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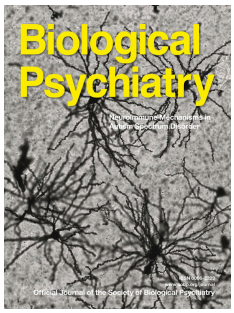

Clinical presentation of a complex neurodevelopmental disorder caused by mutations in *ADNP*

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Abstract

Background: In genome-wide screening studies for *de novo* mutations underlying autism and intellectual disability, mutations in the *ADNP* gene are consistently reported amongst the most frequent. *ADNP* mutations have been identified in children with autism spectrum disorder co-morbid with intellectual disability, facial features and deficits in multiple organ systems. However, a comprehensive clinical description of the Helsmoortel-Van der Aa syndrome is lacking.

Methods: We identified a worldwide cohort of 78 individuals with likely disruptive mutations in *ADNP* from January 2014 to October 2016 through systematic literature search, by contacting collaborators, and through direct interaction with parents. Clinicians filled in a structured questionnaire on genetic and clinical findings to enable genotype-phenotype correlations. Clinical photographs and specialist reports were gathered. Parents were interviewed to complement the written questionnaires.

Results: We report on the detailed clinical characterization of a large cohort of individuals with an *ADNP* mutation and demonstrate a distinctive combination of clinical features, including mild to severe intellectual disability, autism, severe speech and motor delay and common facial characteristics. Brain abnormalities, behavioral problems, sleep disturbance, epilepsy, hypotonia, visual problems, congenital heart defects, gastrointestinal problems, short stature and hormonal deficiencies are common co-morbidities. Strikingly, individuals with the recurrent p.Tyr719* mutation were more severely affected.

Conclusions: This overview defines the full clinical spectrum of individuals with *ADNP* mutations, a specific autism subtype. We show that individuals with mutations in *ADNP* have many overlapping clinical features, distinctive from other autism/ID syndromes. In addition, our data show preliminary evidence of a genotype-phenotype correlation.

Keywords: Genetics; autism; intellectual disability; neurodevelopmental disorder; ADNP; Helsmoortel-Van der Aa syndrome

Short title: Clinical consequences of *ADNP* mutations

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Introduction

Autism Spectrum Disorder (ASD) is a condition defined by deficits in social interaction, communication and selected behaviors (1). Each aspect of the disorder may vary in presentation, range and severity, cumulating in a broad clinical spectrum. The frequency of the disorder is under continuous debate, but may affect up to 1.5% of the population (2). Although a genetic contribution to its etiology has been firmly demonstrated (3), it took the introduction of trio-based whole exome sequencing (WES) to truly accelerate substantially the identification of ASD genes. In these studies, individuals are screened along with their parents, enabling the unbiased detection of *de novo* mutations in large ASD cohorts (4-6). These initiatives are complemented by targeted resequencing of larger cohorts (7). Studies in ASD cohorts co-morbid with intellectual disability (ID) collectively demonstrate an unprecedented genetic heterogeneity of ASD, with no single gene responsible for more than a fraction of the total population. Several of the identified genes appear to cluster in a subset of cellular networks, including networks enriched for chromatin remodeling and synaptic functioning (5, 8). Overlap between ASD genes and genes causative for other neurodevelopmental disorders, including ID and seizures, is common (9, 10).

Despite the high heterogeneity and observed molecular overlap, there is preliminary evidence for the existence of clinical ASD subtypes. For instance, mutations in the chromatin remodeler *CHD8* cause an ASD/ID subtype with specific physical characteristics, such as macrocephaly and significant gastrointestinal problems (11, 12). In contrast, individuals with a mutation in *DYRK1A*, a gene duplicated in Down syndrome, have ASD/ID, microcephaly, intrauterine growth retardation, febrile seizures in infancy, impaired speech, stereotypic behavior, hypertonia and a distinctive facial gestalt (13). Yet, the clinical delineation of ASD/ID syndromes has lagged behind their respective molecular definition. Since possible future treatment may be based upon targeting the underlying molecular defect rather than on

the basis of the clinical presentation, it is of primary importance to define autism subtypes correctly at the molecular level (14).

ADNP was one of the most frequently mutated genes across multiple recent WES and targeted molecular inversion probe (MIPs) sequencing studies in ASD/ID cohorts (6, 7). The *ADNP* gene plays a role in embryonic development, especially during the time of neuronal tube closure and is involved in chromatin remodeling (15-18). Based on the first ten individuals identified with *ADNP*-related ASD/ID, *ADNP* mutations were estimated to explain 1-2/1000 ASD/ID cases and some shared clinical features were suggested (19). Since that time, a number of case reports have expanded the phenotype of the Helsmoortel-Van der Aa syndrome (OMIM 615873) (20-23). Here, we describe the clinical details of a cohort of 78 individuals from 16 countries with a likely disruptive mutation in *ADNP*. We herewith define a novel subtype of ASD/ID and at the same time, present evidence for a significant genotype-phenotype correlation.

Methods and Materials

Participants

The study was performed at the University of Antwerp, Belgium. Individuals were identified through exome sequencing in our own center or gathered from genetic centers worldwide offering exome-wide or targeted genetic screening in a clinical or a research setting. Additional individuals were collected on the website http://humandiseasegenes.com/adnp/. A minority of the individuals were previously described in case reports as cited above (19-23). All individuals were enrolled between Jan 1, 2014 and Oct 1, 2016. Inclusion required a clinical geneticist-confirmed diagnosis of a nonsense or frameshift mutation in the *ADNP* gene and presence of clinical information in at least 3 domains, including demographics,

development, craniofacial features and behavior. Essentially all mutations were identified by next generation sequencing of individuals with autism and/or developmental delay often in combination with additional syndromic features. In part the *ADNP* mutations were identified in individuals in preassembled ID/autism cohorts that were subjected to trio based whole exome sequencing or targeted MIP sequencing as described in Helsmoortel *et al.* (19). The remainder of our cohort was assembled from the individuals in whom an *ADNP* mutation was diagnosed after genetic testing using either neurodevelopmental gene panels or trio based whole exome sequencing. After the identification of a causative *ADNP* mutation in an individual, their clinical geneticist asked for consent to be included in this study. In each case the mutation that was identified using next generation sequencing was independently verified using Sanger sequencing either in our own or in the referring laboratory. Individuals carrying a missense mutation in *ADNP* were excluded from this study. All gene annotations have been made according to NM_015339.2 (hg19). Approval for this study was obtained from the Ethics Committee of the Antwerp University Hospital. Pictures were only published if the parents provided written informed consent on behalf of their child.

Procedures

Collaborating physicians were asked to fill out an extensive questionnaire with clinical and molecular information about the individuals they had identified and assessed. We specifically asked for the results of the test the individuals had been subjected to, including but not limited to IQ-test and ADOS test. Medical specialist reports and MRI data were collected and systematically re-evaluated in order to refine the interpretation of the findings. In order to compare the data that were collected in various parts of the world, not in all cases using the same tests and terminology, we curated all incoming data and recontacted the collaborating clinicians to harmonize the medical information. The *ADNP*kids Facebook community (24)

helped us contact clinicians and parents, in order to complete and verify the details of the clinical information.

