

Comprehensive review on the molecular genetics of autosomal recessive primary microcephaly (MCPH)

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Review

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Abstract

Primary microcephaly (MCPH) is an autosomal recessive sporadic neurodevelopmental ailment with a trivial head size characteristic that is below 3–4 standard deviations. MCPH is the smaller upshot of an architecturally normal brain; a significant decrease in size is seen in the cerebral cortex. At birth MCPH presents with non-progressive mental retardation, while secondary microcephaly (onset after birth) presents with and without other syndromic features. MCPH is a neurogenic mitotic syndrome nevertheless pretentious patients demonstrate normal neuronal migration, neuronal apoptosis and neural function. Eighteen MCPH loci (MCPH1–MCPH18) have been mapped to date from various populations around the world and contain the following genes: *Microcephalin*, *WDR62*, *CDK5RAP2*, *CASC5*, *ASPM*, *CENPJ*, *STIL*, *CEP135*, *CEP152*, *ZNF335*, *PHC1*, *CDK6*, *CENPE*, *SASS6*, *MFS2A*, *ANKLE2*, *CIT* and *WDFY3*, clarifying our understanding about the molecular basis of microcephaly genetic disorder. It has previously been reported that phenotype disease is caused by MCB gene mutations and the causes of this phenotype are disarrangement of positions and organization of chromosomes during the cell cycle as a result of mutated DNA, centriole duplication, neurogenesis, neuronal migration, microtubule dynamics, transcriptional control and the cell cycle checkpoint having some invisible centrosomal process that can manage the number of neurons that are produced by neuronal precursor cells. Furthermore, researchers inform us about the clinical management of families that are suffering from MCPH. Establishment of both molecular understanding and genetic advocating may help to decrease the rate of this ailment. This current review study examines newly identified genes along with previously identified genes involved in autosomal recessive MCPH.

1. Introduction

Microcephaly is a rare neurodevelopmental disorder defined by biparietal diameter (BPD) based on a Brazilian study where small head size is below 3–4 standard deviations and the cerebral cortex region of the brain is reduced in size (Araujo Junior *et al.*, 2014). Microcephaly patients may or may not have mild to severe mental retardation as well as squat physique and seizures or hereditary hearing loss (Darvish *et al.*, 2010). It can be classified into two forms; primary microcephaly (at birth) or secondary microcephaly (onset after birth) with and without other syndromic features (Cowie, 1960). Primary microcephaly is a prenatal developmental neurogenic disorder whereas secondary microcephaly is associated with progressive neurodegenerative disease.

Autosomal recessive primary microcephaly (MCPH) is a neurogenic mitotic disorder with normal neuronal migration, neuronal apoptosis and neural function of affected patients. There are various genetic and environmental causes including chromosomal aberrations, dented DNA as a consequence of incorrect mitotic spindle alignment, impulsive chromosomal abridgment, maternal overconsumption of alcohol, congenital infections, drugs taken during pregnancy, brain injury, metabolic disorders such as alaninuria or reaction to teratogenic remedies and substances taken during pregnancy (Darvish *et al.*, 2010; Faheem *et al.* 2015). Metabolic disorders often cause secondary rather than primary microcephaly and are often associated with additional symptoms and clinical signs (Von der Hagen *et al.*, 2014). Inquiries of metabolic screening, if necessary, must primarily focus on maternal phenylketonuria, phosphoglycerate dehydrogenase paucity and Amish fatal microcephaly (2-ketoglutaric aciduria) as a secondary basis of microcephaly (Kelley *et al.* 2002). Rare metabolic causes of primary microcephaly include serine biosynthesis defects, sterol biosynthesis disorders, mitochondriopathies and congenital disorders of glycosylation (Von der Hagen *et al.*, 2014).

The incidence of MCPH ranges from 1:30,000–1:250,000 per live-birth depending on the population (Zaqout *et al.* 2017). In Asian and Arab populations where consanguineous marriages are mutual, MCPH is more common than in whites (Muhammad *et al.*, 2009). Worldwide MCPH has been reported in excess of 300 families and distinct patients; often with only sparse phenotype descriptions. Separate from intellectual infirmity (IQ between 30 and 70–80), hyperactivity and devotion deficit, dialog deferral, and a tapered slanting forehead, MCPH patients ordinarily do not have any auxiliary neurological ciphers (Passemard *et al.*, 2009; Kaindl *et al.*, 2010; Bhat *et al.*, 2011; Kraemer *et al.*, 2016). To date, for MCPH 18 loci and residing genes are found to have mutations and are described in Figure 1, and their cytogenic locations are summarized in Figure 2 (Faheem *et al.*, 2015; Zaqout *et al.*, 2017). The most common causes of MCPH are biallelic mutations in ASPM (68.6%), followed by those in the WDR62 gene (14.1%) and MCPH1 gene (8%). More genetic loci are still expected to exist given the lack of mutations in known loci in approximately 50–75% of western Europeans or North Americans with MCPH and approximately 20–30% of Indians or Pakistanis with MCPH (Verloes *et al.*, 1993; Sajid Hussain *et al.*, 2013).

The up-to-date knowledge on the molecular genetics of MCPH, including the newly notorious locus with its gene (MCPH1–MCPH18), is expansively discussed in this review article. We review the corresponding genes and the proteins encoded by these genes, their probable role in the emerging brain (Table 1) and discuss mutations of these genes found in the Pakistani population (Table 2). In addition, the potential for these genes to perform various cognitive roles during human brain evolutionary processes is discussed.

2. Molecular genetics

(i) MCPH1 (microcephalin)

MCPH1 encodes the important regulator of chromosome condensation (BRCT–BRCA1 C-terminus) the ‘Microcephalin protein’. The Microcephalin protein consists of three BCRT domains and conserved tandem repeats of phospho-peptide interacting amino acids (Faheem *et al.*, 2015; Pulvers *et al.*, 2015). This gene is located on chromosome 8p23 comprising 14 exons, 835 amino acids, and with a genome size and molecular weight of 241,905 bp and 92,877 Da, respectively. It exists in three isoforms obtained after splicing and an open reading frame (ORF) of approximately 8032 bp (Venkatesh *et al.*, 2013; Faheem *et al.*, 2015). The Microcephalin protein, being a pleiotropic factor, imparts its significant effect in neurogenesis; it regulates the division of neuroprogenitor cells and prevents them from exhaustion, that is, from microcephaly. It is the significant regulator of telomere integrity and involved in the DNA damage repair mechanism. It also acts as a tumour suppressor in several human cancers, in germline functions and performs its function in brain development and in the regulation of cerebral cortex size (Venkatesh *et al.*, 2013; Pulvers *et al.*, 2015). Some studies have reported that the MCPH1 gene is involved in brain size determination and as a positive selector for primate lineage (Montgomery *et al.*, 2014). It was considered that MCPH1 may be a common denominator in the pathway of causing microcephaly enclosing the spectrum of both environmental and genetic causes. It leads to the drastic reduction of brain size, especially in the region of cerebral cortex, and short stature. Magnetic resonance imaging

(MRI) of patients revealed the existence of cerebral deformations, such as the gyral pattern observed in the brain and corpus callosum hypoplasia. It is caused by premature switching of symmetric neuroprogenitors to asymmetric division. Mutation in MCPH1 causes mis-regulated chromosomal condensation, genomic instability and delayed de-condensation post mitosis (Liu *et al.*, 2016). Successful experiments for MCPH1 mouse models reported mis-regulated mitotic chromosome condensation, deficiency in DNA repair, defective spindle orientation and a reduced skull size with approximately 20% reduction in body weight (Zhou *et al.*, 2013; Faheem *et al.*, 2015).

