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Supplemental Data

NFIB Haploinsufficiency Is Associated with Intellectual Disability and Macrocephaly

Ina Schanze, Jens Bunt, Jonathan W.C. Lim, Denny Schanze, Ryan J. Dean, Marielle Alders, Patricia Blanchet, Tania Attié-Bitach, Siren Berland, Steven Boogert, Sangamitra Boppudi, Caitlin J. Bridges, Megan T. Cho, William B. Dobyns, Dian Donnai, Jessica Douglas, Dawn L. Earl, Timothy J. Edwards, Laurence Faivre, Brieana Fregeau, David Genevieve, Marion Gérard, Vincent Gatinois, Muriel Holder-Espinasse, Samuel F. Huth, Kosuke Izumi, Bronwyn Kerr, Elodie Lacaze, Phillis Lakeman, Sonal Mahida, Ghayda M. Mirzaa, Sian M. Morgan, Catherine Nowak, Hilde Peeters, Florence Petit, Daniela T. Pilz, Jacques Puechberty, Eyal Reinstein, Jean-Baptiste Rivière, Avni B. Santani, Anouck Schneider, Elliott H. Sherr, Constance Smith-Hicks, Ilse Wieland, Elaine Zackai, Xiaonan Zhao, Richard M. Gronostajski, Martin Zenker, and Linda J. Richards

SUPPLEMENTAL DATA

Supplemental Note: Case Reports.

Proband 1 *p.(Arg37*)*

This 16-year old male proband is the oldest child from a family of three. He has a healthy brother and sister. His sister had a surgical correction of a ventricular septum defect.

The proband was born after an uncomplicated pregnancy at a gestational age of 41 weeks by vacuum extraction with a low birth weight of 2750 g. There was a motor and speech developmental delay, which was assigned to agenesis of the corpus callosum, resulting in an intellectual deficit (verbal IQ is 60; non-verbal IQ is 80). Other health complaints were: obstipation (in early childhood), bilateral hernia inguinalis at young age (between first and second year of his life). He suffered from frequent recurring ear infections due to an anatomically abnormal Eustachian tube, resulting in some hearing loss for which no hearing aids are needed. He has difficulties in falling asleep. Facial dysmorphic features of this proband are: he has a relatively large head circumference of 60.5 cm (+3.76 SD) with a height of 173 cm (+0.2 SD) and a weight of 56 kg (-0.42 SD). He has a large forehead with a high frontal hairline, with a frontal tuft, down slanting palpebral fissures, a high nasal bridge, a small columella, small ears, a relatively large mouth with a high palate, some small fissures in the tongue, and a large pointing chin. There is a pectus excavatum with widely spaced nipples. There is no abdominal wall defect but the navel shows laxity. The hands are small. The feet are large; there is bilateral pes planus, a sandal gap and broad halluces. There is no hypo- or hyperpigmentations, apart from 10 to 20 simple naevi on the back and one capillary malformation (“haemangioma”) on the back side of the upper left arm.

Proband 2 *p.(Arg89*)*

This 7-year old male proband is the youngest of two children from a non-consanguineous family. He has a healthy sister, as well as two healthy maternal half-sisters. The proband was born following an uncomplicated pregnancy. However, his mother has a history of drug use. He was born full term with a birth weight of 3997 g. At age 6 months, his OFC was noted to be above the 95th percentile. He was subsequently noted to have developmental delays. He first walked at about 1 year of age, but his language development was more delayed; his first words were between 1.5 and 2 years. His speech is limited to single words and short phrases. He was enrolled in speech therapy in early childhood. The proband also has several behavioral problems including anger control problems suggesting possible oppositional-defiant behavior. His attention span is described as being short. He has a history of episodic tachycardia and underwent a cardiology evaluation that included an electrocardiogram which was normal, with no further intervention. On last assessment at age 6 years and 8 months, his height was 124.9 cm (+0.58 SD), weight 23 kg (-0.02 SD) and occipito-frontal circumference was 57.2 cm (+3.94 SD). Examination revealed macrocephaly with dolichocephaly, broad and prominent forehead, bifid tip of the uvula, mildly flat mid-face, flat nose, and fullness of the nasolabial folds. Thumbs and great toes were mildly broad, with a mild sandal-gap toe deformity. Skin examination revealed two hyperpigmented macules and several nevi over the trunk and extremities. Brain MRI at age 1 year and 9 months showed macrocephaly with a prominent forehead, mildly in folded gyri in the perisylvian regions but otherwise normal gyral pattern, increased perivascular spaces in the caudal basal ganglia, diffusely thin white matter, and a thin corpus callosum. He also had mild cerebellar tonsillar ectopia, but no Chiari malformation.

Proband 3 *p.(Lys114Thr)*

This proband was almost 7 years old when he presented to the neurogenetics clinic and was at that time diagnosed with ASD, Attention Deficit Hyperactive Disorder, Global Developmental Delay affecting language, cognition, fine and gross motor skills. He was the product of a twin pregnancy that was conceived via in−vitro fertilization and was otherwise uncomplicated. He was born to non−consanguineous parents; a 36-year-old mother and 30-year-old father. His birth weight was 2499 g and he was born via emergency C-section secondary to late decelerations. He had multiple otitis media and required pressure equalizing tubes. He was diagnosed with obstructive sleep apnea at 1 year old and esotropia of the right eye both resolved with intervention. He walked at 18 months old, used a mature pincer grasp after 1 year old and started using words around 4.5 years old.

His twin sister shows unremarkable development. Maternal history is significant for migraines and vasovagal syncope and there is a paternal history of learning disabilities. Extended family history is notable for hypertension, anxiety, diabetes mellitus, anxiety, depression, prostate and breast cancer, thyroid disease, delayed language, learning disabilities and ASD. His clinical exam revealed weight 43.6 kg (+4.17 SD), height 137.0 cm (+2.80 SD) and OFC 58 cm (+5.17 SD). He has wide spaced teeth, flat nasal bridge and small almond shaped eyes. A MRI of the brain is normal. Fragile X DNA testing and SNP microarray were normal. Whole exome sequencing also revealed a variant of uncertain significance c.950A>G (p.Q317R), in the SHANK3 gene (NM_033517.1). Inheritance unknown as his father was unavailable.

