Autosomal recessive primary microcephaly due to ASPM mutations: An update

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Autosomal recessive primary microcephaly due to *ASPM* mutations: an update

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ABSTRACT

Autosomal recessive microcephaly or MicroCephaly Primary Hereditary (MCPH) is a genetically heterogeneous neurodevelopmental disorder characterized by a reduction in brain volume, indirectly measured by an occipitofrontal circumference (OFC) 2 standard deviations or more below the age- and sex-matched mean (-2SD) at birth and -3SD after 6 months, and leading to intellectual disability of variable severity. The Abnormal SPindle-like Microcephaly gene (*ASPM*), the human ortholog of the *Drosophila melanogaster* 'abnormal spindle' gene (*asp*), encodes ASPM, a protein localized at the centrosome of apical neuroprogenitor cells and involved in spindle pole positioning during neurogenesis. Loss-of-function mutations in *ASPM* cause MCPH5, which affects the majority of all MCPH patients worldwide.

Here, we report 47 unpublished patients from 39 families carrying 28 new *ASPM* mutations, and conduct an exhaustive review of the molecular, clinical, neuroradiological and neuropsychological features of the 282 families previously reported (with 161 distinct *ASPM* mutations). Furthermore, we show that ASPM-related microcephaly is not systematically associated with intellectual deficiency and discuss the association between the structural brain defects (strong reduction in cortical volume and surface area) that modify the cortical map of these patients and their cognitive abilities.

Background

Primary microcephaly (PM) refers to a group of autosomal recessive or dominant disorders characterized by a reduction in brain growth starting *in utero*, intellectual disability (ID) of variable severity, and the absence of extra-CNS malformations (Kaindl et al., 2009; Thornton & Woods, 2009). The worldwide incidence of PM varies from 1:30,000 to 1:250,000 live births, depending on the geographic origin and mode of ascertainment (Komai, Kishimoto, & Ozaki, 1955; Morris et al., 2016; Van Den Bosch, 1959). MCPH (Microcephaly primary hereditary) refers to a subtype of PM in which patients display an isolated primary microcephaly with nearly-normal brain cytoarchitecture, originally called "microcephalia vera". Nowadays, MCPH also includes primary microcephaly with cortical malformations. Clinically, MCPH is defined by an occipitofrontal circumference (OFC) that is 2 standard deviations (SD) or more below the age- and sex-matched mean at birth, and 3 SD or more below the mean after 6 months of age. MCPH might be detected from the 2nd trimester of pregnancy by ultrasound scan (Woods & Parker, 2013).

MCPH is genetically heterogeneous: an MCPH phenotype has been associated with mutations in at least 18 genes, MCPH1-18. Among them, *ASPM* (MCPH5 locus) is the most frequently mutated gene reported (Verloes, Drunat, Gressens, & Passemard, 2013). Mutations in the remaining 17 genes cause less than 40% of the reported diagnosis. Many MCPH families have not been ascribed to any of the known genes, suggesting that additional MCPH genes are still to be discovered.

The Abnormal SPindle-like Microcephaly gene (*ASPM*; MIM# 605481) is the human ortholog of the *D. melanogaster* 'abnormal spindle' gene (*asp*) and maps at the 1q31.3 locus (Bond et al., 2002; Jamieson, Fryns, Jacobs, Matthijs, & Abramowicz, 2000; Pattison et al., 2000). Four

isoforms have been described for the *ASPM* gene (Kouprina et al., 2005). The full length *ASPM* gene contains 28 exons and encodes a 3477 amino-acid protein localized to the spindle pole during metaphase and to the midbody during cytokinesis (Higgins et al., 2010; Kouprina et al., 2005; Paramasivam, Chang, & LoTurco, 2007). ASPM plays a crucial role in the division of neural progenitor cells by keeping them cycling, promoting symmetric proliferative divisions at the expense of asymmetric neurogenic divisions (Fish, Kosodo, Enard, Paabo, & Huttner, 2006). Different mouse models in which *Aspm* is knocked out reproduce the microcephaly observed in humans and show a reduction in cortical surface area (Capecchi & Pozner, 2015; Pulvers et al., 2010). The mechanisms underlying Aspm microcephaly in mice are an increase in cell cycle duration in neural progenitors, many of which exit the cell cycle, thereby leading to the premature exhaustion of the neural progenitor pool, and a subsequent increase in the production of neurons of the lower cortical layers along with a reduction in upper layer neuron production (Capecchi & Pozner, 2015). Whether these mechanisms also explain microcephaly in humans is still unknown.

The ASPM protein (Figure 1) contains an amino-terminal ASH (ASPM, SPD-2, Hydin) domain with a putative microtubule-binding function, found in proteins associated with cilia, flagella, the centrosome and the Golgi complex (Schou, Morthorst, Christensen, & Pedersen, 2014), an Actin Binding Domain (ABD) comprising two calponin homology (CH) domains that bind one actin monomer in the filament (Stradal, Kranewitter, Winder, & Gimona, 1998), a series of repeated calmodulin-binding IQ domains, an Armadillo-like domain, and a carboxy-terminal region of unknown significance. Although ASPM is highly conserved across species, the variability of its calmodulin-binding IQ repeats is of peculiar interest: The human protein displays 81 calmodulin-binding IQ repeats at positions 1273 to 3234, whereas there are 61

calmodulin-binding IQ repeats in mice and 24 calmodulin-binding IQ repeats in *Drosophila* (Bond et al., 2002; Kouprina et al., 2005; Kouprina et al., 2004). Although still a topic of debate, it has been proposed that the expansion of the cerebral cortex depends on the number of calmodulin-binding IQ repeats (Bond et al., 2002; Bond & Woods, 2006; Kouprina et al., 2005; Kouprina et al., 2004; Ponting & Jackson, 2005).

In vitro experiments have shown that the N-terminal portion of ASPM, encoded by the first seven exons, is sufficient to induce ASPM localization to the spindle pole during metaphase, whereas the C-terminal domain, encoded by the last three exons, is required for its localization to the midbody during cytokinesis (Kouprina et al., 2005; Paramasivam et al., 2007).

