced with two sgRNAs targeting *DNAJC30*. *DNAJC30* deletion efficiency was confirmed by immunoblotting. **(E)** Cell growth was monitored in *DNAJC30*-deleted cells compared to the control group. **(F–J)** Effects of *DNAJC30* deletion on mitochondrial functions in cells were evaluated by measuring the oxygen consumption rate (OCR) on the Seahorse XFe Extracellular Flux Analyzers (F–G), mitochondrial membrane potential (ΔΨm) by TMRE staining (200 nM) (H), relative cellular ROS level by Carboxy-H2DCFDA staining (10 μM) (I) and relative mitochondrial ROS level by MitoSox Red staining (5 μM) (J). **(K)** OXPHOS Complex I enzyme activity was directly detected in mitochondria isolated from *DNAJC30*-deleted and control cells by ELISA assays.

p.W8X) identified by whole genome sequencing. He also showed acute bilateral optic atrophy and subacute central vision loss with abnormal T2 hyperintensity within the right cerebral peduncle, dorsal midbrain in periaqueductal gray matter, and dorsal medulla [see Supplementary Data (Individual 2) for the detailed clinical course].

To verify the effect of this mutation on *DNAJC30* expression, we constructed plasmids expressing DNAJC30 wildtype (WT) coding sequences (CDS) and c.24G>A mutation containing CDS (c.24G>A), and confirmed the c.24G>A mutation site by Sanger sequencing (Fig. 1A). Consistent with the *in-silico* predictions, c.24G>A mutation failed to express DNAJC30 protein in 293 T cells (Fig. 1B, right panel), despite over two-thousand-fold transcript overexpression (Fig. 1C) and high transduction efficiency (Fig. 1B, left panel).

We established *DNAJC30*-deleted 293 T cells using CRISPR-cas9. After successful depletion of DNAJC30 protein expression (Fig. 1D), we first demonstrated that DNAJC30 is required for normal 293 T cell growth (Fig. 1E). Depletion of *DNAJC30* inhibited cell growth, presumably attributed to mitochondrial dysfunction due to the lack of functional DNAJC30 protein in the cells.

We then tested the effects of *DNAJC30* deletion on mitochondrial functions. Indeed, *DNAJC30* depletion caused a decrease in oxygen consumption rate concerning basal, ATP production, and maximum conditions (Fig. 1F, G), consistent with the previous report regarding the role of DNAJC30 in mitochondrial function demonstrated in neocortical neurons of *Dnajc30*−/− mice.[1] In addition, through specific mitochondria staining assays, we found that *DNAJC30* depletion decreased mitochondrial membrane potential (Fig. 1H; Fig. S2A) while increasing cellular and mitochondrial ROS levels (Fig. 1I, J; Fig. S2B, C). DNAJC30 was first reported to interact with the ATP-synthase machinery and facilitate ATP synthesis in neocortical neurons.[1] However, in a recent study of *DNAJC30*-related LHOH, pathogenic *DNAJC30* variants in the patients affected with LHON are reported to cause a complex I defect. Further proteomics data revealed that the mutations in *DNAJC30* resulted in impaired repair of specific subunits of mitochondrial complex I (Complex I (CI) N-module proteins (CI^HIGH)), which are supposed to rely on DNAJC30 for their disassembly and subsequent degradation.[2] Therefore, we next tested the enzyme activity of OXPHOS complexes derived from *DNAJC30*-deleted 293 T cells using a cell-free reaction system. Interestingly, we demonstrated that *DNAJC30* depletion specifically decreased enzyme activity of complex I but not complex IV or V (Fig. 1K; Fig. S2D, E), which is consistent with the results demonstrated by the proteomics data from a similar *DNAJC30*-deleted HEK cell experiment.[2] Lastly, we did not observe significant differences in OXPHOS complex protein levels in *DNAJC30*-deleted cells (Fig. S2F).

Overall, we identified a homozygous nonsense germline variant in *DNAJC30* as a previously unknown cause of mitochondria-related disorders. The consequence of this sequence change likely led to the complete loss of DNAJC30 function. This case highlights a rare cause of mitochondria-

related LHON with Leigh-like conditions with extended mutation spectrums and phenotypic presentations. To our knowledge, this is the first case reported to involve biallelic truncating mutations in gene *DNAJC30*.