Proband 4 *p.(Lys126Glu)*

This male proband was the second of two children of a non-consanguineous, healthy Caucasian couple with unremarkable family history. At conception, the mother was aged 27 and the father was 35 years old. He was born at 39 weeks' gestation after an uneventful pregnancy with a length of 50 cm (-0.06 SD), a weight of 3080 g (+0.77 SD), and an OFC of 39.5 cm (+2.2 SD). He presented with neonatal hypotonia and right cryptorchidism. Developmental milestones were delayed. He started to walk at 2 years old. Speech was delayed with first words around 3 years old. He needed specialized education from the age of 6 onwards.

Currently aged 32, he has mild to moderate intellectual disability and lives in a social care institution. He is able to communicate with short sentences and perform manual tasks. Reading, writing and calculation skills have not been acquired. He is friendly and sociable but suffers from anxiety when faced with change.

On clinical examination, his height was 192.5 cm (+2.25 SD), weight 65 kg -0.49 SD) and OFC 63 cm (+5.52 SD). He has a marfanoid habitus with arachnodactyly, scoliosis, pes planus and sandal gap toes. There is elbow laxity and mild contractures of the fingers flexors. Facial dysmorphism includes brachycephaly, hypertelorism and down-slanting palpebral fissures. Skin examination shows multiple naevi and atrophic scars. He needs optical correction because of nystagmus, strabismus, and astigmatism. ECHO is normal. A cerebro-medullar CT-scan was performed in infancy showing moderate diffuse cerebral atrophy, a cyst of septum pellucidum, and a dorsal meningocele. Because of his intellectual disability and marfanoid habitus, karyotype, array-CGH 44K, testing of *FMR1*, *FBN1*, *FBN2*, *TGBR1* and *TGFBR2* genes were performed and showed normal results.

Proband 5 *p.(Leu132Pro)*

The proband is an 8-year-old male who is the second child of his nonconsanguineous parents. He was born at 40 weeks gestational age to his then 38-year-old mother after an uneventful pregnancy. His birth measurements were normal but frontal bossing was noted. His gross motor development was on time but his speech and fine motor skills were delayed. He has macrocephaly with a prominent occiput (+2.17 SD). Head shape is somewhat dolichocephalic and there is mild frontal bossing at the upper forehead. The paternal head circumference is 59 cm (greater than the 98th percentile). Ears are mildly cupped. He has malar flatness, arched eyebrows with synophrys, a mildly smoothed philtrum, a thin upper vermilion border, and a bifid uvula. He has a translucent skin, thin extremities, and a mildly low muscle tone. Currently, at the age of 8, he has been diagnosed with borderline ID, ASD with sensory integration disorder, aggressive and obsessive behaviors, developmental coordination disorder and attention deficit hyperactivity disorder (ADHD)-combined type. His brain MRI revealed a small rostrum of the corpus callosum. He has sudden unexplained headaches that clear easily with acetaminophen. The proband has undergone chromosomal microarray, *PTEN* sequencing, fragile X testing, sequencing of *TGFBR1* and *TGFBR2*, sequencing of *NSD1*, testing for carbohydrate deficient glycoprotein syndrome, glutaric acid study, and biochemical testing for MPS, all of which were normal.

Probands 6a and 6b *p.(Asn254*)*

Proband 6a is a 33-year-old Jewish female of Sephardi/Ashkenazi origin, the first child of healthy non-consanguineous parents. She was born at term at 2960 g for weight. She had normal tonus and no feeding difficulties, and gross motor development was normal. Yet, fine motor skills and language were delayed. She was evaluated several times and found to have mild developmental delay. There were neither sleeping problems nor seizures; vision and hearing are intact, except for strabismus. She has a normal neurological exam, and normal EEG. She did not have CT or MRI of the brain. She completed 12 years in small classes in the public education system, and is currently working in the public education system as an aid to a girl with special needs. She did not have hospital admissions and her general health is good. At age 26 she gave birth to a male with the same medical condition (P6b). She currently lives at her parents' home with her son and is rather independent. Focused physical examination revealed an overweight female with mild facial dysmorphism including small, hypoteloric eyes, and strabismus. Her son was born at term, via C-section at weight 3900 g. After birth a low muscle tonus with joint laxity were observed and motor milestones were mildly delayed. He has significant language delay and studies in small class at the public-school system. Gross neurological examination as well as vision and hearing were normal. He has similar facial features to his mother.

Proband 7 *p.(Ile355Serfs*48)*

The proband is a 3-year-old girl with developmental delay, hypotonia and ventriculomegaly. Prenatally, she was diagnosed with ventriculomegaly. The ventricles were noted to be enlarged at around 30 weeks of gestation by the prenatal ultrasound. Because of ventriculomegaly, she was born by C-section at 38 weeks of gestation. Her birth weight was 3020 g (-0.77 SD) and length was 48 cm (-0.81 SD). Head circumference at birth was 37cm +1.22 SD). Ventriculomegaly was confirmed postnatally by brain MRI, although surgical intervention was not recommended. In addition, delayed myelination for age was noted. She has experienced several episodes of ear infections, and head CT demonstrated bilateral cholesteatomas. She underwent bilateral tympanomastoidectomy, ear tube placement and adenoidectomy surgeries. She has been diagnosed with bilateral conductive hearing loss. She also has had hypermetropia/hyperopia since 12 months old.

Her development has been mildly delayed. She smiled responsively and laughed at 3 months. She started sitting independently at 8 months and walking independently at 19 months. Her speech and language development has also been delayed. Her first words were at 12 months. At 28 months, she had about 50 words but continued to have poor enunciation and mostly syllables that are interpreted by the context. No developmental regression was noted. The most recent MRI brain at 20 months revealed two small nodules of gray matter heterotopia along the frontal horns of the lateral ventricles. In addition, there were subtle irregularity and thickening along the posterior perisylvian cortex, right greater than left, which raises the possibility of polymicrogyria. Stable prominence of the lateral and third ventricles was demonstrated. No seizures have been observed. Chromosome SNP microarray was unremarkable. Family history is non-contributory.