(ii) MCPH2 (WDR62)

The WDR62 gene encodes the ‘WD repeat-containing protein 62’, located on chromosome 19q13.12, comprising 35 exons, 1518 amino acids, with a genomic size of 50,230 bp and a molecular mass of 165,954 Da, it has four known spliced isoforms (Faheem *et al.*, 2015; Pervaiz & Abbasi, 2016). WDR62 consists of a WD40 domain, a JNK docking domain and a MKK7 binding domain. It is a scaffold protein involved in the pathway of the c-Jun N-terminal kinase (Bastaki *et al.*, 2016) being highly expressed in neuronal precursors and within post mitotic neurons of the developing brain and in the ventricular and sub ventricular zone in the forebrain region (Pervaiz & Abbasi, 2016). This protein plays a significant part in the formation of several cellular layers in the cerebral cortex region during embryogenesis. It plays a compelling role in the proliferation and migration of neurons and in the duplication of centrioles that are dependent on mother centrioles (Sgourdou *et al.*, 2017). Several findings revealed that WDR62 functions are somehow similar to ASPM which is another MCPH gene. Moreover, studies revealed that the WDR62 gene, as it plays an important part in brain cortical development, is involved in human brain evolutions indicated by dramatic inflation in the size of the cerebral cortex (Pervaiz *et al.*, 2016). Mutation in WDR62 can lead to a wide range of disorders including: microcephaly, cognitive disability, cortical malformations and multiple transcript variants by alternative splicing. The majority of mutations found in WDR62 revealed that these are responsible for approximately 10% of cases of microcephaly. Homozygous or heterozygous mutations in WDR62 both cause MCPH with or without cortical malformations (Naseer *et al.*, 2017). Patients with these mutations have a head circumference ranging from normal to severe. MRI of patients with these mutations showed numerous cortical malformations such as pachygyria, impulsivity, polymicrogyria, aggression, hypoplasia of corpus callosum, delayed psychomotor development, simplified gyral patterns, mental retardation with reduced head size and lissencephaly (Farag *et al.*, 2013; Faheem *et al.*, 2015; Bastaki *et al.*, 2016). Disruption of WDR62 in a mouse model altered the late neurogenesis neocortical progenitors proliferation process which further depicts asymmetric centrosome inheritance abnormalities leading to microcephaly in mice, and impaired mitotic cycle progression, causing temporary arrest at pro metaphase, as well as defects in spindle pole localization of WDR62 (Farag *et al.*, 2013; Sgourdou *et al.*, 2017).

(iii) MCPH3 (CDKRAP2)

The CDK5RAP2 gene is located on chromosome 9q33.2, comprising 39 exons, 1893 amino acids with a molecular mass of 215,038 Da (Graser *et al.*, 2007). This gene is also known as

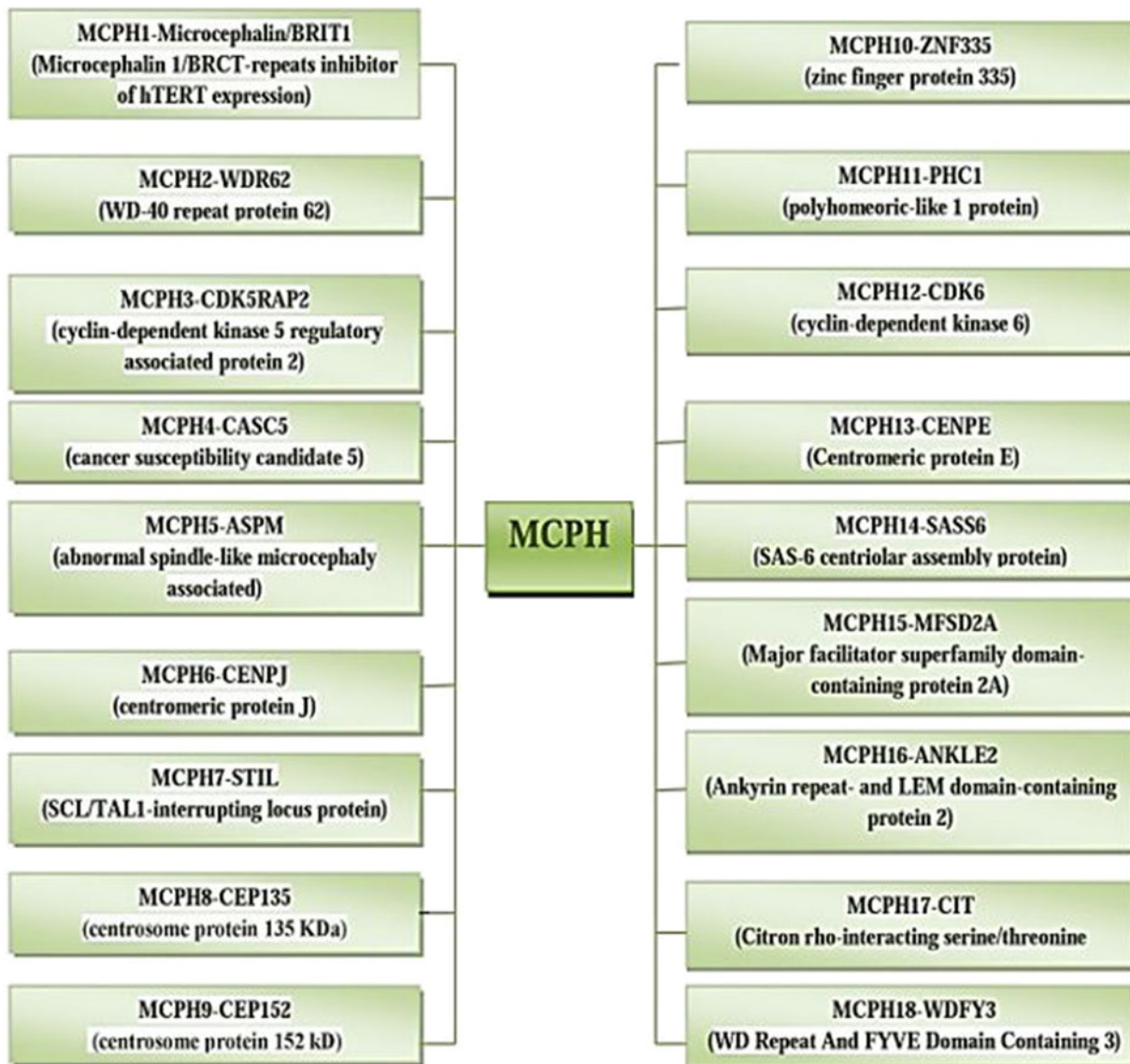


Fig. 1. MCPH loci and residing genes responsible for microcephaly.

C48, Cep215 and MCPH3. Cnn-1N is a small motif present at the N-terminal of a group of centrosome or spindle pole body associated proteins, such as Mto1 and Pcp1 from *Schizosaccharomyces pombe*, *centrosomin* from flies and CDK5RAP2 from mammals (Megraw *et al.*, 1999; Verde *et al.*, 2001; Flory *et al.*, 2002; Venkatram *et al.*, 2004; Conduit *et al.*, 2014). The CDK5RAP2 gene encodes a regulator of CDK5 activity which is localized in the Golgi complex and centrosome in cells and in the cerebral cortex of the brain (Faheem *et al.*, 2015). It interacts with pericentrin and CDK5R1 and plays an important role in microtubule nucleation and centriole engagement. CDK5RAP2 being a part of the pericentriolar material is crucial for the microtubule organization capacity of the centrosome. Patients exhibiting the primary fibroblasts had a drastic reduction in the amount of CDK5RAP2 and showed nuclear and centrosomal abnormalities and an increased frequency of alteration in cell size and migration. In addition, researchers also identified interplay of CDK5RAP2 with the constituents of the Hippo pathway, the transcriptional regulator TAZ and MASTI kinase. This finding enabled

us to understand the mechanism of the Hippo pathway, revealing its role in the regulation of centrosome number. Higher levels of TAZ and Yup in fibroblast patients are observed but none of the other genes involved in the Hippo pathway have been seen to be downregulated. Modifications observed in the Hippo pathway constituents could consequently alter cellular properties and centrosomal deficiencies in patients with affected fibroblasts. This could further be relevant to MCPH controlling brain size and development (Sukumaran *et al.*, 2017). Robustly connected with microtubules, centrosome and Golgi apparatus, CDK5RAP2 is mainly found inside the neural progenitors of the ventricular and sub ventricular areas of the developing brain; it has also been discovered in glial cells and early born neurons and is gradually downregulated when brain maturation occurs. In spindle checkpoint regulation CDK5RAP2 also imparts its effect. The damage to CDK5RAP2 shows chromosomal segregation and spindle checkpoint protein expression issues via binding in HeLa cells (Faheem *et al.*, 2015).

