Mutation update of the *ASPM* gene causing autosomal recessive primary microcephaly

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Key Words: ASPM, centrosome, primary microcephaly, MCPH, brain development, brain imaging, intellectual disability

ABSTRACT (180-200 words)

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) of below -2SD at birth and -3SD after 6 months, and leading to intellectual disability of variable severity. The Abnormal SPindle-like Microcephaly gene (*ASPM*) is the human ortholog of the *D. melanogaster* 'abnormal spindle' gene (*asp*), and 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 represents the majority of all MCPH patients worldwide.

Here, we report 51 unpublished patients from 42 families carrying 28 new *ASPM* mutations and review the molecular, clinical and neuropsychological features of 280 reported families (160 distinct *ASPM* mutations). Furthermore, we discuss structural brain defects that highlight a strong reduction in cortical volume and surface area, which differentially affect various brain regions, thus modifying the cortical map of these patients.

Background

Primary microcephalies (PM) refer to a group of autosomal recessive disorders characterized by a reduction in brain growth beginning *in utero*, intellectual disability (ID) of variable severity, normal stature and absence of extra CNS malformations (Kaindl, et al., 2009; Thornton and Woods, 2009). A subgroup of patients with PM were defined as MCPH (Microcephaly, primary, hereditary), which initially address the definition of microcephalia vera, i.e. isolated primary microcephaly with a nearly normal brain cytoarchitecture. Nowadays, MCPH also includes primary microcephaly with cortical malformations. 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, et al., 1955; Morris, et al., 2016; Van Den Bosch, 1959). Clinically, PM is defined by an occipitofrontal circumference (OFC) 2 standard deviations (SD) below the age- and sex-matched mean at birth and below 3 SD after 6 months of age. PM can be detected from the 2nd trimester of pregnancy by ultrasound scan (Woods and Parker, 2013).

PM is genetically heterogeneous: a MCPH phenotype has been associated with mutations in at least 17 genes, MCPH1-17 (Verloes, et al., 2013). Among them, *ASPM* (MCPH5; MIM #608716) is the most frequent mutated gene reported. Mutations in the other 16 known MCPH genes cause less than 40% of the reported diagnosis. Many MCPH families remain without any diagnosis in known MCPH genes, suggesting that new genes are still to be discovered.

Although numerous patients have been reported (Abdel-Hamid, et al.; Abdel-Hamid, et al., 2016; Ahmad, et al., 2016; Akbariazar, et al., 2013; Al-Gazali and Ali; Ariani, et al., 2013; Bond, et al., 2002; Bond, et al., 2003; Darvish, et al., 2010; Desir, et al., 2006; Desir, et al., 2008; Gul, et al., 2006; Gul, et al., 2007; Halsall, et al.; Hashmi, et al.; Hu, et al., 2014; Kousar, et al., 2010; Kumar, et al., 2004; Muhammad, et al., 2009; Nakamura, et al., 2015; Nicholas, et al., 2009; Papari, et al., 2013; Passemard, et al., 2009a; Pichon, et al., 2004; Rump, et al.; Saadi, et al., 2009; Sajid Hussain, et al., 2013; Shen, et al., 2005; Tan, et al., 2014; Wang, et al., 2017), the developmental phenotype of these patients is documented in only a minority of case. As many patients were ascertained in countries where access

to neuroimaging, neurocognitive and behavioral assessments are difficult, this has often precluded correlation studies between neuroanatomical anomalies and ID or epilepsy. Intellectual disability (Passemard, et al., 2009a) and epilepsy (Shen, et al., 2005) are the most frequently reported clinical findings in patients with *ASPM* mutations.

The Abnormal SPindle-like Microcephaly gene (*ASPM*; MIM *605481) is the human ortholog of the *D. melanogaster* 'abnormal spindle' gene (*asp*) and maps on chromosome 1q31.3 (Bond, et al., 2002; Jamieson, et al., 2000; Pattison, et al., 2000). Four isoforms have been described in *ASPM* gene (Kouprina, et al., 2005). The *ASPM* full length 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, et al., 2007). ASPM plays a crucial role in cell division of neural progenitor cells by keeping them cycling, promoting symmetric proliferative divisions at the expense of asymetric neurogenic divisions (Fish, et al., 2006). Different mouse models of *Aspm* knock-out recapitulate the microcephaly observed in Human and show a reduction in cortical surface area (Capecchi and Pozner, 2015; Pulvers, et al., 2010) and thickness (Capecchi and Pozner, 2015). Mechanisms underlying Aspm microcephaly in mice are an increase in the cell cycle duration of the neural progenitors many of which exit the cell cycle, thereby leading to a premature exhaustion of the neural progenitors' pool, a subsequent increase of lower layers neurons production and a reduced production of neurons in the upper cortical layers (Capecchi and Pozner, 2015). Whether these mechanisms also explain microcephaly in human 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, centrosome and Golgi complex (Schou, et al., 2014), an Actin Binding Domain (ABD) comprising 2 calponin homology (CH) domains able to bind one actin monomer in the filament (Stradal, et al., 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, a peculiar interest lies in the variation of its IQ repeats: The human protein displays 81 IQ repeats at positions 1273 to 3234,

whereas there are 61 IQ in mouse and 24 IQ in drosophila (Bond, et al., 2002; Kouprina, et al., 2005; Kouprina, et al., 2004). Although still debated, the assumption that the expansion of the cerebral cortex would depend on the number of IQ repeats has been proposed (Bond, et al., 2002; Bond and Woods, 2006; Kouprina, et al., 2005; Kouprina, et al., 2004; Ponting and 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 at the spindle pole during metaphase, whereas the C-terminal domain encoded by the last three exons is required for its localization at the midbody during cytokinesis (Kouprina, et al., 2005; Paramasivam, et al., 2007).

Here, we report on 51 new patients (42 families) followed within the EuroMicro network, including 28 novel *ASPM* mutations, and present an exhaustive overview of all published affected individuals with *ASPM* mutations along with their molecular, clinical, radiological and neuropsychological features.