She was referred to the genetics clinic at 33 months of age. Her height was +0.28 SD, weight was +0.20 SD, and head circumference was +4.72 SD. Her physical examination revealed macrocephaly, broad forehead, frontal bossing, midface hypoplasia, and thin upper lip. She was also noted to have low muscle tone.

Probands 8a and 8b *225kb Del*

The two sisters of 6 and 8 years are in foster care. Their biological mother has learning difficulties, but was not tested genetically. The elder sister (8b) had a cardiac murmur ascribed to mild supravalvular pulmonary stenosis that disappeared within two years. She had slight motor delay but marked language delay, and she fulfilled criteria for ASD at age 4. Cerebral MRI showed asymmetric cranium and a slightly larger left hemisphere. At age 4.5 she was put in foster care, and was then weaned off diapers, learned to run and her language improved quickly. At age 8 years, her cognitive level was tested and it was determined that she has learning disabilities, borderline to but not fulfilling criteria for mild intellectual disability, and she is able to read. She also has problems with attention and social interaction. At 8 years of age, her height is +0.56 SD and OFC is +2.71 SD. She has small ears with attached ear lobule and retrognathia. Both sisters have everted lower lip, small alae nasi and fetal fingerpads on thumbs. They both learned to ride a bike at age 5.5.

The younger sister (8a), of whom less childhood information is available, had a reactive attachment disorder that was obvious from early age. Her early development is said to be better than her elder sister. She had delayed speech. She had a systolic heart murmur, and a small muscular ventricular septal defect without haemodynamic significance was found. Motor development was mentioned as delayed at age 4. She has a pleasing personality and less social problems, and does not have autistic features. She has attention deficit and her cognitive levels are clearly delayed with learning disability, mild intellectual disability is not excluded and new assessment is planned. Her height at age 6 is +1.46 SD, and her OFC +3.35 SD.

Proband 9 *284kb Del*

The male proband was the first child of unrelated healthy Caucasian parents without family history. Pregnancy was marked by gestational diabetes and ultrasound monitoring showed hydramnios and decreased fetal movements. He was born at 37 weeks of gestation by caesarean section due to breech presentation, biometric parameters were 50 cm (-0.06 SD) for length, 3500 g (-0.1 SD) for weight, and 37 cm (+0.68 SD) for OFC. He presented a psychomotor delay with severe hypotonia: walking at 3 years of age, first words at 2 years of age, and first sentences at 5 years of age. On clinical examination at the age of 7 years, parameters were: height 131 cm (+1.7 SD), weight 22 kg (-0.41 SD), and OFC 55 cm (+2.17 SD). He presented mild intellectual deficiency (mostly slowness), sleep disturbance, chronic constipation, diffuse articular hyperlaxity, scoliosis treated by orthopaedic corset, and velvet skin. He needed special education at 6 years of age. He had minor facial dysmorphic features such as long and flat face, up-slanting palpebral fissures, malar hypoplasia, opened mouth, and high arched palate. Fragile X syndrome screening, brain MRI at age 2 years and a half, and 4 years and a half and heart ultrasounds were normal.

Proband 10a and 10b *312kb Del*

Proband 10a was first seen as an 11 year old girl with mild intellectual disability, a large head (59.5 cm; +6.01 SD), high forehead, anteverted nares and a broad nasal tip. No cause was identified. She had a history of histiocytosis in her skull; at age 7 years she was treated for this condition with radiotherapy and made a full recovery. She coped within the regular school system with extra help but was significantly less academic than her parents and siblings. She was reviewed at age 20 years. Further investigations were again undertaken but no cause found. She had brief employments in retail but finds routine work difficult. She re-presented at 25 years of age when she was 21 weeks pregnant.

Proband 10b was the daughter of proband 10a. She was born by normal delivery with a birth weight 3.4 kg (-0.05 SD) and OFC 37 cm (+1.22 SD). There were no neonatal problems and her early milestones were normal. At 3 months the baby strongly resembled her mother facially with anteverted nares and a high forehead. Her weight and length were on 50th centiles but OFC on 90th centile. She made normal progress in the first few months, she sat at six months, bottom shuffled at 13 months and walked at 22 months. At 2 years 9 months she is speaking in sentences. Her height remains on the 50th centile, her weight is on 91th centile but her OFC (55.5 cm) is +4.60 SD above the mean. She required surgery for bilateral inguinal hernias, ligation of a PDA and blocked tear ducts.

Proband 11 *1.5 Mb Del*

The 8 years 4 months old boy is the only child of non-consanguineous Caucasian parents. He was referred for delayed language and pervasive development disorder. His mother had a personal and familial history of breakdown. He was born eutrophic after an uneventful pregnancy, at 39 weeks of gestation. He had feeding difficulties during the neonatal period. Motor milestones were not delayed, the boy was able to walk at 15 months. By contrast, language was severely delayed, however, by 8 years old the proband was no longer impaired. He also had attention deficit and anxiety disorder. Electroencephalography showed no sign of seizure. At examination, dysmorphic features included down-slanting palpebral fissures, deep-set eyes, blepharophimosis, broad nasal tip, thin upper lip and small ears without lobule. The boy also had brachydactyly, finger pads and transverse palmar crease. Height corresponds to +0.62 SD, weight to -0.04 SD and OFC to +0.15 SD.

