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Brief Report

De Novo Truncating Mutations in the kinetochore-microtubules attachment gene *CHAMP1* Cause Syndromic Intellectual Disability

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Abstract

A rare syndromic form of intellectual disability with impaired speech was recently found associated with mutations in *CHAMP1* (chromosome alignment-maintaining phosphoprotein 1), the protein product of which is directly involved in microtubule-kinetochore attachment. Through whole-exome sequencing in six unrelated non-consanguineous families having a sporadic case of intellectual disability, we identified six novel *de novo* truncating mutations in *CHAMP1*: c.1880C>G p.(Ser627*), c.1489C>T; p.(Arg497*), c.1876_1877delAG; p.(Ser626Leufs*4), c.1043G>A; p.(Trp348*), c.1002G>A; p.(Trp334*) and c.958_959delCC; p.(Pro320*). Our clinical observations confirm the phenotypic homogeneity of the syndrome, which represents therefore a distinct clinical entity. Besides, our functional studies show that CHAMP1 protein variants are delocalized from chromatin and are unable to bind to two of its direct partners, POGZ and HP1. These data suggest a pathogenic mechanism of the *CHAMP1*-associated intellectual disability syndrome mediated by direct interacting partners of CHAMP1, several of which are involved in chromo/kinetochore-related disorders.

Key Words: Intellectual disability, *CHAMP1*, microcephaly, *POGZ, HP1,* kinetochores, microtubules

Division of neural progenitor cells is one of the key processes, which, together with neuronal polarization and differentiation, shapes the complex architecture of the developing human brain [Sakakibara, et al., 2013]. Microtubules play an important part in these different morphogenetic processes, and notably in cell division, where they contribute to the partition of chromosomes in daughter cells by empowering chromosome movements and by linking chromosomes to spindles through binding to kinetochores [Duro and Marston, 2015]. Chromosome segregation accuracy is highly dependent on attachment of sister kinetochores to microtubules from opposite spindle poles [Tanaka and Watanabe, 2008]. Such a phenomenon requires the coordinated action of numerous proteins acting directly on the microtubule-binding interface of kinetochores [Itoh, et al., 2011], correcting improper microtubule-kinetochore attachments [Ruchaud, et al., 2007], and controlling the quality of these attachments [Musacchio and Salmon, 2007]. Mutations in microtubule-related genes, including *KIF5C*, *KIF2A*, *DYNC1H1* and *TUBG1*, have been shown to cause malformations of cortical development associated with severe intellectual disability (ID) and/or microcephaly [Poirier, et al., 2013].

Following a first finding from a large cohort study [Rauch, et al., 2012], Hempel *et al.* [Hempel, et al., 2015] documented recently five ID cases with severe impaired speech associated with mutations in *CHAMP1* (chromosome alignment-maintaining phosphoprotein 1; MIM# 616327), the last subtelomeric gene from the long arm of chromosome 13 (chr13:114,314,490-114,327,328, GRCh37/hg19). *CHAMP1* contains two untranslated exons and one translated exon encoding an 812-amino-acid zinc-finger protein, which is directly involved in microtubule-kinetochore attachment.

CHAMP1 protein product is highly conserved across vertebrates and contains three characteristic repeat motifs named WK, SPE, and FPE [Itoh, et al., 2011].

Through a multicenter collaborative study on sporadic cases with ID, we were able to identify six new *de novo* variants very likely affecting the function of *CHAMP1* in children with ID. The six affected children presented here were enrolled in three different programs or centers investigating the molecular basis of intellectual disabilities (Table 1): the Western France consortium HUGODIMS (French acronym standing for "Projet inter-régional Français des Hôpitaux Universitaires du Grand Ouest pour l'exploration par approche exome des causes moléculaires de Déficience Intellectuelle isolée ou syndromique Modérée à Sévère"; proband F1-II.1), the Wellcome Trust Sanger Institute British program Deciphering Developmental Disorders (DDD; patients F2-II.1, F5-II.1 and F6-II.1), and the American Baylor Miraca Genetics Laboratory (BMGL) clinical exome testing cohort (patients F3-II.1 and F4-II.1). Regarding both HUGODIMS and DDD, probands were included together with their biological parents, following a trio strategy, whereas the diagnostics protocol defined by the BMGL required sampling of the patient only and testing of parents for variants of interest. Written informed consent for inclusion in the study and consent for the publication of photographs was obtained for all patients. The study has been approved by the CHU de Nantes-ethics committee (number CCTIRS: 14.556). Clinical evaluation was performed for each patient by at least one expert clinical geneticist; it revealed very similar phenotypic features shared by all six affected individuals: severe or moderate ID, hypotonia, absent or very poor speech, microcephaly (3/6) ranging from -2SD to -4SD, and facial dysmorphy (Fig. 1, Table 1, Supp. Methods and Supp. Table S1). For all patients, brain MRIs were considered normal or with nonspecific findings. Routine clinical genetic and metabolic screenings were negative. Detailed observations are reported in Supp. Methods and Supp. Table S1.