Mutations

Reported mutations

From the original discovery of *ASPM* mutations (Bond, et al., 2002) to January 2017, 160 mutations have been reported. These reports have been collected using the PubMed library. The terms “ASPM”, “MCPH5”, “MCPH”, “autosomal recessive microcephaly”, “microcephaly primary hereditary” and “microcephalic dwarfism” have been used as key words. *ASPM* mutations have been identified in 634 affected individuals belonging to 280 families. All the mutations are depicted on Figure 1 and summarized in Supp. Table S1. These mutations are spread all along the coding sequence. No intragenic CNV have been reported. Large deletion encompassing more than *ASPM* have been reported Described mutations include 2 large deletions encompassing several exons/introns (1.3%), 83 nucleotide substitutions (73 exonic, 10 intronic), 62 deletions (60 exonic, 2 intronic) and 9 duplications/insertions predicting a premature stop codon (nonsense, frame shift mutations: 46.8% and 41.6% respectively), intronic or exonic splice site mutations that interfere or are predicted to interfere with correct splicing (9%), and a few missense mutations (1%). These mutations are already available on The Leiden Open Variation Database (http://databases.lovd.nl/shared/genes/ASPM). 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. Very few studies have been conducted to verify this hypothesis except for two mutations located in exon 24 (c.9754del; pArg3252Glufs*10) and in intron 25 (c.9984+1G>T; p.?) (Higgins, et al., 2010; Kouprina, et al., 2005). In both cases, western blot analysis revealed an ASPM protein, truncated for the frame shift mutation, and with a similar size as the full length for the splice site mutation (start of traduction from an upstream cryptic splice donor site), but with a significant decreased expression of mutated ASPM at the mitotic spindle pole. Among the 160 mutations described so far, three mutations are recurrently observed. The c.3978G>A mutation (allele frequency = 18%), is only reported in Turkish and Pakistani

families (60 families). The c.9557C>G mutation is reported exclusively in Pakistan (7 families). Both mutations suggest a funder effect, whereas the c.7782_7783del mutation, which represents 4% of all mutations, is reported in families from different geographic origin (Europe, Africa and Asia) and is also found in our series with a high allele frequency (14%). This latter may correspond to a hotspot mutation.

Unreported mutations, methods of identification, cohort

Molecular analysis of was performed within our European Network "EuroMicro" (including five partners in France, Belgium, Germany, Switzerland, UK), since 2007 until January 2017 in patients referred for typical MCPH, primary microcephaly with cortical malformation or microcephalic primordial dwarfism. The unique inclusion criteria was an OFC below -2SD at birth (or within the first month of life in case of skull hematoma secondary to delivery and -3SD after 6 months of age, whatever their stature. Exclusion criteria were: 1) context of anoxo-ischemia at birth, 2) 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 bp of intron/exon boundaries of the *ASPM* gene were screened for variants either by Sanger Sequencing or Next Generation sequencing. Pathogenicity of the variants was assessed using the Alamut Software (Interactive Biosoftware, Rouen, France).

Altogether, we genotyped 51 patients from 42 unrelated families. 19 index cases were born to consanguineous parents. Genotyping identified 22 published and 28 unpublished variants (Table 1 and Figure 1). These new variants included 17 frameshift mutations, 9 nonsense mutations and 2 splicing mutations. The molecular data are available in Table 1 and Figure 1. All mutations were declared in the Leiden Open Variation Database (databases.lovd.nl/shared/genes/ASPM).

Epidemiology, phenotype

Epidemiology: 685 patients have been reported: (51 from our series + 634 from literature) from 322 (42+280) families. Among those whose gender is available, there is 227 (30+197) males and 183 (20+163) females (Sex ratio M/F = 1,2). Most families came from middle-east: Pakistan (164 families), Saudi Arabia (18), Egypt (2+16) and Iran (13). 43 (12+31) families come from Europe and 2 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 25% of patients during childhood). The auxological data of our series and those of the literature are summarized in Figure 3 for OFC, height, and weight at birth and after 6 years of age. For our series, the standard deviations (SD) were calculated. For literature cases, we used the SD values given 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 the literature (2 families, (Desir, et al., 2008; Hu, et al., 2014)), whereas microcephaly may be observed from the 2nd trimester of pregnancy, as shown in our series for 21 families. Interestingly, most patients do not exhibit the criteria of microcephalic primordial dwarfism since their height is rarely below -4 SD at birth or postnatally. The reduced OFC growth kinetics with height being little affected, as shown in Figure 3, characterizes *ASPM*-mutated patients.

Development and clinical features: Walking without support was acquired around 21 months of age (+/- 12, ranging from 10 to 66 months; n=23/48 from our series and n= 20/601 from the literature). More precisely, 52% walked prior age 18 months of age. Available data related to verbal skills are scarce and heterogeneous in the literature, yet language seems to be delayed. 20% of patients from our series (n=5/25) were able to make sentences beyond 3 years of age. Behavior disorders, such as hyperkinesia, impulsiveness and aggressiveness are reported in 17 in our series and 13 patients of the

literature. Neurological examination may show pyramidal syndrome or even spasticity (n=4 in our series and n=4 in literature). Ataxia, tremor have not been reported. Seizures were reported in 46 patients (11/51, i.e. 21.5% in our series and n=37 in 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, #30, #34, #35.1 and #35.2). Malformations are rare and do not present a recurrent pattern: scoliosis (2 families: patients #35.1, #35.2 and #40), middle ear hypoplasia (1 patient: #19), preaxial polydactyly (1 patient: (Ahmad, et al., 2016)), unilateral cystic kidney (1 patient: (Passemard, et al., 2009b)), tricuspid insufficiency (1 family: (Ariani, et al., 2013))]. Deafness (1 patient (Darvish, et al., 2010) , Guillain Barré syndrome (1 patient (Passemard, et al., 2009b))nystagmus (patient #24.3) and familial retinitis pigmentosa (patient #21)have been reported. Fatal issues have been reported three times in the literature, one patient died after an acute myeloid leukemia (Al-Gazali and Ali) and two children (3 and 9 years old) died without any reported explanation (Abdel-Hamid, et al.; Hashmi, et al.). Co-occurrence of two unrelated genetic diseases has been also reported in two patients: one had a deletion of the *STS* gene (Abdel-Hamid, et al.), the other one exhibited an oculo-cutaneous albinism (Abdel-Hamid, et al.). However, these associations do not seem more frequent than in the general population.

Cognition: Major prognosis factor of microcephaly is of course based on intellectual abilities. Although intellectual disability (ID), was systematically reported in patients with *ASPM* mutations, from mild to severe, neuropsychological assessment has been only performed in 35/624 patients in the literature (i.e. 5.6%, Figure 4). Among these 35 patients, 23 were assessed by one of the Wechsler scales to measure a full scale Intellectual Quotient (IQ), the remaining 12 by various motor and language skill assessment allowing to estimate a developmental quotient (DQ). The average total IQ was 54 $\pm$ 8 (ranging from 40 to 71). The average DQ was 47 $\pm$ 22 (ranging from 30 to 104). In our series, among 37 children aged of 3 years and more, psychological evaluation was not possible for 8 children living outside Europe. Among the remaining 29 patients, 12 could undergo psychological assessment. Two evaluations were not conclusive. The total average IQ of the 6 children that performed Wechsler

assessment was 57 ± 8 (ranging from 50 to 68). The DQ average of the 4 remaining children was 39 ± 12 (ranging from 30 to 56).

