HGGA, Volume *5*

Supplemental information

Biallelic *NDC1* variants that interfere with ALADIN binding are associated with neuropathy and triple A-like syndrome

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Supplemental methods

Whole exome sequencing

Family 1: Trio exome capturing was carried out using Agilent SureSelect Target Enrichment Clinical Research Exome V2 (Agilent Technologies, Santa Clara, CA, USA). Sequencing (paired-end 150bp) was performed by the Illumina HiSeq 4000 platform (Illumina, San Diego, CA, USA). Data was demultiplexed by Illumina Software CASAVA. Reads are mapped to the genome (build hg19/GRCh37) with the program BWA (reference: http://bio-bwa.sourceforge.net/). Variants are detected with the Genome Analysis Toolkit (reference: http://www.broadinstitute.org/gatk/). Subsequently, variants were filtered with the Alissa Interpret software package (Agilent technologies) on quality (read depth ≥10), frequency in databases (≥1% in 200 alleles in dbSNP, ESP6500, the 1000 Genome project or the ExAC database) and location (within an exon or first/last 10 bp of introns). Variants were further selected based on three inheritance models (de novo autosomal dominant, autosomal recessive and X-linked recessive).

Family 2: For whole exome sequencing exonic sequences were enriched from the individual (2-I) using NimbleGen SeqCap EZ human exome library v2.0 and sequenced on a HiSeq2000 (Illumina) with read length of paired-end 2x100 bp and average coverage of >50 fold. FASTQ-files (FASTA format sequences bundled with their quality data) were aligned to the human GRCh37.p11 (hg19) reference sequence using the BWA-MEM V.0.7.1 aligner. Co-segregations of the NDC1 mutation in family F2 was verified by Sanger sequencing using an ABI 3130XL genetic analyzer and BigDye Terminator Cycle Sequencing Kit 1.1 (Applied Biosystems).

Family 3 & Family 4: The samples from family 3 underwent sequencing at Yale Center of Genome Analysis (YCGA) using illumina NovaSeq6000 sequencer and the IDT xGen Exome V2 kit. Family 4's samples were processed at Beijing Genomics Institute (BGI) with the MGISEQ-2000 sequencer.

WES data was analyzed in Phoenix Children's Hospital (PCH). The sequenced reads were aligned to the reference genome, GRCh37, using BWA-MEM and further processed using GATK Best Practice workflows, which include duplication marking, indel realignment and base quality recalibration. Single-nucleotide variants and small indels were called with GATK HaplotypeCaller and annotated using ANNOVAR , dbSNP(v138), 1000 Genomes (August 2015), NHLBI Exome Variant Server (EVS) and the Exome Aggregation Consortium v3 (ExAC). MetaSVM and CADD (v1.3) algorithms were used to predict deleteriousness of missense variants (D-Mis, defined as MetaSVM-deleterious or CADD ≥ 20). Inferred LoF variants consist of stop-gain, stop-loss, frameshift insertions/deletions, canonical splice sites and start-loss. LoF and deleterious missense mutations were considered 'damaging'.

The recessive variants were filtered for rare (MAF ≤ 10−3 across all samples in 1000 Genomes, EVS and ExAC) variants that exhibited high-quality sequence reads (pass GATK variant score quality recalibration) and had a minimum of 8 total reads for the proband. Only LoF variants (stop-gain, stop-loss, canonical splice-site, frameshift indels and start-loss), D-Mis (MetaSVM = D or CADD ≥ 20) and non-frameshift indels were considered potentially damaging to protein function.

De novo variants were called using the TrioDenovo program and filtered using stringent hard cutoffs[33]. These filters include: MAF ≤ 5 × 10−4 in ExAC; a minimum of 10 total reads, 5 alternate allele reads, and a minimum 20% alternate allele ratio in the probands. If alternate allele reads ≥10 or, if alternate allele reads were <10, a minimum 28% alternate ratio; a minimum depth of 10 reference reads and alternate allele ratio < 3.5% in parents; and exonic or canonical splice-site variants.

