The American Journal of Human Genetics, Volume *106*

Supplemental Data

Bi-allelic Loss-of-Function Variants in *NUP188* Cause a Recognizable Syndrome Characterized by Neurologic, Ocular, and Cardiac Abnormalities

Alison M. Muir, Jennifer L. Cohen, Sarah E. Sheppard, Pavithran Guttipatti, Tsz Y. Lo, Natalie Weed, Dan Doherty, Danielle DeMarzo, Christina R. Fagerberg, Lars Kjærsgaard, Martin J. Larsen, Patrick Rump, Katharina Löhner, Yoel Hirsch, David A. Zeevi, Elaine H. Zackai, Elizabeth Bhoj, Yuanquan Song, and Heather C. Mefford

SUPPLEMENTARY TEXT

Supplemental Note: Case Reports:

Individuals 1 and 2 are female siblings (Family 1), born to non-consanguineous parents of Ashkenazi Jewish descent. For individual 1, ventriculomegaly was noted in the third trimester of an otherwise unremarkable pregnancy; prenatal karyotype was normal. At birth, she was noted to have mild hypotonia with a weak suck, dysmorphic facies, micrognathia, small palpebral fissures, congenital cataracts, unilateral preaxial polydactyly and bilateral camptodactyly of fingers 3, 4 and 5, broad great toes, and mild contractures of the knees and elbows. Brain MRI revealed agenesis of the corpus callosum, colpocephaly, and elongated right orbit and globe; echocardiogram showed patent ductus arteriosus and bicuspid aortic valve. Sleep study showed aspects of central hypoventilation. She had progressive microcephaly and developed increasing respiratory insufficiency; she passed away at 5 months of age. An extensive workup revealed normal results for the following laboratory tests: genome-wide microarray, Fanconi breakage studies, very long chain fatty acids, 7-dehydrocholesterol, plasma amino acids, urine organic acids, *BCOR* sequencing, and transferrin isoelectric focusing. Her sibling, individual 2, had ventriculomegaly noted at 20 weeks' gestation; prenatal karyotype and chromosome array were normal. At birth, she was noted to have mildly dysmorphic features, long fingers, bilateral club feet, bilateral cataracts, hypotonia; echocardiogram revealed partial anomalous pulmonary venous return;

MRI showed thin corpus callosum, premature myelination pattern and mild ventriculomegaly. She also had central hypoventilation with progressive respiratory failure and passed away at 5 weeks of age. Autopsy of individual 2 revealed streak ovaries, polysplenia, diffuse gliosis and patchy depletion of Purkinje cells.

Individual 3 was born to non-consanguineous parents of Ashkenazi Jewish descent. Jewish carrier screening performed during the pregnancy was negative. The family history was significant for a mother with tetralogy of Fallot, repaired as a child. Her prenatal course was unremarkable and she was born late pre-term (36 1/7) by C-section due to premature labor in breech presentation. At birth, length was 43.5 cm (15%ile), weight was 2.205 kg (20%ile), and head circumference was 30.5 cm (10%ile). She presented with hypotonia, micrognathia, microphthalmia, optic atrophy, congenital cataracts, cleft palate with bifid uvula, congenital hearing loss, pancytopenia, and hypoglycemia. She required an oro-gastric tube for feeding. She developed hypothyroidism, seizures, and respiratory insufficiency requiring mechanical ventilation. She had progressive microcephaly. Physical examination at 3 months of age demonstrated weight of 4.26 kg (10%ile), length of 56.5 cm (50%ile), and head circumference of 33.5 cm (50%ile for 38 weeks), skull asymmetry with small anterior fontanelle that was laterally displaced to right, bitemporal narrowing, cataracts, epicanthal folds, palpebral fissure length 2 STD below mean, flattened

left pinna with pit on lobe, wide nasal bridge, wide nose, microretrognathia, cleft palate, generalized hypotonia, normally spaced nipples, fixed flexion at the left fifth proximal interphalangeal, overlapping toes, transitional palmar creases, and three out of ten whorls on dermatoglyphics. MRI showed marked cerebral and cerebellar white matter volume loss with ventriculomegaly and hypoplastic corpus callosum. Spine, abdominal and pelvic ultrasounds were normal. Initial testing included 7-dehydrocholesterol, *KAT6B* sequencing for Odho syndrome, and Fanconi breakage studies, which all resulted negative. Genome wide microarray showed regions of homozygosity on chromosomes 1 and 12 (Arr[GRCh37] (1-22,X)x2, 1p21.1p13.3(103,161,471-109,257,747)x2 hmz, 12q21.31q21.33(84,825,399-90,396,110)x2 hmz), likely consistent with identity by descent.

Individual 4 is the first of four children born to unrelated parents; mild ventriculomegaly was noted prenatally. She had severe hypotonia, short stature, dysmorphic features, myopia. She developed chronic respiratory problems, microcephaly, spasticity and myoclonic epilepsy and was suspected of having a neurodegenerative disease. She died at 2 years 7 months of pneumonia. Her sister, **individual 5**, was the fourth of four children and had a suspected brain malformation prenatally. Postnatal MRI revealed thin corpus callosum, delayed myelination pattern and reduced white matter. She had respiratory insufficiency

with severe respiratory infections during the first weeks of life and died of respiratory arrest at 3 weeks of age.

Individual 6 was the product of *in vitro* fertilization; pregnancy was complicated by polyhydramnios. She was noted to have short palpebral fissures, camptodactyly of the fingers and toes, congenital cataracts, cleft palate and hypotonia at birth. She developed seizures. MRI showed hypoplastic brain with wide ventricles. She died at 1 month of sepsis, pulmonary hypertension and chylothorax. Autopsy revealed pulmonary capillary hemagiomatosis and streak ovaries.