Neuropsychological assessment by Wechsler tests is a universally accepted tool (translated in many languages) that allows comparisons between patients from different countries. These patients exhibit mild to moderate intellectual deficiency. For patients with moderate to severe ID, who have an IQ below Wechsler's threshold (45), we performed developmental quotient assessment, with specific tests (Stanford Binet, Leiter-R scales) that are not always internationally available, and therefore only relevant in a population sharing the same language. For patients assessable by Wechsler scales, we have shown significant differences between scales (verbal comprehension, nonverbal performance, working memory or processing speed) in our patients. Total IQ is thus a poor reflect of the abilities of these children, as it may underestimate specific skills and/or hide specific deficits. Moreover, long-term memory, fundamental for learning, can only be assessed by specific tests such as children's memory scale (CMS) or Wechsler memory scale for adults, which are rarely performed. We have previously shown that long-term memory was spared in 5 patients with *ASPM* mutation, despite their intellectual deficiency, suggesting that they are able to learn (Passemard, et al., 2016).

Brain MRI: Brain magnetic resonance imaging was performed in 37/51 patients from our series (73%) and reported in 50/634 patients (8%) in the literature. Most frequent anomalies were: gyral simplification in 71/87 cases (n=26/37, i.e. 70% in our series and n= 45/50, i.e. 90% in literature), corpus callosum abnormality (shape, size,..) in 33/87 cases (n=7/37, i.e. 19% in our series and n= 26/50, i.e. 52% in literature), and middle to moderate cerebellar and/or pontic hypoplasia in 24/87 cases (n=4/37 in our series, i.e. 11% and n= 20/50, i.e. 40% in literature). Some atypical features were also described: polymicrogyria in 3 cases (patient #23.1 with extensive bilateral posterior polymicrogyria and (Marchal, et al.; Passemard, et al., 2009a)), syringomyelia (patient #17.1), and major vermis and cerebellar atrophy (patient #21). Such neuroradiological features are often undiscriminating for diagnosis (Figure 5), since they are not specific either of MCPH, nor of a specific type of MCPH.

Conventional imaging is thus not sufficiently informative enough to orientate the diagnosis, or to predict prognosis, except if it shows migration disorders associated to microcephaly, such as polymicrogyria, that may increase the epilepsy risk. Brain volume reduction in Human provide evidences for early neuronal and glial production defects. Polymicrogyria show that migration disorders are associated to proliferation defects in ASPM microcephaly. To give new insights in ASPM specific brain defects, two different approaches of the cortical structure should now to be considered: the structural brain imaging and the neuropathological study on postmortem cases. Indeed, we have shown that the 50% -average reduction in brain volume is caused by a major reduction of the cortical grey and white matter volumes contrasting with a relative preservation of the volume of the brainstem and cerebellum (Passemard, et al., 2016). This massive reduction of cortical volume and cortical surface preferentially affects the neocortex and spares the hippocampus and mesiotemporal cortici (involved in the long term memory tasks), concordant with the preserved mnesic functions of these patients (Passemard, et al., 2016).

Genotype-phenotype correlation

The vast majority of ASPM mutations probably result in a loss of function of ASPM. Moreover, most of *ASPM* mutations are private. Thus, genotype-phenotype correlations are difficult to make. However, the available data are still few and highlight the absolute requirement of deeper characterization of this rare disease.

Future prospects

Major efforts have been made on the molecular diagnosis of MCPH and NGS implementation in clinical diagnosis has identified *ASPM* mutations as the major cause of MCPH worldwide. The high number of

patients reported is counterbalanced by the dramatic lack of finely tuned clinical description, neurocognitive investigations, and the absence of large scale studies between anomalies of brain morphology and neurodevelopmental, cognitive or behavioral correlates. Hence, we have only limited knowledge of the real intellectual abilities of these patients, the natural history of their cognitive abilities, their functional cortical organization and their cortical map. The autonomy and social insertion of these patients as adults, as well as genetic counseling for the families would benefit from a better knowledge of brain structural and cognitive characteristics.

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

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463 **Tables:**

464

Ref	Origin	Location	DNA HGVnomenclature	Protein HGVnomenclature	Protein effect
#1	Moroccan	Exon 3	c.1850_1853del	p.Thr617Lysfs*30	Frameshift
#2	Belgian		c.1932del	p.Phe645Serfs*23	Frameshift
#3	Moroccan	Exon 4	c.1943_1944insC	p.Ile649Asnfs*3	Frameshift
#4	?	Exon 9	c.2638G>T	p.Glu880*	Nonsense
#5	French	Intron 10	c.2936+2T>C	p.?	Splicing
#6	Moroccan	Exon 13	c.3185_3189del	p.Asn1062Argfs*28	Frameshift
#7	Congolese	Exon 13	c.3269dup	p.Asp1091*	Nonsense
#8	?	Intron 15	c.3741+3A>G	p.?	Splicing
#9	European	Exon 18	c.4250_4251del	p.Tyr1417*	Nonsense
#10	?	Exon 18	c.4732C>T	p.Arg1578*	Nonsense
#11	Spanish	Exon 18	c.4806T>G	p.Tyr1602*	Nonsense
#12.1 #12.2	Egyptian	Exon 18	c.4992_4996dup	p.Arg1667Ilefs*12	Frameshift
#9	European	Exon 18	c.5590_5591del	p.Leu1864Serfs*2	Frameshift
#13	Cameroon	Exon 18	c.5886_5887del	p.Leu1963Glufs*9	Frameshift
#14.1 #14.2	Moroccan Moroccan	Exon 18	c.5940del	p.Tyr1981Ilefs*13	Frameshift
#15 #16	? Turkisch	Exon 18	c.6513dup	p.Val2172Serfs*7	Frameshift
#17.1 #17.2 #4	French French ?	Exon 18	c.6568C>T	p.Gln2190*	Nonsense
#18	Tunisian	Exon 18	c.6658C>T	p.Gln2220*	Nonsense
#17.1 #17.2	French	Exon 18	c.6919C>T	p.Gln2307*	Nonsense
#19	French	Exon 18	c.6920_6921del	p.Gln2307Leufs*10	Frameshift
#20	Italian	Exon 18	c.7744del	p.Ile2582Serfs*34	Frameshift
#5	French	Exon 18	c.7753G>T	p.Glu2585*	Nonsense
#21	French	Exon 18	c.8599delinsAT	p.Gln2867Ilefs*5	Frameshift
#22	?	Exon 18	c.8700_8702delinsCC	p.Lys2900Asnfs*38	Frameshift
#23.1 #23.2 #24.1 #24.2 #24.3	Egyptian Egyptian Moroccan Moroccan Moroccan	Exon 18	c.8702del	p.His290Leufs*37	Frameshift
#13	?	Exon 20	c.9069_9075del	p.His3023Glnfs*2	Frameshift
#21	French	Exon 23	c.9446_9447del	p.Arg3149Metfs*17	Frameshift
#8	?	Exon 28	c.10369del	p.Glu3457Lysfs*13	Frameshift