For the X-linked hemizygous variants, we filtered for rarity (MAF ≤ 5 × 10−5 across all samples in 1000 Genomes, EVS and ExAC) and high-quality heterozygotes (pass GATK variant score quality recalibration, a minimum of 8 total reads, genotype quality score ≥20, mapping quality score ≥40, and a minimum 20% alternate allele ratio in the proband if alternate allele reads ≥10 or, if alternate allele reads were <10, a minimum 28% alternate ratio).

The Autozygosity mapping results for families 3 and 4 indicate that c.1706C>T (p.(Ser569Leu)) and c.1720G>A (p.(Ala574Thr)) variants are located within 11.35 Mb and 9.5 Mb regions of homozygosity, respectively.

Supplemental note: case reports

Case report family 1: Two affected siblings were born to Turkish consanguineous healthy parents after uneventful deliveries (**Figure 1A**). The oldest affected female sibling (individual 1 - I) presented with a delay in motor development from birth. She started walking around 2 years of age. Speech development was normal, although from the beginning nasality was observed. Her total IQ was measured at 75. Since birth, she experiences progressive difficulties with chewing and swallowing. She cried without tears and experiences choking episodes daily. Last examination at the age of 11 years showed fasciculation and atrophy of the tongue, facial weakness, nasal speech, dysarthria and dysmorphic features including down slanting palpebral fissures, and a thin long nose (**Figure 1B**). The skin was pale and thin with visible superficial veins. Further examination revealed generalized hypotonia, moderate distal > proximal limb muscle weakness, intact sensibility diminished symmetric deep tendon reflexes (DTRs), abnormal Trendelenburg gait pattern with feet in exorotation. No signs of autonomic dysfunction, besides alacrima, were observed. The patient reported fatigue, occasionally chest pain, and uses a wheelchair for longer distances. Brain magnetic resonance imaging (MRI), obtained at the age of 4 years, showed normal neuroanatomy. Electromyography (EMG) showed a pure motor demyelinating polyneuropathy with secondary axonal injury, with motor nerve conduction velocities around 25-30 m/sec without evidence of blocks. Muscle computer tomography (CT) showed normal musculature of neck and shoulder musculature, mild atrophy of the pelvis muscles, moderate atrophy of the hamstrings, and severe atrophy with fatty infiltration of the gastrocnemius and soleus muscles. Because of dysphagia, fluoroscopic swallowing studies were performed and showed slower passage through the upper esophageal sphincter but no overt achalasia. Respiratory and heart function are normal. Morning cortisol level was normal at 11 years old.

Her younger brother (individual 1 – II) showed a similar disease course, and similar facial features, but is less severely affected than his sister (**Figure 1B**). At the age of 10 years, his facial weakness and nasal speech were less pronounced, he could walk longer distances wearing an ankle-knee orthosis, but often complained of pelvic pain. He walked unsteadily with Trendelenburg gait and limited heel lifting. He had milder swallowing difficulties compared to the sister, but also cried without tears. Neurologic examination showed facial and upper and lower limb weakness, with intact sensibility and decreased DTRs. The skin was of normal pigmentation. Respiratory and heart functions were normal. Unlike his sister, he developed behavioral problems characterized by angry outbursts and aggressive behavior. His MRI brain at the age of 3 years was normal. EMG showed demyelinating polyneuropathy with secondary axonal injury. His Morning cortisol level was also normal.

Genomic analysis family 1: SNP arrays showed multiple regions of homozygosity (ROH) and a maternally inherited 215 kb duplication on 3q13 (arr[hg19] 3q13.2(112940053_113154905)x3). The

duplication includes a part of *BOC*, *WDR52* and *WDR52-AS1*. This genomic copy number variant is considered a variant of unknown significance, most likely unrelated to the phenotype. It is not frequent in control databases, but there are no disease-associated genes within this locus. Diagnostic exome sequencing using the in house ID panel in trio and followed by full exome analysis did not identify predicted deleterious homozygous variants in both siblings explaining the phenotype (**Table S2**).