Although a large number of patients with *ASPM* mutations have been reported (Abdel-Hamid et al., 2016b; Ahmad et al., 2016; Akbariazar et al., 2013; Al-Gazali & Ali, 2010; Ariani et al., 2013; Bond et al., 2002; Bond et al., 2003; Darvish et al., 2010; Desir, Abramowicz, & Tunca, 2006; Desir, Cassart, David, Van Bogaert, & Abramowicz, 2008; Gul et al., 2006; Gul et al., 2007; Halsall, Nicholas, Thornton, Martin, & Geoffrey Woods, 2010; Hashmi et al., 2010; Hu et al., 2014; Kousar et al., 2010; Kumar, Blanton, Babu, Markandaya, & Girimaji, 2004; Muhammad et al., 2009; Nakamura et al., 2015; Nicholas et al., 2009; Papari et al., 2013; Passemard, Kaindl et al., 2009; Pichon, Vankerckhove, Bourrouillou, Duprez, & Abramowicz, 2004; Rump et al., 2016; Saadi et al., 2009; Sajid Hussain et al., 2013; Shen et al., 2005; Tan et al., 2014; Wang, Khan, Han, & Zhang, 2017), their developmental phenotype has been documented only in a minority of cases. However, ID (Passemard,

Kaindl et al., 2009) and epilepsy (Shen et al., 2005) are the most frequently reported clinical findings in patients with *ASPM* mutations.

In the present review, we report 47 new patients (39 families), followed within the EuroMicro network, and present an exhaustive overview of all individuals with *ASPM* mutations described in the literature since the gene was identified, along with their molecular, clinical, radiological and neuropsychological features. In particular, this review reveals that microcephaly linked to ASPM is not always associated with ID.

Mutations

Reported mutations

From the original discovery of *ASPM* mutations (Bond et al., 2002) to July 2017, 161 mutations have been reported. Reports were collected using the PubMed library. The terms "ASPM", "MCPH5", "MCPH", "autosomal recessive microcephaly", "microcephaly primary hereditary" and "microcephalic dwarfism" were used as key words. No intragenic copy number variations have been reported in Decipher; only large rearrangements encompassing more than the *ASPM* gene have been reported. The 161 *ASPM* mutations have been identified in 638 affected individuals belonging to 282 families. All the mutations are depicted in Figure 1 and summarized in Supp. Table S1. These mutations are spread all along the coding sequence and include 147 exonic variations, 12 intronic variations and 2 large deletions encompassing several exons/introns. Exonic variations (n=147) include73 nucleotide substitutions (leading to 69 nonsense mutations, 2 putative splicing mutations and 2 missense mutations),65 deletions of 1 to few nucleotides (leading to 61 frameshift mutations and 4 nonsense mutations) and 9 duplications or insertions of one nucleotide

(leading to frameshift mutations). Intronic variations (n=12) include 10 nucleotide substitutions and 2 deletions of one nucleotide (all predicted to interfere with correct splicing). Frameshift and splice-site mutations are predicted to result in unstable RNA that would be degraded by nonsense-mediated RNA decay or in truncated protein synthesis. However, few experiments have been carried out to verify this hypothesis except for two mutations located in exon 24 (c.9754del; pArg3252Glufs*10) and in intron 25 (c.9984+1G>T; predicting the removal of the intron 25 splice donor site) (Higgins et al., 2010; Kouprina et al., 2005). In the first case, western blot analysis has revealed the presence of a truncated protein. In the second case, although the size and localization of ASPM were not affected, only weak expression of the protein was detected at the spindle pole. Among the 161 mutations described so far, three mutations recur frequently. The c.3978G>A mutation (allele frequency = 18%) has been specifically reported in Turkish and Pakistani families (60 families). The c.9557C>G mutation has been reported exclusively in Pakistan (7 families). Both mutations suggest a founder effect. In contrast, the third mutation (c.7782_7783del), which represents 4% of all alleles, is reported in families of different geographic origins (Europe, Africa and Asia) and is also found in the present study with a high allele frequency (17%). It may therefore correspond to a hotspot mutation.

Unreported mutations, methods of identification, cohort

Molecular analysis was performed within our “EuroMicro” European Network (including five partners in France, Belgium, Germany, Switzerland and the UK), between 2007 and 2017, using samples from patients referred for typical MCPH, primary microcephaly with cortical malformation or microcephalic primordial dwarfism. The unique inclusion criterion was an

OFC lower than 2 SD below the age- and sex-matched mean at birth and lower than 3 SD below the mean after 6 months of age, irrespective of the patient's stature. Exclusion criteria were: 1) a context of anoxia-ischemia at birth, 2) a diagnosis of infectious or toxic fetopathy, or 3) major associated malformations suggestive of syndromic microcephaly.

Mutation analysis was performed on DNA extracted from peripheral blood leucocytes using standard procedures. The coding sequence +/- 25 base pairs of intron/exon boundaries of the *ASPM* gene were screened for variants either by Sanger Sequencing or Next Generation Sequencing.

In total, we genotyped 47 patients from 39 unrelated families. 15 index cases were born to consanguineous parents. Genotyping identified 18 published and 28 unpublished variants (Table 1 and Figure 1). The new variants included 17 frameshift mutations, 9 nonsense mutations and 2 splicing mutations, all likely resulting in truncated protein products. Using Alamut Software (Interactive Biosoftware, Rouen, France), the pathogenicity of the identified variants was predicted to result in a loss of ASPM function in all cases, even though functional tests are needed to definitively determine their real consequences. Likewise, the presence of additional variants in the genome cannot be ruled out. The molecular data are shown in Table 1 and Figure 1. All mutations have been declared in the Leiden Open Variation Database (databases.lovd.nl/shared/genes/ASPM).

Epidemiology, phenotype

Epidemiology: 685 patients have been reported so far: (47 from the present study + 638 in the literature) from 321 (39+282) families. Among those whose sex has been described, 229 (28+201) are males and 182 (19+163) are females (Sex ratio M/F = 1.3). Most families come

from the Asian subcontinent and middle-east: Pakistan (167 families), Saudi Arabia (18), Egypt (2+16) and Iran (13); 47 (16+31) families are from Europe and 3 from the Americas (Figure 2).

Growth: Although affected patients are described as "microcephalic", accurate growth parameters (especially OFC) are poorly documented in the literature (reported in less than 3% of patients at birth and only for 26% of patients during childhood). Auxological data for the present study and those reported in the literature are summarized in Figure 3, and include OFC, height, and weight at birth and after 6 years of age. For our European series, SD was calculated according to Sempé (Sempé M, 1979). For cases published previously, we used the SD values provided by the authors. When only absolute values were available, we used WHO Child Growth Standards and WHO Reference 2007.

