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ORIGINAL ARTICLE

Phenotypes and genotypes in non-consanguineous and consanguineous primary microcephaly: High incidence of epilepsy

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Abstract

Background: Primary microcephaly (PM) is defined as a significant reduction in occipitofrontal circumference (OFC) of prenatal onset. Clinical and genetic heterogeneity of PM represents a diagnostic challenge.

Methods: We performed detailed phenotypic and genomic analyses in a large cohort (n = 169) of patients referred for PM and could establish a molecular diagnosis in 38 patients.

Results: Pathogenic variants in *ASPM* and *WDR62* were the most frequent causes in non-consanguineous patients in our cohort. In consanguineous patients, microarray and targeted gene panel analyses reached a diagnostic yield of 67%, which contrasts with a much lower rate in non-consanguineous patients (9%). Our series includes 11 novel pathogenic variants and we identify novel candidate genes including *IGF2BP3* and *DNAH2*. We confirm the progression of microcephaly over time in affected children. Epilepsy was an important associated feature in our PM cohort, affecting 34% of patients with a molecular confirmation of the PM diagnosis, with various degrees of severity and seizure types.

Conclusion: Our findings will help to prioritize genomic investigations, accelerate molecular diagnoses, and improve the management of PM patients.

KEYWORDS
brain developmental disorders, consanguinity, epilepsy, Mendeliome, primary microcephaly, rare disease

1 | INTRODUCTION

Microcephaly is a clinical condition referring to a small head size. It is assessed by measuring the occipitofrontal circumference (OFC) (Rollins et al., 2010). The OFC is a surrogate for brain volume, microcephaly reflecting a small brain, mostly a small neocortex (Cox et al., 2006). Most subjects with an OFC smaller than 3 standard deviations (SDs) below the mean for sex and age, have intellectual deficiency, and some of them present with additional neurological deficits like epilepsy or paresis (Létard et al., 2018). The prevalence of such additional deficits remains unclear and no systematic study of epilepsy in microcephaly has been reported.

Recognized causes of microcephaly are very heterogeneous. Hundreds of syndromes have been described with microcephaly as a feature. Microcephaly can be attributed to environmental causes (e.g., infections, teratogens, perinatal hypoxia, hypoglycemia) or genetic causes. Primary microcephaly (PM) refers to a prenatal defect of brain volume development, and secondary microcephaly consists of progressive atrophy of an initially normal brain, usually starting after birth (Woods & Basto, 2014). In spite of some overlap, these two groups are fairly separated, with distinct patterns of brain growth deceleration (Boonsawat et al., 2019; Shaheen et al., 2019). PM is divided into syndromic and non-syndromic. Patients with primary, non-syndromic microcephaly typically present with a mild phenotype consisting of mild to a moderate intellectual disability only. The relatively high recurrence risk in siblings of an affected child, and the high rate of consanguinity in these families, indicate that a large proportion of cases is inherited as autosomal recessive. This phenotype is referred to as microcephaly primary hereditary (MCPH). The MCPH brain is small but its architecture is well conserved (Woods & Basto, 2014). Genetic heterogeneity in MCPH is striking, with 27 genes reported so far, 24 of which show autosomal recessive inheritance (Table S1). Additionally, *RRP7A* was recently associated with MCPH in a consanguineous Pakistani family (Farooq et al., 2020). Many of the MCPH proteins are recruited at the centrosome (Vertii et al., 2016) or cilium, or act in cell-cycle dynamics, and are expressed during corticogenesis (Zaqout et al., 2017). Some syndromes associate microcephaly with short stature and/or dysostoses, for example, Seckel syndrome or microcephalic osteodysplastic primordial dwarfisms, or with congenital diabetes (Duerinckx & Abramowicz, 2018). The large

genetic heterogeneity of PM and rarity of many pathogenic variants make gene prioritization and variant interpretation difficult.

PM has mostly been studied in highly consanguineous populations. Studies conducted in consanguineous families of Pakistani (Gul et al., 2006; Roberts et al., 2002; Sajid Hussain et al., 2013) and Iranian origin (Darvish et al., 2010) reported *ASPM* pathogenic variants as the most prevalent cause of MCPH, followed by *WDR62*. A later study in a mainly consanguineous population used Mendeliome or exome sequencing combined with autozygosity mapping, and found pathogenic variants in PM genes in 24% of the patients, *ASPM* being the most prevalent disease-causing gene (Shaheen et al., 2019). All these studies tested consanguineous families of Middle Eastern origin, and it is not clear whether their results can be extrapolated to other, or non-consanguineous, populations (Verloes et al., 1993). A few studies in non-consanguineous patients reported a large genetic heterogeneity, with a few variants in *ASPM* and none in *WDR62*, but were limited by small numbers (Boonsawat et al., 2019; Rump et al., 2016).

