

Multi-omics framework for diagnosing *NAXE* deficiency

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Background

- NAXE* encodes **NAD(P)HX epimerase**, which repairs damaged NADH/NADPH back to functional cofactors¹.
- This pathway sustains mitochondrial energy metabolism and prevents toxic NAD(P)HX accumulation^{1,2}.
- NAXE* deficiency** (MIM #617186) is an ultra-rare autosomal recessive disorder.
- Features: developmental delay, recurrent encephalomyopathic crises, seizures, ataxia, neurodegeneration².
- Onset usually in childhood, often triggered by fever/illness, with variable outcomes².

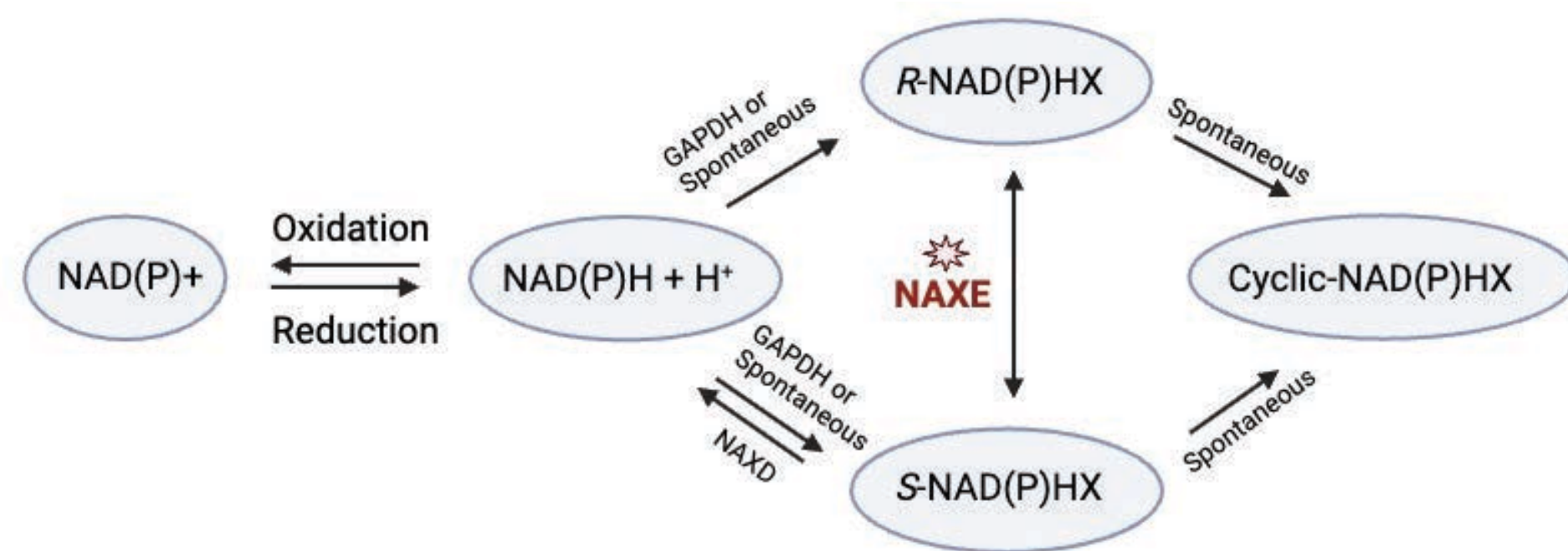
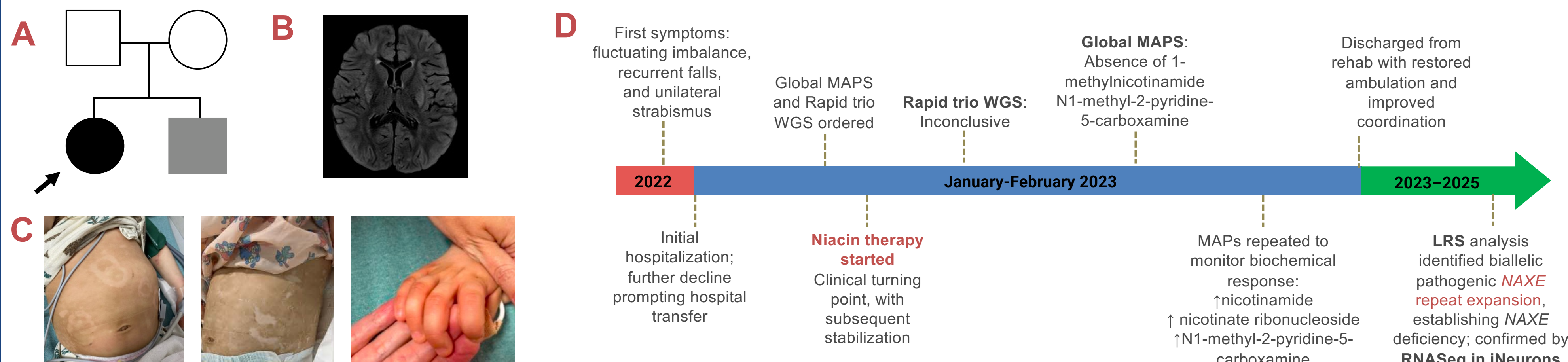