Statistical Analysis

Associations between reported clinical features were systematically tested in a pair-wise analysis using one-way ANOVA, Pearson correlation and Fisher Exact tests, depending on the nature of the variables. A listing of all 170 variables included in our analysis is provided in Table S1. For one-way ANOVA, features for which only a single level was available, were excluded. If ANOVA resulted in significant results ($p<0.05$), post-hoc TukeyHSD testing was applied to identify significant differences in mean. For Fisher Exact tests, a minimal of two levels per tested category, and at least 10 records per tested condition were required. In case either category contains three or more levels, p-values were calculated using Monte-Carlo simulation using 10,000 replicates. Association between demographic features, including gender and age and clinical features was analyzed similarly. Additionally, we evaluated the presence of genotype-phenotype correlations. First, the three most frequent mutations were analyzed separately: p.Tyr719* (17 individuals), p.Leu831Ilefs*82/p.Asn832Lysfs*81 (14 individuals) and p.Arg730* (5 individuals). Subsequently, mutations were grouped according to gene location: in the N-terminus (25 individuals), at the center of the gene (49 individuals) and in the C-terminus (4 individuals). Finally, we analyzed mutations per domain. For each analysis, prevalence or extent of all individual clinical features was compared between the selected sub-cohort and the remaining individuals. Multiple testing correction was performed via the false discovery rate (FDR) method (Qvalue add-on package in R, version 2.6.0 (25)). All calculations were carried out in the software package in R (version 3.3.1 (26)). Significant correlations are indicated at the appropriate results section.

Results

We included 78 individuals with a disruptive mutation in *ADNP*, including 44 males and 34 females (Figs. 1,2). The mean age of our cohort is eight years and two months, with a range of 1-40 years. Individuals were from 44 clinics in 16 countries. Parental consanguinity was not reported and no siblings were diagnosed with a mutation in *ADNP*. Five individuals have non-identical healthy twin siblings. We found 46 unique mutations on the DNA level, of which 25 were nonsense and 21 frameshift (Supplemental Table S2). All but three mutations were located in the fifth and last exon of the *ADNP* gene and predicted to escape nonsense mediated decay. On the protein level, three mutations were present in five or more individuals, including the p.Tyr719* mutation. Sixty-eight mutations in our cohort were confirmed *de novo*, eight mutations were of unknown inheritance and two C-terminal mutations were inherited.

Pre- and perinatal observations and congenital abnormalities

Most children were born at term (mean gestational age 38.7 weeks, range 30-42 weeks). Mean maternal and paternal age at birth is 30 and 32 years, respectively. Intra-uterine growth retardation was not reported. Overall, birth weight, height and head circumference were within normal ranges (Supplemental Table S3, Supplemental Fig. S1-A-C).

Six individuals (12.5%) were born with renal anomalies (narrow ureters, bilateral vesico-ureteral reflux which was surgically repaired; Table 1). Reported hand and feet abnormalities were nonspecific, including fetal finger pads, clinodactyly, small fifth fingers, brachydactyly, single palmar crease, sandal gap, pes planus, long or broad halluces and syndactyly of the 2nd and 3rd toe. Twenty-five percent had nail abnormalities such as thin or small nails, or hypoplastic nails of the fifth digit. Some had widely spaced nipples, pectus excavatum, pectus carinatum or combined excavatum/carinatum deformity. One child had a submucous cleft

palate. Two of the children were born with metopic craniosynostosis, and one of them needed surgery. Six children had plagiocephaly, of whom three wore a cranial molding helmet.

Failure to thrive in early childhood was noted in a number of individuals. Some of them appeared to have severe cardiac problems, requiring open heart surgery. Thirty-eight percent had one or more congenital cardiac defects. These were diverse: atrial septal defect, patent ductus arteriosus, patent foramen ovale, mitral valve prolapse, ventricular septal defect and other cardiovascular malformations such as a right aortic arch, dysplastic aortic valve, tetralogy of Fallot, ductus arteriosus aneurysm, quadricuspidal aortic valve, aortic ectasia and a mild pulmonary valve stenosis were found. (Fig. 3-A)

Facial appearance

Individuals shared similar facial features, including a prominent forehead with a high anterior hairline, a wide and depressed nasal bridge, and a short nose with full, upturned nasal tip (Fig. 4, Supplemental Table S4). One third of the individuals had downslanted palpebral fissures and prominent eyelashes. Ear malformations were observed in nearly half of individuals. Abnormalities included small or dysplastic, low-set and posteriorly rotated ears. The philtrum was long in 39.3% of study cohort. Seventy percent of individuals had a thin upper lip, often combined with an everted lower lip and a pointed chin that appears more pronounced at younger age (Fig. 5). One third have widely spaced teeth.

Growth and endocrine system

Twenty-three percent of the individuals have short stature (height <-2SD, range 2-23 years old; Supplemental Table S3, Supplemental Fig. S1-E). Nine individuals had hormonal deficiencies (Table 1). Two of these had isolated growth hormone deficiency, four had hypothyroidism and three a combination of both hormonal deficiencies. One 29-year-old woman had a narrow thorax with breast hypoplasia. Signs of early puberty were present in

three of ten individuals older than six for whom information was available; one boy and one girl had pubic hair growth at the age of seven and eight years, and one girl had menarche at eight years of age.

Development and neurology

Fifty-two percent of the individuals in this cohort present with severe intellectual disability at the age of assessment, 36% have a moderate disability and 12% have a mild disability. Developmental delay is present in all individuals, with motor delay being one of the key features. The average age to sit up independently is 12.8 months (cohort range 6 to 60 months, normal range 4 to 9 months (27), Supplemental Fig. S2-A). Delayed age of walking independently (after 18 months of age (27), Supplemental Fig. S2-B) was observed in 86.8% of the children, with average age of 2 years and 5.5 months (cohort range 15 to 72 months).

Interestingly, individuals with a p.Tyr719* mutation start walking at 3.5 years, significantly later than the 2 years and 2 months of the remainder of the cohort ($p<0.0001$, One Way Anova). Seventy-eight percent of the children had hypotonia, while hypertonia was present in three children. Standing unassisted for long periods of time or walking long distances is difficult for many of the children. The walking pattern can be abnormal, e.g., broad-based or tip-toe gait, foot slap. Six children learned walk with support between the ages of 5.5 and 8 years, after many years of physiotherapy. A minority were not able to walk at the time of last evaluation.

Another key feature is speech delay which presents in 98.6% of individuals. Mean age of first words was 30 months (cohort range 7 to 72 months, as opposed to a normal range of 12 to 18 months, Supplemental Fig. S2-C). Nineteen percent have no language development at all. Apparent loss of acquired abilities has been reported in 12 children for skills like speaking, counting, riding a bicycle or being toilet trained. Eighty-one percent of the children have a

considerable delay in bladder training and many are still not toilet-trained when approaching puberty.

Sixteen percent have seizures, including absence seizures, focal seizures with reduced awareness, epilepsy with Continuous Spike and Waves during Slow Wave Sleep (CSWS), or unclassified seizures. At least five children are reported with breath holding spells. Some of them were hospitalized for multiple cyanotic episodes causing an acute life-threatening event.

Autistic features, behavior and sleep

Ninety-three percent of the individuals present with autistic features (Fig. 3-B). Sixty-seven percent of them have been reported to have a clinical diagnosis of ASD. They have a strong sensory interest illustrated by putting fingers or objects in their mouth, or being attracted to lights or water. Repetitive use of objects, hand and finger mannerisms, stereotyped movements like rocking back and forth or hand flapping are common. Some present with echolalia. Sixty-seven percent have also been diagnosed with sensory processing disorder. A high pain threshold is reported in 63.6% of individuals. Interestingly, all individuals with a p.Tyr719* mutation are included in this group ($p=0.0003$, Fisher Exact Test).

Although parents report that 88% of the children are overall happy and friendly, behavioral problems are reported in 77.6% of them. Several present with obsessive compulsive behavior, mood disorder, a high anxiety level, temper tantrums, self-injurious and (verbally) aggressive behavior. Forty-four percent of the individuals are hyperactive or easily distracted. About one third of them have a diagnosis of Attention Deficit Hyperactivity Disorder (ADHD). Several individuals take behavior-regulating medication like methylphenidate or atypical antipsychotics like risperidone or olanzapine to help control behavioral disturbances, particularly aggression.

Sleep problems are present in 65.2%. Some of them are extremely anxious, with struggles falling asleep and frequent night-time awakenings. Some were treated with melatonin. Many individuals have a low daytime activity level or excessive daytime sleepiness; a minority have sleep apnea.