Table 1 – Novel ASPM mutations identified in our cohort, according to HGVS nomenclature recommendations and using the sequence NM_018136.4 as a reference.
#: patient's number in our series. Siblings are indicated as #1.1, 1.2 …

Ref	Sex	OFC at birth (SD)	Length at birth (SD)	Weight at birth (SD)	Age at last follow-up (y)	OFC at last follow-up (SD)	Length at last follow-up (SD)	Weight at last follow-up (SD)	Walk < 1.5y	First sentences < 3y	Epilepsy (if yes, age of onset, year)	Brain MRI (age)	Intellectual assessment (test/age)	Others features
#1	M	-5.9	NA	NA	5	-11.6	-3.2	-1.2	no	no	no	slight cortical atrophy	NA	behavioral disorders
#2	M	-3.5	+0.1	-0.7	5.5	-5.9	-0.6	NA	yes	yes	NA	NA	TIQ = 64 (5.8y)	NA
#3	F	-5.8	-4.9	-3.6	0.6	-4	0	-1	-	-	no	gyral simplification; thin corpus callosum; subcortical T2-weight images hypersignal	-	hyperpigmentation spot
#4	M	-2.7	-1.5	-0.8	1.7	-6.1	-2.5	-2.8	no	-	no	gyral simplification	-	behavioral disorders
#5	M	-2.3	-0.1	+1	20	-3.7	-0.9	0	no	NA	no	NA	NA	NA
#6	F	NA	NA	-1.5	7	-10.6	2.3	-2.9	no	no	no	gyral simplification; corpus callosum hypoplasia	NA	Congenital hip dislocation
#7	M	-5	-2.2	-1.9	7	-8.5	-1.1	-1	NA	NA	NA	NA	NA	NA
#8	M	-2.4	-0.6	-0.4	1.8	-5.4	+0.1	-0.7	no	-	no	said as normal (0.1y)	-	NA
#9	F	-3.1	-1.2	-0,5	4.5	-5.5	-0.4	-0.6	NA	NA	NA	gyral simplification	TIQ = 50 (5y)	NA
#10	F	-3.7	-0.1	-0.7	19	-7.3	-0.1	-0.7	yes	no	yes (14)	gyral simplification, mild ventricle enlargement; scaphocephaly (0.8y)	NA	behavioral disorders
#11	F	-1,5	-0.5	-1	2	NA	0	NA	NA	-	no	gyral simplification; arachnoid cyst in the posterior fossa; enlarged Wirshow Robin spaces (1.7y)	-	NA
#12.1	F	NA	NA	0	7	-7.5	-3	-2.5	NA	NA	no	gyral simplification, mild ventricle enlargement, thin corpus callosum and brainstem (7y)	DQ = 56 (Stanford Binet)	NA
#12.2	M	NA	NA	0	0.1	-3	+2.5	-0.5	NA	-	no	gyral simplification, ventricle and pericerabral spaces enlargement; thin corpus callosum and brainstem; myelination delay in T2 weight-images (0.3y)	-	NA
#13	M	-3.5	-2.3	-1.7	5	-5.2	-0.6	-1.1	yes	no	no	said as normal	NA	NA
#14.1	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
#14.2	M	NA	NA	NA	9	-4	0	0	NA	NA	yes (0.5)	NA	NA	NA
#15	F	-3.3	-1.2	-1.8	5.5	-4	+1	+0.2	NA	NA	no	gyral simplification; enlarged subarachnoid spaces, mega cisterna magna (0.5y)	NA	NA
#16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
#17.1	M	-6.7	NA	NA	15	-5.4	NA	NA	no	no	yes (7)	slight left cerebal atrophy; **syringomyelia** (6y)	NA	behavioral disorders
#17.2	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	no	NA	NA	NA
#18	M	-2.7	-1	+0.3	1	-6	+2	+1	-	-	no	said as normal (1y)	-	behavioral disorders
#19	F	-6.2	-0.9	-0.3	2,5	-6.4	-4.4	-2.5	no	-	yes (1.5)	gyral simplification, thin corpus callosum, pineal cyst, large arachnoid cyst in the posterior fossa (0.3y)	-	left middle ear hypoplasia, behavioral disorders
#20	M	-3.1	-1.3	-0.6	7	-5	-1.4	-1.7	yes	no	yes (6)	gyral simplification (7y)	34 (Leiter-R scale)	no
#21	M	NA	NA	NA	45	-5.4	NA	NA	no	no	yes (7)	gyral simplification; mild ventricle enlargement; mega cisterna magna; T2 -weighted images hyper signal of temporal poles; thin brainstem; **major vermis and cerebellar atrophy** (45y)	NA	retinitis pigmentosa
#22	F	-1,6	+2.1	NA	15	-3.2	-1	+1.4	yes	yes	yes (15)	said as normal	TIQ = 50 (15)	NA
#23.1	M	NA	NA	0	4	-9.6	-3.6	-2.9	no	NA	no	thick frontal gyri, gyral simplification; thick corpus callosum; **extensive bilateral posterior polymicrogyria**	NA	spastic tetraplegia
#23.2	M	NA	NA	0	6	-8	-1	-1	NA	no	no	thick frontal gyri, gyral simplification; thick corpus callosum; T2 -weighted images hyper signal of temporal poles	DQ = 36 (Stanford Binet)	behavioral disorders