Scientists have determined that the 'an' homozygous mutation in Hertwig's anemia within the CDK5RAP2 gene results in

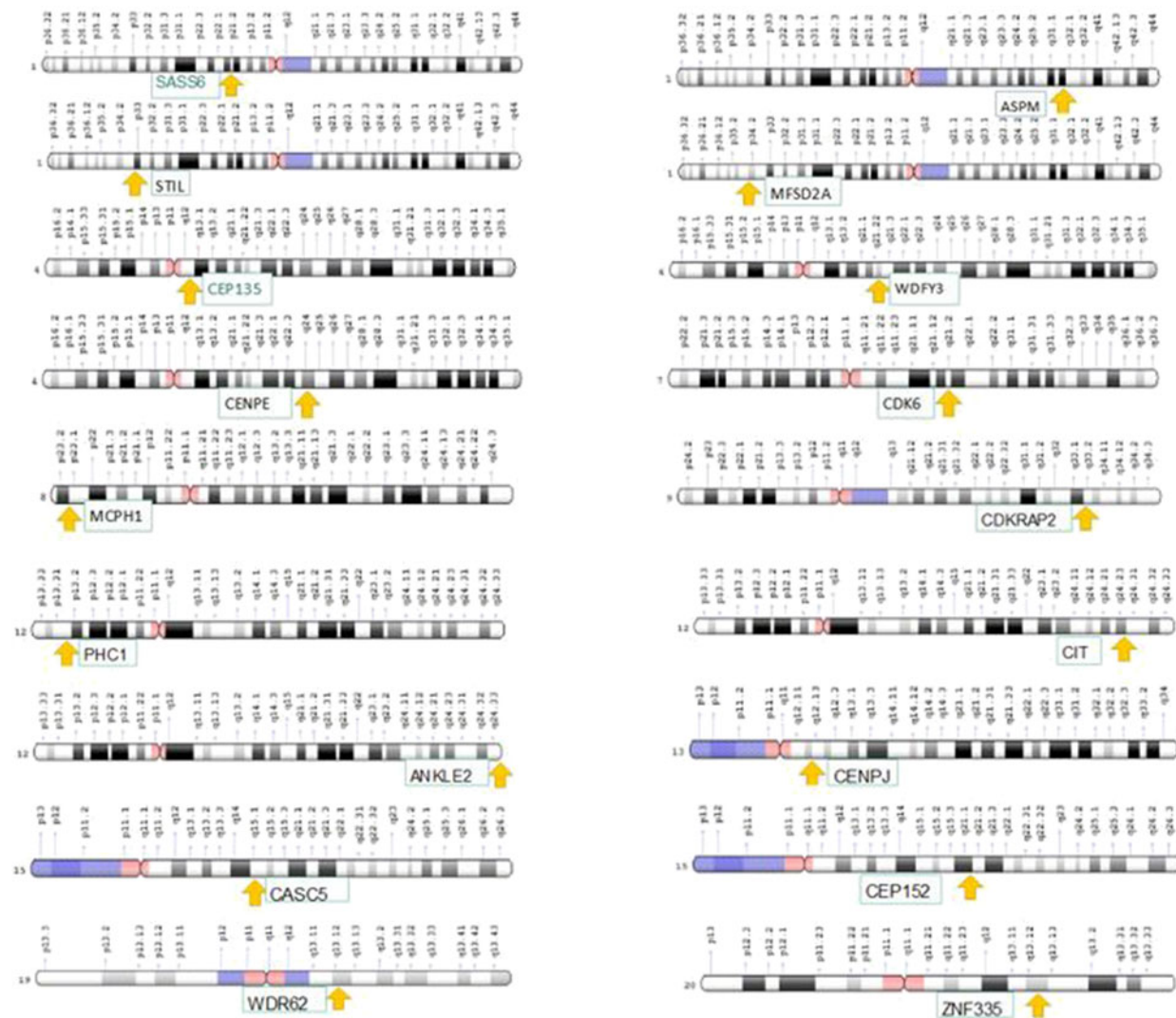


Fig. 2. Cytogenic location of MCPH1–18 genes.

deletion of exon four. Mutant mice exhibited microcephaly symptoms along with hypoplasia of brain regions – cortex and hippocampus – and hematopoietic phenotype. Mutant mice neuronal progenitors confirmed proliferative and survival defects and underwent apoptosis. The impaired centrosomal characteristic and altered mitotic spindle orientation in neuronal progenitors are consequences of CDK5RAP2 mutation (Lizarraga *et al.*, 2010).

(iv) MCPH4 (CASC5)

CASC5 is a protein coding gene, located on chromosome 15q15.1, comprising 27 exons, 2342 amino acids, with a molecular mass of 265 kDa. It is also known as hKNL-1, D40, Spc7, MCPH4, CT29, AF15Q14, PPP1R55 and hSpcl05. In cells, the CASC5 gene is present in the nucleoplasm and in the brain the CASC5 gene is present in the cerebral cortex (Petrovic *et al.*, 2014). The protein encoded by this gene is an important part of the multiprotein assembly that is used for the proper development of kinetochore-microtubule coupling and chromosome separation. It's a conserved scaffold protein that is used for proper kinetochore assembly, checkpoint functioning and spindle assembly. Unlike

other MCPH proteins that are present around the centrosome, the CASC5 protein is used for coupling of chromatin with the mitotic apparatus and also interacts with BUB1, which properly manages the spindle assembly checkpoint. Any reduction in CASC5 protein causes chromosome misalignment and drives the cell into the mitosis (Saadi *et al.*, 2016). Mutated CASC5 disrupts hMIS12, which is vital for correct chromosome alignment and segregation. Frequently this gene is part of the metaphase chromosome kinetochore and elevated mitotic index in patient cells designated mitotic arrest within the cells carrying the mutation. Lobulated and fragmented nuclei have also been identified in addition to micronuclei inside the affected cells. Moreover, altered DNA harms reactions with high peaks of γ H2AX and 53BP1 seen in mutated cells as compared to control fibroblasts. These studies confirm the role of CASC5 in primary microcephaly (Szczepanski *et al.*, 2016).

(v) MCPH5 (ASPM)

ASPM is located on chromosome 1q31.3, comprising 62,567 bp, 3477 amino acids, 28 exons divided into 10,906 ORFs

Table 1. Functions of MCPH genes causing microcephaly.

Locus	Chromosome	Genes	Inheritance	Function
MCPH1	8p23.1	Microcephalin	AR	Involved in chromosomal condensation, cell cycle checkpoint and DNA damage response Reduced MCPH1 enhances the production of early born neurons, which comprise deep layers (IV–VI), and reduces late-born neurons, which produce the thinner outer cortex layer (II–III).
MCPH2	19q13.12	WDR62	AR	Microtubule, spindle organization and kinetochore association Neurogenesis, spindle orientation and cerebral cortical development, proliferation and migration of neuronal precursors, mutation in WDR62 affects its role in proliferating and migrating neural precursors and causes severe brain malformations
MCPH3	9q33.2	CDKRAP2	AR	Centriole duplication, regulates microtubule function, spindle organization and kinetochore association Mutation in CDK5RAP2 reduces the progenitor pool/decreases the number of neurons and reduces cell survival
MCPH4	15q15.1	CASC5	AR	Vital for the spindle checkpoint of the mitotic cycle, CASC5 underscores the role of kinetochore integrity in the proper volumetric development of the human brain
MCPH5	1q31.3	ASPM	AR	Orientation of mitotic spindles during embryonic neurogenesis, centriole duplication, spindle organization and kinetochore association ASPM mutations can decrease the size of the brain by influencing the orientation of the mitotic spindle
MCPH6	13q12.12-q12.13	CENPJ	AR	Controls centriole length/microtubule function, spindle organization and kinetochore association Its deletion causes an increased incidence of multiple spindle poles, apoptosis and mitosis arrest
MCPH7	1p33	STIL	AR	Apoptosis regulator/cell cycle progression, centriole duplication, and ciliogenesis, its mutation in Zebra fish causes an embryonic lethal defect, and STIL knockout mice (Sil ^{-/-}) exhibit numerous developmental abnormalities Decreased size/defective midline neural tube
MCPH8	4q12	CEP135	AR	Maintains organization/structure of the centrosome, centriole duplication, CEP135 Knockdown showed decreased growth rate/disorganized microtubules
MCPH9	15q21.1	CEP152	AR	Centriole duplication/shape and cell/polarity/motility, conversion of glutamine into proline disturbs potential coiled-coiled protein domain/reduced head size
MCPH10	20q13.12	ZNF335	AR	Progenitor cell division/differentiation, neurogenesis, neuronal migration and spindle orientation Mutated ZNF335 gene causes degeneration of neurons, knockdown of ZNF335 caused a small brain size with an absent cortex/disrupted proliferation and differentiation of neuronal cells
MCPH11	12p13.31	PHC1	AR	Regulates cell cycle, and controls DNA damage response PHC1 mutation highlights the role of chromatin remodelling in the pathogenesis of primary microcephaly
MCPH12	7q21.2	CDK6	AR	Controls cell cycle/organizes microtubules, spindle organization and kinetochore association CDK6 mutation affects apical neuronal precursor cells proliferation, reduces the progenitor pool, decreases neuronal production and causes primary microcephaly
MCPH13	4q24	CENPE	AR	Microtubule, spindle organization and kinetochore association Mutation in these gene leads to the dysregulation of a large number of genes involved in cell cycle, causes defective DNA repair and cell cycle with reduced proliferative activity
MCPH14	1p21.2	SASS6	AR	Centrioles formation through procentriole complex In the PISA domain of SAS-6 gene, the replacement of threonine with isoleucine at position 62 causes primary microcephaly
MCPH15	1p34.2	MFSD2A	AR	Protein encoded by MFSD2A gene is transmembrane, the encoded protein plays important role in blood–brain barrier and is also important for normal brain function and growth When the homozygous mutation occurs in the MFSD2A gene at the location of 1p34 then it causes problems in the activity of the blood–brain barrier, which leads to severe primary microcephaly
MCPH16	12q24.33	ANKLE2	AR	Highly involved in proliferation of neuroprogenitor cells during initial brain development and biogenesis of CNS ANKLE2 mutated gene leads to impaired brain development that reduces the brain

(Continued)

Table 1. (Continued.)