Cerebral imaging

In this cohort, MRI of the brain was performed in 75.6% of the individuals. Fifty-six percent of them appeared to have cerebral abnormalities, including atypical white matter lesions, delayed myelination, cortical dysplasia or atrophy, perinatal hypoxic ischemic encephalopathy, hydrocephalus, and hippocampal hypoplasticity (Fig. 3-C).

MRI images of five individuals were studied in detail. The following abnormalities were seen in multiple individuals: underdevelopment of the frontal lobes with simplified gyral pattern of the cortex and occasional hypoplasia of the bulbus olfactorius and chiasma opticum; a thin and/or short, underdeveloped corpus callosum and inferior vermis hypoplasia; abnormal, often asymmetric opercularization of the Sylvian fissure with sometimes abnormal overlying cortex; dilatation of the lateral ventricles, mostly in the frontal areas and dilated perivascular spaces of Virchow-Robin in the cerebral white matter (Fig. 6).

Gastrointestinal problems

Eighty-three percent of the individuals have feeding or gastrointestinal problems, mainly gastro-esophageal reflux, frequent vomiting and constipation (Fig. 3-D). A few have excessive appetite. At the age of assessment, 20.9% of the individuals were overweight and 7.5% were obese, according to standard WHO classification (28). Two individuals have Crohn's disease, one of them with a positive familial history. Oral movement problems, with implications for feeding and speech, are common (45.6%), and significantly more common in individuals with mutations in the NLS and C-terminal of this domain (p=0.0004, Fisher Exact

Test). Problems drinking liquids or aspiration difficulties were frequent. Eight individuals were fed by gastrostomy tube (G-tube) in early childhood. The individuals suffering from gastrointestinal problems present more often with sleep disturbances (p= 0.0005, Fisher Exact Test).

Visual problems

Visual problems were present in 73.6% of the individuals, especially hypermetropia (40.3%) and strabismus (49.2%), but also myopia and astigmatism (Fig. 3-E). Many of them are prescribed glasses. Forty-one percent of the individuals have a diagnosis of cerebral visual impairment (CVI). Ophtalmologic defects are diverse: ectropion, coloboma, congenital cataracts, nystagmus. Some have an everted or notched eyelid, or mild ptosis, the latter particularly in individuals with mutations in the NLS and C-terminal of this domain (p=0.0004, Fisher Exact Test).

Additional problems

Musculoskeletal problems were common (Fig. 3-F). In addition to joint hypermobility, mild scoliosis was present in some individuals. Four had hip problems. Thirty-four percent of the males had unilateral or bilateral cryptorchidism; two had bilateral inguinal hernias. Fifty-one percent of the individuals have recurrent infections. Many of the children experienced chronic otitis media requiring ventilation tubes. Some of them (11.7%) were diagnosed with mild hearing loss in childhood. Two children have hearing aids for sensorineural hearing loss. Ear-Nose-Throat problems, including narrow ear canals, laryngomalacia and sleep apnea, were present in 32.1% of the individuals.

Discussion

Individuals with mutations in *ADNP* present with mild to severe intellectual disability, autistic features and a delay in language and motor development (Table 1). In addition, the syndrome

may be accompanied by a wide range of medical conditions, including very frequent (>75%) gastrointestinal and feeding problems, hypotonia, and behavioral disturbances. Frequent co-morbidities (50-75%) include visual problems, brain malformations, sleep disturbances, hand/foot and musculoskeletal abnormalities, and frequent infections. Common (25-50%) associated features include congenital heart disease, otorhinolaryngologic problems and urogenital defects. Up to 25% of individuals have hormonal deficiencies, short stature or seizures. The clinical symptoms of Helsmoortel-Van der Aa syndrome show partial overlap with other genetic syndromes that include developmental delay and ASD, as evidenced by genetic testing of our cohort for disorders like Angelman, Prader-Willi, Kleefstra, Smith-Magenis or Rett syndromes prior to the diagnosis of an ADNP mutation. As we not have access to the full clinical data of all individuals in the screening cohorts from which our cohort was assembled, we cannot determine to what extent a possible ascertainment bias has influenced the clinical presentation of the syndrome.

A striking element is the presence of mutational hot spots. The p.Tyr719*, p.Leu831Ilefs*82/p.Asn832Lysfs*81 and p.Arg730* mutation each occurred independently in at least five individuals. Interestingly, we found evidence for a genotype-phenotype correlation. We noticed for instance that individuals with a p.Tyr719* mutation walked later and have a higher pain threshold than the individuals with other mutations. Individuals with mutations in and C-terminal of the NLS domain more often had ptosis or oral movement problems than individuals with mutation elsewhere in the gene. Our findings encourage further investigations on larger study cohorts to unveil possible additional genotype-phenotype correlations. We did not find any evidence for gender, IQ-level or age-specific correlations.

Social media is increasingly used by parents to connect with each other and with scientists. This has certainly been the case for this syndrome (24). These interactions helped us to collect

genetic and clinical information and the parent's experiences providing us with important new insights into symptoms, daily struggles and challenges. While consensus has to grow what level of evidence is required to include parental observations of this type in a scientific publication, some of these hypotheses have been successfully tested in follow-up studies. As an example, the recently reported early teething in individuals with an *ADNP* mutation started as a parental observation (29).

Through a careful and structured comparison of the clinical symptoms of 78 individuals with a mutation in the *ADNP* gene, we delineated the clinical presentation of this specific subtype of autism. Our synthesis is indispensable in the decision-making process for caretakers and relatives. Moreover, it will significantly improve the interpretation of the clinical relevance of novel rare variants in the gene. The main limitation of our study is the relative young age of our study cohort. Long-term follow-up studies are necessary to define the developmental path of individuals with a mutation in *ADNP*. While to date most cases have been found on a genotype-first basis, a specific combination of features like ID, ASD, speech and motor delay and additional problems may emerge to screening for *ADNP* mutations in cohorts including older individuals. Finally, this clinical delineation can be used to monitor effects of potential future treatment, when available.

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Figure Legends

Figure 1: Schematic illustration of *ADNP* and its functional domains.

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Frontal and lateral views. Note the prominent forehead with high anterior hairline, the wide and depressed nasal bridge, and short nose with full, upturned nasal tip. Informed consent has been obtained for publication of all images present in this paper. (Individual numbers from Supplemental Table S2 corresponding to the pictures: A=49, B=34, C=44, D=21, E=17, F=63, G=28, H=29, I=45, J=11, K=38, L=15, M=48, N=50, O=60, P=36, Q=58, R=33, S=51, T=39, U=42, V=31, W=41, X=10, Y=70, Z=27).

Figure 5: Individuals at different ages showing evolution with age.

(A) Individual 1 at age 10m, 15m, 29m, 4y, 6y;

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Figures

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Tables

Table 1. Clinical features of the reported individuals with mutation in the *ADNP* gene.