Microcephaly related to *ASPM* mutations has exceptionally been reported during pregnancy in 2 families (Desir et al., 2008; Hu et al., 2014). In the present study, microcephaly was detected during the third trimester of pregnancy in 23 cases (Table 2) and from the second trimester in 2 cases. However, the impact of these early forms on intellectual prognosis is variable (see details for patients # 8, 9, 13, 20 and 30 in Table 2 and the paragraph on "cognition").

Two features characterize the growth of *ASPM*-mutated patients: the reduction of OFC growth kinetics with age and preserved growth in height from birth, as shown in Figure 3.

Development and clinical features: Walking without support is acquired around 20 months of age (+/- 11 months, range 10 to 66 months; data available for n=32/42 in the present study and for n=20/605 in the literature - children aged 18 months or more). 59% walked prior to or at 18 months of age. Available data related to verbal skills are scarce and heterogeneous in the literature, yet language acquisition seems to be delayed. 17% of patients from the present study were able to make full sentences at 3 years of age (data available for n=30/37 - children aged 3 years or more). Behavioral disorders, such as hyperkinesia, impulsiveness and aggressiveness, were observed in 16 patients from the present study and 17 from the literature. Neurological examination may show pyramidal syndrome or even spasticity (n=4 in the present study and n=7 in the literature). Ataxia and tremors have not been reported. Seizures have been reported in 47 patients (10/47, i.e. 21% in the present study and n=37 in the literature). They appeared during childhood (not before 6 months of age), and were usually sensitive to antiepileptic drugs. Some patients show hypo- and/or hyperpigmented spots (6 patients: #3, #25, #27, #30, #32.1 and #32.2). Malformations are rare and do not present a recurrent pattern: scoliosis (2 families: patients #32.1, #32.2 and #37), middle ear hypoplasia (1 patient: #19), preaxial polydactyly (1 patient: (Ahmad et al., 2016)), unilateral cystic kidney (1 patient: (Passemard, Titomanlio et al., 2009)), tricuspid insufficiency (1 family: (Ariani et al., 2013))]. Deafness (1 patient (Darvish et al., 2010), Guillain-Barré syndrome (1 patient (Passemard, Titomanlio et al., 2009)) and nystagmus (patient #24.3) have been reported or noticed in the present study. Fatal issues have been reported three times in the literature: one patient died after acute myeloid leukemia (Al-Gazali & Ali, 2010) and two children (3 and 9 years old) died without any reported explanation (Abdel-Hamid et al., 2016; Hashmi et al., 2016). The co-occurrence

of two unrelated genetic diseases has been shown in 3 patients: one with a deletion of the *STS* gene (Abdel-Hamid et al., 2016), one with oculocutaneous albinism (Abdel-Hamid et al., 2016), and one with familial retinitis pigmentosa due to *CLN3* mutations (patient #21) .

Cognition: A major prognostic factor to take into account in microcephaly is, naturally, intellectual ability. Although ID, from mild to severe, has been systematically reported in patients with *ASPM* mutations neuropsychological assessment was performed in only 35/628 patients in the literature (i.e. only 5.6%, Figure 4). Among these 35 patients, a Full Scale Intellectual Quotient (FSIQ) was available for only 24 patients. The mean FSIQ was 54 $\pm$ 8 (range 40 to 71). Despite their ID, we have previously shown that long-term memory in these patients is spared, suggesting that they are able to learn (Passemard et al., 2016). The remaining 11 were assessed by various motor and language skill assessments, which allowed the developmental quotient (DQ) to be estimated, with a mean value of 46 $\pm$ 23 (range 30 to 104).

In the present study, among 36 children aged of 3 years or more at the last examination, psychological evaluation was not possible for 11 children living outside Europe. Among the remaining families (25 patients), the parents of 14 children agreed to a neuropsychological assessment and their children cooperated. Wechsler tests were first proposed to all patients. These tests are universally accepted tools (translated into many languages) that allow for comparisons between patients from different countries. Six patients were unable to take the Wechsler tests. Therefore, neuropsychologists proposed developmental quotient (DQ) assessment, using specific tests (Stanford Binet, Borel-Maisonny, Bayley) that

are not always internationally available, and only relevant in populations sharing the same language. The mean DQ was 58 ± 15 (range 34 to 95, Figure 4B and Table 2), similar to scores seen in the literature.

Eight patients were able to perform the different subtests of the Wechsler scales. As shown in Figure 4 and Table 2, the mean FSIQ was 64 ± 10 (range 50 to 82). As compared to the 24 FSIQ values reported in the literature, the FSIQ of patients in the present study was significantly higher (Figure 4A, $p < 0.05$, un-paired T-test). The "age" factor could explain such a difference. Indeed, our patients are younger (average = 6.6y) than those described in the literature (average = 11.3y). These patients may face problems with maintaining their cognitive abilities with time, as tasks become more and more difficult, or may reach the upper limit of their abilities earlier, during childhood or adolescence. The precocity of diagnosis and of appropriate rehabilitation, whose effectiveness also depends on the age factor, would also influence intellectual prognosis. Surprisingly, for 4 out of 5 children who underwent detailed neuropsychological assessment (patients #2, 8, 13 and 34, Table 2), at least one subtest, verbal comprehension and/or nonverbal performance, was in the normal range, suggesting learning disabilities rather than ID. For three patients (patients # 2, 8 and 34), a difference of 20 points or more between scores was highlighted, leading to a diagnosis of dyspraxia. Furthermore, one child (patient #13; 5.5y) obtained scores within the normal range or the low average on all subtests and the FSIQ, thus excluding ID (Table 2 and Figure 4). To our knowledge, this is the first report of *ASPM*-mutated patients with normal intelligence.

Brain MRI: Brain magnetic resonance imaging was performed in 39/47 patients from the present study (83%) and has been reported in 50/638 patients (8%) in the literature. The

most frequent anomalies were: gyral simplification in 71/89 cases (67% of the present study and 90% of those examined in the literature), corpus callosum abnormalities (shape, size etc.) in 38/89 cases (31% of the present study and 52% in the literature), and middle to moderate cerebellar and/or pontine hypoplasia in 26/89 cases (15% of the present study [including obvious vermis and cerebellar atrophy in patient #21, who developed a late onset ceroid lipofuscinosis, typically known to induce such atrophy] and 40% in the literature). Such neuroradiological features are often undiscriminating in terms of diagnosis (Figure 5), since they are not specific either to MCPH or to a specific type of MCPH. Some atypical features have also been described: polymicrogyria in 3 cases (patient #23.1 with extensive bilateral posterior polymicrogyria and (Marchal et al.; Passemard, Kaindl et al., 2009)), and syringomyelia (patient #17.1). Conventional imaging is thus not informative enough to orient diagnosis or to predict prognosis, except if it shows migration disorders associated with microcephaly, such as polymicrogyria, that may increase the risk of epilepsy. The reduction of brain volume in humans provides evidence for early neuronal and glial defects. The existence of polymicrogyria show that migration disorders are associated with proliferation defects in ASPM microcephaly.