Proband 12 *1.6Mb Del*

The proband is a 32-year-old male. His biological family history is unknown because he was adopted at birth. At three months of age he presented with macrocephaly (OFC +4.46 SD) leading to a CT scan showing agenesis of the corpus callosum. An MRI done at 21 months of age confirmed the diagnosis of complete agenesis of the corpus callosum. A follow up MRI performed when the proband was 21 years old also showed complete ACC with associated parallel orientation of the lateral ventricles, colpocephaly, Probst bundles, and prominence of the third ventricle. Seizures were suspected at one point, but an extended EEG was unrevealing, and he had no recurrence. This proband was delayed developmentally; at 21 months he was walking on his knees and did not begin to walk on his feet until 28-30 months of age. He also had difficulties with balance. He received physical therapy up to age 12, and currently attends occupational, speech and language, and psychological therapy once a week. He has never had any problems with his vision or hearing. He is somewhat insensitive to pain and has dysregulated core temperature with associated hyperhydrosis. He has elevated blood pressure (140/90) for which he takes medication. He has diagnoses of ADHD and learning disability. He has also impulse control problems and trouble gauging emotion in others. He took Ritalin up to age 14, then Adderall 40 mg 1x/day. Other medications include Prozac 40 mg 1x/day and Depakote ER 500 mg 1x/day. Upon a neurological exam at 21 years of age, his upper and lower limb reflexes were abnormal as well as his extraocular movements. At age 21, his head circumference was 61.5 cm. At age 22, he had a full-scale IQ of 88, a Verbal IQ of 95 and a Performance IQ of 79.

Proband 13 *2.4Mb Del*

This 7 year 10 months old girl was the first of two children of a non-consanguineous, healthy couple with unremarkable family history. The girl was born after an uneventful pregnancy at 41 weeks of gestation with a length of 56 cm (+2.38 SD), weight of 4030 g (+1.32 SD), and an OFC of 37 cm (+1.22 SD). She was reported to have recurrent infections including several pneumonias and frequent otitis media during early childhood. Initial developmental milestones were reported normal, but speech delay became apparent at the age of 24 months. At the age of 3 years global developmental delay, intellectual disability and behavioral abnormalities (temper tantrums, aggression) were diagnosed. The proband's speech improved with speech therapy; receptive language skills appeared to be less delayed than expressive speech. She attended a school for special needs, and was reported to be friendly, sociable and diligent. At the age of 6 years 5 months, a global IQ of 45 was determined by formal testing using HAWIK. An MRI of the brain at the age of 3 years 8 months revealed complete agenesis of corpus callosum and slightly enlarged cerebral ventricles.

On clinical examination at the age of 7 years and 10 months, her height was 137 cm (+2.68 SD), her weight was 43.9 kg (+3.82 SD), and her head circumference was 57 cm (+4.39 SD). Slight facial anomalies were noted including down-slanting palpebral fissures, sparse lateral eyebrows, high and narrow nasal bridge, flat philtrum, thin upper and thick lower lip, small ears, and slightly retrognathia (Figure 1A). Moreover the proband showed an inverted nipple on the left side, long fingers and toes, flat feet, and bilateral sandal gaps. She had muscular hypotonia but no specific neurological deficits.

Proband 14 *4.3Mb Del*

The proband was a 17 year old female and the only child of non-consanguineous Caucasian parents. She had four maternal and paternal healthy half-siblings. She was born at term after an uneventful pregnancy; her birth weight was 3200 g (-0.58 SD). She was a slow feeder and had recurrent infections in infancy. She reached all early motor developmental milestones appropriately. However, her speech development was delayed and she also had articulation difficulties. Bilateral mid-ear effusions were treated with grommets, and she also had a tonsillectomy. At the age of 5 years she developed bilateral esotropia, which was surgically corrected on three occasions. She was also prescribed glasses for hypermetropia. She had mild learning difficulties and an attention deficit, and needed some extra support in a mainstream school. She displayed episodes of challenging behavior. When assessed at the age of 8 years and 11 months, her height and weight were on the 25th centile and her OFC on the 75th centile. She had bilateral esotropia, significant epicanthic folds, short narrow palpebral fissures and sparse lateral eyebrows (Figure 1A). Her ears were small with flat upper helices and the right was low set. She had a small nose, narrow nasal bridge, a small mouth and an everted lower lip. Her hands showed mild radial deviation of the 4th distal phalanges, broad thumbs and fetal finger pads. Her 2nd toes were longer than her halluces, and she had very short 4th and 5th metatarsals. There was mild hypertrichosis on her lower back and lower legs. Her abdomen was distended, but there was no evidence of organomegaly. Muscular tone was reduced. At a subsequent assessment at the age of 16 years and 11 months she was post pubertal. Her height was on the 75th centile, and her weight and OFC were between the 50th and 75th centile. Additional features included mild shortening of the 4th and 5th metacarpals. She also had a systolic murmur on cardiac auscultation. An echocardiogram revealed a bicuspid aortic valve and mild tricuspid valve regurgitation. The distal limb anomalies were similarly present in her mother who was otherwise healthy and probably represent a familial form of Brachydactyly Type E.

Proband 15 *4.9 Mb Del*

The proband was the second child of healthy, non-consanguineous Caucasian parents. His birth weight was 3200 g at a gestational age of 40 weeks. He had feeding difficulties and had crying spells for several months. A developmental delay became apparent at the age of 9 months due to little social interaction and a mild delay in motor development. He had trigonocephaly with surgical correction at the age of 1 year. Speech development was severely delayed until the age of 5, despite intensive speech therapy. He suffered from recurrent ear infections and had unilateral cryptorchidism. At the age of 4 years old he was diagnosed with ASD by an expert multidisciplinary team. He had auditory sensitivity, was very anxious and had a psychotic episode at the age of 8 years old. He had a mild developmental delay, a friendly personality with an impairment of social interaction and very little eye contact. Clinical examination at the age of 10 shows facial dysmorphism with short and narrow palpebral fissures, sparse eyebrows and epicanthic folds. He has a long philtrum, a high narrow palate and small low set ears. He had a distended abdomen with weak abdominal muscles and normal body proportions.