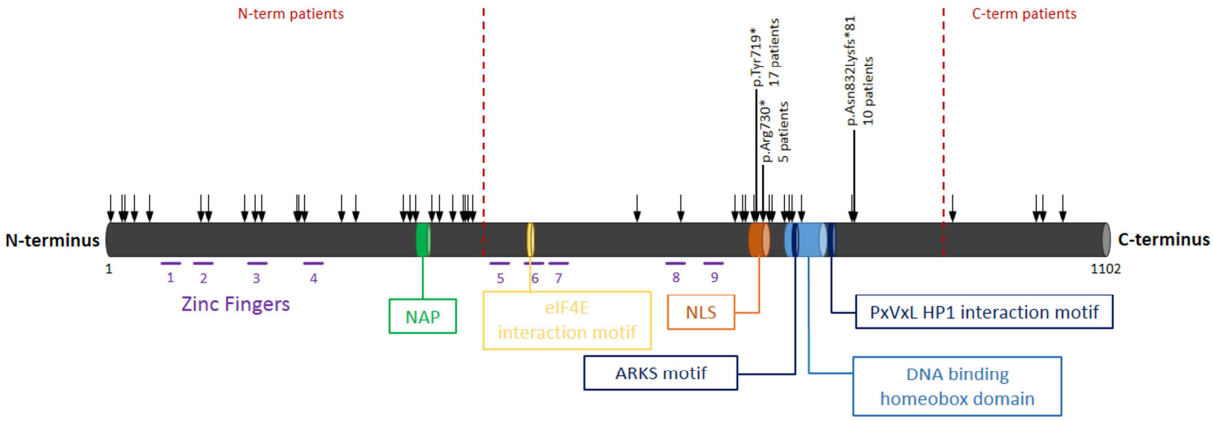
General information		
Age at examination (range)	1-40y (mean 8y 2m)	78/78
Gender F:M	34:44	78/78
Gestational age (weeks)	38.7	70/70
Age father at time of birth	32.1y	65/65
Age mother at time of birth	29.8y	67/67
Mutation information		
De novo *ADNP* mutation	97.1%	68/70
Nonsense mutation	56.4%	44/78
Frameshift mutation	43.6%	34/78
Growth		
Short stature (< -2SD)	23.2%	16/69
Neurodevelopmental features		
Developmental delay / Intellectual disability (ID)	100.0%	73/73
Mild ID	12.3%	9/73
Moderate ID	35.6%	26/73
Severe ID	52.1%	38/73
Motor delay	95.9%	71/74
Sitting independently (mean age)	1.1y	58/58
Walking independently	86.8%	66/76
Walking independently (mean age)	2.5y	64/64
Speech delay	98.6%	70/71
First words (age)	2.5y	49/49
No speech	19.4%	14/72

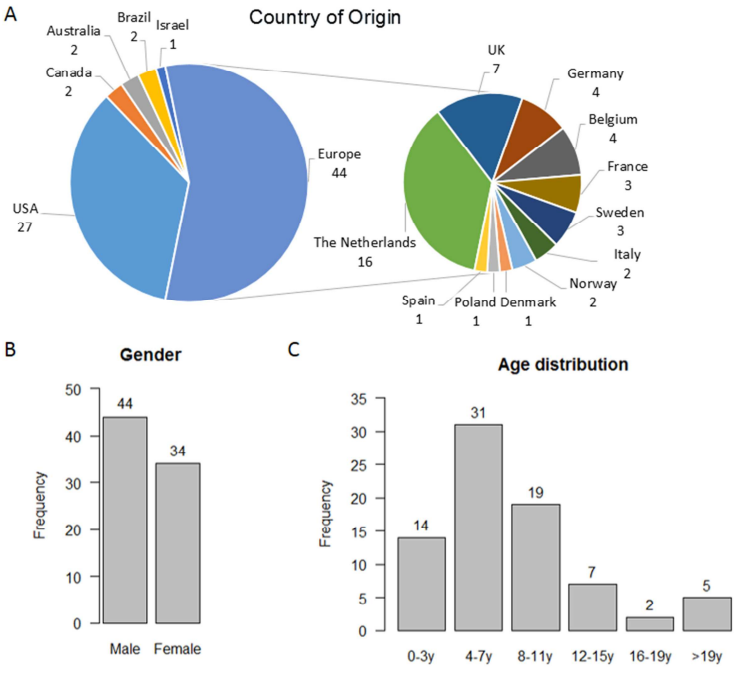
Autism Spectrum Disorder including autistic features	92.8%	64/69
ADHD	43.9%	25/57
Loss of skills	20.3%	12/59
Bladder training delay	81.1%	43/53
Feeding and gastrointestinal problems	83.3%	60/72
Gastroesophageal Reflux (Disease)	58.5%	38/65
Constipation	49.3%	34/69
Oral movement problems	45.6%	26/57
Lack of satiation	41.5%	22/53
Problems swallowing liquids	32.2%	19/59
Frequent vomiting	29.5%	18/61
Aspiration difficulties	21.4%	12/56
Gastrostomy tube (G-tube)	12.7%	8/63
Obesity	7.5%	5/67
Neurological problems and behavior		
Hypotonia	78.3%	54/69
Hypertonia	3.8%	3/78
Seizures	16.2%	12/74
Cerebral imaging - structural brain abnormalities	55.9%	33/59
Wide ventricles	29.4%	15/51
Corpus Callosum Underdevelopment	18.4%	9/49
Cerebral atrophy	17.8%	8/45
Delayed myelination	8.9%	4/45
White matter lesions	7.5%	4/53
Cortical dysplasia	3.8%	2/52
MRI brain abnormalities· unspecified	36.2%	17/46
Behavioral problems	77.6%	38/49

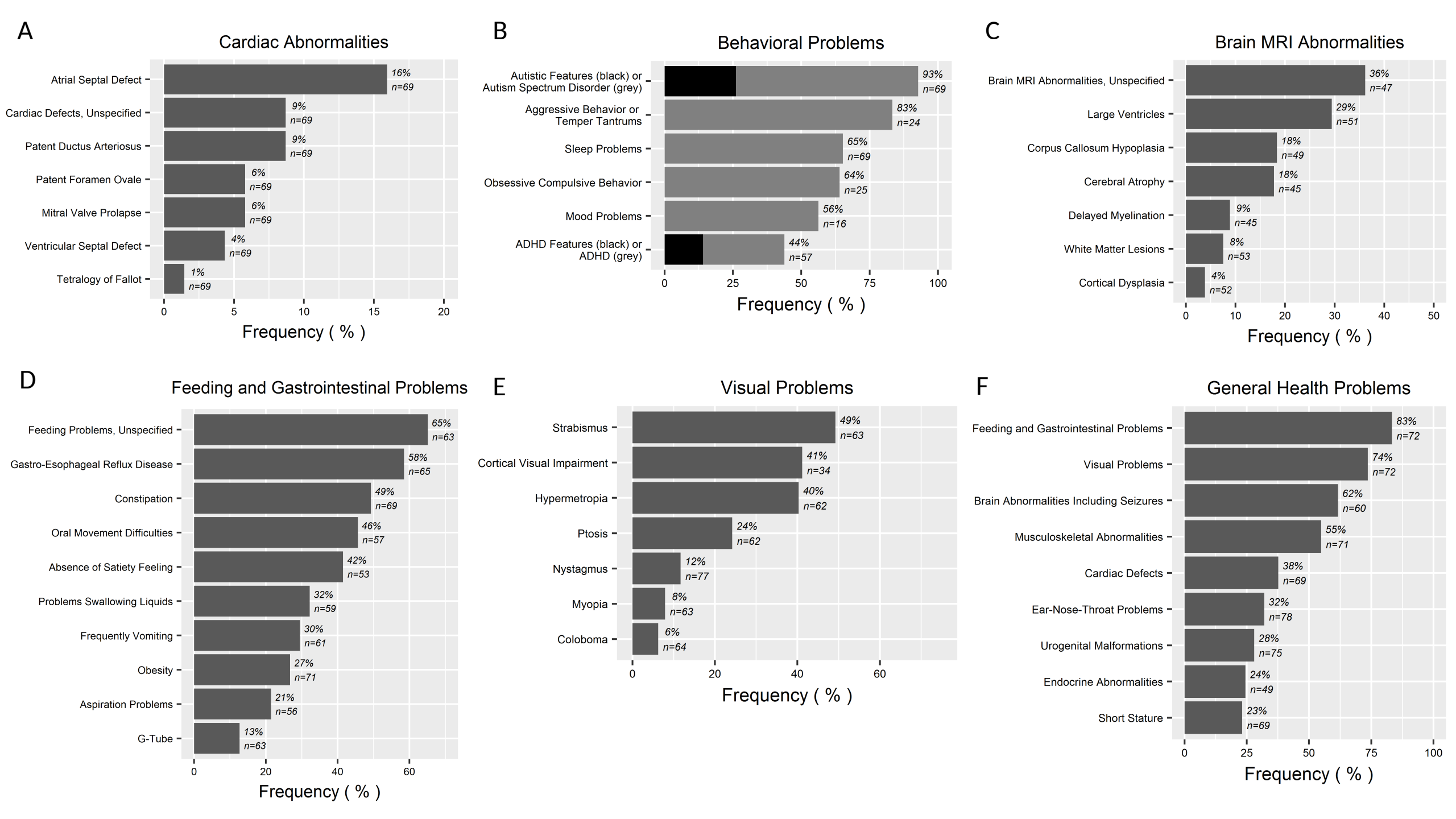
Temper tantrums/aggression	83.3%	20/24
Obsessive compulsive behavior	64.0%	16/25
Mood disorder	56.3%	9/16
Self-injurious behavior	20.0%	2/10
Insensitivity to pain	63.6%	35/55
Sensory Processing Disorder	66.7%	28/42
Sleep problems	65.2%	45/69
Visual system	73.6%	53/72
Strabismus	49.2%	31/63
Cerebral Visual Impairment (CVI)	41.2%	14/34
Hypermetropia	40.3%	25/62
Ptosis	24.2%	15/62
Nystagmus	11.7%	9/77
Myopia	7.9%	5/63
Colobomata	5.6%	4/72
Ear-Nose-Throat (ENT) system	32.1%	25/78
Narrow hearing canal	87.5%	7/8
Frequent otitis media	85.7%	12/14
Hearing tubes	73.3%	11/15
Hearing loss	11.7%	7/60
Obstructive Sleep Apnea Syndrome (OSAS)	6.6%	5/76
Cardiovascular system	37.7%	26/69
Atrial Septal Defect	15.9%	11/69
Patent Ductus Arteriosus	8.7%	6/69
Mitral Valve Prolaps	5.8%	4/69
Patent Foramen Ovale	5.8%	4/69
Ventricular Septal Defect	4.3%	3/69