Figure 1: NAXE maintains NAD(P)H balance by repairing damaged derivatives (R- and S-NAD(P)HX) and prevents accumulation of toxic cyclic-NAD(P)HX. Loss of NAXE activity causes toxic NADHX accumulation and energy failure.

Clinical Presentation

- Case:** 3-year-old female presented with ataxia, encephalopathy, regression, mucositis, rash, dystonia, and spasticity.
- Challenge:** Initial clinical short read genome sequencing was non-informative despite a suggestive phenotype.



Metabolic Clues to *NAXE* Deficiency

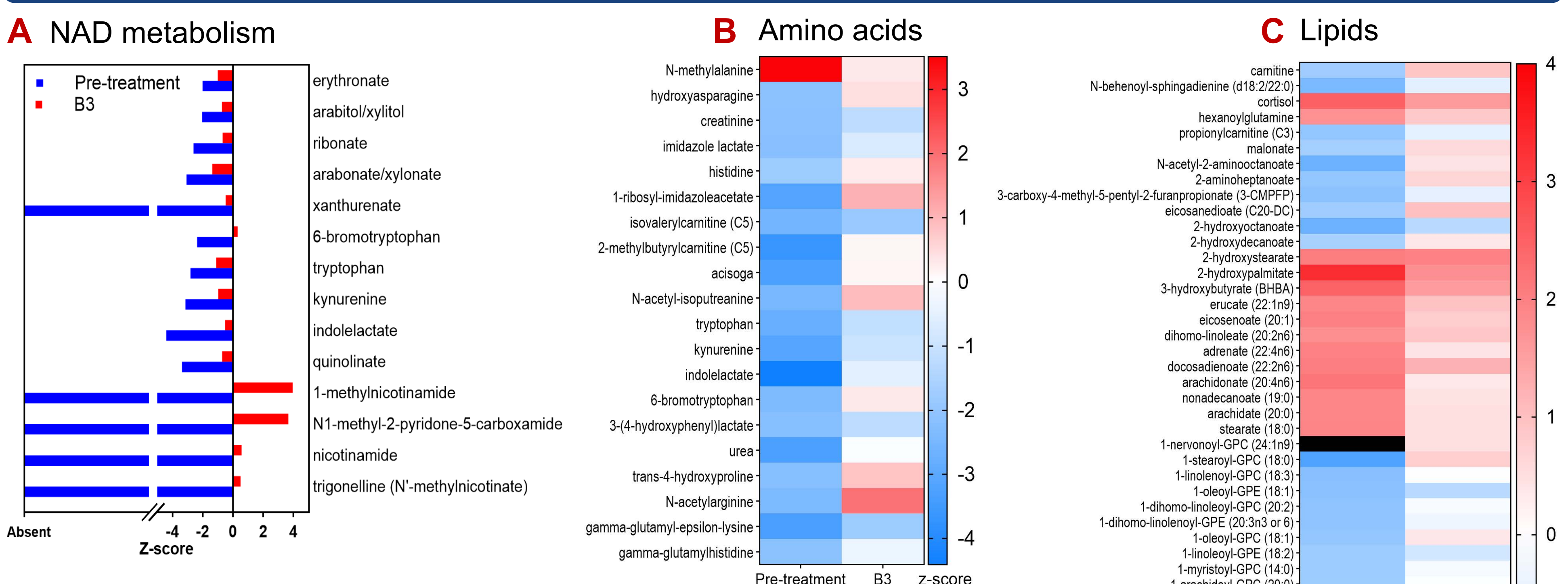


Figure 3 (A-C): Untargeted metabolomics revealed a NAD⁺ depletion signature with secondary PPP/redox stress, rescued by niacin.