Fetal case *p.(Ser356Leu)*

This case is a 29-gestational week male fetus of non-consanguineous parents. His mother has a manic-depressive illness. The pregnancy was obtained by insemination. At the first trimester, ultrasound revealed cervical hygroma. The karyotype performed on chorionic villi was normal (46, XY), as well as early echocardiography. Following ultrasounds showed an incomplete regression of the nuchal hygroma. At 22 weeks of gestation short corpus callosum, pulmonary sequestration and facial dysmorphism were noted. A CGH array was performed on amniotic fluid sample and revealed no genomic alteration. After genetic counselling, the couple asked for termination of pregnancy that was performed at 29 weeks according with the French Law. Fetal examination showed a male fetus with growth parameters at the high limits of standards for weight (95^{th} centile) and head circumference (90^{th} centile), and normal height (50^{th} centile). Facial dysmorphism included high and narrow forehead, down-slanting palpebral fissures, synophris, and hypertrichosis of face and upper chest. He had low set thumbs, broad halluces and hypoplastic nails. Autopsy showed pulmonary sequestration with two pulmonary lobes on both side, and revealed an auricular septal defect. Neuropathological examination showed a brain weight at 95^{th} centile and a short but thick corpus callosum with no other brain malformation.

Supplemental Figures

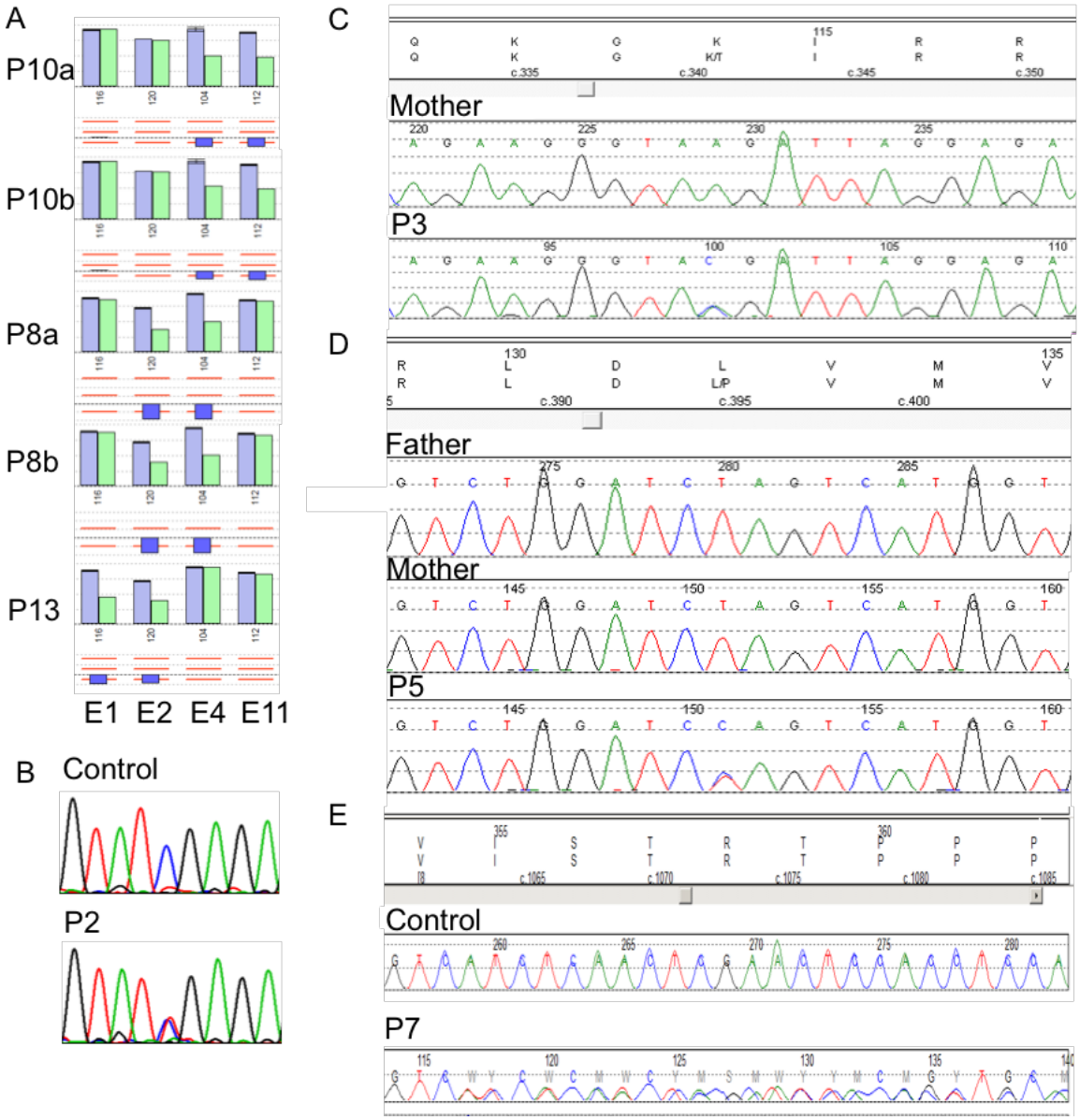

Figure S1 Additional information for *NFIB* deletion and sequence variation

(A) MLPA results for individuals 10a (mother) and 10b (daughter) showing a heterozygous deletion of exons 4 and 11 indicating the intragenic deletion breakpoint between exons 2 and 4. The intragenic deletion in individuals 8a and 8b was validated by MLPA analysis showing a heterozygous deletion of exons 2 and 4 but not of exons 1 and 11. MLPA result for individual 13 showing a heterozygous deletion of exons 1 and 2 also indicates the intragenic deletion breakpoint between exons 2 and 4. (B) Electropherogram demonstrating the heterozygous nucleotide exchange c.265C>T in exon 2 of the *NFIB* gene in individual P2, but not in a control individual. (C) Electropherogram demonstrating the heterozygous nucleotide exchange c.341A>C in exon 3 of the *NFIB* gene in P3, but not in his mother. (D) Electropherogram demonstrating the heterozygous nucleotide exchange c.376A>G in exon 5 of *NFIB* in individual P5 but not in his parents. (E) Electropherogram demonstrating the heterozygous nucleotide change c.1063_1076del in exon 8 in individual P7, but not in a control individual.