By applying a whole-exome sequencing (WES) strategy to the first trio (Family 1), we identified the *de novo* nonsense heterozygous variant NM_032436.2:c.1880C>G; p.(Ser627*) in *CHAMP1* in patient F1-II.1 (Supp. Fig. S1), which was verified by Sanger sequencing (Supp. Fig. S2). We did not identify any X-chromosomal, compound heterozygous or homozygous candidate variants in known ID genes. From the BMGL independent cohort of 5300 individuals undergoing clinical WES, we identified two additional patients (patients F3-II.1 and F4-II.1) having a truncating variant in *CHAMP1* (NM_032436.2:c.1876_1877delAG; p.(Ser626Leufs*4) and c.1043G>A; p.(Trp348*)). We validated these variants by Sanger sequencing, and parental genotyping confirmed their *de novo* origin (Supp. Fig. S2). Three further patients recruited from the DDD cohort exhibited a *de novo* nonsense *CHAMP1* variant verified by capillary sequencing (Supp. Fig. S2): c.1002G>A (p.(Trp334*)) in patient F2-II.1, c.958_959delCC (p.(Pro320*)) in patient F5-II.1 and c.1489C>T (p.(Arg497*)) in patient F6-II.1. None of the six mutations was present either in over 6,500 in-house exomes or in any public variant databases, save c.1489C>T (p.(Arg497*) which is referenced in dbSNP and ClinVar (rs782397980;RCV000191999.2). Two of these patients (patients F2-II.1 and F6-II.1) were previously reported without clinical description as part of a large cohort study on ID [Deciphering Developmental Disorders, 2015].

Our six patients show a phenotype very similar to the patients reported by Hempel *et al.* who already noted phenotypic similarities with cases of subtelomeric 13q deletions reported in copy number variation databases [Hempel, et al., 2015]. The syndromic form of ID due to *CHAMP1* mutations might be difficult to recognize clinically, but association of neonatal hypotonia with motor delay, severe speech impairment, facial anomalies (round face, facial hypotonia, hypertelorism) and microcephaly should lead to evoke this diagnosis.

CHAMP1 expression is consistent with a role in ID. In mice, proteins levels are detected in a few organs only, including brain, and also thymus, testis, and ovary (Supp. Fig. S3). *In situ* hybridization with a probe to mouse *Champ1* reported in EMAGE database showed expression in the thymus, gonads and developing brain at embryonic day (E) 14.5. Expression was seen in the ventricular zone and the cortical plate, hereby consistent with a putative role in progenitor cells [Richardson, et al., 2014]. All *de novo* mutations identified in our patients and in the five ones reported by Hempel *et al.* (2015) create a premature stop codon located in the only coding exon of *CHAMP1,* exon 3b, and affect residues highly conserved across species. Interestingly, the resulting truncated proteins all lack the C-terminal region containing three zinc-finger domains (Supp. Fig. S1), which region is crucial for CHAMP1 localization to mitotic chromatin [Itoh, et al., 2011].