- NAD metabolism: Pre-treatment shows NAD⁺ depletion with absent 1-methylnicotinamide and N1-methyl-2-pyridone-5-carboxamide (normally seen in 99% of samples), restored after B3 therapy. This depletion–restoration pattern parallels NAXE/NAXD NAD(P)HX repair defects^{3,4}. [Figure 3A]
- Amino acids (Trp → Kynurenine): Altered Trp/Kyn ratio indicate blocked *de novo* NAD synthesis; niacin salvage rescues pathway. [Figure 3B]
- Lipids / Redox (PPP): Disturbed NADPH/redox and lipid balance pre-treatment normalize after niacin therapy. [Figure 3C]
- Therapy: Niacin therapy stabilized clinical symptoms and corrected most metabolic derangements, though lipid abnormalities persisted, highlighting the complex metabolic role of NAXE³.

Biallelic GGGCC repeat expansion in promoter region of *NAXE*

- Discovery with long-read sequencing: Identified biallelic GGGCC promoter repeat expansions in *NAXE* - proband (90, 233 repeats) and genotype-positive younger brother (152, 199 repeats; early treatment, prodromal only).
- Methylation profiling: Hypermethylation at the repeat expansion in the proband and the brother compared to lower levels in the mother and father.
- Long-read RNA-seq: Markedly reduced to absent *NAXE* mRNA expression in the proband.

