Mutation in CEP135 causing primary microcephaly and subcortical heterotopia

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To the Editor

Autosomal recessive primary microcephaly (MCPH) is characterized by congenital microcephaly (>2–3 standard deviations [SD] below the mean for age and gender) and intellectual disability without additional syndromic features (Alcantara & O’Driscoll, 2014). Up to now, 18 genes have been linked to MCPH, all of them involved in the neurogenesis of radial glia cells as the primary progenitor cells of the developing cerebral cortex (Jayaraman, Bae, & Walsh, 2018). Most of these genes encode centrosomal proteins involved in centriole biogenesis (WDR62, CDK5RAP2, CASC5, ASPM, CENPJ, STIL, CEP135, CEP152, SASS6) (Jayaraman et al., 2018). Others are involved in DNA replication and repair, kinetochore function, transmembrane or intracellular transport, autophagy, or cell polarity (Jayaraman et al., 2018).

Barkovic et al. provide a comprehensive review on malformations of cortical development (MCD), a large heterogenous group of defects in cerebral cortex formation, resulting from dysfunctional neurogenesis, neuronal migration, and postmigrational development (Barkovich, Guerrini, Kuzniecky, Jackson, & Dobyns, 2012). MCPH was classified as group IA, representing a reduced size of the cerebral cortex due to reduced proliferation, generally without gross morphological abnormalities (Barkovich et al., 2012). Nonetheless, cases with structural changes have been reported, for example, cortical malformations and subcortical heterotopia in WDR62 patients and periventricular heterotopia in MCPH1 (Nicholas et al., 2010; Trimborn et al., 2004; Yu et al., 2010).

Several MCD are predominantly characterized by clusters of neurons unable to migrate to their proper position in the cortex, referred to as heterotopic gray matter brain malformation (HET) (Oegema, Barkovic, Mancini, Guerrini, & Dobyns, 2019). Periventricular nodular heterotopia (PNH) is the most common subtype, formed by nodules in the wall of the lateral ventricles (Oegema et al., 2019). Recently, a new classification for the less common subcortical heterotopias (SUBH) was introduced (Oegema et al., 2019). SUBH are considered a different disease entity, defined as heterotopic gray matter located in the white matter between the lateral ventricles and the cortex (Oegema et al., 2019).

Mutations in the Centrosomal Protein 135 gene (CEP135) are a very rare cause of primary microcephaly (MCPH8, OMIM 614673), since only three families have been reported without brain-MRI, and detailed information on brain morphology have been lacking. Here, we report a patient presenting with epilepsy as new feature in CEP135 related disease and provide the first brain-MRI images identifying subcortical heterotopia as underlying cerebral malformation.

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The female patient is the only child of healthy unrelated German parents. Microcephaly had already been diagnosed prenatally. The girl was born spontaneously at term without complications after an otherwise uneventful pregnancy. Head circumference at birth was 31 cm (1.5 cm <third percentile, −3.6 SD) and 46.5 cm (2.5 cm <third percentile, −5 SD) at age 8 years. Her early motor and speech development were delayed, but she finished regular school and her overall IQ was 79 (WISC-IV at age 15 years). First complex-partial seizures started at age 10 years, which initially were well-controlled by Oxcarbazepine.

At age 18 years, she suffered from generalized tonic–clonic-seizures requiring hospitalization. Her head circumference was 48.5 cm (3.5 cm <third percentile, −6.5 SD), height 165.3 cm (33rd percentile), and weight 69.5 kg (87th percentile) at 18 years of age. Apart from a sloping forehead and a long thin nose, no dysmorphisms were apparent. Brain-MRI displayed marked bilateral nodular heterotopia in the peritrigonal regions, defined as group 1a according to the most recent HET classification of Oegema et al. (Figure 1) (Oegema et al., 2019). No further structural abnormalities were apparent, especially gyral pattern and myelination were unremarkable.

Consecutively, whole exome sequencing was performed as previously described (Pergande et al., 2019) (see Supplementary Material). We identified a novel homozygous frameshift mutation (c.3211A>T; p. Lys1071*) in CEP135 (NM_025009.4). The mutation was confirmed by Sanger Sequencing, and both parents are heterozygous carriers. The variant has not been reported in any database and was classified as pathogenic (PVS1, PM2, PM3) according to the ACMG classification (Richards et al., 2015). In addition, two heterozygous variants in the FAT4 gene (NM_024582.4), known to cause autosomal-recessive syndromic periventricular nodular heterotopia were apparent (Alders et al., 2014). But as both variants in FAT4 were inherited form the healthy mother and the reported FAT4-phenotypes including the pattern of brain malformation and pathognomonic facial features did not match our patient, they were not considered disease causing. A missense variant c.12778G>A (p.Asp4260Asn) was detected in HUWE1 (NM_031407.6), variants in HUWE1 are known to cause dominant X-linked intellectual disability of the Turner Type (OMIM: 309590). For HUWE1 patients multiple brain-MRI reports are available, none of them depicting cortical brain malformations, and in animal models, no structural nervous system anomalies were reported (Moortgat et al., 2018; Vandewalle et al., 2013). Thus, we considered this variant to be a less probable cause of the subcortical nodular heterotopia compared to the CEP135 variant. Nonetheless, it may act as a disease modifier for the intellectual disability depending on the degree of X-chromosome inactivation. No further likely pathogenic variants in genes previously associated with MCPH or MCD were identified, nor were convincing novel candidate-genes found (see Supplementary Material). Still a modifying effect of the reported secondary variants on the phenotype together with other unknown genetic disease modifiers has to be considered.