Tetralogy of Fallot	1.4%	1/69
Cardiac defect· unspecified	8.7%	6/69
Urogenital system	28.0%	21/75
Cryptorchidism	34.3%	12/35
Renal anomalies	12.5%	6/48
Small genitalia	5.4%	4/74
Endocrine system	24.5%	12/49
Early puberty	30.0%	3/10
Thyroid hormone problems	15.2%	7/46
Growth hormone deficiency	10.9%	5/46
Musculoskeletal system	54.9%	39/71
Joint hypermobility	37.7%	23/61
Scoliosis	17.2%	11/64
Hip problems (hip dysplasia, Perthes' disease, dislocated hips)	7.5%	4/53
Thorax abnormalities	22.2%	12/54
Pectus excavatum	14.8%	8/54
Pectus carinatum	5.6%	3/54
Narrow thorax	1.9%	1/54
Abnormal skull shape	13.9%	10/72
Plagiocephaly	8.3%	6/72
Trigonocephaly	2.8%	2/72
Brachycephaly	4.2%	3/72
Hand and foot abnormalities	62.3%	43/69
Finger abnormalities (prominent distal phalanges, prominent interphalangeal joints, polydactyly, interdigital webbing, 2-3 toe syndactyly, 5th finger clinodactyly, small fifth finger or absent distal phalanx of fifth finger, tapering fingers, brachydactyly, broad fingers, fetal fingertip pads)	46.3%	31/67

Single palmar crease	10.8%	7/65
Nail anomalies	25.0%	14/56
Sandal gap	19.6%	11/56
Toe abnormalities (broad halluces, 2-3 toe syndactyly, brachydactyly)	10.8%	7/65
Other		
Early teeth	71.1%	32/45
Frequent infections	50.7%	35/69
Widely spaced nipples	20.4%	11/54
Umbilical/inguinal hernia	8.5%	5/59






A
Cardiac Abnormalities
Atrial Septal Defect
16%
n=69
Cardiac Defects, Unspecified
9%
n=69
Patent Ductus Arteriosus
9%
n=69
Patent Foramen Ovale
6%
n=69
Mitral Valve Prolapse
6%
n=69
Ventricular Septal Defect
4%
n=69
Tetralogy of Fallot
1%
n=69
0
5
10
15
20
Frequency (%)
B
Behavioral Problems
Autistic Features (black) or Autism Spectrum Disorder (grey)
93%
n=69
Aggressive Behavior or Temper Tantrums
83%
n=24
Sleep Problems
65%
n=69
Obsessive Compulsive Behavior
64%
n=25
Mood Problems
56%
n=16
ADHD Features (black) or ADHD (grey)
44%
n=57
0
25
50
75
100
Frequency (%)
C
Brain MRI Abnormalities
Brain MRI Abnormalities, Unspecified
36%
n=47
Large Ventricles
29%
n=51
Corpus Callosum Hypoplasia
18%
n=49
Cerebral Atrophy
18%
n=45
Delayed Myelination
9%
n=45
White Matter Lesions
8%
n=53
Cortical Dysplasia
4%
n=52
0
10
20
30
40
50
Frequency (%)
D
Feeding and Gastrointestinal Problems
Feeding Problems, Unspecified
65%
n=63
Gastro-Esophageal Reflux Disease
58%
n=65
Constipation
49%
n=69
Oral Movement Difficulties
46%
n=57
Absence of Satiety Feeling
42%
n=53
Problems Swallowing Liquids
32%
n=59
Frequently Vomiting
30%
n=61
Obesity
27%
n=71
Aspiration Problems
21%
n=56
G-Tube
13%
n=63
0
20
40
60
Frequency (%)
E
Visual Problems
Strabismus
49%
n=63
Cortical Visual Impairment
41%
n=34
Hypermetropia
40%
n=62
Ptosis
24%
n=62
Nystagmus
12%
n=77
Myopia
8%
n=63
Coloboma
6%
n=64
0
20
40
60
Frequency (%)
F
General Health Problems
Feeding and Gastrointestinal Problems
83%
n=72
Visual Problems
74%
n=72
Brain Abnormalities Including Seizures
62%
n=60
Musculoskeletal Abnormalities
55%
n=71
Cardiac Defects
38%
n=69
Ear-Nose-Throat Problems
32%
n=78
Urogenital Malformations
28%
n=75
Endocrine Abnormalities
24%
n=49
Short Stature
23%
n=69
0
25
50
75
100
Frequency (%)