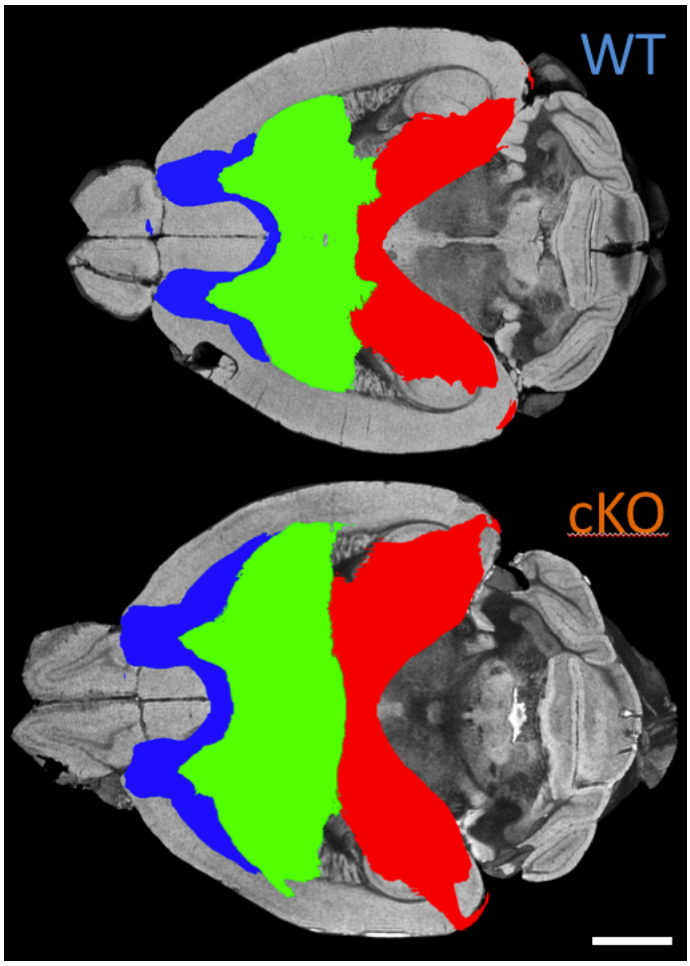

Figure S2 Additional information for tractography in mouse model.

Streamline density maps depicting the interhemispheric connectivity across the corpus callosum in an exemplar wildtype (WT; top) and dorsal telencephalon specific *Nfib* conditional knockout (cKO; bottom) mouse brain. The maps were derived from a probabilistic (iFOD2) diffusion fiber tractogram, seeded in the genu (green), body (blue) and splenium (red) of segments of the corpus callosum, respectively. Scale bar is 3 mm.

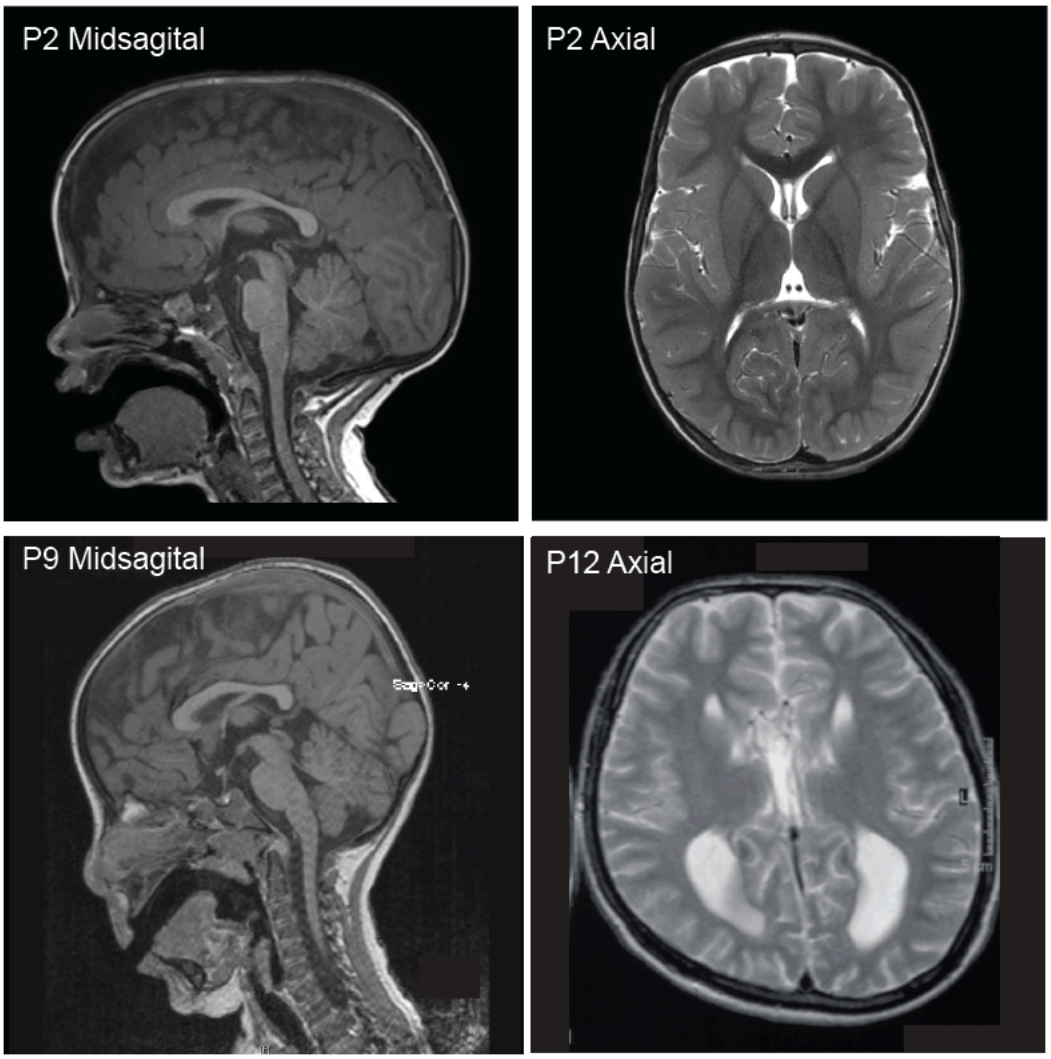

Figure S3 Additional brain imaging of individuals.