Because we have a whole-exome and not a whole genome sequencing, it is not possible to exclude the low probability of a digenic inheritance of deep intronic variants or structural variants in a second gene modifying the phenotype (Posey et al., 2017).

Homozygous truncating mutations in CEP135 have first been identified in two Pakistani families with primary microcephaly, learning disability, speech impairment, and sloping forehead (Farooq et al., 2016; Hussain et al., 2012). In addition, a two-year-old boy was reported presenting with primordial dwarfism, spasticity, and developmental delay. His dysmorphic features included microcephaly, scoliosis, hypotelorism, sloping forehead, small face, and broad nose.
Our stop mutation in CEP135 is involved in centrosomal microtubule assembly by serving as a linker protein directly connecting the central hub protein HsSAS-6 to the outer microtubule (Lin et al., 2013). It consists of an N-terminal microtubule interacting domain, a central CENPJ interacting domain, and a C-terminal HsSAS-6 interacting domain (Lin et al., 2013). Our stop mutation in CEP135 presumably results in a loss of the last sixth coiled-coil domains and is predicted to be deleterious as it covers part of the region responsible for interaction with HsSAS-6 (MCPH14, OMIM 616402). In addition, the transcript is predicted to undergo nonsense mediated decay (NMD) which would lead to a complete loss of function. The milder microcephaly and subcortical heterotopia could represent a hypomorphic allele manifestation in our case. The premature stop codon near the C-terminus of CEP135 might only lead to partial NMD with residual activity in contrast to the previously complete loss-of-function mutations causing a more severe microcephaly.

Loss of CEP135 leads to disorganized interphase and multiple and fragmented centrosomes with disorganized microtubules (Hussain et al., 2012; Lin et al., 2013; Ohta et al., 2002). This disrupts cell division but also causes disordered neuronal cell polarity and basal body formation, which is essential for neuronal migration (Jana et al., 2018). CEP135 is not the very first MCPH gene being linked to neuronal migration defects. Interestingly, mutations of WDR62 (MCPH2, OMIM 604317) are reported in patients manifesting microcephaly and malformations of cerebral cortical architecture and subcortical heterotopia (Yu et al., 2010). Our patient presents microcephaly and solid nodular HET in the region of the peritrigonal optic pathway posterior to the deep gray nuclei (group 1a). The same pattern has been described by Oegema et al. in a patient with CENPJ mutations (MCPH6, OMIM 608393), which closely interacts with CEP135 in centrosome formation (Lin et al., 2013; Oegema et al., 2019; Tang, Fu, Wu, Hsu, & Tang, 2009). In addition, SUBH 1a was associated with TUBB (CDCBM6, OMIM 615771) coding for the microtubular subunit tubulin-beta, and KATNB1 (LIS6, OMIM 616212) which forms the microtubule-severing protein katanin together with KATNB1 and localizes to the centrosome (Breuss et al., 2012; Mishra-Gorur et al., 2014; Oegema et al., 2019). All these genes are involved in the asymmetrical division of neuronal progenitor cells and disruption leads to impaired neuronal proliferation and migration (Breuss et al., 2012; Insolecera, Bazzi, Shao, Anderson, & Shi, 2014; Mishra-Gorur et al., 2014).

Here, we report the first patient with a homozygous CEP135 nonsense mutation and subcortical heterotopia; thus CEP135 mutations shall be added to the genetic differential diagnosis of subcortical nodular heterotopia of the peritrigonal region (SUBH 1a). Furthermore, our findings emphasize the effect of CEP135 related centrosomal disruption on neuronal migration in human brain development.

CONFLICT OF INTEREST
All authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

AUTHORS’ CONTRIBUTIONS

Daniel Bamborschke: Analysis and interpretation of data, drafting the manuscript. Hülya-Sevcan Daimagüler: Production and interpretation of data. Andreas Hahn: Clinical evaluation of the patient, reviewing the manuscript for intellectual content. Muhammad S. Hussain: Analysis and interpretation of data, reviewing the manuscript for intellectual content. Peter Nürnberg: Reviewing the manuscript for intellectual content. Sebahattin Cirak: Conception and design, analysis and interpretation of data, reviewing the manuscript for intellectual content.

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(Shaheen et al., 2019). All of these cases presented severe microcephaly with a head circumference of −10 to −13 standard deviation (SD). Our patient has milder primary microcephaly (−6.5 SD) and mild learning disabilities.

Noteworthy in our case are the novel features: epilepsy and subcortical heterotopia group 1a associated with CEP135 mutations. Our patient has milder primary microcephaly (−6.5 SD) and mild learning disabilities. Our stop mutation in CEP135 presumably results in a loss of the last sixth coiled-coil domains and is predicted to be deleterious as it covers part of the region responsible for interaction with HsSAS-6 (MCPH14, OMIM 616402). In addition, the transcript is predicted to undergo nonsense mediated decay (NMD) which would lead to a complete loss of function. The milder microcephaly and subcortical heterotopia could represent a hypomorphic allele manifestation in our case. The premature stop codon near the C-terminus of CEP135 might only lead to partial NMD with residual activity in contrast to the previously complete loss-of-function mutations causing a more severe microcephaly.

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