Locus	Chromosome	Genes	Inheritance	Function
				size as a result of decreased number of neuroblasts, reduced mitosis, less cell proliferation and increased apoptosis This improper development of brain contributes to MCPH16 due to recessive mutation in gene
MCPH17	12q24.23	CIT	AR	CIT is critical for the development of normal brain size, and it is also essential for the successful completion of cytokinesis Mutation in CIT causes MCPH by homozygous missense variants in the kinase domain that can lead to the loss or activation of citron kinase protein
MCPH18	4q21.23	WDFY3	AD	Required for the organization of misfolded proteins that are degraded by autophagy in body Acts as a ligand for some molecules involved in the selective macroautophagy in the degradation of the midbody ring Mutation in gene leads to abnormal cerebral cortex size and other neurodevelopmental disorders

AD: Autosomal dominant; AR: Autosomal recessive; MCPH: Autosomal recessive primary microcephaly.

(Saunders *et al.*, 1997; Ponting, 2006). It is present on spindle poles and centrosomes during mitosis. The domains of ASPM includes an N-terminus, an 81 IQ (isoleucine–glutamine) domain and a Calponin homology domain (Craig & Norbury, 1998; Bond *et al.*, 2002; Kouprina *et al.*, 2005) and a C-terminus without any domain weighing about 220 kDa (Bond *et al.*, 2003). The orthologs of ASPM include almost 20 different species. The cerebral cortex, the ganglia and the mouse neocortex show the expression of the gene during the process of neurogenesis (Bond *et al.*, 2003; Paramasivam *et al.*, 2007). ASPM downregulation is a result of neurosphere differentiation (Thornton & Woods, 2009). ASPM plays a role in pole organization through activation of kinesin-14 and CDK5RAP2 in normal cells (Tungadi *et al.*, 2017). The most important and crucial role of the ASPM gene is to perform cytokinesis during meiosis. ASPM plays a fundamental role in organizing microtubules and focusing spindle poles during mitosis (do Carmo Avides & Glover, 1999). ASPM knockdown mediated by Morpholino results in the reduction of head size in different species such as Zebra fish (Kim *et al.*, 2011). The reduced surface area and increased thickness of white matter are consequences of loss of ASPM, while preserving the memory of patients in contrast to its intellectual disabilities (Passemar *et al.*, 2016). ASPM knockdown effects could be reduced by CITK overexpression and CITK microcephaly phenotype is a result of spindle orientation (Gai *et al.*, 2016). The similarities of WDR62 and ASPM includes the same location and physical interaction during interphase mediated by CEP63. Loss of WDR62 and ASPM in the developing brain induces shortage of cilia and centrosomes. Both genes determine the fate of cells and localize CPAP to the centrosome (Jayaraman *et al.*, 2017). Different types of mutations have been observed in the ASPM gene in 33 families from Pakistan (Gul *et al.*, 2006a). These mutation types include deletion, substitution, duplication and variation in the intrinsic region (Muhammad *et al.*, 2009; Saadi *et al.*, 2009). The ASP gene in mutant form was first discovered in *Drosophila* (do Carmo Avides & Glover, 1999). Different experiments were performed to study mutations in ASPM. One study examined the developing cerebral cortex in two mutant mouse lines and concluded that the reduced size of the brain was a consequence of ASPM mutation (Riparbelli *et al.*, 2002). Using whole-genome sequencing, a mutation in exon 16 in the ASPM gene was seen, and non-syndromic microcephaly with altered IQ number was reported.

(vi) MCPH6 (CENPJ)

CENPJ is present on chromosome 13q12.2, is comprised of 40,672 bp, 1338 amino acids, 17 exons distributed in 5187 bp ORFs, weighing approximately 153 kDa (Saunders *et al.*, 1997). The domains of CENPJ include protein phosphorylation domains, five coiled-coil domains (CCDs), and the C-terminal domain has 21 G-box repeats and a leucine zipper motif (Hung *et al.*, 2000; Bond *et al.*, 2005). The gene holds its position within the centriole. The CENPJ gene has almost 204 orthologs. CENPJ carries out microtubule assembly in the centrosome by causing nucleation and depolymerization of microtubules (Hung *et al.*, 2004). CENPJ also maintains centriole integrity with rearrangement of microtubules (Kirkham *et al.*, 2003). During neurogenesis, the frontal cortex neuro-epithelium expresses the CENPJ gene (Bond *et al.*, 2005). Damage to the structure of the centrosome causes cell arrest in mitosis and is caused by low levels of the CENPJ protein (Cho *et al.*, 2006). CENPJ knockdown increases the rate of multiple spindle poles, apoptosis and mitosis arrest and loss of centrioles in *Drosophila* (Koyanagi *et al.*, 2005; Basto *et al.*, 2006). *Drosophila* flies without centrioles die at an early age due to loss of cilia or flagella (Stevens *et al.*, 2007). The total number of mutations in the CENPJ gene never exceeded five. The first mutation was discovered in a Brazilian family, the second and third mutations belonged to a Pakistani family (Bond *et al.*, 2005) and the fourth was discovered in people suffering from Seckel syndrome (Al-Dosari *et al.*, 2010). Studies showed that in the third mutation, four consecutive nucleotide units (TCAG) are deleted from around 19 bp downstream of exon 11 resulting in frameshifting and premature termination of protein (Gul *et al.*, 2006b).

(vii) MCPH7 (STIL)

STIL is located on chromosome 1p33, comprising 63,018 bp with an ORF of 5225 bp, 20 exons, 1287 amino acids and a molecular weight of 150 kDa (Kumar *et al.*, 2009; Kaindl *et al.*, 2010). It has recently been reported that the human oncogene SCL/TAL1 locus is thoroughly conserved in vertebrate species, and is involved in regulation of toxic susceptibility in PC12 cells through the sonic hedgehog (Shh) pathway. Knockdown of STIL expression by RNAi showed no effect on survival of proliferating PC12 cells

Table 2. Reported mutations of MCPH1–MCPH18 genes.