Midsagital and axial MRI images of P2, P9 and P12.

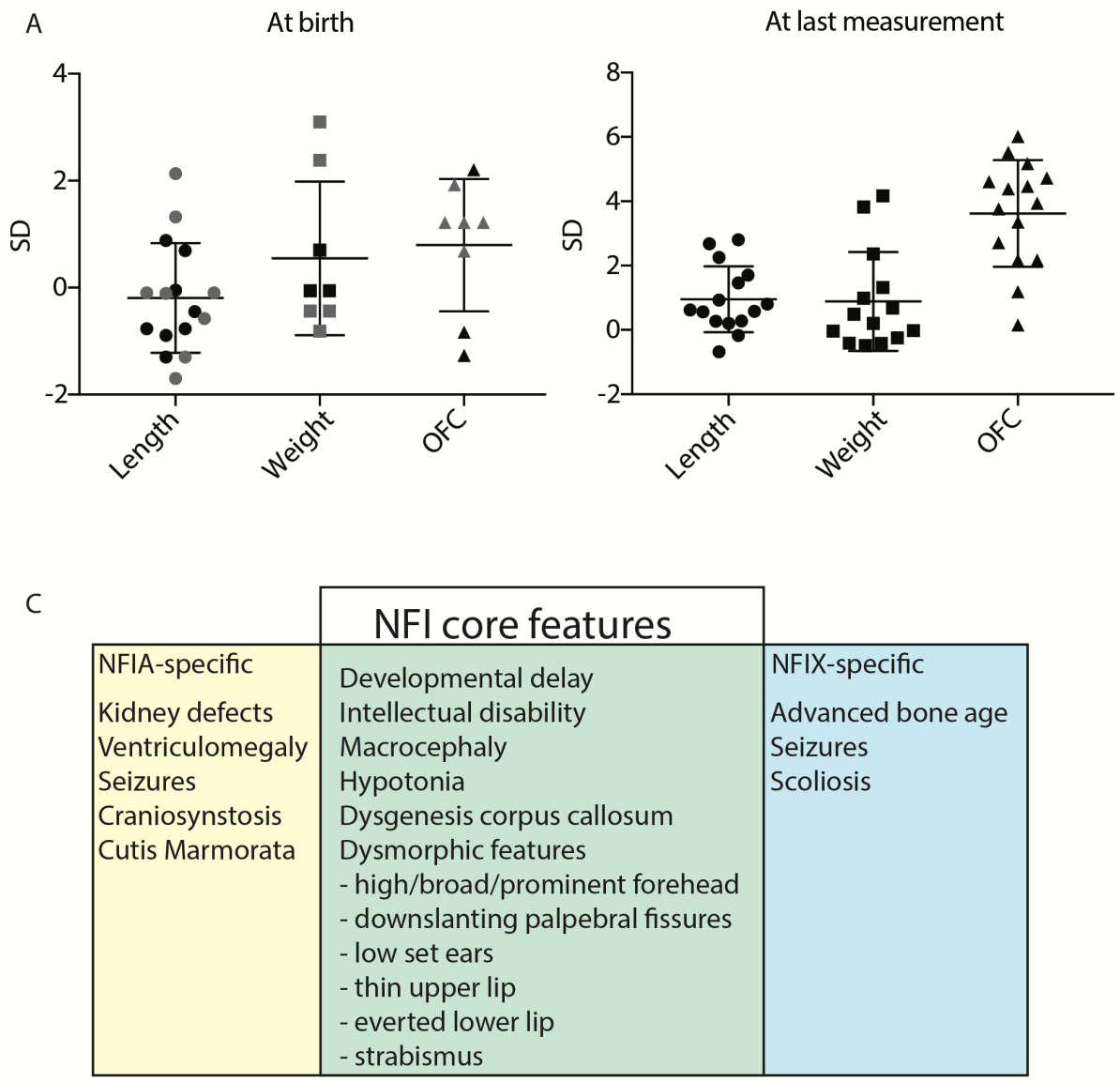

Figure S4 Clinical characteristics.

Dotplot representing the standard deviation (SD) of the growth parameters at birth (A) and at last measurement (B) based on information represented in Table 1. Grey dots represents individuals born prior to term or without this information available. (C) Schematic representation of the overlapping and distinct features of individuals with *NFIA*, *NFIB* or *NFIX* heterozygous sequence variants or deletions. Features in italic are reported in less than half the cases in literature.

Table S1: Primers used for cloning mutation

mutNFI_clon_F	TGCTGGTTATTGTGCTGTCTCA
mutNFI_clon_R	GGGGCGGAATTTACGTAGC
mutNFIB_K114T_F	GACCAGAAGGGTACGATTAGGAGGATCG
mutNFIB_K114T_R	CGATCCTCCTAATCGTACCCTTCTGGTC
mutNFIB_K126E_F	GACAGGCAGACGAAGTCTGGCGTCTGGATC
mutNFIB_K126E_R	GATCCAGACGCCAGACTTCGTCTGCCTGTC
mutNFIB_L132P_F	CAGGATCACCATGACTTGATCCAGACGCCAGAC
mutNFIB_L132P_R	GTCTGGCGTCTGGATCAAGTCATGGTGATCCTG
mutNFIB_S356L_F	CGCACAGTGTCATCTTAACTCGAACTCCAC
mutNFIB_S356L_R	GTGGAGTTCGAGTTAAGATGACACTGTGCG