Genotype-phenotype correlation

The vast majority of *ASPM* mutations are nonsense or frameshift mutations, predictive of the synthesis of a truncated protein. No IQ is available for patients carrying the two missense mutations (Gul, 2006; Darvish, 2010; Ahmad, 2016; Kraemer 2016). Most *ASPM* mutations are private. Moreover, intra-familial variability is frequent. In our opinion, the available data

are still too scarce to make any correlations, underlining the need for better characterization of this rare disease.

Future prospects

Major efforts have been made for the molecular diagnosis of MCPH, and the implementation of NGS in clinical diagnosis has identified *ASPM* mutations as the principal cause of MCPH worldwide. Although the vast majority of *ASPM* mutations likely result in the loss of function of the protein product, systematic functional studies are still required to prove the pathogenicity of the variants, and not only refine our knowledge of the roles of this protein but determine whether all mutations have the same consequences or whether some have differential impacts on the cell. The high number of patients reported is also countered by the drastic lack of fine clinical descriptions, neurocognitive investigations and large-scale studies correlating anomalies of brain morphology with neurodevelopmental, cognitive and behavioral characteristics. Hence, we have only limited knowledge regarding the real intellectual abilities and their natural history in these patients, or their functional cortical organization and cortical maps. To obtain new insights into ASPM-specific brain defects, two different approaches to cortical structure should be considered: structural brain imaging and the neuropathological study of post mortem cases. Indeed, we have shown that the 50% average reduction in brain volume is caused by a major reduction of cortical grey and white matter volumes that are in contrast to the relative preservation of the volume of the brainstem and cerebellum (Passemard et al., 2016). This massive reduction in cortical volume and cortical surface preferentially affects the neocortex and

spares the hippocampus and mesiotemporal cortices, involved in long-term memory tasks, concordant with the preserved mnesic functions of these patients (Passemard et al., 2016). The autonomy and social insertion of these patients as adults as well as genetic counseling for their families would benefit from improved knowledge of the structural and cognitive characteristics of the brain.

Many biological questions remain regarding the mechanisms underlying ASPM-microcephaly in humans. Mouse models have confirmed that the *Aspm* gene plays a major role in cortical expansion, promoting the symmetric proliferative divisions of neural progenitors. It is now crucial to better understand the consequences of *ASPM* mutations, not only in terms of neuronal production in affected patients, but also in terms of the specification/differentiation of these neurons, their connectivity and obviously their function. Improving synaptic plasticity in these patients to enhance their cognitive abilities is a future scientific challenge.

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Figure Legends:

Figure 1: Location of the known and novel mutations identified in the human *ASPM* gene and corresponding domains in the protein.

The mutations reported previously are shown on the upper arch. Mutations identified in the present study but already known are indicated by #. Novel mutations from the present study are indicated directly on exons. Various symbols represent missense, nonsense, splice and frameshift mutations as indicated. Allelic frequency (AF) is indicated if > 2%. Abbreviations: AF= allelic frequency; ASH = ASPM,SPD-2,Hydin; CH= calponin homology.

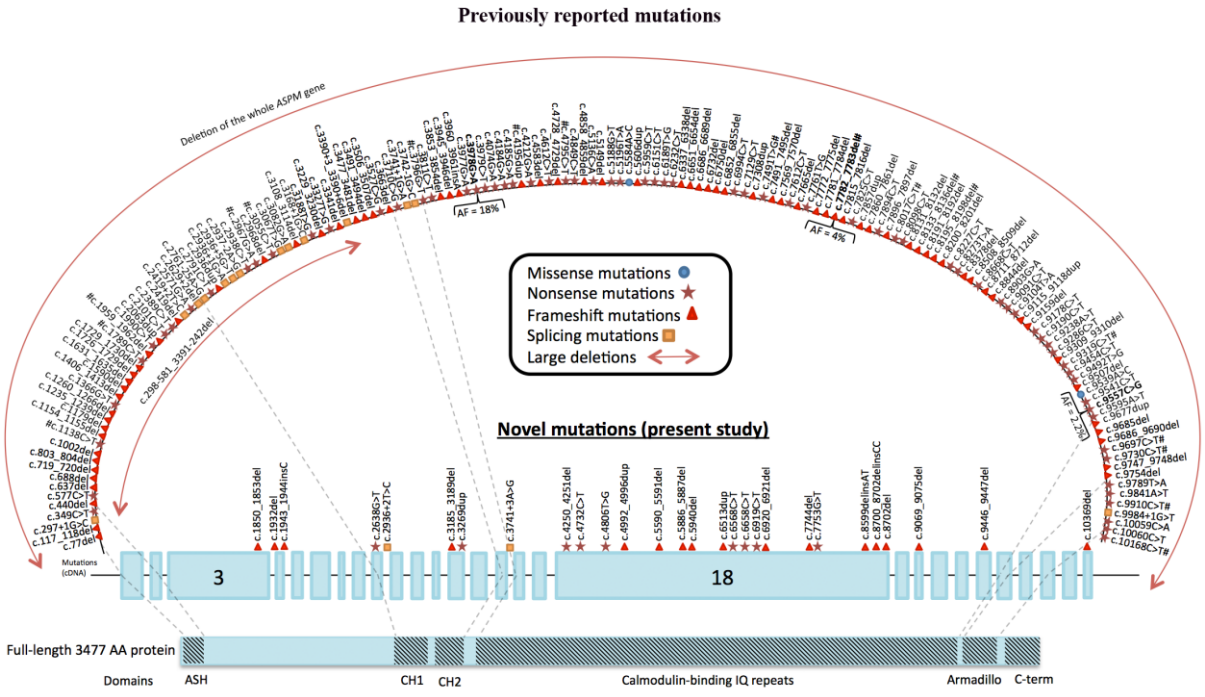


Figure 1

Figure 2: Geographical distribution of families with *ASPM* mutations.

The indicated number corresponds to the number of families per country or region.

Total number of families = 321 (11 families of unknown origin).