A
B
C
D
E
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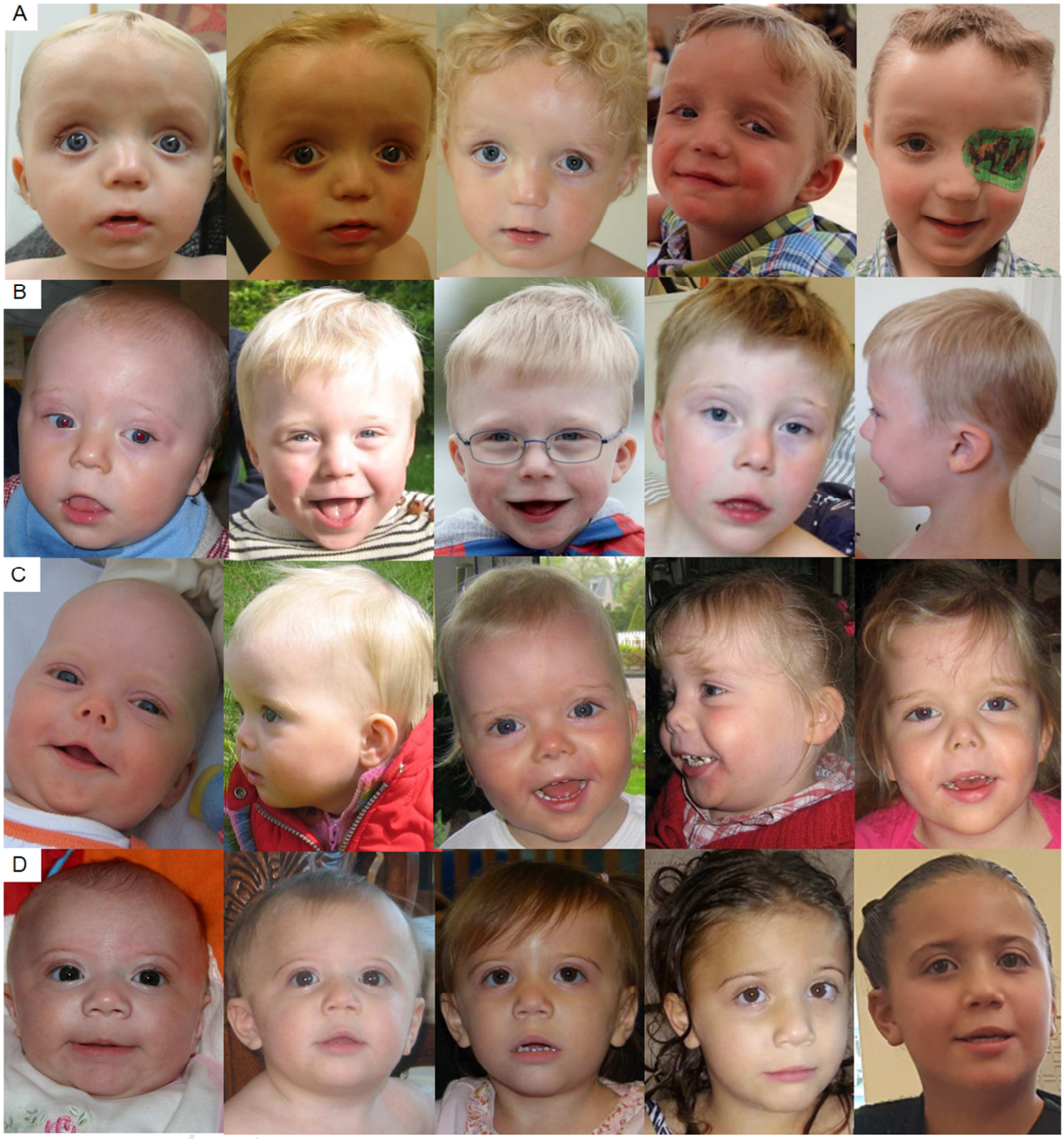
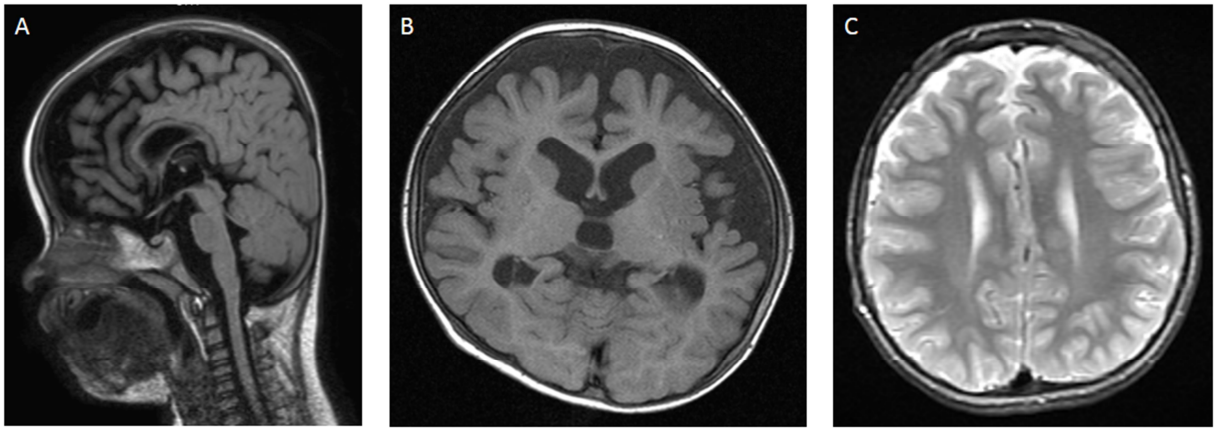

A
B
C
D



Clinical Presentation of a Complex Neurodevelopmental Disorder Caused by Mutations in *ADNP*

Supplemental Information

Supplemental Tables

Table S1. Parameters used for statistical analyses

Epidemiology
Country of lab
Country of Origin
Gender
Age (months) - last observation
Age father (years) - at time of birth
Age mother (years) - at time of birth
Part of twin
Growth
At birth: duration gestation (weeks)
Weight, g
Weight, SD
Length, cm
Length, SD
OFC, cm
OFC, SD
Post-natal - age last measurements (yr)
Weight, SD
Height, SD
OFC, SD
BMI
Short stature (< -2SD)
Development
Delay/ ID : severe; moderate; mild
Motor delay
Sitting (months)
Walking independently
Walking independently from the age of (months)
Speech delay
No speech (nonverbal child)
Speech - first words (months)
Loss of skills
Autism
ADHD
Behavioral problems
Mood disorder

Aggressive behavior
Self-injurious behavior
Temper tantrums
Obsessive compulsive behavior
Social behavior
Asocial behavior (reserved, avoids people)
Friendly behavior
Feeding problems
Gastrointestinal problems
Feeding G-Tube
GERD or Reflux
Oral movement difficulties
Oral drinking liquid problems
Satiety problems: does not seem to "get full"
Aspiration difficulties
Obesity
Frequent vomiting
Constipation
Hypotonia
Hypertonia
Seizures
Ear-Nose-Throat
Hearing loss
Narrow hearing canal
Hearing tubes
Frequent otitis media
Eye defects
Hypermetropia
Strabismus
Ptosis
Hypertelorism
CVI
Myopia
Nystagmus
Craniofacial features
Coarse face
High hairline
Low hairline
Abnormal hair thickness
Prominent forehead
Eversion/notch eyelid
Prominent eyelashes
Thick eyebrows
Sagging periorbital skin
Narrow palpebral fissures
Upward slant palpebral fissures
Downward slant palpebral fissures
Small chin

Pointed chin
Wide nasal bridge
Narrow nasal bridge
Low nasal bridge
Upturned nasal tip (anteverted nares)
Broad nasal tip
Broad nasal base
Short nose
Broad philtrum
Long philtrum
Short philtrum
Smooth philtrum
Large mouth
Thin upper vermillion
Thick lower vermillion
Drooping lower lip
Cleft palate / submucous cleft
Widely spaced teeth
Teeth problems
Low set or posteriorly rotated ears
Malformed ears
Small ears
Trunc and limbs
Thick neck
Scoliosis
Widely spaced nipples
Pectus
Cryptorchidism (uni/bilateral)
Small genitalia
Umbilical/inguinal hernia
Hand abnormalities
Single palmar crease
Thumb abnormalities
Finger abnormalities
Broad fingers
Broad halluces
Fetal finger pads
Brachydactyly
Fetal finger pads
Hyperlaxity
Sandal gap
Nail anomalies
Feet abnormalities
Toe abnormalities
Flat feet
Cardiac abnormalities
Cardiac

Atrial Septal Defect
Mitral Valve Prolapse
Ventricular Septal Defect
Patent Foramen Ovale at birth
Patent Ductus Arteriosus at birth
Tetralogy of Fallot
Other cardiac defect
Neuroradiology
MRI brain abnormality
Delayed myelination
Wide ventricles
Callosal body underdevelopment
Cerebral atrophy
White matter lesions
Cortical dysplasia
Other MRI brain abnormalities
Brain abnormalities including seizures
Therapy
Speech therapy
Physical therapy
Opposite effects of medication
Antiepileptic therapy
Other
Renal anomalies
Dentition (normal; delayed prim / delayed permanent)
Early teeth
Early puberty
Frequent infections
Sleep problems
Obstructive Sleep Apnea Syndrome
Bladder training delay
Hormonal deficiencies
Insensitivity to pain
Sensory Processing Disorder
Growth hormone deficiency
Thyroid hormone problems
Orthopedic and muscular system abnormalities
Skull abnormalities
Hip problems