MCPH1–Microcephalin			
74C-G transversion	Ser25-to-ter (S25X) substitution	Exon2	Jackson <i>et al.</i> (2002)
1-bp insertion (427insA)	Truncated protein of 146 a.a	Exon5	Trimborn <i>et al.</i> (2004)
150- to 200-kb deletion	–	Exon1–6	Garshasbi <i>et al.</i> (2006)
1-bp insertion (566insA)	Frameshift mutation	Exon6	Darvish <i>et al.</i> (2010)
147C-G transversion	His49-to-gln (H49Q) substitution	Exon3	Darvish <i>et al.</i> (2010)
215C-T transition	Ser72-to-leu (S72L) substitution	Exon3	Darvish <i>et al.</i> (2010)
302C-G transversion	Ser101-to-ter (S101X) substitution	Exon4	Farooq <i>et al.</i> (2010)
MCPH2–WDR62			
4-bp deletion (c.4205delTGCC)	Val1402GlyfsTer12	Exon31	Bilguvar <i>et al.</i> (2010)
c.1576G-A substitution	Glu52-to-Lys (E526 K) substitution	Exon12	Bilguvar <i>et al.</i> (2010)
G-to-C transversion	Try224-to-Ser (W224S) substitution	Exon6	Bilguvar <i>et al.</i> (2010)
C-to-T transition	Glu to termination substitution (Q470X)	Exon11	Bilguvar <i>et al.</i> (2010)
17-bp deletion	Gly1280AlafsTer21	Exon30	Bilguvar <i>et al.</i> (2010)
1313G-A transition	Arg438-to-His (R438H) substitution	Exon10	Nicholas <i>et al.</i> (2010)
1-bp duplication (4241dupT)	Premature termination	Exon31	Nicholas <i>et al.</i> (2010)
1531G-A transition	Asp511-to-Asn (D511N) substitution	Exon11	Nicholas <i>et al.</i> (2010)
1-bp insertion (3936insC)	Premature termination	Exon30	Yu <i>et al.</i> (2010)
1-bp deletion (363delT)	Premature termination	Exon4	Yu <i>et al.</i> (2010)
193G-A transition	Val65-to-Met (V65M) substitution	Exon2	Yu <i>et al.</i> (2010)
1-bp deletion (2083delA)	–	Exon17	Murdock <i>et al.</i> (2011)
2-bp deletion (2472_2473delAG)	–	Exon23	Murdock <i>et al.</i> (2011)
MCPH3–CDK5RAP2			
c.243 T-A transversion	Ser81-to-Ter (S81X) substitution	Exon4	Bond <i>et al.</i> (2005)
c.246 T-A transversion	Tyr82-to-Ter (Y82X) substitution	–	Hassan <i>et al.</i> (2007)
c.15A-G transition	–	Intron26	Bond <i>et al.</i> (2005)
c.700G-T transversion	Glu234-to-Ter (E234X) substitution	Exon8	Pagnamenta <i>et al.</i> (2012)
c.4546G-T transversion	Glu1516-to-Ter (E1516X) substitution	–	Lancaster <i>et al.</i> (2013)
c.4672C-T transition	Arg1558-to-Ter (R1558X) substitution	–	Lancaster <i>et al.</i> (2013)
5-bp deletion (c.524_528del)	Gln175ArgfsTer42	Exon7	Tan <i>et al.</i> (2014)
MCPH4–CASC5			
6125G-A transition	Met2041-to-Ile (M2041I) substitution	Exon18	Genin <i>et al.</i> (2012)
MCPH5–ASPM			
2-bp deletion (719-720delCT)	Premature termination 15 codons downstream	Exon3	Bond <i>et al.</i> (2002)
7-bp deletion (1258-1264delTCTCAAG)	Premature termination 31 codons downstream	Exon3	Bond <i>et al.</i> (2002)
7761 T-G transversion	Immediate truncation	Exon18	Bond <i>et al.</i> (2002)
1-bp deletion (9159delA)	Premature termination four codons downstream	Exon21	Bond <i>et al.</i> (2002)
9178C-T transition	Gln3060-to-Ter (Q3060X) substitution	Exon21	Kumar <i>et al.</i> (2004)
3978G-A transition	Trp1326-to-Ter (W1326X) substitution	Exon17	Kumar <i>et al.</i> (2004)
349C-T transition	Arg117-to-Ter (R117X) substitution	–	Kumar <i>et al.</i> (2004)
6189 T-G transversion	Tyr2063-to-Ter (Y2063X) substitution	Exon18	Shen <i>et al.</i> (2005)
1-bp insertion (4195insA)	Premature termination	Exon6	Saadi <i>et al.</i> (2009)
2389C-T transition	Arg797-to-Ter (R797X) substitution	Exon18	Saadi <i>et al.</i> (2009)
2-bp deletion	7781delAG	–	Saadi <i>et al.</i> (2009)

(Continued)

Table 2. (Continued.)

MCPH1–Microcephalin			
MCPH6–CENPJ			
1-bp deletion (17delC)	Thr6fsTer3	–	Leal <i>et al.</i> (2003); Bond <i>et al.</i> (2005)
1-bp deletion (c.18delC)	Ser7ProfsTer2	Exon 2	Sajid Hussain <i>et al.</i> (2013)
3704A-T transversion	Glu1235-to-Val (E1235 V) substitution	Exon16	Bond <i>et al.</i> (2005)
4-bp deletion (3243delTCAG)	–	Exon11	Gul <i>et al.</i> (2006 b)
IVS11-1G-C	–	Intron11	Al-Dosari <i>et al.</i> (2010)
2462C-T transition	Thr821-to-Met (T821M) substitution	Exon7	Darvish <i>et al.</i> (2010)
MCPH7–STIL			
3715C-T transition	Gln1239-to-Ter (Q1239X) substitution	Exon18	Kumar <i>et al.</i> (2009)
1-bp deletion (3655delG)	–	Exon18	Kumar <i>et al.</i> (2009)
G-to-A transition	–	Intron16	Kumar <i>et al.</i> (2009)
c.2392 T-G transversion	Leu798-to-Trp (L798W) substitution	Exon14	Papari <i>et al.</i> (2013)
c.453 + 5G-A transition	Asp89GlyfsTer8	Intron5	Kakar <i>et al.</i> (2015)
MCPH8–CEP135			
1-bp deletion (970delC)	Gln324SerfsTer2	Exon8	Hussain <i>et al.</i> (2012)
c.1473 + 1G-A transition	Glu417GlyfsTer2	Intron11	Farooq <i>et al.</i> (2016)
MCPH9–CEP152			
A-to-C transversion	Gln265-to-Pro (Q265P) substitution	–	Guernsey <i>et al.</i> (2010)
C-to-T transition	Arg987-to-Ter (R987X) substitution	–	Guernsey <i>et al.</i> (2010)
MCPH10–ZNF335			
3332G-A transition	Arg1111-to-His (R1111H) substitution	Exon20	Yang <i>et al.</i> (2012)
MCPH11–PHC1			
c.2974C-T transition	Leu992-to-Phe (L992 F) substitution		Awad <i>et al.</i> (2013)
MCPH12–CDK6			
c.589G-A transition	Ala197-to-Thr (A197 T) substitution	Exon5	Hussain <i>et al.</i> (2013)
MCPH13–CENPE			
c.2797G-A transition	Asp933-to-Asn (D933N) substitution	–	Mirzaa <i>et al.</i> (2014)
c.4063A-G transition	Lys1355-to-Glu (K1355E) substitution		Mirzaa <i>et al.</i> (2014)
MCPH14–SASS6			
c.185 T-C transition	Ile62-to-Thr (I62 T) substitution	Exon3	Khan <i>et al.</i> (2014)
MCPH15–MFS2A			
c.476C-T transition	Thr159-to-Met (T159M) substitution	–	Guemez-Gamboa <i>et al.</i> (2015)
c.497C-T transition	Ser166-to-Leu (S166L) substitution	Exon 10	Guemez-Gamboa <i>et al.</i> (2015)
c.1016C-T transition	Ser339-to-Leu (S339L) substitution	–	Alakbarzade <i>et al.</i> (2015)
MCPH16–ANKLE2			
c.2344C-T transition	Gln782-to-Ter (Q782X) substitution	Exon11	Yamamoto <i>et al.</i> (2014)
c.1717C-G transversion	Leu573-to-Val (L573 V) substitution	Exon10	
MCPH17–CIT			
c.317G-T transversion	Gly106-to-Val (G106 V) substitution	Exon4	Li <i>et al.</i> (2016)
c.376A-C transversion	Lys126-to-Gln (K126Q) substitution	–	Li <i>et al.</i> (2016)
c.689A-T transversion	Asp230-to-Val (D230 V) substitution	–	Li <i>et al.</i> (2016)
c.1111 + 1G-A transition	Gly353_371delinsAla	Intron9	Shaheen <i>et al.</i> (2016)
c.29_38delATCCTTTGGA	Asn10MetfsTer15	Exon2	Harding <i>et al.</i> (2016)

(Continued)

Table 2. (Continued.)