#24.1	F	NA	NA	NA	22	-3.5	0	NA	NA	NA	no	NA	NA	NA
#24.2	F	NA	NA	NA	13	-6.8	0	NA	NA	NA	no	NA	NA	NA
#24.3	M	NA	NA	NA	7	-7.8	0	-2	no	no	no	NA	NA	nystagmus
#25	F	-6.6	NA	-1.7	0.6	-7	-1	-1.5	-	-	no	gyral simplification, thin corpus callosum and white anterior commissure (0.8y)	-	hyperpigmentation spot
#26	M	NA	NA	NA	10	-7.5	-1.1	-1.6	NA	NA	NA	NA	QD = 30 (20y)	behavioral disorders
#27	M	+1.2 ???	NA	NA	12	-7.5	NA	NA	NA	NA		NA	NA	NA
#28	M	-5.5	-4.7	-3	2.7	-8.7	NA	-1.6	no	-	no	gyral simplification; anterior pachygyria; mega cisterna magna; short splenium of the corpus callosum and cerebellum; relative large mamillary corpus (3y)	-	behavioral disorders
#29	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
#30	M	-3.5	+0.1	-1.2	13	-9	-2.2	-2.1	no	no	yes (1.5)	microlissencephaly; corpus callosum hypoplasia	NA	hypo/hyperpigmentation spots, behavioral disorders
#31	F	-2	-0.6	+1.6	5	-5.5	NA	NA	yes	no	NA	gyral simplification	NA	behavioral disorders
#32.1	M	-3.5	NA	NA	6	-4.4	NA	NA	yes	no	NA	gyral simplification	TIQ = 68 (4,5y)	pyramidal signs, hyperactivity
#32.2	F	-3.3	-0.1	+0.6	9	-6.5	-0.6	-1	yes	no	yes (NA)	pachygyria	TIQ < 50 (6y)	hyperactivity
#33	M	NA	NA	NA	3	-5.3	NA	NA	yes	no	no	microcephalia vera	NA	behavioral disorders
#34	M	-4.3	-2.1	-1.7	3.5	-5.8	-1.8	-1.2	yes	no	no	gyral simplification	TIQ = 55	hypo/hyperpigmentation spots
#35.1	F	-3.3	-1.7	-1.9	21.5	-5.2	-1.5	-0.6	yes	no	no	gyral simplification; vermis hypoplasia (9y)	NA	scoliosis, hyperpigmentation spot, inverted nipples
#35.2	F	-2	-2.7	-0.8	23.8	-4.8	-0.9	-0.7	yes	no	no	slight cortical atrophy (5y)	NA	scoliosis, hypopigmentation spot, inverted nipples
#36.1	M	NA	NA	NA	2.7	-5.4	-0.5	-0.6	NA	-	no	gyral simplification	-	NA
#36.2	F	-4.1	-1.7	-0.8	0.2	-5.7	-0.8	-1.8	-	-	no	NA	-	NA
#37	M	-2	NA	NA	5	-4.4	NA	NA	yes	no	no	gyral simplification; enlarged Wirshow Robin spaces; mega cisterna magna (5y)	NA	behavioral disorders
#38	F	-2.4	-0.6	+0.2	1.4	-5.1	-0.9	-0.7	-	-	no	gyral simplification; thin corpus callosum; enlarged Wirshow Robin spaces (0.3y)	-	behavioral disorders
#39	M	-3	-1.3	-1.2	16	-3.7	NA	NA	yes	NA	NA	dysplasia?	NA	NA
#40	F	-3	-0.6	-0.8	13	-6.1	-1.6	-1.3	no	no	yes (NA)	gyral simplification; scaphocephaly; enlarged Wirshow Robin spaces; mild enlarged ventricles (11y)	NA	scoliosis
#41	M	-3.9	-1.5	-0.6	1.8	-5.9	-1.4	-3.2	yes	-	no	gyral simplification (fetal)	-	NA
#42	F	+1 ???	NA	NA	12	-7.4	NA	NA	NA	NA	NA	NA	NA	NA

Table 2 – Clinical and radiological features of patients of our cohort (42 families, 51 patients). #: patient's number in our series. Siblings are indicated as #1.1, 1.2 …

Location	DNA HGVnomenclature	Protein HGVnomenclature	Protein effect	References	Origin
	/	Whole gene deletion	Large deletion	Passemard et al., 2009	French
Exon 1	c.77del	p.Gly26Alafs*42	Frameshift	Nicholas et al., 2008 Kousar et al., 2009 Passemard et al., 2009	European Pakistani French
Exon 1	c.117_118del	p.Leu41Glnfs*30	Frameshift	Tan et al., 2014	Spanish
Intron 1	c.297+1G>C	p.?	Splicing	Darvish et al., 2010	Iranian
Intron 1 - Intron 13	c.298-581_3391-242del	in-frame deletion of exon 2 to exon 13	Large deletion	Nicholas et al., 2008	European
Exon 2	c.349C>T	p.Arg117*	Nonsense	Bond et al., 2003 Kumar et al., 2004	Pakistani Turkish
Exon 2	c.440del	p.Lys147Argfs*54	Frameshift	Nicholas et al., 2008	European
Exon 3	c.577C>T	p.Gln193*	Nonsense	Nicholas et al., 2008	European
Exon 3	c.637del	p.Ile213Tyrfs*47	Frameshift	Tan et al., 2014	Somalia
Exon 3	c.688del	p.Glu230Asnfs*30	Frameshift	Abdel-Hamid et al., 2016b	Egyptian
Exon 3	c.719_720del	p.Ser240Cysfs*16	Frameshift	Bond et al., 2002	Pakistani
Exon 3	c.803_804del	p.Lys268Serfs*4	Frameshift	Tan et al., 2014	Mexican
Exon 3	c.1002del	p.Val335*	Nonsense	Muhammad et al., 2009	Pakistani
Exon 3	c.1138C>T	p.Gln380*	Nonsense	Tan et al., 2014 #25	Saudi Kuwaiti
Exon 3	c.1154_1155del	p.Glu385Valfs*3	Frameshift	Nicholas et al., 2008	European
Exon 3	c.1179del	p.Asn394Ilefs*4	Frameshift	Nicholas et al., 2008	European
Exon 3	c.1235_1239del	p.Lys412Thrfs*5	Frameshift	Ahmad et al., 2016	Pakistani
Exon 3	c.1260_1266del	p.Gln421Hisfs*32	Frameshift	Bond et al., 2003 Gul et al., 2006 Kousar et al., 2009	Pakistani
Exon 3	c.1366G>T	p.Glu456*	Nonsense	Bond et al., 2003 Nicholas et al., 2008 #26	Turkish Turkish Turkish
Exon 3	c.1406_1413del	p.Asn469Ilefs*9	Frameshift	Nicholas et al., 2008	European
Exon 3	c.1590del	p.Val531*	Nonsense	Nicholas et al., 2008	European
Exon 3	c.1631_1635del	p.Tyr544Serfs*9	Frameshift	Desir et al., 2006 Passemard et al., 2009 #27	Turkish French Turkisch
Exon 3	c.1726_1729del	p.Lys576Alafs*10	Frameshift	Tan et al., 2014	European
Exon 3	c.1729_1730del	p.Ser577Argfs*33	Frameshift	Bond et al., 2003	Yemenite
Exon 3	c.1789C>T	p.Arg597*	Nonsense	Abdel-Hamid et al., 2016b #20	Egyptian Italian
Exon 4	c.1959_1962del	p.Asn653Lysfs*14	Frameshift	Bond et al., 2003 Nicholas et al., 2008 Tan et al., 2014 Abdel-Hamid et al., 2016 #28	Saudi European Saudi Egyptian Kuwaiti
Exon 4	c.1990C>T	p.Gln664*	Nonsense	Bond et al., 2003	Pakistani
Exon 5	c.2053dup	p.Asn688Lysfs*5	Frameshift	Rump et al., 2016	?
Exon 5	c.2101C>T	p.Gln701*	Nonsense	Kousar et al., 2009	Pakistani
Exon 6	c.2389C>T	p.Arg797*	Nonsense	Passemard et al., 2009 Saadi et al., 2009 Rump et al., 2016 #29	Algerian Algerian Algerian ?