Table S2. List of mutations identified in the reported individuals (NM_015339.2 (hg19))

cDNA	Protein	gDNA	CADD score*	Patient ID	Frequency
c.1A>G	p.Met1?	g.49520533T>C	24.6	48	1
c.118C>T	p.Gln40*	g.49518637G>A	37	11	1
c.190dupA	p.Thr64Asnfs*35	g.49518565insT	34	64	1
c.339delC	p.Phe114Serfs*47	g.49510912delG	21.2	21	1
c.372_373delGT	p.Ile125*	g.49510878_49510879delAC	33	54	1
c.484C>T	p.Gln162*	g.49510767G>A	36	47	1
c.517C>T	p.Arg173*	g.49510734G>A	36	17	1
c.539_542delTTAG	p.Val180Glyfs*17	g.49510709_49510712delCTAA	34	28, 39	2
c.646 C>T	p.Arg216*	g.49510605G>A	36	20	1
c.651_655delAGAGA	p.Glu218*	g.49510596_49510600delTCTCT	29	68	1
c.673C>T	p.Arg225*	g.49510578G>A	35	42, 66	2
c.790C>T	p.Arg264*	g.49510461G>A	36	73	1
c.819delC	p.Lys274Asnfs*31	g.49510432delG	25.2	62	1
c.1026_1027insT	p.Val343Cysfs*56	g.49510224_49510225insA	33	23	1
c.1033C>T	p.Gln345*	g.49510218G>A	37	65	1
c.1046_1047delTG	p.Leu349Argfs*49	g.49510204_49510205delCA	32	13	1
c.1102C>T	p.Gln368*	g.49510149G>A	37	80	1
c.1134T>G	p.Tyr378*	g.49510117A>C	23.5	50	1
c.1184_1190delAGTCTGC	p.Gln395Leufs*11	g.49510061_49510067delGCAGACT	34	60	1
c.1211C>A	p.Ser404*	g.49510040G>T	37	2	1
c.1216delC	p.Gln406Serfs*2	g.49510035delG	33	59	1
c.1222_1223delAA	p.Lys408Valfs*31	g.49510028_49510029delTT	32	3	1
c.1235delT	p.Leu412Profs*10	g.49510016delA	31	52	1
c.1754dupA	p.Asn585Lysfs*2	g.49509497dupT	26.5	75	1
c.1930C>T	p.Arg644*	g.49509321G>A	37	9	1

cDNA	Protein	gDNA	CADD score*	Patient ID	Frequency
c.2089C>T	p.Gln697*	g.49509162G>A	39	36	1
c.2129delC	p.Pro710Glnfs*6	g.49509122del	35	41	1
c.2129dupC	p.Ser711Lysfs*24	g.49509122dupG	34	29	1
c.2153_2165delCTTACGAGCAAAT	p.Thr718Argfs*6	g.49509086_49509098delATTTGCTCGTAAG	35	4	1
c.2156dup	p.Tyr719*	g.49509095dupT	32	5, 10, 27, 38, 44, 55, 70, 79	8
c.2157C>A	p.Tyr719*	g.49509094G>T	22.9	57, 58, 63	3
c.2157C>G	p.Tyr719*	g.49509094G>C	21.9	12, 16, 26, 34, 49, 56,	6
c.2188C>T	p.Arg730*	g.49509063G>A	36	18, 25, 45, 69, 74	5
c.2206dupA	p.Ser736Lysfs*2	g.49509045dupT	28.4	51	1
c.2213C>G	p.Ser738*	g.49509038G>C	43	71	1
c.2251delGinsTAAA	p.Val751*	g.49509000delCinsTTTA	38	31	1
c.2268_2269insT	p.Lys757*	g.49508982_49508983insA	35	53	1
c.2287delT	p.Ser763Profs*9	g.49508964delA	35	46	1
c.2310delT	p.Leu771*	g.49508941delA	32	33	1
c.2491_2494delTTAA	p.Leu831Ilefs*82	g.49508757_49508760delTTAA	35	6, 8, 15, 78	4
c.2495_2499delATAAA	p.Asn832Serfs*4	g.49508752_49508756delTTTAT	35	61	1
c.2496_2499delTAAA	p.Asn832Lysfs*81	g.49508752_49508755delTTTA	35	1, 14, 19, 24, 30, 32, 35, 40, 67, 72	10
c.2808delC	p.Tyr936*	g.49508443delG	16.42	7	1
c.3047dupA	p.Ala1017Glyfs*6	g.49508204dupT	35	37	1
c.3069_3072delAGAG	p.Arg1023Serfs*3	g.49508179_49508182delCTCT	35	43	1
c.3170T>A	p.Leu1057*	g.49508081A>T	47	22	1

* CADD score is Phred-scaled

Table S3. Growth in individuals with Helsmoortel-Van der Aa syndrome

Growth													
	Mean		**Mean z-score**		**z-score < -2 (%)**			**z-score > +2 (%)**			**Number of patients**		
	F	**M**	**F**	**M**	**F**	**M**	**Total**	**F**	**M**	**Total**	**F**	**M**	**Total**
Birth weight (g)	2965.8	3155.5	-0.3	0.4	10.0%	6.1%	7.9%	0.0%	9.1%	4.8%	30	33	63
Birth length (cm)	48.9	49.5	-0.2	0.3	12.5%	0.0%	6.3%	0.0%	0.0%	0.0%	24	24	48
Birth OFC (cm)	34.7	34.5	0.5	0.1	12.5%	0.0%	6.5%	0.0%	0.0%	0.0%	16	15	31
Weight at last observation	NA	NA	0.4	0.7	0.0%	5.9%	3.2%	10.3%	5.9%	7.9%	29	34	63
Length at last observation	NA	NA	-0.4	-0.8	22.6%	23.7%	23.2%	0.0%	2.6%	1.4%	31	38	69
OFC at last observation	NA	NA	-0.4	-0.3	10.7%	5.9%	8.1%	0.0%	0.0%	0.0%	28	34	62

Growth parameters at birth and at last observation. OFC = Occipital Frontal Circumference; F = Female; M = Male

Table S4. Facial features of the reported individuals with mutation in the *ADNP* gene

Facial feature	Frequency	Total
Thin upper vermillion	70.3%	45/64
Prominent forehead	65.6%	42/64
Wide nasal bridge	50.0%	33/66
High hairline	50.0%	33/66
Short nose	49.2%	31/63
Malformed ears	48.5%	32/66
Upturned nasal tip	46.7%	28/60
Everted lower lip	45.5%	25/55
Long philtrum	39.3%	22/56
Thick lower vermillion	36.4%	20/55
Downward slant palpebral fissures	33.3%	20/60
Widely spaced teeth	34.6%	18/52
Broad nasal base	29.6%	16/54
Broad nasal tip	26.3%	15/57
Teeth problems	24.5%	13/53
Narrow palpebral fissures	24.1%	14/58
Large mouth	23.2%	13/56
Low nasal bridge	20.6%	13/63
Eversion/notch eyelid	19.0%	12/63
thick eyebrows	18.3%	11/60
Coarse face	16.9%	11/65
Prominent eyelashes	16.7%	10/60
Broad philtrum	16.1%	9/56
Low-set or posteriorly rotated ears	14.7%	10/68
Abnormal hair thickness	14.0%	8/57
Sagging periorbital skin	13.6%	8/59
Small ears	11.6%	8/69
Thick alae nasi	11.3%	6/53