Case report family 2: Two affected girls were born in a consanguineous Turkish family after uncomplicated pregnancies. The older girl (individual 2-I) presented at age 13 years with mild growth failure (skeletal height at 10. percentile) and developmental delay. Examination showed nasal speech, myopathic face, tongue fasciculation, peripheral neuropathy, distal muscle weakness, and the development of 'stork legs'. She had reduced amplitudes of motor neuron conduction velocities, and EMG recordings showed loss of motor units. Individual 2-I presented with mild hyperreflexia but missing achilles tendon reflexes. Her esophageal peristalsis was moderately delayed. X-ray revealed advanced bone age by 2 years, hypoplastic end phalanx of dig. V and minus variant of the ulna. Her younger affected sister (2-II) showed mild short stature (skeletal height at 10. percentile) and milder developmental delay, and attends normal school. She has similar neurological features including nasal speech, tongue fasciculations, peripheral neuropathy, distal muscle weakness and reduced motor neuron conduction velocities. She presents with normoreflexia but missing Achilles tendon reflexes and does not have achalasia. Both girls have hypolacrima, but no other autonomic disturbances and normal ACTH levels. In individual 2-II short ACTH stimulation test at age 10 years showed normal basal and stimulated cortisol levels. Both girls had no clinical or laboratory sign for mineralocortiocoid deficiency. In addition, both patients were reported with limited heart rate variability, hyperkeratosis and mineral deficiency of the bones.

Case report family 3: A 27-year-old female from Iran was clinically diagnosed with Triple-A syndrome (individual 3 -II). She was the fifth child from a consanguineous marriage, born at term via caesarian section with no complications during pregnancy or delivery (**Figure 1A**). Patient's symptoms began in the first year of life, with recurrent vomiting. She was diagnosed with achalasia and underwent surgery at the age of 3 years. She was globally developmentally delayed and did not walk and talk until she was 3.5 years old. After she began walking, her parents noticed limping, which progressively worsened. She developed progressive swallowing difficulties, which reached its maximum intensity at the age of 26 years. Her parents reported no episodes of hypoglycemic seizures or shock, although her EEG showed mild slowing and focal epileptiform features at age two.

Physical examination revealed an alert, young woman with intellectual disability, underweight and of short stature. Bilateral facial muscle weakness was present, with a high-arched palate, nasal speech, dysphagia, decreased gag reflex, muscle atrophy, decreased strength, increased deep tendon reflexes, gait abnormality, and coxa vara deformity (**Figure 1B**). There were no signs of autonomic disturbance, including abnormal pupillary reflex, diminished heart rate variability, or orthostatic hypotension. Diagnostic tests were positive for urolithiasis, and bone deformities. Laboratory tests showed no signs of adrenal insufficiency. The second son in this family (individual 3-I) experienced relatively similar problems. He was also diagnosed with the same condition after a history of vomiting, recurrent aspiration and acute pneumonitis, developmental delay, urolithiasis and growth disorder (**Figure 1B**).

*Genomic analysis family 3***:** SNP array in individual 3-II showed a normal hybridization pattern. The *SMN* copy number was normal.

Case report family 4: This family includes one affected boy that was born at 39 weeks of gestation with growth measurements within the normal range (**Figure 1A,** individual 4-I). After birth, he presented with severe developmental delay. He started walking at 3 years of age and is non-verbal. During the first years of life he had seizures described as atonic/tonic episodes or head drops and jerks at the time of waking. Electroencephalogram (EEG) recordings showed epileptic activity in the fronto-central regions. He had swallowing difficulties, requiring soft texture foods, and cortical blindness. Neurologic examination showed brisk deep tendon reflexes without obvious muscle weaknesses and increased muscle tone and contractures at knees, elbows and one ankle. He did not show signs of autonomic disturbances or adrenal insufficiency. Clinical evaluation at 11 years revealed scoliosis, tapered fingers, preauricular tag and obesity (>97thp). In addition, he had avascular necrosis of the left hip. Brain MRI showed mild prominence of the lateral ventricles and extra-axial CSF spaces and nonspecific prominence of some perivascular spaces with subtle decrease in cerebellar volume.

References

1. Huang, G., Zhan, X., Zeng, C., Zhu, X., Liang, K., Zhao, Y., Wang, P., Wang, Q., Zhou, Q., Tao, Q., et al. (2022). Cryo-EM structure of the nuclear ring from Xenopus laevis nuclear pore complex. Cell Res *32*, 349-358. 10.1038/s41422-021-00610-w

10.1038/s41422-021-00610-w [pii].