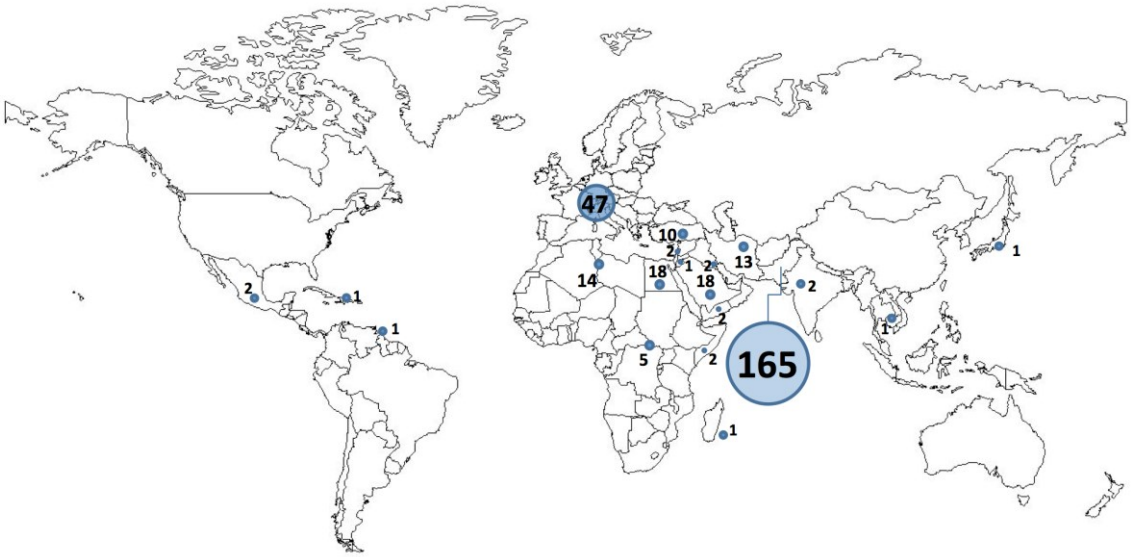
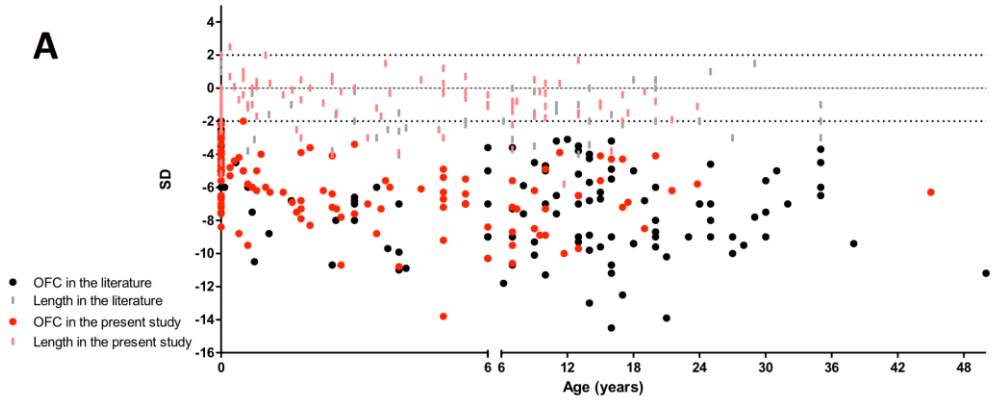


Figure 2

Figure 3: OFC, length and weight measurements in patients with *ASPM* mutations in the present study and in the literature

A- OFC and length measurements: Red symbols depict patients from our cohort while black symbols correspond to patients in the literature. Dots represent OFC and lines represent length. Each symbol represents an individual measurement. A single individual may have several measurements at different ages. OFC = occipitofrontal circumference.

B- OFC, length and weight averages at birth and after 6 years



B

	At birth	After 6 years
OFC	**- 4.4 ± 1.6 SD** (ranging from -2 to -8.4 SD) n= 35/47 in the present study n= 19/634 in the literature	**-8.2 ± 2.8 SD** (from -3.1 to -14.5 SD) n= 25/26 children aged 6 years or more in the present study n= 155/601 children aged 6 years or more in the literature
Length	**-1.2 ± 1.3 SD** (from -5.2 to +2 SD) n= 34/47 in the present study n=17/634 in the literature	**-1.4 ± 1.7 SD** (from -5.8 to +2 SD) n= 23/26 children aged 6 years or more in the present study n=32/601 children aged 6 years or more in the literature
Weight	**-0.9 ± 1.3 SD** (from -4.3 to +1.5 SD) n= 36/47 in the present study n= 18/634 in the literature	**-0.9 ± 1.1 SD** (from -2.5 to +2.3 SD) n= 22/26 children aged 6 years or more in the present study n= 22/601 children aged 6 years or more in the literature

Figure 3

Figure 4: Intellectual abilities of patients with *ASPM* mutations

A- Full-scale IQ of *ASPM*-mutated patients in the literature and in the present study. Unpaired T-test*p<0.05)

B- Developmental quotient of *ASPM*-mutated patients

FSIQ= full scale intellectual quotient; DQ = developmental quotient.