MCPH1–Microcephalin			
c.753 + 3A-T	Asp221Ter	Intron7	Basit <i>et al.</i> (2016)
MCPH18–WDFY3			
c.7909C-T transition	Arg2637-to-trp (R2637W) substitution	–	Kadir <i>et al.</i> (2016)

following increased cell death in differentiated neurons after drug analysis. In case of overexpression of STIL in proliferating cells toxic susceptibility is increased but it causes no effect in mature neurons (Szczepanski *et al.*, 2016). Biochemical, cell biology and biophysical analysis reported that STIL retains a central short CCD, which suggests a critical role in oligomerization, centrosomal localization and protein interaction. Protein interaction is mediated by the central intrinsically disordered region of STIL, comprising 400–700 residues, just like CPAP interaction during centriole duplication. Generally, STIL is a disordered protein retaining three structured regions: N-terminal domain possessing 1–370 residues, 1062–1148 residues identified as a STIL ANA-2 motif and a short segment of 718–750 that give rise to a CCD. Size exclusion chromatography suggested that the CCD peptide comprises α helices that form a quaternary structure. Repeating patterns of hydrophobic, hydrophilic and charged residues present at specified locations to form a CCD. Recent studies revealed that two regions retaining eight hydrophobic residues exist in this domain and that these regions mediate oligomerization of CCD. Within this domain hydrophobic residues form axes occupied by hydrophilic residues promoting hydrophobic interactions that are an important part of protein structure and stability. Two leucine residues, L718 and L736, identified in this hydrophobic region lead to oligomerization of the CCD, and L736 has a greater role in this process. Further inspection revealed that mutations in these residues did not affect the secondary structure but alter the quaternary configuration which leads to malfunction of STIL. The CCD has a key role in oligomerization and promoting STIL function in self-interaction, centriolar replication, embryogenesis and in development of cilia. Current studies report that interaction of one helix of STIL with a PLK4 PB3 domain occurs via hydrophobic residues in the CCD and promotes centriole duplication. Phosphorylation of STIL by PLK4 facilitates STIL and SAS-6 protein interaction. Note that the L736 residue is critically involved in the interaction with PLK4. The hydrophobic core in the CCD of STIL imparts oligomerization building dimers and tetramers, which are analogues of its *Drosophila* ortholog Ana-2 and *Caenorhabditis elegans* centrosomal protein SAS-6 (David *et al.*, 2016). Clinical and molecular genetic studies revealed that MCPH is rarely caused by STIL mutation (2.2%). The expression study demonstrated that STIL is critically involved in early forebrain development that may be associated with the Shh signalling pathway (Mouden *et al.*, 2015).

(viii) MCPH8 (CEP135)

The CEP135 gene is located on chromosome 4q12, comprising 26 exons and 1140 amino acids, it encodes a centrosomal protein that is a reserve helical protein detected throughout the cell cycle at the centrosomes giving greater strength to the centrosomes. Recent studies revealed that CEP135 in *Drosophila* plays a crucial role in central microtubule pair assembly in sperm

axoneme and asymmetric cell division of neuroblasts. Intriguingly, in *Homo sapiens* and *Drosophila* microtubule binding sites have been mapped to the N-terminal. Fluorescence microscopy, cryo-electron analysis, including biochemical analysis, elucidated that *in vitro* development of microtubule bundles is induced by the interaction of CEP135 with tubulin, protofilaments and microtubules. A microtubule binding site has been detected between 96–108 residues and this segment integrated with positively charged surface patch 2; this was discovered in an atomic CEP135 model study, which suggested that the basic amino acids of the microtubule binding domain mediate interactions with the outer surface of the microtubule that is negatively charged (Hilbert *et al.*, 2016). Further studies of major microtubule binding sites discovered a segment of 13 amino acids comprising 96–108 residues leading to microtubule binding activity of CEP135-N. Within this segment three lysine residues, K101, K104 and K108, were identified that contribute highly positive electrostatic surface potential of patch 2. These three lysine residues play a critical role in efficient microtubule bundling or cross linking by CEP135-N. Binding ability of two or more microtubules of CEP135-N leads to the development of microtubule triplets or joining of adjacent triplets within the microtubule wall. In such a way, the centrosomal protein CEP135 plays a key role in the biosynthesis of centrosomes which control the cell (Hilbert *et al.*, 2016).

(ix) MCPH9 (CEP152)

CEP152 is a protein coding gene located on chromosome 15q21.1, comprising 149,368 bp, 38 exons, 1710 amino acids, with a molecular mass of 195,626 Da (Jamieson *et al.*, 2000). The CEP152 gene is also known as Asterless, KIAA0912, SCKL5 and MCPH9 (Dzhindzhev *et al.*, 2010). In cells this gene is localized in centrosomes and in the brain it is localized in the cerebral cortex (Faheem *et al.*, 2015). Centrosomal protein 152 contains (KW-0175) a CCD (KW-9994), also known as a Heptad Repeat pattern including five different conserved protein sequences that lead to the coiled-coil protein domain formation, these are formed of 234–490, 615–664, 700–772, 902–993 and 1170–1241 amino acids (Kalay *et al.*, 2011). The protein of this gene organizes the microtubules of cells and has a very important role in shaping the cell, polarity, receptivity and cellular division. The protein localized in the microtubule and centrosome interacts with PLK4, CENPJ (via N-terminus), CINP, CDKRAP2, WDR62, CEP63, CEP131 and DEUP1 (Cizmecioglu *et al.*, 2010; Dzhindzhev *et al.*, 2010; Kalay *et al.*, 2011; Firat-Karalar & Stearns, 2014). The interaction of CEP152 with CEP63, CDK5RAP2 and WDR62 form a bit by bit assembled complex at the centrosome creating a ring close to the parental centriole. CEP152 plays an important role in centrosome duplication/shape and cell/polarity/motility, and also functions as a molecular scaffold that facilitates the interaction of CENPJ and PLK4, two

molecules that play an important role in centriole configuration (Cizmecioglu *et al.*, 2010). It is suggested to take PLK4 away from PLK4:CEP92 complexes in early G1 daughter centrioles and to reposition PLK4 at the outer boundary of a new forming CEP152 ring structure. It also plays an essential role in deuterosome-mediated centriole amplification that can form many centrioles. Overexpression of CEP152 can drive amplification of centrioles (Dzhindzhev *et al.*, 2010). The CEP152 (human) gene is an ortholog of the *Drosophila* asterless (*asl*) gene, comprising 72,835 bp and 1710 amino acids and with a protein with a molecular weight of 152 kDa. A mutation in the CEP152 protein that converts glutamine into proline is assumed to be pathogenic and can disrupt the potential coiled-coiled protein domain, which results in reduction of head size. Similarly, a greater head size reduction was also seen in compound heterozygous females compared with missense homozygous females. The truncated protein suggests a nonsense-mediated decay of the mutated transcript; these findings have also shown that throughout a functional assay to determine subcellular localization that the wild-type CEP152–GFP fusion protein can be found in γ -tubulin co-structures. Mutant CEP152 flagged with GFP failed to co-localize with the γ -tubulin, which furthermore substantiate pathogenicity caused by this mutation (Guernsey *et al.*, 2010).

(x) *MCPH10 (ZNF335)*

ZNF335 is a protein coding gene located on chromosome 20q13.12, comprising 24,258 bp, 28 exons, 1342 amino acids, with molecular mass of 144,893 Da (Deloukas *et al.*, 2001). The ZNF335 gene is also known as NIF-1, NIF-2 and MCPH10 (Mahajan *et al.*, 2002; Garapaty *et al.*, 2008). In the cell this gene is localized in the nucleus and in the brain it is localized in the cerebral cortex (Faheem *et al.*, 2015). ZNF335 codes for the zinc finger protein 335 and has a repeat domain and a zinc finger C2H2-type (IPR013087) domain (Klug, 1999). The ZNF335 protein is a part or an associated component of some histone methyltransferase complexes and is also involved in regulating transcription through recruitment of these complexes to gene promoters and via the nuclear hormone receptor it also increases ligand dependent transcriptional activation. In addition, it is also very important for proliferation and self-renewal of neural progenitor cells by controlling the regulation of specific genes that are involved in brain development, including REST, and it is also involved in regulating the expression of genes that are involved in somatic development, for example, lymphoblast proliferation. This gene also encodes a component of the vertebrate-specific trithorax H3K4-methylation chromatin remodelling complex that controls central nervous system (CNS) gene expression and cell fate (Yang *et al.*, 2012). ZNF335 is very important for progenitor cell division/differentiation; mutation in this gene leads to the degeneration of neurons, and knockdown of this gene causes a reduction in the size of the brain with a lack of cortex formation as well as disrupted differentiation and proliferation of neuronal cells (McLean *et al.*, 2017). Due to a mutation in ZNF335, MCPH10 was identified in an affected member of an Arab Israeli family. The mutation was identified in exon 20 as a homozygous 3332G>A transition, which resulted in an Arg1111 to His (R1111H) substitution at the conserved residue in the 13th zinc-finger domain. This mutation was found at the final point of a splice donor site that disrupts normal splicing, which leads to the formation of unusually large transcripts that contain intron 19 and 20 with a proposed premature termination

sequence (Yang *et al.*, 2012). Levels of ZNF335 protein is severely reduced in patient cells. Various studies regarding this gene have revealed that ZNF335 is attached to the chromatin remodelling complex (analogue to the TrxG (trithorax) complex in *Drosophila*) involving H3K4 methyltransferase, which can control the expression of important genes in various pathways. nBAF is a neural-specific chromatin regulatory complex, any mutation in this complex disrupts the proliferation of cells, indicating that it can also lead to microcephaly. Deficiency of the ZNF335 genes leads to the degeneration of neurons, which makes it much more critical in contrast to the other microcephaly syndromes that are related to postnatal survival. Studies of *in vitro* and *in vivo* mouse models revealed that the knockdown of ZNF335 causes a severe reduction in the size of the brain, which has no cortex, and disturbs the differentiation and proliferation of nerve cells (Yang *et al.*, 2012).