Supplemental Figures

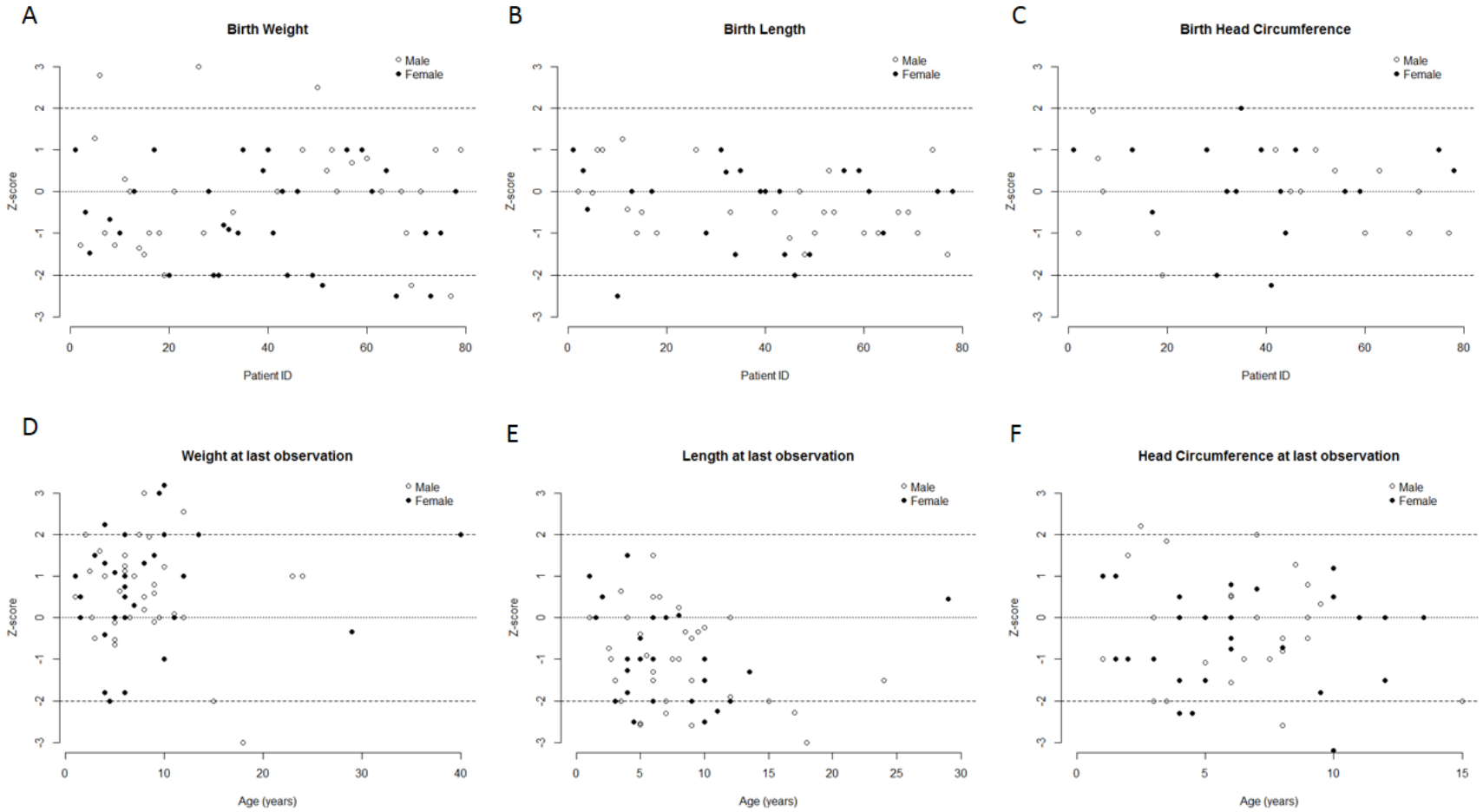


Figure S1. Growth in individuals with Helsmoortel-Van der Aa syndrome

Growth z-scores for height, weight and head circumference in male and female individuals at birth (A-C) and at last observation (D-F).

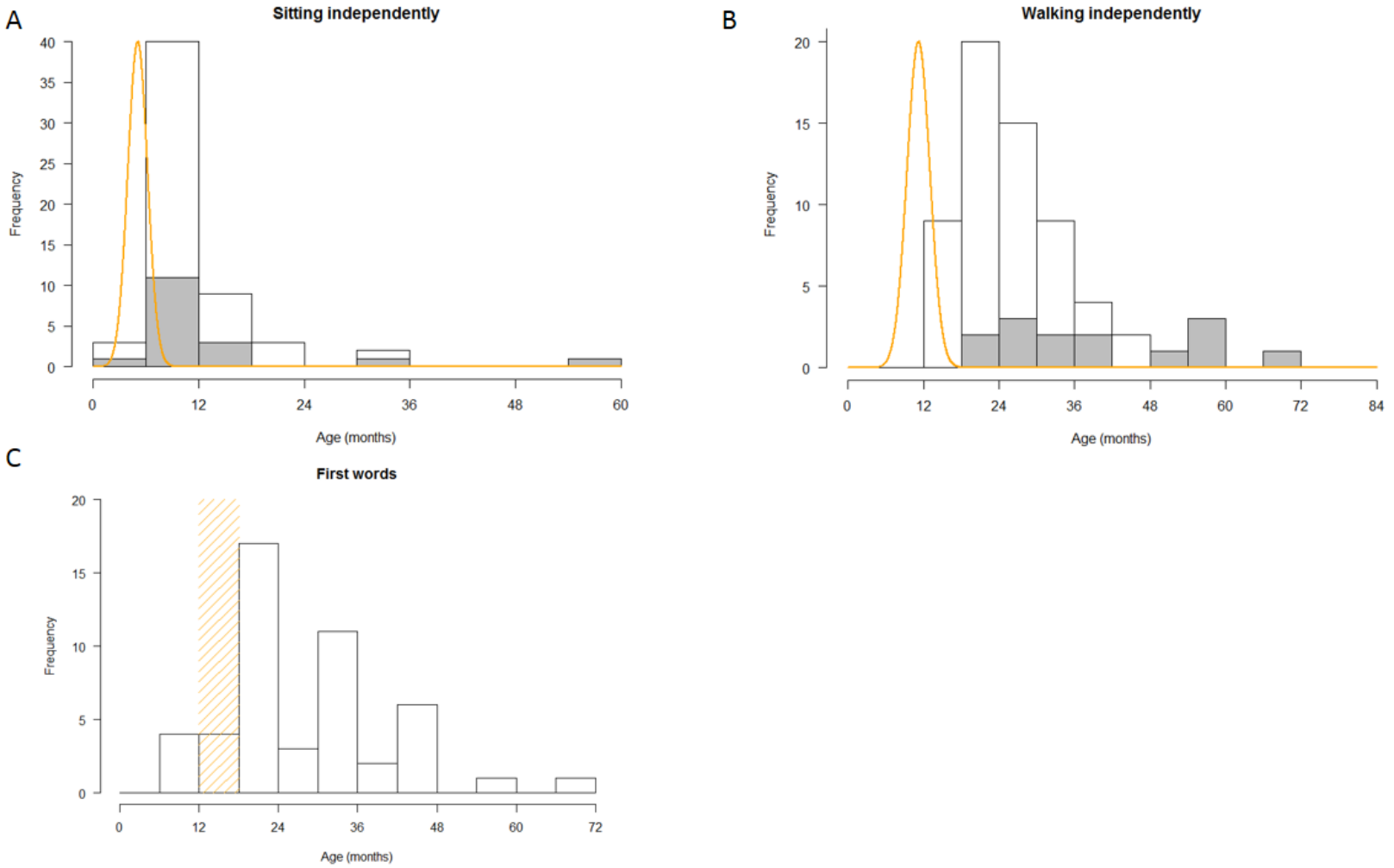


Figure S2. Developmental milestones

(A) Sitting independently. In orange: WHO reference cohort[1]. Grey area: Individuals with p.Tyr719* mutation; (B) Walking independently. In orange: WHO reference cohort[1]. Grey area: Individuals with p.Tyr719* mutation; (C) First words. Shaded area: Typically developing children.

[1] The WHO Child Growth Standards. 2017. http://www.who.int/childgrowth/.