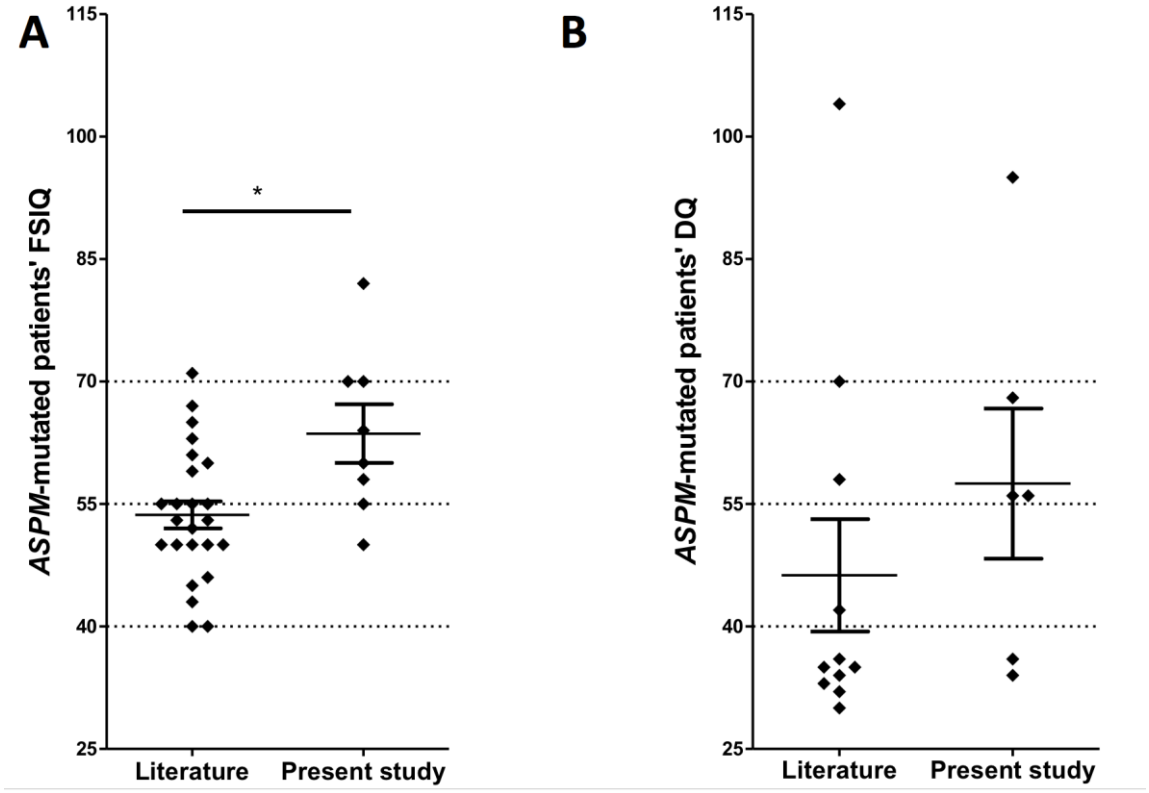


Figure 4

Figure 5: Typical and atypical neuroradiological features of ASPM-related primary microcephaly

A: patient #26 (3 years), B: patient #8 (3.7 years), C: patient #23.1 (4.3 years) and D: age-matched control, (4 years). From left to right: Sagittal T1- / coronal T1- / axial T1- / coronal T2-weighted images.

Drastic reductions in the volume of both hemispheres affecting the white matter and cerebral cortex and gyral simplification are the main features of ASPM-related primary microcephaly (A, B and C) as compared to age-matched controls. *A contrario*, the volume of the cerebellum is preserved, as shown in the coronal view. Unilateral or bilateral polymicrogyria may be associated with ASPM-related primary microcephaly, as shown in C (white arrows).

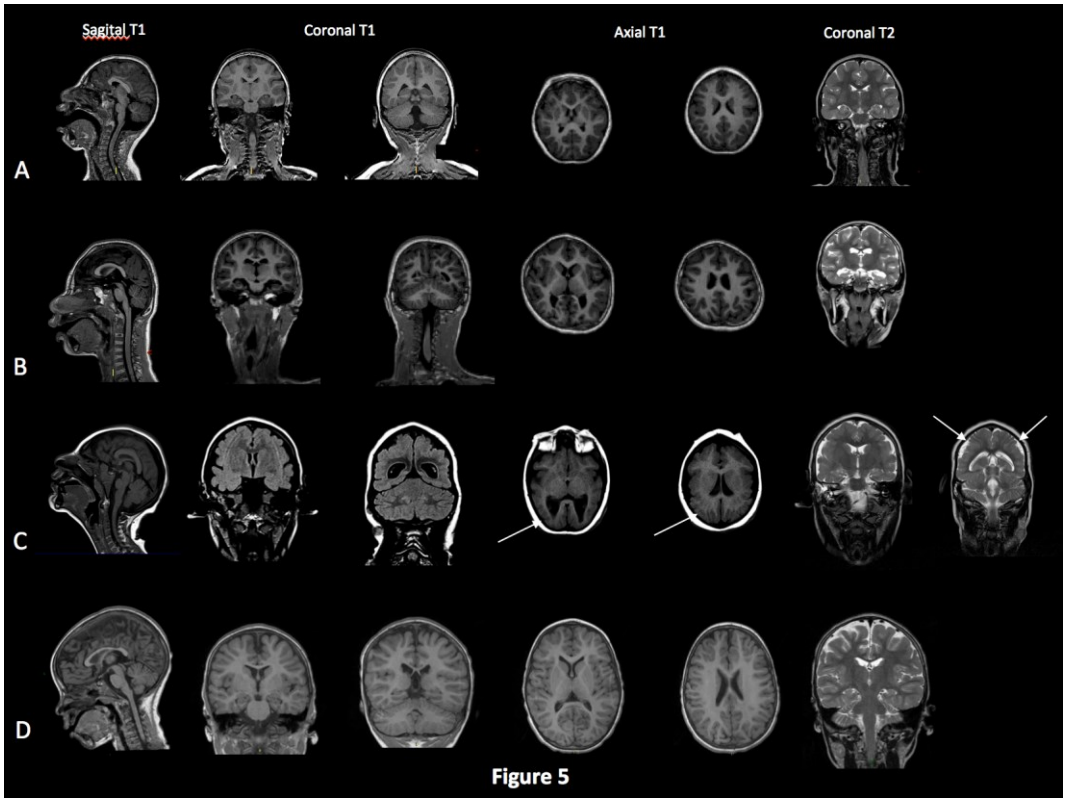


Figure 5

Tables:

Table 1 – Novel ASPM mutations identified in our cohort, according to HGVS nomenclature recommendations and using the sequence NM_018136.4 as a reference.