A few studies reported epilepsy to be associated with PM (Bhat et al., 2011; Dohrn & Bolaños, 2019; Nardello et al., 2018; Passemard et al., 2009; Rodríguez et al., 2019; Shen et al., 2005; Zombor et al., 2019). These patients were described to have generalized tonic-clonic seizures that were controlled with antiepileptic drugs (AED) (Bhat et al., 2011; Passemard et al., 2009). In the present study, we describe the clinical features of a large cohort of PM patients, 40 consanguineous and 129 non-consanguineous. We report the pathogenic variants identified, including 11 novel pathogenic variants. We provide detailed clinical information on all probands in which a molecular cause was identified with a special focus on epilepsy and identify three novel candidate genes.

2 | PATIENTS AND METHODS

2.1 | Patients

One-hundred and sixty-nine unrelated probands were referred for investigation of PM to our genetic center between 2001 and 2018. Inclusion criteria consisted of an OFC smaller than 2 SD below age- and sex-related mean at birth or smaller than 3 SD after one year of age, based on established growth charts (Rollins et al., 2010), and no evidence of perinatal infection or substance use in the mother, nor maternal phenylketonuria. Inclusion and exclusion criteria are listed in Table S2. Clinical information was obtained through the referring geneticist or child neurologist. Peripheral blood from the patients and their parents was collected for DNA extraction and genetic analysis.

2.2 | Molecular analysis

Successive analyses were performed in a strategy that evolved over the study period: standard karyotyping or comparative genomic hybridization (CGH), Sanger sequencing of *ASPM* and *WDR62* (after microsatellite and/or 11K SNP microarray genotyping in consanguineous families), and later next-generation sequencing consisting of a 14-gene panel by capture, than exome sequencing of the proband and targeted analysis. The affected sib's exome was sequenced in 5 families and the unaffected parents' exome was sequenced in 11 other families.

Patients' DNA samples from the exome cohort were enriched for exonic sequences and patients' DNA samples from the gene panel cohort were enriched for exonic sequences of 14 PM genes (*ASPM*, *KNL1*, *CDK5RAP2*, *CENPJ*, *CEP135*, *CEP152*, *MCPH1*, *ORC1*, *ORC4*, *ORC6*, *PCNT*, *STIL*, *TRMT10A*, and *WDR62*). For exome sequencing, the DNA capture kit and the sequencing platform varied according to the time of the analysis. The different sequencing platforms were Beijing Genomics Institute, China (Illumina HiSeq2000), AROS applied biotechnology, Denmark (Illumina HiSeq 2000), and BRIGHTcore Brussels Interuniversity Genomics High Throughput core, Brussels, Belgium (Illumina HiSeq 1500). The DNA capture kits used were Illumina TruSeq Exome Target, NimbleGen SeqCap EZ v3, NimbleGen SeqCap EZ v5, Agilent SureSelect All Exon v1, and Agilent SureSelect All Exon v5. For the gene panel cohort, exonic sequences were enriched using SeqCap EZ Choice NimbleGen Roche, and sequencing was performed on a MiSeq Illumina sequencer at the molecular genetic laboratory of Erasme Hospital, Brussels, Belgium. For gene panel and exome sequencing, all the raw sequences were aligned to the reference genome GRCh37 using BWA algorithm version 0.7.12 (Li & Durbin, 2009), duplicated reads were then marked using Picard version v1.119 (https://broadinstitute.github.io/picard/), alignment quality was improved using the GATK (DePristo et al., 2011) realigner, and base recalibrator version 3.3, and finally, variants were called using GATK Haplotype Caller version 3.3. The resulting variant set was annotated using SnpEff v4.1 (Cingolani et al., 2012), dbNSFP 2.8 (Liu et al., 2011), and filtered using the Highlander software (https://sites.uclouvain.be/highlander/).