Recently, three missense variants in the *DNAJC30* gene in the homozygous state (p.Y51C, p.P78S, and p.L101Q) were reported in individuals of Eastern European ancestry affected with LHON in a recessive inheritance.[2] Historically, LHON has been predominantly caused by specific point mutations in mitochondrial DNA with maternal inheritance. Interestingly, the patient cohort with *DNAJC30*-associated LHON (arLHON) demonstrated a similar phenotypic pattern of mitochondrial LHON, including adult-onset visual loss, incomplete penetrance, male predominance, and idebenone responsivity.[2] The missense variants identified in these affected individuals were shown to cause complex I repair defects without disruption of complex IV and V activities. In addition, these missense variants do not affect DNAJC30 expression at the RNA and protein levels compared to controls.[2] In contrast, our patients experienced markedly earlier onset of symptoms containing rapid progression of bilateral optic atrophy and severe motor difficulties (hand tremors, involuntary movements of arms, leg pains, and incoordination), consistent with mitochondria-related Leigh-like disease. The homozygous nonsense variant detected in our patient likely results in a complete loss of DNAJC30 function. To date, this nonsense variant has not been linked to any diseases. It has only been observed in less than 10 heterozygotes in Latino/Admixed American population in gnomAD, the largest general population database. Correspondingly, our *in vitro* functional assessment showed that c.24G>A mutation caused a complete loss of *DNAJC30* expression in cells, resulting in disadvantaged cell growth and a series of mitochondrial dysfunction, including decreased oxygen consumption rate, reduced mitochondrial membrane potential, and increased cellular ROS and mitochondrial ROS. Moreover, we evaluated complex I, IV and V enzyme activity in *DNAJC30*-deleted 293 T cells and demonstrated that *DNAJC30* depletion specifically affected complex I enzyme activity, consistent with the previous report about the defects caused by the missense *DNAJC30* variants identified in arLHON.[2] Of note, due to the limited access to the primary patient samples, 293 T cells were used as a cell model to evaluate the impact of *DNAJC30* deletion on cell growth and mitochondrial functions. Alternatives such as neuronal lineage-related cell lines and/or primary brain cell culture may be more desired for further understanding of the role of DNAJC30 in neurological function regression.

Interestingly, *DNAJC30* is also one of the genes deleted in the multisystem developmental disorder WS.[1,3] Homozygous deletion of *Dnajc30* in mice caused hypofunctional mitochondria, decreased neocortical pyramidal neurons, and altered behaviors that mimic WS.[1] Our patient carries a homozygous nonsense *DNAJC30* variant which is equivalent to homozygous deletion in terms of DNAJC30 functionality. Our patient has an abnormal brain MRI and clinical symptoms suggesting mitochondrial disorders. However, clinically, heterozygous carriers of both parents are healthy. In

addition, several heterozygous truncated variants in *DNAJC30* are observed in the general population database (e.g. gnomAD), in which the individuals are considered phenotypically normal in general. These observations suggest that the loss of one *DNAJC30* allele is not sufficient to cause disease in human individuals, consistent with the previous report that heterozygous *Dnajc30* deletion in mice does not cause significant morphological defects compared to WT mice.[1] Nonetheless, following the current understanding of the potential effects of *DNAJC30* defects on the mitochondrial functionalities, our patient has started on riboflavin 100 mg and coenzyme Q10 (CoQ10) 10 mg/kg orally a day to support the Mitochondrial Complex I and III functions. Further elucidation of the biological functions of DNAJC30 in human brain development will help to fully illuminate the course of *DNAJC30*-related disease and progress therapeutic considerations.

Author contributions

J.C. and M.S. conceived and designed the project. C.S., K.W., W.L., C.Z., J.C. and M.S. conducted experiments and/or data analysis. A.S., K.P. and M.S. provided case reports for the patients. J.C. partially sponsored the project. S.C., K.W., J.C. and M.S. wrote the manuscript and all the authors provided feedback.

Conflict of interests

The authors declare no competing interests.

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Appendix A. Supplementary data

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