(xi) *MCPH11 (PHC1)*

The PHC1 gene is located on chromosome 12p13.31 and encodes a protein containing 1004 amino acids with a molecular mass of 105,534 Da. The PHC1 gene is also known as Early Developmental Regulator 1, HPH1, EDR1, RAE28, PH1 and MCPH11 (Chavali *et al.*, 2017). MCPH11 is a disease that is caused by a homozygous mutation in the PHC1 gene on chromosome 12p13. PHC1 takes part in the regulation of the cell cycle, and its mutation highlights the role of chromatin remodelling in the pathogenesis of primary microcephaly (Awad *et al.*, 2013). Enhanced geminin expression was observed in cells with a PHC1 mutation. A core component of canonical PRC1 is PHC1 as a genetic basis for MCPH11 in a family with reported consanguineous homozygous missense PHC1 variants. The functional analysis of this pathogenic variant in patient cells revealed lower PHC1 expression with lower genome wide H2Aub1 levels and impaired recruitment of PHC1 to loci of DNA damage and repair (Srivastava *et al.*, 2017). Interestingly, the MCPH11 PHC1 variant essentially had an impact on neural development in affected individuals, potentially highlighting a sensitivity of the developing brain to dysregulation of H2Aub1 modification exchange. As PHC1 regulates the cell cycle, PHC1 mutation highlights the role of chromatin remodelling in the pathogenesis of primary microcephaly (Srivastava *et al.*, 2017; Triglia *et al.*, 2017).

(xii) *MCPH12 (CDK6)*

CDK6 encodes a protein of the cyclin dependent protein kinase (CDK) family, located on chromosome 7q21.2 with a genome size of 231,707 bp, 326 amino acids, 10 exons and a molecular weight of 36,983 Da. The CDK6 protein controls the mechanism of the cell cycle and plays a very significant role in differentiation of different cell types. A mutated CDK6 gene or knockdown cells cause distorted nuclei disorganization of the spindles and microtubule and supernumerary centrosomes, whereas a decrease in proliferation was also observed in patients. Mutation in cells also disturbs apical neuronal precursor cell proliferation, which leads to the imbalance between symmetric and asymmetric cell division that causes progenitor cell depletion that could be the basis for reduction in neuronal cell production that finally leads to primary microcephaly (Hussain *et al.*, 2013). In a recent study, most related genes that code for CDK6 were found to have a homozygous single nucleotide substitution, 589G>A. This mutation leads to altered mitosis because during the mitosis

process patient primary fibroblasts failed to recruit CDK6 to the centrosome and it also affects cellular localization (Grossel *et al.*, 1999). Moreover, this study was also substantiated by another study that revealed reduced proliferation capacity of CDK6 patient primary fibroblasts and knockdown cells (Grossel & Hinds, 2006).

(xiii) MCPH13 (CENPE)

CENPE is a protein coding gene located on chromosome 4q24, comprising 492,604 bp and 2701 amino acids. This gene encodes a protein that has signal transducer activity and sequence specific DNA binding activity. It also has transcription factor activity. Domains of this gene include Ploop_NTPase, Kinesin_motor_dom, CENPE, Kinesin_motor_CS and Kinesin-like FAM. MCPH is a heterogeneous autosomal recessive disorder. In last four years studies there has been increased knowledge about the new mutated genes involved in MCPH and intense work is done at both the clinical and cellular level to determine disease mechanisms. The functions of proteins encoded by WDR62, CASC5, PHC1, CDK6, CENP-E, CENP-F, CEP63, ZNF335, PLK4 and TUBGPC genes, have been added to the complex network of critical cellular processes that play an important role in brain growth and size. In a male child with the mutated CENPE gene and who had microcephaly, his head was seen to start squeezing at the age of 5 and after some years he died. The boy also had dysmorphic facial features, including, prominent nose and sloping forehead. His sister of 3 years old also had microcephaly and also showed the disease phenotype. Up until now various mutations have been found in CENPE (Ahmad *et al.*, 2017).

(xiv) MCPH14 (SASS6)

The SAS-6 gene is located on chromosome-1p21.2, comprising 49,552 bp, 657 amino acids, 17 exons and a mass of 74,397 Da. The SAS-6 gene encodes a protein known as Spindle assembly abnormal protein-6 homolog, which is a coiled-coil protein. The gene is found within the cytoplasm, cytoskeleton, microtubule organizing centre, centrosome, cytoskeleton and centrioles. The most critical and fundamental role of the SAS-6 gene is to help in centriole formation by producing a procentriole. Centrioles play a crucial role in cilia and flagella and so are of much importance and their formation is strongly linked to cell duplication and centrosome replication (Gupta *et al.*, 2015; Arquint & Nigg, 2016). To ensure genome integrity, centriole replication is essential. SAS-6 self-assembles into a cartwheel structure, and is able to undergo dimerization and oligomerization thus supporting centriole formation. The most primitive pathway for assembling centrioles involves seven major constituents: STIL, γ -tubulin, Plk4, CPAP, Cep135, Cep135 and hSAS6.

After attachment of Plk4, Cep135 assembles at the parental centriole position, then phosphorylated STIL and hSAS-6 gather and through a positive feedback mechanism form a complex with CPAP and initiate the formation of the procentriole (Ohta *et al.*, 2002; Gopalakrishnan *et al.*, 2010). HsSAS-6 located in the cytoplasm gathers around the centriole and forms a complex with Cep135 to initiate cartwheel structure organization by oligomerization by passing through G1/S phase. G2/S phase leads to the stability of hSAS-6 (Strnad *et al.*, 2007; Keller *et al.*, 2014). A family was selected from Dera Ismail Khan, an urban area in the province of Pakistan, Khyber Pakhtunkhwa. A pedigree

analysis of five generations provided a result of four persons diagnosed with several physiological and mental issues resulting from consanguineous marriages. Out of four, two were girls, aged 3.5 and 5 years, and two were men, aged 42 and 50. All four experienced mental retardation, low level IQ (between 20–40), pronunciation issues and even walking problems. By performing several tests including tomography, STR, SNP, genome-wide linkage analysis and whole-genome analysis etc., it was concluded that the SAS-6 gene was responsible for this condition, which was determined to be primary microcephaly. In the PISA domain of the SAS-6 gene, the replacement of threonine with isoleucine at position 62 causes primary microcephaly. Microcephaly linked with hSAS-6 is an autosomal recessive disease. The drastic impact of hSAS-6 deficiency on centriole formation also affects cell division thus disturbing normal neurogenesis and ultimately affecting brain nourishment (Khan *et al.*, 2014).

(xv) MCPH15 (MFSD2A)

MFSD2A, also known as MCPH15 and NLS1, encodes a transmembrane protein required for brain uptake of omega-3 (Docosahexaenoic acid) and different long-chain fatty acids (lysophosphatidylcholine) (Alakbarzade *et al.*, 2015; Guemez-Gamboa *et al.*, 2015). This gene is localized on chromosome 1p34.2, comprising 14 exons, 543 amino acids, with a genome size of 14,857 bp and a molecular weight of 60,170 Da. MFSD2A is mainly localized to the cytosol, plasma membrane and cytoplasmic bodies in the human brain as well as other cells. The MFSD2A gene is also present in the cerebral cortex (endothelial cells, neuronal cells, etc) and is an essential component of the blood-brain barrier (Reiling *et al.*, 2011). MFSD2A is evolutionarily conserved from teleost fish to humans. Mutation in the MFSD2A gene causes microcephaly leading to a brain volume that is smaller than usual. High expression of MFSD2A is observed in the blood-brain barrier of both humans and mice. Reduced brain DHA levels along with reduced LPC uptake and microcephaly as the most prominent phenotype is reported in MFSD2A-deficient mice (Nguyen *et al.*, 2014). As supported by these studies, LPC is essential for normal development of human and mice brains (Quek *et al.*, 2016). Most affected people have delayed speech and language skills, and delayed motor skills (standing, sitting and walking). Slim, sloping forehead, behavioural issues and short stature compared to others in their family was also observed. Two distinctive homozygous missense mutations were seen to occur in the MFSD2A gene in affected individuals from two consanguineous families from northern Africa with autosomal recessive microcephaly causing early death (Guemez-Gamboa *et al.*, 2015). Homozygous missense mutations occurring in the MFSD2A gene were also seen in affected individuals belonging to a consanguineous Pakistani family. It was also seen that morpholino knockdown of orthologous MFSD2A in Zebra fish brought about early postnatal lethality, microcephaly and blood-brain barrier interruption. Likewise, it was also found that MFSD2A-null mice had a roughly 40% expansion in plasma levels of LPC compared to controls with debilitated take-up of LPC into the brain (cerebrum) (Guemez-Gamboa *et al.*, 2015).