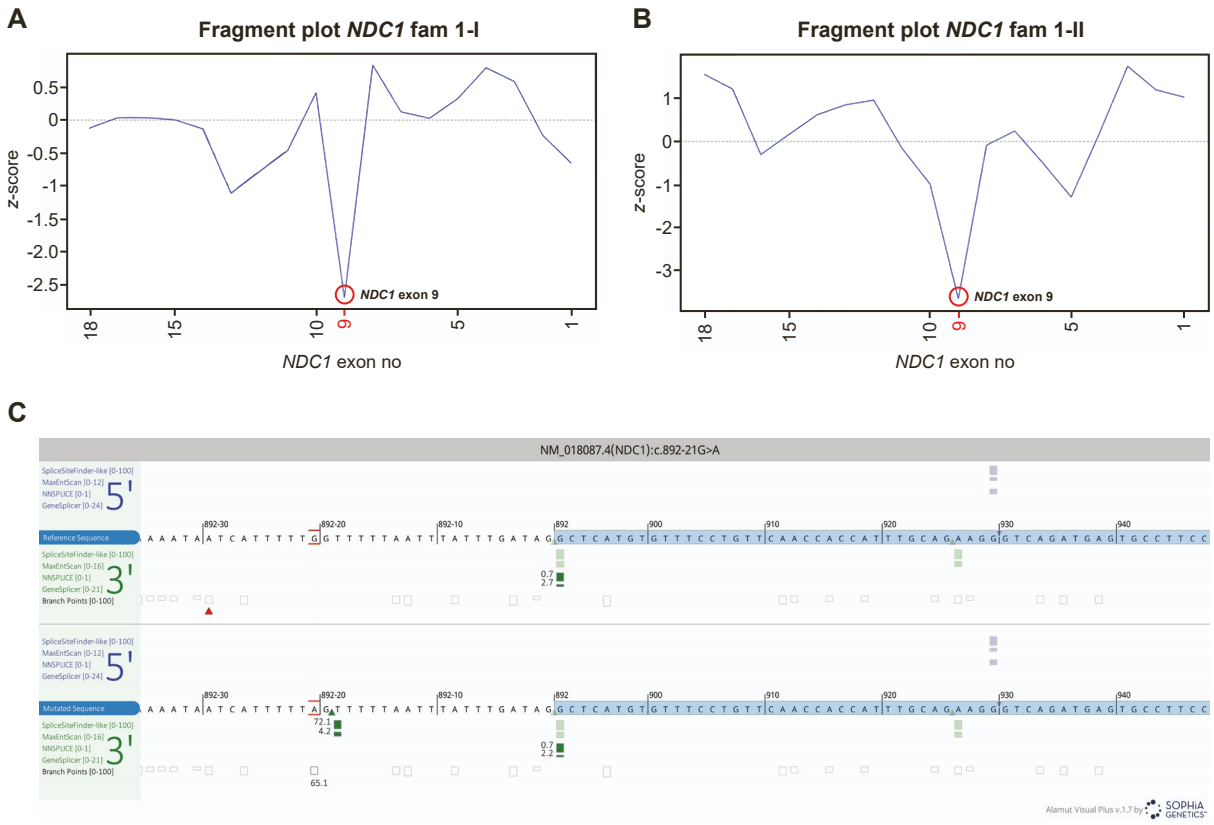

Figure S1. Downregulation of NDC1 exon 9 identified with RNA-seq. (A,B) Fragment plots *NDC1* of individual 1-I (A) and individual 1-II (B). In both individuals *NDC1* exon 9 is the only downregulated exon (red circle). (C) Alamut software splice prediction shows that the *NDC1*:c.892-21G>A variant results in an AG dinucleotide in the polypyrimidine tract.