Table S2: *In silico* predictions of consequence of NFIB sequence variants

Amino acid change	**p.(Arg37*)**	**p.(Arg89*)**	**p.(Lys114Thr)**	**p.(Lys126Glu)**	**p.(Leu132Pro)**	**p.(Asn254*)**	**p.(Ile355Serfs*48)**	**p.(Ser356Leu)**
HG19 chr9 position	14307441	14307285	14307210	14307174	14307155	14150191	14120603-14120622	14120617
Nucleotide change	G->A	G->A	T->G	T->C	A->G	C->CAC	del ATCTCAACTC GAACTCCACC	G->A
PredictSNP2	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious	NA	NA	Deleterious
expected accuracy	0.77	0.93	0.87	0.87	0.87	NA	NA	0.87
CADD	Deleterious	Deleterious	Neutral	Neutral	Neutral	NA	NA	Deleterious
expected accuracy	0.6	0.56	0.56	0.61	0.56	NA	NA	0.8
DANN	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious	NA	NA	Deleterious
expected accuracy	0.66	0.87	0.6	0.71	0.7	NA	NA	0.77
FATHMM	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious	NA	NA	Deleterious
expected accuracy	0.64	0.95	0.62	0.6	0.65	NA	NA	0.83
FunSeq2	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious	NA	NA	Deleterious
expected accuracy	0.65	0.93	0.61	0.61	0.61	NA	NA	0.61
PolyPhen-2 HumDiv	NA	NA	Probably damaging	Probably damaging	Probably damaging	NA	NA	Probably damaging
probability	NA	NA	0.991	0.979	0.997	NA	NA	0.985
PolyPhen-2 HumVar	NA	NA	Probably damaging	Probably damaging	Probably damaging	NA	NA	Probably damaging
probability	NA	NA	0.988	0.982	0.996	NA	NA	0.966
Mutationtaster	Disease causing	Disease causing	Disease causing	Disease causing	Disease causing	Disease causing	Disease causing	Disease causing
PROVEAN	NA	NA	Deleterious	Deleterious	Deleterious	NA	NA	Neutral
SIFT	NA	NA	Damaging	Damaging	Damaging	NA	NA	Damaging

Supplemental Methods

MR image acquisition

Magnetic resonance (MR) images were acquired for both WT (n = 3) and NFIB conditional knockout (n = 4) mouse brains at P110. The MR imaging was conducted using a 16.4 Tesla vertical bore, small animal MRI system (Bruker Biospin, Rheinstetten, Germany; ParaVision v5.0) which was equipped with Micro 2.5 imaging gradient and a 15 mm linear, surface acoustic wave coil (M2M, Brisbane, Australia). The protocol included both fast low-angle shot (FLASH) and diffusion-weighted spin-echo magnetic resonance imaging (dMRI). Prior to MR imaging, each brain was immersed in a proton-free oil, Fomblin Y-VLAC (Y06/6 grade, Solvay, USA); oxygen was actively removed from each sample via vacuum pumping.

Parameters for the FLASH pulse sequence: MTX 654 × 380 × 280, FOV 19.6 × 11.4 × 8.4 mm (0.03 mm isotropic voxels), TR/TE 50/12 ms, flip angle of 30°. No image averaging was used. Acquisition time was 0.7 hours per brain. Parameters for the dMRI pulse sequence: MTX 196 × 114 × 84, FOV 19.6 × 11.4 × 8.4 mm (0.10 mm isotropic voxels), TR/TE 200/23 ms, δ/Δ 2.5/12 ms. Two B0 (b-value = 0 s mm^{-2}) images and 30 diffusion-weighted (b-value = 5000 s mm^{-2}) volumes were acquired for each brain. The 30 diffusion-gradient directions used were evenly distributed over a hemisphere using the electrostatic repulsion method. No image averaging was used. Acquisition time was 15.5 hours per brain.

MR image processing was performed on a high-performance computing cluster with 1600 CPU cores and 8TB of RAM housed at the Queensland Brain Institute. Prior to processing, the diffusion-weighted MR images were de-noised and corrected for bias field inhomogeneity using tools provided by MRtrix (v 3.0; www.nitrc.org/projects/mrtrix). Scalar diffusion metric images were generated via weighted least-squares diffusion tensor estimation, while fibre orientation distribution functions (fODFs) was estimated with MRtrix via a constrained spherical deconvolution (CSD) model using the default pre-processing parameters (maximum spherical harmonic order lmax = 6). The response functions required to generate the fODFs were estimated using an iterative algorithm for single-fiber voxel selection.[81]

Brain measurements

Brain orientation was standardized between mice using an in-house script (MATLAB R2014a, Mathworks, California) to rotate the images to minimize the yaw and roll of each brain relative to the midline fissure. The pitch of each brain was then manually adjusted to ensure that an axial plane would intersect the center of the anterior commissure and the caudal tip of lobule IX of the cerebellar vermis. Measurements were made in either the midsagittal plane, or the coronal plane at Bregma level -2.18 mm.

These measurements included the cortex length and area in the midsagittal plane, and the cortex height in the coronal plane as represented in Figure 4D. The cortex length and area were normalized using the distance between the rostral tip of the cortex (the frontal pole) and the caudal tip of lobule IX of the cerebellar vermis in the midsagittal plane; the cortex height was normalized using the vertical distance between the dorsal edge of the cortex and the ventral edge of the hypothamalus.

Tractography

CSD-based probabilistic tractography, utilizing the iFOD2 algorithm, was performed on each brain.[55] The tractograms consisted of 200 thousand streamlines, and were created using the following parameters: maximum number of attempts = 1 million, fODF cut-off = 0.1, step size = 50 μm, maximum streamline length = 0.5/40 mm. The tractograms were seeded in either the midsagittal anterior commissure, or the midsagittal corpus callosum; the seed regions were contralaterally paired with inclusion regions (offset by several voxels on either side of the midline) to ensure streamlines connected the brain hemispheres. The seed regions were conservative in size in order to avoid seeding streamlines in the surrounding grey matter. Homotopic streamlines connecting the anterior cingulate cortices were also isolated in the corpus callosum tractograms, as was conducted in a previous study.[54]

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