(xvi) MCPH16 (ANKLE2)

Several mutated genes have been identified to play an important role in the regulation of the cell cycle and cell proliferation.

One of them is the ANKLE gene encoding the LEM4 protein, which plays a critical role in nuclear envelope formation through the mitotic phosphorylation of BAF protein during mitosis. Upon mitotic entry, the LEM protein interacts with a chromatin-binding protein known as Barrier to Autointegration. BAF regulates chromatin structure and chromosome segregation in gene expression and development. Recent studies have revealed that the ANKLE2 gene mapped to chromosome 12q24.33, has a size of 938 amino acids and a molecular mass of approximately 10,000 Da. According to current studies its N-terminal tail is followed by a transmembrane domain of approximately 40 amino acids, which is known as the LEM domain and retains two central ankyrin repeats and a cytoplasmic C-terminus. Mutation in this gene leads to abnormal development of the nuclear envelope during mitosis. Mutated ANKLE2 gene leads to impaired brain development and reduces brain size as a result of a decreased number of neuroblasts, reduced mitosis, less cell proliferation and increased apoptosis (Faheem *et al.*, 2015). This improper development of the brain contributes to MCPH16, which is predominantly caused by consanguineous marriages. Clinical review or neuroimaging of patients with MCPH16 reveals symptoms such as reduced brain size, intellectual disability, knee contractures, enlarged posterior horns of the lateral ventricles, adducted thumbs and epilepsy (von der Hagen *et al.*, 2014). Recently, whole-exome sequencing and analysis of genes that are potentially involved in neurological disorders identified compound heterozygous mutations in the ANKLE2 gene in exon 11 (c.23344C-T transition as a result of Gln782 to Ter (Q782X) substitution), and in exon 10 (c.1717C-T as a result of Leu 573 to Val (L573 V) substitution). Analyses of brains from *Drosophila* identified a mutation in *l(l) G0222*, the homolog of the ANKLE2 gene in humans. This mutation results in loss of thoracic bristles and impairs development of sensory organs in clones. Such evidence suggests that genes identified in *Drosophila* clones and variant alleles in *Homo sapien* homologs in families with Mendelian diseases play a critical role in the discovery of disease causing genes and the mechanisms involved in these disorders (Yamamoto *et al.*, 2014).

(xvii) MCPH17 (CIT)

The CIT gene encoding citron rho-interacting serine/threonine kinase is critical for the development of a normal sized brain. CIT has an N-terminal kinase domain and many C-terminal domains that aid interaction between the components of its contractile ring (i.e., Rho A, aniline, actin and myosin) (Harding *et al.*, 2016). This gene is located on chromosome 12q24.23, comprising 50 exons, 2027 amino acids, with a genome size and molecular weight of 191,501 bp and 231,431 Da, respectively. It has four already known isoforms and a 6207 bp ORF. The CIT protein is located in the mitotic cells midbody and cleavage furrow. This gene is conserved in many species including *H. sapiens*, *M. musculus*, *D. melanogaster*, *M. mulatta*, *P. troglodytes*, *X. tropicalis*, *X. tropicalis* and *A. gambiae*. CIT is important in phosphorylation of many compounds, for the maintenance of the structure of the midbody during cell division and in the completion of cytokinesis due to kinase activity (Di Cunto *et al.*, 2000; Li *et al.*, 2016). Homozygous mutation in the kinase domain of CIT causes MCPH by the loss or activation of protein citron kinase (Basit *et al.*, 2016; Harding *et al.*, 2016). Individuals with these mutations exhibit abnormal cytokinesis characterized by deferred mitosis, spindles having multiple poles, chromosomal

instability, aneuploidy, PF3 cycle arrest initiation, massive apoptosis and have multinucleated neurons throughout the cerebral cortex (Li *et al.*, 2016). CITK deficient cells have heightened levels of sensitivity to ionizing radiation and inoperative rehabilitation from radiation-induced DNA damage (Bianchi *et al.*, 2017). Mutations in the CIT gene causes a severe neurological disorder characterized by very small head and individuals with this microcephaly have intellectually disability, axia hypotonia, brisk reflexes, social impairment, thin corpus callosum and reduced cerebral volume and sometimes have a sloping forehead, dysmorphic features and their brain shows gyral patterns (Harding *et al.*, 2016; Li *et al.*, 2016; Shaheen *et al.*, 2016). In rodents CITK is important for the proliferation of neural progenitor cells and male germ cell precursors. CIT knockout mice show ataxia, testicular hypoplasia, lethal seizures, growth deficiencies, severe reduction in brain size and often show simplified gyral patterns and temperaments linked to cytokinesis defects, that is, they have multinucleated neurons throughout the cerebrum and cortex and show massive apoptosis (Harding *et al.*, 2016; Li *et al.*, 2016; Bianchi *et al.*, 2017).

(xviii) MCPH18 (WDFY3)

WDFY3, encoding Alfy protein, performs binding and beta-N-acetylglucosaminylglycopeptide beta-1,4-galactosyltransferase activity. Alfy protein domains are: WD40_repeat domain, WD40/YVTN domain and FYVE type domain. This gene is located on chromosome 8p23, comprising 3526 amino acids, with a genome size and molecular weight of 296,855 bp and 395,258 Da, respectively, and two already known isoforms. WDFY3 is used as a scaffold protein required for the selective self-breakdown of macromolecules such as aggregation-prone proteins. Alfy protein is highly expressed in the developing CNS and crucial for the development of axonal tracts. The protein breakdown is mandatory to provide the proper atmosphere in which to coordinate complicated cell signalling events and to enhance cellular remodelling. The *Wdfy3* gene is suspected to be involved in neurodevelopmental ataxia such as microcephaly and autism. Its homolog is Blue Cheese (*bchs*), which is abundantly expressed in the developing and adult fly CNS. *Wdfy3* dysfunction in mice has subtle effects on migration and proliferation of neural progenitor cells. The affected mice display larger brains as a repercussion of an analogous alteration in the mechanism of radial glial mitoses to symmetric from asymmetric. The other 17 genes of microcephaly follow the autosomal recessive mode of inheritance; however, the WDFY3 gene follows the autosomal dominant mode of inheritance. Heterozygous mutation has been reported in the WDFY3 gene (R2637W), a c.7909C-T transition means tryptophan replaces arginine at the 2637 position. *In vitro* expression studies on *Drosophila* have revealed increased levels of DVL3 upon transfection of *Drosophila* cells with mutant human WDFY3. Increased levels of DVL3 cause abnormal activation of Wnt signalling as well as continued generation of apical progenitor cells without transition to differentiation and generation of the basal progenitor cell layers in the cerebral cortex, thus resulting in impaired cortical development and microcephaly (Jayaraman *et al.*, 2018).

3. Conclusion

Microcephaly is considered to be a rare neurodevelopmental disorder with many underlying causes. Pathogenicity analysis led to

the discovery of 18 genes being a possible reason for this primary neurogenic mitotic syndrome. Recent experiments for successful generation of model animals for MCPH genes opened the door for researchers to further comprehend the pathophysiology and aetiology of MCPH. Improvement in the genotypic and imaging techniques along with organizing patients according to the basis of their genotypic homogeneity would enable us to better predict a correlation between genotype–phenotype. Mutational analysis of patients from different regions of the Middle East, and especially Pakistan, would aid in counselling and diagnostic approaches for MCPH ensuring better health quality for patients. This study has allowed us a better understanding of neuronal production processes by stem cells. The ongoing research projects on MCPH genes will lead us towards better understanding of this rare nonprogressive neuropediatric disorder. Moreover, MCPH genes are strong candidates for brain development and evolutionary studies.

Declaration of interest. None.

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