Exon 6	c.2419del	p.Gly807Glufs*7	Nonsense	Ahmad et al., 2016	Pakistani
Intron 6	c.2419+2T>C	p.?	Splicing	Tan et al., 2014	African
Exon 8	c.2571G>A	p.Trp857*	Nonsense	Tan et al., 2014	?
Intron 8	c.2629+2del	p.?	Splicing	Akbari-Azar et al., 2013	Iranian
Intron 9	c.2761-25A>G	p.?	Splicing	Nicholas et al., 2008	European
Exon 10	c.2791C>T	p.Arg931*	Nonsense	Tan et al., 2014	Spanish
Exon 10	c.2936dup	p.Arg980Alafs*31	Frameshift	Tan et al., 2014	European
Intron 10	c.2936+1G>A	p.?	Splicing	Abdel-Hamid et al.,2016a	Egyptian
Intron10	c.2936+5G>T	p.?	Splicing	Bond et al., 2003	Pakistani
Intron 10	c.2937-2A>G	p.?	Splicing	Kraemer et al., 2016	Pakistani
Exon 11	c.2938C>T	p.Arg980*	Nonsense	Muhammad et al., 2009 Kraemer et al., 2016	Pakistani
Exon 11	c.2967G>A	p.Trp989*	Nonsense	Nicholas et al., 2008 Kraemer et al., 2016 #19	European European French
Exon 11	c.2968del	p.Asp990Thrfs*11	Frameshift	Tan et al., 2014	African
Exon 11	c.3055C>T	p.Arg1019*	Nonsense	Nicholas et al., 2008 Muhammad et al., 2009 Darvish et al., 2010 Nakamura et al., 2015 #10	European Pakistani Iranian Japanese ?
Exon 11	c.3067T>G	p.?	Splicing	Al-Gazali and Ali, 2010	Saudi
Exon 11	c.3082G>A	p.?	Splicing	Bond et al., 2003	Pakistani
Exon 12	c.3108_3114del	p.Val1037Glyfs*13	Frameshift	Abdel-Hamid et al., 2016b	Egyptian
Intron 12	c.3168 + 1G > C	p.?	Splicing	Rump et al., 2016	?
Exon 13	c.3188T>G	p.Leu1063*	Nonsense	Nicholas et al., 2008	Pakistani
Exon 13	c.3229_3230del	p.Lys1077Glufs*14	Frameshift	Darvish et al., 2010	Iranian
Exon 13	c.3327T>G	p.Tyr1109*	Nonsense	Tan et al., 2014	European
Exon 13	c.3341del	p.Lys1114Serfs*3	Frameshift	Abdel-Hamid et al., 2016b	Egyptian
Intron 13	c.3390+3_3390+6del	p.?	Splicing	Tan et al., 2014	Mexican
Exon 14	c.3477_3481del	p.Ala1160Metfs*23	Frameshift	Muhammad et al., 2009	Pakistani
Exon 14	c.3491_3494del	p.Arg1164Leufs*15	Frameshift	Ahmad et al., 2016	Pakistani
Exon 14	c.3506_3507del	p.Val1169Glyfs*15	Frameshift	Darvish et al., 2010	Iranian
Exon 14	c.3527C>G	p.Ser1176*	Nonsense	Bond et al., 2003	Jordanian
Exon 15	c.3663del	p.Arg1221Serfs*13	Frameshift	Bond et al., 2003	Pakistani
Exon 15	c.3710C>G	p.Ser1237*	Nonsense	Nicholas et al., 2008	European
Intron 15	c.3741+1G>A	p.?	Splicing	Nicholas et al., 2008 Darvish et al., 2010	European Iranian
Intron 15	c.3742-1G>C	p.?	Splicing	Hashmi et al.,2016	Saudi
Exon 16	c.3796G>T	p.Glu1266*	Nonsense	Nicholas et al., 2008 Halsall et al., 2010 Ariani et al., 2013 #30	African ? Italian ?
Exon 16	c.3811C>T	p.Arg1271*	Nonsense	Bond et al., 2003 Nicholas et al., 2008 Passemard et al., 2009 Tan et al., 2014	Dutch Indian European Reunion island
Exon 16	c.3853_3854del	p.Asp1285Serfs*32	Frameshift	Tan et al., 2014	Mexican