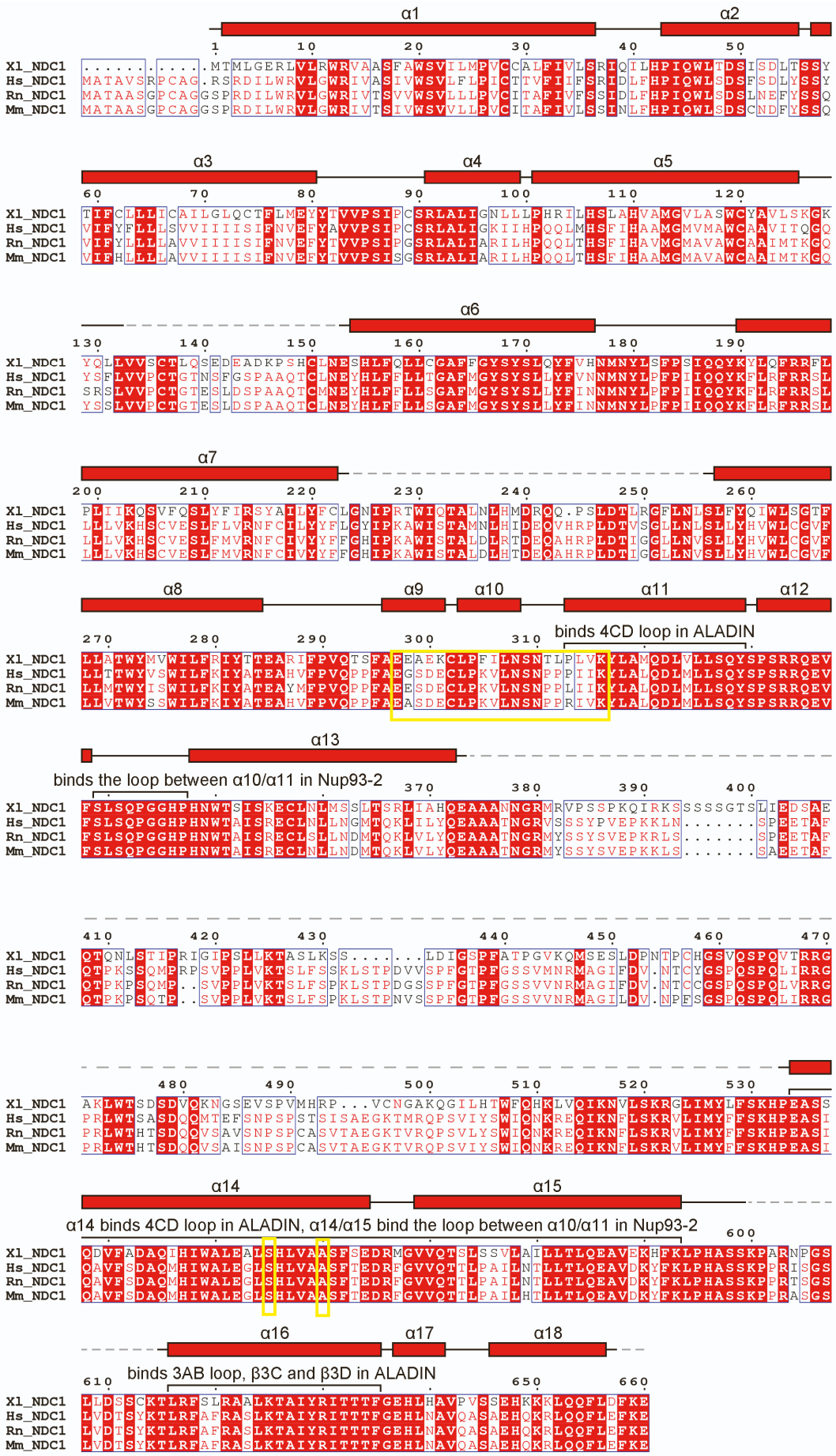

Figure S2. ALADIN – binding sites in the NDC1 protein. The image was adapted from Huang *et al*[1]. The image shows the NDC1 protein sequence from different species Xl: *xenopus laevis*, Hs: *homo sapiens*, Rn: *rattus norvegicus*, Mm: *mus musculus*. The secondary protein structure is depicted above the amino acid sequence. Highly conserved amino acids are depicted in red. The location of the described *NDC1* variants is depicted with yellow boxes.