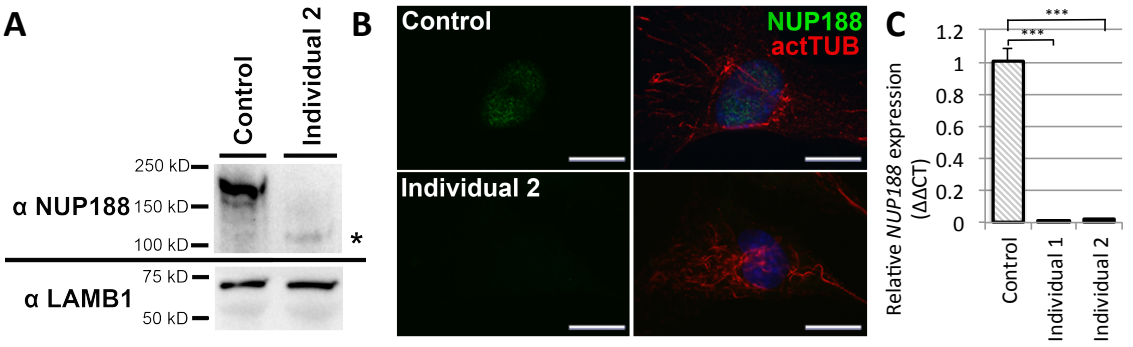

Figure S1: NUP188:p.(Ile302Valfs*7) and NUP188:p.(Tyr1048*) result in loss of full length NUP188; related to Figure 2. (**A**) Western blot of nuclei isolated from Individual 2 and control fibroblasts stained with an antibody against NUP188 reveals full length NUP188 (~188 kDa) is undetectable in Individual 2. A smaller, faint band (*) in Individual 2 but not in Individual I or the control individual is most likely non-specific. LAMB1 is used as a loading control. Individual 2 has compound heterozygous truncating variants NUP188:p.(Ile302Valfs*7) and NUP188:p.(Tyr1048*). (**B**) Immunofluorescent staining of NUP188 in fibroblasts from Individual 2 and a control individual. NUP188 (green) localizes to the nucleus in control fibroblasts but is undetectable in fibroblasts from Individual 2. Acetylated-tubulin antibody (red) was used to identify the cell bodies. Experiment was preformed in triplicate (n=20 cells each) and representative images are shown. Scale bar = 20 μm. (**C**) There is a 98% reduction of *NUP188* transcript in affected individuals compared to controls as measured by Real-time PCR. n=3, *** $P < 0.001$ by two-tailed unpaired Student's t-test.

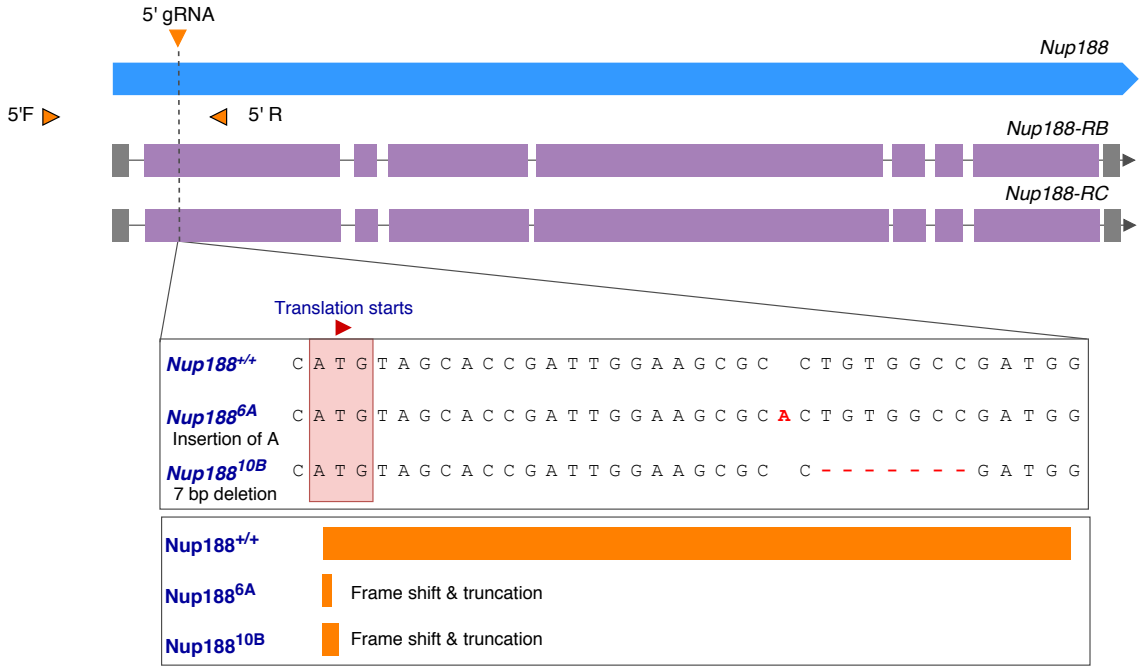

Figure S2: Generation of the knockout alleles for *Nup188*. Representation of the two *Nup188* knockout alleles. *Nup188*^{6A} has an insertion of A and *Nup188*^{10B} has a 7 bp deletion, both resulting in a frame shift and truncation. The *Nup188*^{KO} was generated using the CRISPR/Cas9 technique by non-homologous end joining.