To assess a possible effect of the variants found in our patients, we examined subcellular localization of three of the CHAMP1 protein variants, derived from c.1880C>G, c.1043G>A, c.1876_1877delAG , by expressing them as Green Fluorescent Protein (GFP)-fusion proteins in HeLa cells (Supp. Fig. S4A). We found that, unlike full-length CHAMP1, they do not localize to chromatin in metaphase cells (Supp. Fig. S4B). In interphase cells, full-length CHAMP1 localizes to heterochromatic regions densely stained with DAPI (Supp. Fig. S4B), probably reflecting the interaction with HP1 (heterochromatin protein 1), which is involved in heterochromatin formation. In contrast, two *CHAMP1* mutants (c.1880C>G; p.(Ser627*), and c.1876_1877delAG; p.(Ser626Leufs*4)) showed diffuse nuclear localization (Supp. Fig. S4B). Another *CHAMP1* mutant (c.1043G>A; p.(Trp348*)), which further lacks the middle region containing the FPE and WK motifs (Supp. Fig. S1), localized to the cytoplasm (Supp. Fig. S4), suggesting that this middle region contains a nuclear localization signal. These data suggest that these CHAMP1 truncated mutants cannot localize to chromatin.

To further verify the defects in the CHAMP1 mutants, we investigated the interaction of CHAMP1 with other proteins. CHAMP1 was identified as an interacting protein with Mad2L2 (Mitotic arrest-deficient 2, S.Cerevisiae, Homolog-like-2), which binds to the middle region of CHAMP1 containing the WK motifs [Itoh, et al., 2011]. In addition, HP1 (Heterochromatin protein 1) and POGZ (POGO transposable element with ZNF domain) were found to interact with CHAMP1 by proteomic analysis (K.T., unpublished data), which was confirmed by western blotting analysis (Supp. Fig. S5A). POGZ (pogo transposable element-derived protein with zinc finger domain) was found as an HP1-binding protein together with CHAMP1 [Nozawa, et al., 2010]. CHAMP1, Mad2L2, HP1, and POGZ were also identified as a complex bound to trimethylated histone H3K9 [Vermeulen, et al., 2010]. We determined the interaction domains of CHAMP1 with HP1 and POGZ by *in vitro* binding assays using purified proteins (Supp. Fig. S5B). HP1 was found to bind to the C-terminal fragment of CHAMP1, but not the C-terminal fragment lacking the zinc-finger domains (Supp. Fig. S5C), indicating that HP1 binds to the C-terminal region containing the zinc-finger domains. We also found that the C-terminal fragment of POGZ interacts with the same C-terminal region of CHAMP1 containing the zinc-finger domains (Supp. Fig. S5D). As this region is lacking in all CHAMP1 mutants, these mutants are probably incapable of binding to both HP1 and POGZ. The six mutations observed in the patients lie in regions predicted to be disordered (Supp. Fig. S6) and are located between domains at both termini which would contain two and three zinc-finger respectively, according to three-dimensional modeling of *CHAMP1* protein product (Supp. Fig. S7); the six mutations give all rise to truncation of CHAMP1, removing the three zinc-fingers domain at the C-terminus (Supp. Fig. S7).

Overall, our functional studies show that truncation protein variants of CHAMP1 are likely delocalized from chromatin and unable to bind to two of its direct partners, POGZ and HP1. These

data are not sufficient to fully understand the pathogenic mechanism of the *CHAMP1*-associated ID syndrome, but they allow us to formulate several hypotheses.

First, pathogenicity may result from loss of mitotic function of CHAMP1. This protein is involved in kinetochore–microtubule attachment in a way that maintains chromosome alignment on the metaphase plate [Itoh, et al., 2011]. Therefore, it is plausible that chromosome segregation defects in neuronal progenitor cells may cause this developmental disorder.

Alternatively, the pathogenic mechanism leading to ID may result from impaired CHAMP1 binding to its partners; it was reported indeed that disruption of POGZ also causes mitotic defects, abnormal chromosome segregation, nuclear fragmentation, and disrupted mitotic HP1-alpha localization [Nozawa, et al., 2010]. It is intriguing that *de novo* loss-of-function mutations in *POGZ* have recently been identified in individuals affected by neurodevelopmental disorders with a similar phenotype (ID, microcephaly) [Fromer, et al., 2014; Iossifov, et al., 2012; Neale, et al., 2012]. Our data show that all the CHAMP1 mutants lack the C-terminal region which is required for interaction with POGZ, suggesting that the inability to form complexes with POGZ may affect the *CHAMP1* function. Furthermore, as for *CHAMP1*, heterozygous *de novo POGZ* mutations found in patients with ID give rise to truncations of the C-terminal region , which is required for interaction with CHAMP1 (Supp. Fig. S5D).