Location	DNA HGVS nomenclature	Protein HGVS nomenclature	Protein effect	Families	Origin
Exon 3	c.1850_1853del	p.Thr617Lysfs*30	Frameshift	#1	Moroccan
Exon 4	c.1932del	p.Phe645Serfs*23	Frameshift	#2	Belgian
Exon 4	c.1943_1944insC	p.Ile649Asnfs*3	Frameshift	#3	Moroccan
Exon 9	c.2638G>T	p.Glu880*	Nonsense	#4	French
Intron 10	c.2936+2T>C	p.?	Splicing	#5	French
Exon 13	c.3185_3189del	p.Asn1062Argfs*28	Frameshift	#6	Moroccan
Exon 13	c.3269dup	p.Asp1091*	Nonsense	#7	Congolese
Intron 15	c.3741+3A>G	p.?	Splicing	#8	African
Exon 18	c.4250_4251del	p.Tyr1417*	Nonsense	#9	European
Exon 18	c.4732C>T	p.Arg1578*	Nonsense	#10	French
Exon 18	c.4806T>G	p.Tyr1602*	Nonsense	#11	Spanish
Exon 18	c.4992_4996dup	p.Arg1667Ilefs*12	Frameshift	#12 (n=2)	Egyptian
Exon 18	c.5590_5591del	p.Leu1864Serfs*2	Frameshift	#9	European
Exon 18	c.5886_5887del	p.Leu1963Glufs*9	Frameshift	#13	Cameroonian
Exon 18	c.5940del	p.Tyr1981Ilefs*13	Frameshift	#14 (n=2)	Moroccan
Exon 18	c.6513dup	p.Val2172Serfs*7	Frameshift	#15 #16	Turkish Turkish
Exon 18	c.6568C>T	p.Gln2190*	Nonsense	#4 #17 (n=2)	French French
Exon 18	c.6658C>T	p.Gln2220*	Nonsense	#18	Tunisian
Exon 18	c.6919C>T	p.Gln2307*	Nonsense	#17 (n=2)	French
Exon 18	c.6920_6921del	p.Gln2307Leufs*10	Frameshift	#19	French
Exon 18	c.7744del	p.Ile2582Serfs*34	Frameshift	#20	Italian
Exon 18	c.7753G>T	p.Glu2585*	Nonsense	#5	French
Exon 18	c.8599delinsAT	p.Gln2867Ilefs*5	Frameshift	#21	French
Exon 18	c.8700_8702delinsCC	p.Lys2900Asnfs*38	Frameshift	#22	Moroccan
Exon 18	c.8702del	p.His2901Leufs*37	Frameshift	#23 (n=2) #24 (n=3)	Egyptian Moroccan
Exon 20	c.9069_9075del	p.His3023Glnfs*2	Frameshift	#13	French
Exon 23	c.9446_9447del	p.Arg3149Metfs*17	Frameshift	#21	French
Exon 28	c.10369del	p.Glu3457Lysfs*13	Frameshift	#8	African

Table 2 – Clinical and radiological features of patients of our cohort (39 families, 47 patients).

Ref	Sex	Birth			MCon US	Last follow up				Walk < 1.5 year	First sentence < 3 years	Epilepsy (if yes, age of onset in years)	Abnormalities in brain MRI, except microcephaly (age in years)	Intellectual assessment Test (age in years): score(s)	Others features
		OFC (in SD)	Length (in SD)	Weight (in SD)		Age (in years)	OFC (in SD)	Length (in SD)	Weight (in SD)						
#1	M	-7.5	-1	-1.6	T2	11.7	-10	-5.8	-1.3	no	no	no	slight cortical atrophy	NAv	hyperactivity
#2	M	-4.7	0	-0.9	NAv	9.5	-8.9	-0.2	-0.7	yes	yes	no	NAv	WPPSI-R (5.8): FSIQ=64/ VIQ=81/ PIQ=52	hyperactivity
#3	F	-6.7	-5.2	-3.2	NAv	0.6	-5.8	-1	-1.4	NAp	NAp	no	gyral simplification; thin corpus callosum; subcortical hypersignal on T2-weighted images (0.6)	NAp	hyperpigmentation spot
#4	M	-3.8	-1.5	-0.9	NAv	1.7	-7.5	-2.5	-2.8	no	NAp	no	gyral simplification (0.5)	NAp	behavioral disorders
#5	M	-3.4	0	+1.1	no	20	-4.1	-0.8	+0.9	no	no	no	NAv	NAv	no
#6	F	NA	NA	-1.5	NAv	7	-10.6	-2.2	-2.5	no	no	no	gyral simplification; corpus callosum hypoplasia (age?)	NAv	congenital hip dislocation
#7	M	-6.6	-2.5	-2.2	NAv	7	-9.5	-0.8	-0.8	NAv	NAv	yes (<4)	NAv	NAv	no
#8	M	-3.8	-1	-0.8	T3	3.7	-5.6	+1.5	+0.6	no	no	no	ventricular enlargement, cerebellar hypoplasia, subcortical hypersignal on T2-weighted images, elongated superior cerebellar peduncles (3.7)	WPPSI-III (3.7): FSIQ=60*/ VIQ=74/ PIQ=54/ GCL=85	no
#9	F	-3.8	-1.3	-0.6	T3	4.5	-6.1	+0.5	-0.4	NAv	NAv	no	gyral simplification (0.7)	Test? (5y): FSIQ = 50	no
#10	F	-4.5	-0.2	-0.7	T3	19	-8.5	-0.2	-0.4	yes	no	yes (14)	gyral simplification, mild ventricular enlargement; scaphocephaly (0.8)	NAv	behavioral disorders
#11	F	-2.7	-0.5	-1	NAv	3	-3.4	NAv	NAv	yes	NAv	no	gyral simplification; arachnoid cyst in the posterior fossa; enlarged Virchow-Robin spaces (1.7)	Bayley III (3.5): DQ=95	no
#12.1	F	NAv	NAv	NAv	NAv	7	-7.2	-3.7	-1.9	NAv	NAv	no	gyral simplification, mild ventricular enlargement, thin corpus callosum and brainstem (7)	Stanford Binet (7): DQ = 56	no
#12.2	M	NAv	NAv	NAv	NAv	0.2	-4.8	+2.5	+0.2	NAp	NAp	no	gyral simplification, ventricular and pericerebral space enlargement; thin corpus callosum and brainstem; myelination delay in T2-weighted images (0.3)	NAp	no