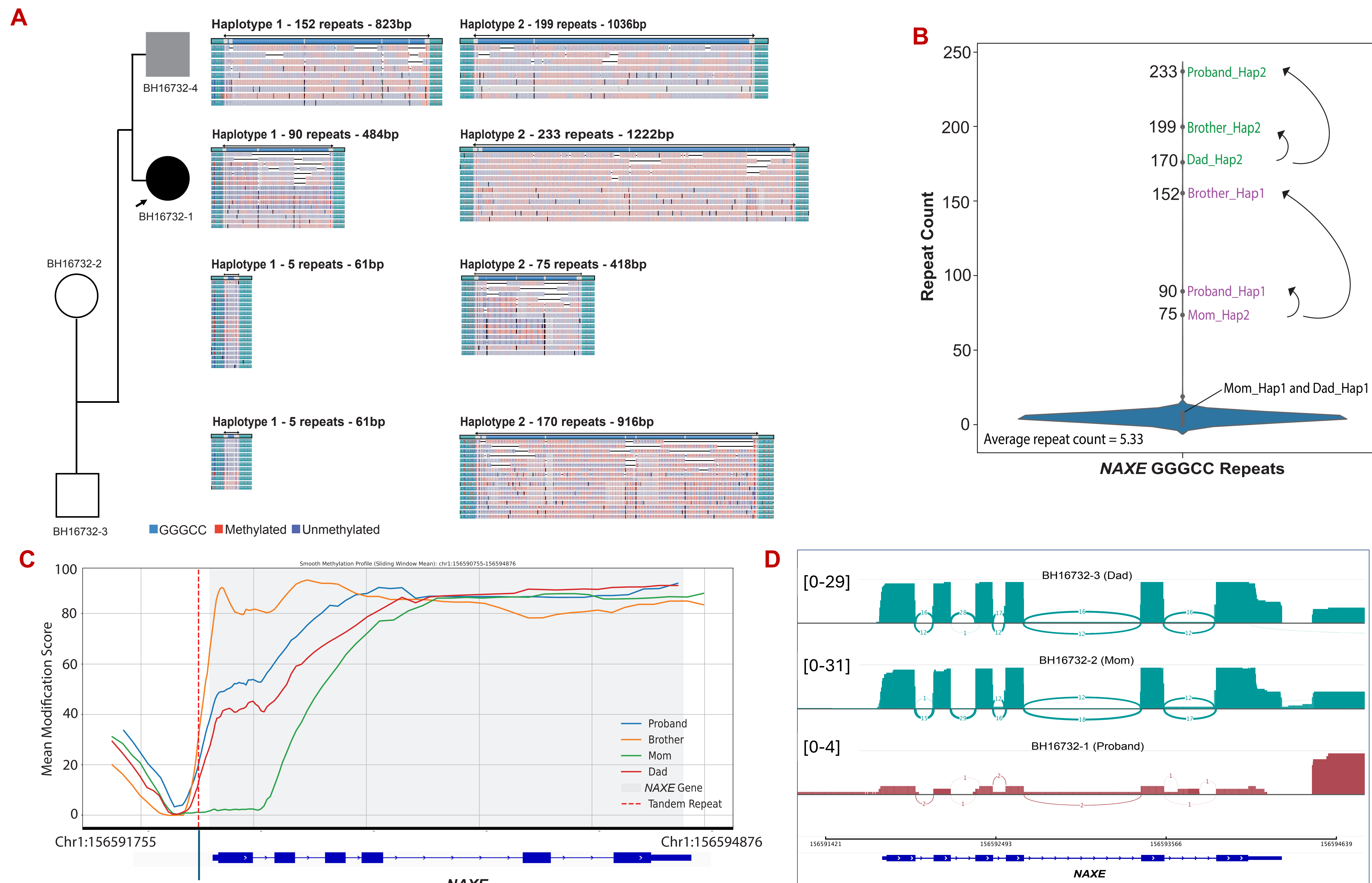
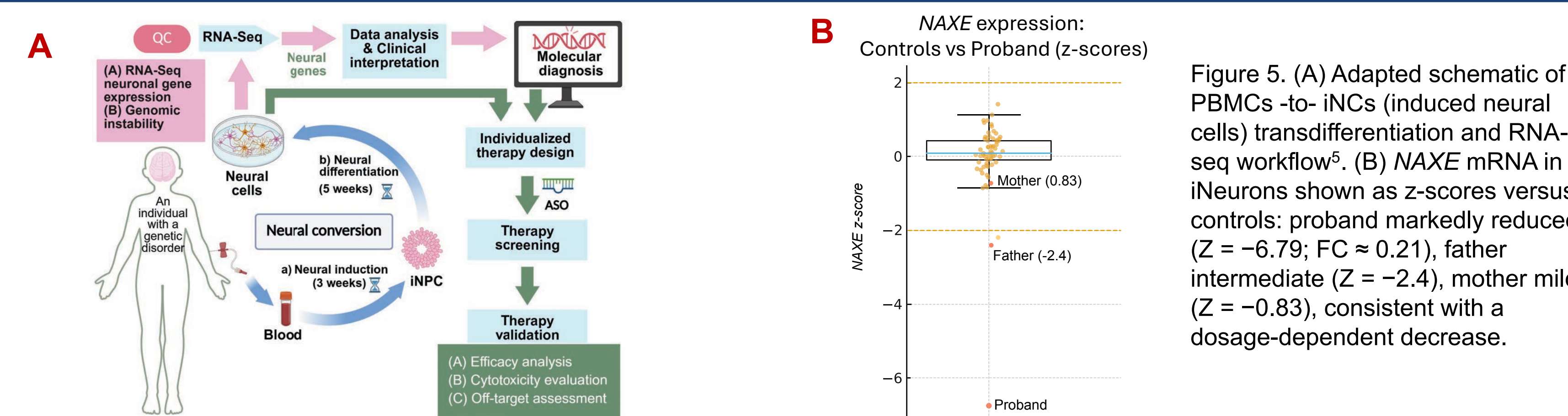


Figure 4 (A-D): Pedigree and long-read sequencing reveal inheritance and phasing of *NAXE* repeat expansions. In (A), the circle indicating the proband is shaded in black; the treated affected brother is gray to indicate a milder, treatment-modulated presentation with prodromal symptoms only. Panels (B-D) depict allele distribution, associated hypermethylation, and loss of mRNA expression in the proband from long-read genomic and transcriptomic analyses. Key: black = affected; gray = confirmed genotype with attenuated phenotype; open = unaffected/unknown.

Modeling *NAXE* Deficiency in iNeurons

- Patient-derived PBMCs were transdifferentiated into induced neural cells (iNCs) to assess neuron-specific gene expression.
- RNA-seq demonstrated markedly reduced *NAXE* mRNA expression in the proband ($Z = -6.79$; $FC = 0.21$ vs controls).
- Findings indicate a dosage-dependent loss of *NAXE* expression in iNeurons.



Multioomic analysis revealed *NAXE* repeat expansions as a novel non-coding cause of a treatable mitochondrial encephalopathy. This case is the second documented to our knowledge with this disease mechanism³. This case emphasizes the power of integrative genomics to uncover hidden mechanisms and enable targeted therapy in rare disease.

ACKNOWLEDGEMENTS

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