Exon 17	c.3945_3946del	p.Arg1315Serfs*2	Frameshift	Passemard et al., 2009 Tan et al., 2014	German European
Exon 17	c.3960_3961insA	p.Val1321Serfs*29	Frameshift	Tan et al., 2014	Spanish
Exon 17	c.3977G>A	p.Trp1326*	Nonsense	Halsall et al., 2010	?
Exon 17	c.3978G>A	p.Trp1326*	Nonsense	Kumar et al., 2004 Gul et al., 2006 Gul et al., 2007 Kousar et al., 2009 Muhammad et al., 2009 Sajid Hussain et al., 2013 Wang et al., 2016 Ahmad et al., 2016	Turkish Pakistani
Exon 17	c.3979C>T	p.Arg1327*	Nonsense	Sajid Hussain et al., 2013 Abdel-Hamid et al., 2016b	Pakistani Egyptian
Exon 18	c.4074G>A	p.Trp1358*	Nonsense	Passemard et al., 2009	?
Exon 18	c.4184G>A	p.Trp1395*	Nonsense	Halsall et al., 2010	?
Exon 18	c.4185G>A	p.Trp1395*	Nonsense	Wang et al., 2016	Pakistani
Exon 18	c.4195dup	p.Thr1399Asnfs*20	Frameshift	Desir et al., 2008 #31 #32.1 #32.2	Moroccan ? Moroccan Moroccan
Exon 18	c.4212G>A	p.Trp1404*	Nonsense	Ahmad et al., 2016	Pakistani
Exon 18	c.4583del	p.Lys1528Argfs*24	Frameshift	Bond et al., 2003	Pakistani
Exon 18	c.4612C>T	p.Arg1538*	Nonsense	Abdel-Hamid et al., 2016b	Egyptian
Exon 18	c.4728_4729del	p.Arg1576Serfs*7	Frameshift	Tan et al., 2014	African
Exon 18	c.4795C>T	p.Arg1599*	Nonsense	Bond et al., 2003 Tan et al., 2014 #33	Pakistani Pakistani ?
Exon 18	c.4849C>T	p.Arg1617*	Nonsense	Papari et al., 2013	Iranian
Exon 18	c.4858_4859del	p.Ile1620Phefs*24	Frameshift	Nicholas et al., 2008	Pakistani
Exon 18	c.5136C>A	p.Tyr1712*	Nonsense	Gul et al., 2007	Pakistani
Exon 18	c.5149delA	p.Ile1717*	Nonsense	Gul et al., 2007	Pakistani
Exon 18	c.5188G>T	p.Glu1730*	Nonsense	Darvish et al., 2010	Iranian
Exon 18	c.5196T>A	p.Cys1732*	Nonsense	Tan et al., 2014	European
Exon 18	c.5584A>C	p.Lys1862Gln	Missense	Darvish et al., 2010 Ahmad et al., 2016	Iranian
Exon 18	c.5606dup	p.His1870Thrfs*26	Frameshift	Kraemer et al., 2016	Pakistani
Exon 18	c.5959C>T	p.Gln1987*	Nonsense	Ahmad et al., 2016	Pakistani
Exon 18	c.6151C>T	p.Gln2051*	Nonsense	Sajid Hussain et al., 2013	Pakistani
Exon 18	c.6189T>G	p.Tyr2063*	Nonsense	Shen et al., 2005	Saudi
Exon 18	c.6232C>T	p.Arg2078*	Nonsense	Passemard et al., 2009	French
Exon 18	c.6337_6338del	p.Ile2113Serfs*11	Frameshift	Nicholas et al., 2008	Pakistani
Exon 18	c.6651_6654del	p.Thr2218Tyrfs*8	Frameshift	Passemard et al., 2009	French
Exon 18	c.6686_6689del	p.Arg2229Thrfs*10	Frameshift	Kousar et al., 2009 Passemard et al., 2009 #29	Pakistani Lebanese ?
Exon 18	c.6732del	p.Tyr2245Thrfs*15	Frameshift	Muhammad et al., 2009	Pakistani
Exon 18	c.6750del	p.Phe2250Leufs*10	Frameshift	Nakamura et al., 2015	Japanese
Exon 18	c.6852_6855del	p.Leu2285Argfs*6	Frameshift	Ahmad et al., 2016	Pakistani

Exon 18	c.6994C>T	p.Arg2332*	Nonsense	Halsall et al., 2010 Wang et al., 2016	Pakistani
Exon 18	c.7129C>T	p.Gln2377*	Nonsense	Ahmad et al., 2016	Pakistani
Exon 18	c.7308dup	p.Val2437Cysfs*14	Frameshift	Tan et al., 2014	Mexican
Exon 18	c.7491T>G	p.Tyr2497*	Nonsense	Abdel-Hamid et al., 2016b #14.1 #14.2	Egyptian Moroccan Moroccan
Exon 18	c.7491_7495del	p.Thr2499Serfs*18	Frameshift	Nicholas et al., 2008	European
Exon 18	c.7569_7570del	p.Glu2525Lysfs*17	Frameshift	Halsall et al., 2010	?
Exon 18	c.7612C>T	p.Gln2538*	Nonsense	Tan et al., 2014	Saudi
Exon 18	c.7665del	p.Ala2556Leufs*4	Frameshift	Tan et al., 2014	European
Exon 18	c.7761T>G	p.Tyr2587*	Nonsense	Bond et al., 2002 Nicholas et al., 2008	Pakistani
Exon 18	c.7772_7775del	p.Lys2591Argfs*24	Frameshift	Hu et al., 2014	Turkish
Exon 18	c.7781_7784del	p.Lys2595Tyrfs*20	Frameshift	Rump et al., 2016	?
Exon 18	c.7782_7783del	p.Lys2595Serfs*6	Frameshift	Nicholas et al., 2008 Passemard et al., 2009 Saadi et al., 2009 Tan et al., 2014 Kraemer et al., 2016 #7 #11 #18 #34 #35.1 #35.2 #36.1 #36.2 #37 #38 #39	Pakistani European Algerian Spanish Cambodian Congolese Spanish Algerian Algerian European European ? ? ? ? Portuguese
Exon 18	c.7815_7816del	p.Glu2605Aspfs*31	Frameshift	Ariani et al., 2013	Italian
Exon 18	c.7825C>T	p.Gln2609*	Nonsense	Tan et al., 2014	European
Exon 18	c.7857dup	p.Glyn2620Thrfs*17	Frameshift	Tan et al., 2014	?
Exon 18	c.7860_7861del	p.Gln2620Hisfs*16	Frameshift	Nicholas et al., 2008	Saudi
Exon 18	c.7894C>T	p.Gln2632*	Nonsense	Muhammad et al., 2009	Pakistani
Exon 18	c.7896_7897del	p.Lys2633Alafs*3	Frameshift	Bond et al., 2003	Pakistani
Exon 18	c.8017C>T	p.Gln2673*	Nonsense	Tan et al., 2014 #35.1 #35.2	Saudi European European
Exon 18	c.8098C>T	p.Arg2700*	Nonsense	Ahmad et al., 2016	Pakistani
Exon 18	c.8131_8132del	p.Lys2711Glufs*12	Frameshift	Nicholas et al., 2008	European
Exon 18	c.8133_8136del	p.Lys2712Leufs*16	Frameshift	Tan et al., 2014 #2	European Belgian
Exon 18	c.8191_8192del	p.Glu2731Lysfs*19	Frameshift	Passemard et al., 2009	French
Exon 18	c.8195_8198del	p.Arg2732Lysfs*4	Frameshift	Passemard et al., 2009 #40	German ?
Exon 18	c.8200_8201del	p.Asn2734Leufs*16	Frameshift	Sajid Hussain et al., 2013	Pakistani
Exon 18	c.8227C>T	p.Arg2743*	Nonsense	Hu et al., 2014	Turkish
Exon 18	c.8273T>A	p.Leu2758*	Nonsense	Passemard et al., 2009	French
Exon 18	c.8378del	p.Met2793Argfs*27	Frameshift	Nicholas et al., 2008	Pakistani