#13	M	-4.7	-2.5	-2	T3	5.5	-6.4	-0.6	-0.4	yes	no	no	white matter hypersignal in T2-weighted images, ventricular enlargement (5.5)	WPPSI-III (5.5): FSIQ=82*/ VIQ=75/ PIQ=90/ GCL=77	no
#14.1	M	NAv	NAv	NAv	T3	9	-8.5	+0.5	NAv	yes	yes	yes (0.5)	NAv	NAv	hyperactivity, enuresis
#14.2	M	NAv	NAv	NAv	T3	13	-6.5	+1.7	NAv	yes	yes	yes (0.5)	NAv	NAv	enuresis
#15	F	-4	-1.3	-1.5	no	5.5	-5.5	+0.7	+1.1	no	no	no	gyral simplification, enlarged subarachnoid spaces, mega cisterna magna (0.5)	NAv	no
#16	M	-6.5	-1.6	-0.7	T3	NAv	NAv	NAv	NAv	NAv	NAv	NAv	NAv	NAp	closed fontanelles before birth
#17.1	M	-8.4	-2.6	-4	T3	17.5	-6.9	-1.5	-1.6	no	no	yes (7)	slight left cerebral atrophy; cervicothoracic syringomyelia (9)	Borel-Maisonny (4.5) : DQ = 56	spastic hemiplegic cerebral palsy, severe behavioral disorders
#17.2	M	-6.6	-2.5	-3.2	T3	17	-7.2	-0.4	-1	no	no	no	no (1)	Borel-Maisonny (4.4): DQ = 68	no
#18	M	-3.8	-1	+0.2	T2&T3	1	-6	+2	+1	yes	NAp	no	no (1)	NAp	behavioral disorders
#19	F	-7.2	-1.1	-0.3	no	3.3	-7	-3	-2.2	no	no	yes (1.5)	gyral simplification, thin corpus callosum, pineal cyst, large arachnoid cyst in the posterior fossa (0.3)	NAv	left middle ear hypoplasia, behavioral disorders
#20	M	-4.3	-0.5	0	T2&T3	7	-5.6	-1.4	-1.7	yes	no	yes (6)	gyral simplification, polymicrogyria in fronto-insular region (7)	Test? (9.3): DQ=34	no
#21	M	NAv	NAv	NAv	NAv	45	-6.3	NAv	NAv	no	NAv	yes (7)	gyral simplification; mild ventricular enlargement; mega cisterna magna; hypersignal of the temporal poles in T2 -weighted images; thin brainstem; major vermis and cerebellar atrophy (45)	NAv	Ceroid lipofuscinosis with identified *CLN3* mutations (retinitis pigmentosa)
#22	F	-2.2	+2	NA	no	15	-4.1	-1.1	+2.3	yes	yes	yes (15)	no	NAv	no
#23.1	M	NAv	NAv	NAv	NAv	4	-10.8	-3.9	-3.1	no	NAv	no	thick frontal gyri, gyral simplification; thick corpus callosum; extensive bilateral posterior polymicrogyria (4.3)	NAv	spastic tetraplegia
#23.2	M	NAv	NAv	NAv	NAv	6	-8.4	-1.1	-0.6	NAv	no	no	thick frontal gyri, gyral simplification; thick corpus callosum. hypersignal of temporal poles in T2-weighted images (6.5)	Test? (age?): DQ = 36	behavioral disorders
#24.1	F	NAv	NAv	NAv	no	17	-4.3	-2	-1.2	yes	no	no	NAv	NAv	no
#24.2	F	NAv	NAv	NAv	T3	9	-6.2	-1.7	-1.9	yes	no	no	NAv	NAv	no

#24.3	M	NAv	NAv	-4.3	T3	7	-8.7	NAv	-2	no	no	no	Pachygyria	NAv	vertical nystagmus
#25	F	-7.6	NAv	-1.7	T3	0.6	-9.5	-1.2	-2	NAp	NAp	no	gyral simplification (0.6)	NAp	hypertonia, hyperpigmentation spot
#26	M	-7	-4.5	-3	T3	2.7	-10.7	NAv	-1.6	no	NAp	no	gyral simplification; anterior pachygyria; mega cisterna magna; short splenium of the corpus callosum and cerebellar hypoplasia; relatively large mammillary bodies (3)	NAp	behavioral disorders, pyramidal syndrome, oral dyspraxia
#27	M	-4.7	0	-1.3	no	13	-9.7	-1.6	-1.3	no	no	yes (1.5)	Pronounced gyral simplification (microlissencephaly?); corpus callosum hypoplasia	NAv	hypo/hyperpigmentation spots, behavioral disorders
#28	F	-2.2	-0.8	+1.5	T3	5	-4.9	-0.3	-0.3	yes	no	no	gyral simplification (2)	NAv	behavioral disorders
#29	M	NAv	NAv	NAv	no	3	-7.6	0	-0.8	yes	no	no	no (2)	NAv	behavioral disorders
#30	M	-5.6	-2	-1.8	T3	3.5	-8.8	-1.2	-1.2	yes	no	no	gyral simplification (2.6)	WPPSI-III (3.5): FSIQ=55/ VIQ=62/ PIQ=57/ GCL=65	hypo/hyperpigmentation spots
#31	M	-3.8	-0.5	-0.1	T3	2.3	-6.2	-0.9	-0.2	yes	NAp	no	gyral simplification, thin corpus callosum (0.1)	NAp	hypotonia, behavioral disorders
#32.1	F	-4	-1.9	-1.8	no	21.5	-6.2	-1.9	-0.3	yes	no	no	gyral simplification; vermis hypoplasia (9)	Test? (12):FSIQ =70	scoliosis, hyperpigmentation spot, inverted nipples
#32.2	F	-2.2	-2.4	-0.6	no	23.8	-5.8	-1.1	-0.4	yes	no	no	slight cortical atrophy (1)	Test? (10):FSIQ =70	scoliosis, hypopigmentation spot, inverted nipples
#33.1	M	-5.6	-2.5	-2.4	T3	2.7	-7.8	-0.2	-1.1	yes	NAp	no	gyral simplification (0.2)	NAp	abnormality of helix
#33.2	F	-4.9	-1.9	-0.9	T3	0.2	-5.3	+0.7	-0.4	NAp	NAp	no	gyral simplification; thin corpus callosum (0.2)	NAp	abnormality of helix
#34	M	-2.9	-1	-1.1	NAv	11.3	-3.9	+0.3	+0.1	yes	no	no	gyral simplification; enlarged Virchow-Robin spaces; mega cisterna magna (5)	WPPSI-IV (7.2): FSIQ=58*/ VCI=86/ FRI=63/ WMI=56/ PSI=66	behavioral disorders
#35	F	-3.1	-0.8	+0.1	T3	1.4	-6.3	-0.4	-1	NAp	NAp	no	gyral simplification; thin corpus callosum; enlarged Virchow-Robin spaces (0.3)	NAp	behavioral disorders
#36	M	-4.3	-1.3	-1.3	NAv	16	-4.3	-3.8	-2.5	yes	yes	no	hypersignal of the thalamus in T2-weighted images (13.8)	NAv	no
#37	F	-4	-0.8	-0.9	no	13	-6.5	-1.5	-1	no	no	paroxysmal events	gyral simplification, scaphocephaly; enlarged Virchow-Robin spaces, mild enlarged ventricles (11)	NAv	scoliosis
#3	M	-	-	-0.8	T3	3.6	-	-	-2.5	ye	no	no	gyral simplification (fetal)	NAv	NAv

8		5.2	1.5				7.3	1.7		s					
#39	F	-3.1	-2.2	+0.1	T3	7.4	-7.3	-0.8	-0.5	yes	no	no	no (2)	NAv	coarse facial features, retrognathia