While *CHAMP1* may be haploinsufficient, with loss of function of a single copy of the gene sufficiently impairs function to cause disease, dominant-negative or gain-of-function effects of the mutations observed on CHAMP1 protein activity cannot be ruled out. All the nonsense or frameshift mutations identified are located in the only coding exon of the gene and they might therefore escape nonsense-mediated mRNA decay (NMD). In such case, the mutated mRNAs would be

expected to be stable and the resulting truncated proteins functional. This hypothesis is corroborated by the normal phenotype of mice heterozygous for *Champ1* deletions (K.I. and K.T., unpublished data). On the other hand, mice homozygous for the same deletions die soon after birth (K.I. and K.T., unpublished data). The reason for the death is under investigation, but restricted expression of Champ1 in mice including brain (Supp. Fig. S3) implies death could be secondary to neurological impairment. Interestingly, HP1β-/- mice also die in the neonatal period with abnormal cerebral cortex development and reduced proliferation of neuronal precursors [Aucott, et al., 2008].

In conclusion, our clinical and experimental observations confirm the phenotypic homogeneity of the rare ID syndrome described by Hempel *et al*. and caused by *de novo* truncating mutations in *CHAMP1* [Hempel, et al., 2015]. These findings expand the phenotypic spectrum of chromo/kinetochore disorders, several of which are caused by mutations in direct interacting partners of *CHAMP1*. The identification of *CHAMP1* as a novel ID gene will increase the diagnostic yield of exome or genome sequencing in individuals with severe ID, and our data suggest that deciphering the function of *CHAMP1* partners would very likely help to elucidate the pathophysiology of some neurodevelopmental disorders.

The reported sequence variants NM_032436.2:c.1880C>G, p.(Ser627*); c.1002G>A, p.(Trp334*); c.1876_1877delAG, p.(Ser626Leufs*4); c.1043G>A, (p.(Trp348*); c.958_959delCC, p.(Pro320*); and c.1489C>T, (p.(Arg497*) have been deposited in the Leiden Open Variation Database at http://www.lovd.nl/CHAMP1 under accession numbers 0000077846, 0000077847, 0000077848, 0000077981, 0000077984, and 0000077985.

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Figure legend

Figure 1. Frontal facial photographs of the six patients with *CHAMP1* de novo variants included in the study. **a**: Patient F1-II.1, **b**: Patient F2-II.1, **c**: Patient F3-II.1, **d**: Patient F4-II.1, **e**: Patient F5-II.1, and **f**: Patient F6-II.1. Note the clinical similarities, including a round face, hypertelorism, and a large mouth.

Table 1. Detailed clinical features of patients with *CHAMP1* mutation

	Patient F1-II.1 (France)	Patient F2-II.1 (UK)	Patient F3-II.1 (USA)	Patient F4-II.1 (USA)	Patient F5-II.1 (UK)	Patient (UK)
Gender	Male	Male	Female	Male	Female	Female
Developmental delay/intellectual disability	+ (severe)	+ (severe)	+ (severe)	+ (severe)	+ (moderate)	+ (seve
Failure to thrive	+	-	-	+	-	-
Delay in walking	+	+	+	+	+	+
Speech delay	+	+	+	+	+	+
Speech abilities	none	none	very limited	none	very limited	very lin
Behavioural anomalies	-	-	+	+	+	-
Seizures	-	-	+	-	-	ND
Skeletal abnormalities	+	+	+	+	+	-
Facial dysmorphism	+	-	+	+	+	+
Eye anomalies	+	+	+	+	ND	+
Feeding difficulties	+	-	ND	+	+	ND
CHAMP1 mutation (NM_032436.2)*	c.1880C>G, p.(Ser627*)	c.1002G>A, p.(Trp334*)	c.1876_1877delAG; p.(Ser626Leufs*4)	c.1043G>A, p.(Trp348*)	c.958_959delCC, p.(Pro320*)	c.1489 p.(Arg4

* Nomenclature HGVS V2.0 according to mRNA reference sequence NM_032436.2. Nucleotide numbering uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1.