Exon 18	c.8508_8509del	p.Lys2837Metfs*34	Frameshift	Bond et al., 2003 Gul et al., 2007 Nicholas et al., 2008 Muhammad et al., 2009 Sajid Hussain et al., 2013 Ahmad et al., 2016	Pakistani
Exon 18	c.8668C>T	p.Gln2890*	Nonsense	Muhammad et al., 2009 Sajid Hussain et al., 2013	Pakistani
Exon 18	c.8711_8712del	p.Gln2904Argfs*15	Frameshift	Tan et al., 2014	Spanish
Exon 19	c.8844del	p.Lys2979Argfs*7	Frameshift	Nicholas et al., 2008	European
Exon 19	c.8903G>A	p.Trp2968*	Nonsense	Tan et al., 2014	Saudi
Exon 21	c.9091C>T	p.Arg3031*	Nonsense	Darvish et al., 2010 Tan et al., 2014	Iranian Saudi
Exon 21	c.9104T>A	p.Leu3035*	Nonsense	Tan et al., 2014	African
Exon 21	c.9115_9118dup	p.Tyr3034Serfs*3	Frameshift	Gul et al., 2006	Pakistani
Exon 21	c.9159del	p.Lys3053Asnfs*5	Frameshift	Bond et al., 2002 Bond et al., 2003 Kousar et al., 2009	Pakistani
Exon 21	c.9178C>T	p.Gln3060*	Nonsense	Kumar et al., 2004 Nicholas et al., 2008 Tan et al., 2014	Turkish Indian Spanish
Exon 21	c.9190C>T	p.Arg3064*	Nonsense	Bond et al., 2003 Nicholas et al., 2008 Abdel-Hamid et al., 2016b Kraemer et al., 2016	Pakistani European Egyptian
Exon 21	c.9238A>T	p.Lys3080*	Nonsense	Gul et al., 2006	Pakistani
Exon 21	c.9286C>T	p.Arg3096*	Nonsense	Darvish et al., 2010	Iranian
Exon 22	c.9309_9310del	p.Arg3103Serfs*20	Frameshift	Tan et al., 2014	European
Exon 22	c.9319C>T	p.Arg3107*	Nonsense	Muhammad et al., 2009 Passemard et al., 2009 Darvish et al., 2010 #38	Pakistani French Iranian ?
Exon 23	c.9454C>T	p.Arg3152*	Nonsense	Tan et al., 2014	European
Exon 23	c.9492T>G	p.Tyr3164*	Nonsense	Kousar et al., 2009 Muhammad et al., 2009 Sajid Hussain et al., 2013 Wang et al., 2016 Ahmad et al., 2016	Pakistani
Exon 23	c.9507del	p.Ile3170Leufs*9	Frameshift	Passemard et al., 2009	French
Exon 23	c.9539A>C	p.Gln3180Pro	Missense	Gul et al., 2006 Kraemer et al., 2016	Pakistani
Exon 23	c.9541C>T	p.Arg3181*	Nonsense	Abdel-Hamid et al., 2016b	Egyptian
Exon 23	c.9557C>G	p.Ser3186*	Nonsense	Bond et al., 2003 Gul et al., 2006 Muhammad et al., 2009 Sajid Hussain et al., 2013 Tan et al., 2014 Wang et al., 2016	Pakistani
Exon 23	c.9595A>T	p.Lys3199*	Nonsense	Muhammad et al., 2009	Pakistani
Exon 24	c.9677dup	p.Cys3226Trpfs*5	Frameshift	Muhammad et al., 2009	Pakistani
Exon 24	c.9685del	p.Ile3229Leufs*6	Frameshift	Nicholas et al., 2008	Pakistani

Exon 24	c.9686_9690del	p.Ile3229Serfs*10	Frameshift	Passemard et al., 2009	Lebanese
Exon 24	c.9697C>T	p.Arg3233*	Nonsense	Muhammad et al., 2009 Tan et al., 2014 Abdel-Hamid et al., 2016b #41	Pakistani Saudi Egyptian Portuguese
Exon 24	c.9730C>T	p.Arg3244*	Nonsense	Gul et al., 2007 Muhammad et al., 2009 Sajid Hussain et al., 2013 Tan et al., 2014 #36.1 #36.2	Pakistani Pakistani Pakistani African ? ?
Exon 24	c.9747_9748del	p.Tyr3250Glnfs*14	Frameshift	Nicholas et al., 2008	Pakistani
Exon 24	c.9754delA	p.Arg3252Glufs*10	Frameshift	Bond et al., 2003 Al-Gazali and Ali, 2010	Yemenite Saudi
Exon 24	c.9789T>A	p.Tyr3263*	Nonsense	Nicholas et al., 2008 Sajid Hussain et al., 2013	Pakistani
Exon 25	c.9841A>T	p.Arg3281*	Nonsense	Desir et al., 2006 #42	Turkish Turkish
Exon 25	c.9910C>T	p.Arg3304*	Nonsense	Tan et al., 2014 #33	European ?
Intron 25	c.9984+1G>T	p.?	Splicing	Bond et al., 2003	Pakistani
Exon 26	c.10059C>A	p.Tyr3353*	Nonsense	Gul et al., 2007	Pakistani
Exon 26	c.10060C>T	p.Arg3354*	Nonsense	Halsall et al., 2010	?
Exon 27	c.10168C>T	p.Arg3390*	Nonsense	Abdel-Hamid et al., 2016b	Egyptian

Supp. Table S1- Reported *ASPM* mutations (n=160), according to HGVS nomenclature recommendations and using the sequence NM_018136.4 as a reference. #: patient's number in our series. Siblings are indicated as #1.1, 1.2 …

Figure Legends:

Figure 1: Location of the different know and novel mutations on *ASPM* gene and representation of the differents domains of corresponding ASPM protein.

Reported mutations are illustrated on a circle arc at the top of the figure. Mutations identified in our cohort but already published are indicated by a sharp (#). Our novel mutations are aligned just above blue boxes that represent exons. Mutations are depicted using different symbols according to the type of mutations (missense, nonsense,and small deletion) as shown on the caption. Allelic frequency (AF) is indicated if > 2%. Abreviations: AF= allelic frequency; ASH = ASPM; SPD-2,Hydin; CH= calponin homology.

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 = 322 (21 families with unknown or multiple origin).

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

A- OFC and length measurements: Red symbols depict patients from our cohort as compared to black symbols corresponding to reported patients. Dots represent OFC and lines represent length. Each symbol represent an individual measurement. One single individual may have several measurements. OFC = occipitofrontal circumference.

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

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

Red symbols depict patients from our cohort as compared to black symbols corresponding to reported patients. Empty lozenges represent measurement of developmental quotient; full lozenges represent the measurement of full scale intellectual quotient (IQ).

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

A: patient #22, B: patient #18, C: patient #12 and D: age-matched Control. From left to right: Sagital T1 / coronal T1 / axial T1 / coronal T2-weight images.

Drastic reduction in volume of both hemispheres affecting 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 control. *A contrario*, volume of the cerebellum is preserved as shown by the coronal view. Unilateral or bilateral polymicrogyria may be associated to *ASPM*-related primary microcephaly as shown in C (white arrows).