2.3 | Sanger sequencing

PCR primers were designed for exons and flanking intronic sequences using the ExonPrimer software (http://ihg.helmholtz-muenchen.de/ihg/ExonPrimer.html). All exons and flanking intronic regions of the candidate genes were sequenced by the Sanger method using the Big Dye Terminator cycle sequencing kit v2 (Applied Biosystems), and analyzed

on a 3130 Genetic Analyzer sequencing machine (Applied Biosystems). Sequences were analyzed *in silico* for variants using Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The PCR primers used for the *VPS13B* deletion encompassed respectively exon36 (PCRa, Forward: GAGATATA TCATGTTCAGGCATCC, Reverse: CACAAACCAGAAAT GTCCTCATC), exon43 (PCRb, Forward: GGCAGACAGCT GCCAAAC, Reverse: GTGCAGAAGAAATGAATCCCC), and the border of the deletion (PCRc, Forward: GATGGGCA AGTGAAGAGAGGA, Reverse: ACAGGCACACAAGTGC AGAA).

2.4 | Exome sequencing variant classification

ACMG guidelines (Richards et al., 2015) were followed for variant pathogenicity classification. Variants were filtered for quality criteria (pass GATK (DePristo et al., 2011) standard filter, read depth ≥10), allelic frequency (based on the maximum minor allele frequency found in GnomAD Karczewski et al., 2020and for functional impact (nonsynonymous or splice junction effect, using snpeff_effect from SnpEff Cingolani et al., 2012The selected variants in known PM genes were classified as Pathogenic (class 5) or Likely Pathogenic (class 4) according to the ACMG guidelines (Richards et al., 2015), and we considered variants of unknown significance in interesting candidate genes, that is, genes not yet associated with human microcephaly. We looked for homozygous, compound heterozygous, and *de novo* variants in both consanguineous and non-consanguineous families. Familial segregation was checked using Sanger sequencing in order to demonstrate *trans* configuration in the autosomal recessive cases and *de novo* inheritance in the autosomal dominant cases. All variants reported in this manuscript have been submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/, ClinVar accession SCV001481940 to SCV001481968).

2.5 | Cohort and diagnostic workflow description

One-hundred and sixty-nine unrelated PM probands were studied, among whom 40 (24%) were born to consanguineous parents. The ethnic origin of the patients was mostly Western European, but some originated from Northern Africa (mainly Morocco), Turkey or the Middle East (mostly in the consanguineous families). The preliminary step of microarray identified a pathogenic CNV in one patient. The 168 remaining patients underwent a diagnostic workflow that is represented in Figure 1a. A first step consisted of genotyping consanguineous patients for homozygosity mapping using microsatellite analysis and/or SNP arrays, followed by Sanger sequencing of *ASPM* or *WDR62* when the locus was found homozygous, or direct *ASPM* and *WDR62* Sanger sequencing in non-consanguineous patients. This approach identified the causal genotype in 17 patients (10%). Of the remaining 151 patients, 60 received gene panel sequencing including 14 PM genes, 32 exome sequencing, and no further analysis was performed in 59, mostly because the patients were lost to follow-up. Gene panel sequencing identified a conclusive cause in 9 patients (15%), and exome sequencing in 11 patients (34%). The exome analysis furthermore identified qualifying variants in candidate genes in four patients (13%), see Figure 1a.

3 | RESULTS

3.1 | A molecular diagnosis is identified in 67% of consanguineous and 9% of non-consanguineous patients

In 38 patients, including 27 consanguineous and 11 non-consanguineous patients, a molecular cause of PM could be established. In particular, exome sequencing led to a diagnosis in 9 of 20 consanguineous patients (45%) and 2 of 12 non-consanguineous patients (17%). In five consanguineous families, the exome from an affected sib was sequenced in addition to the exome from the proband. A molecular cause of PM could be established in three of these five families (60%).

The distribution of the molecular causes found in the 38 patients is represented in Figure 1b. MCPH genes were incriminated in 29 patients (76%), and other PM genes in 8 patients (21%). A CNV was identified in one patient (3%), referred early in our study with a normal standard karyotype. In the overall cohort, the most prevalent gene was *ASPM* in 10 patients (26%), followed by *WDR62* in 7 patients (18%). The more commonly mutated genes in the consanguineous cohort were *ASPM* (26%), *KNL1 (aka. CASC5)* (19%), and *MCPH1* (11%), and in the non-consanguineous cohort *WDR62* (45%), *ASPM* (27%), and *CEP152* (18%), as shown respectively in Figure 1c.1 and 1c.2.

Patients with *ASPM* pathogenic variants had homozygous (seven patients, 70%) or compound heterozygous (three patients, 30%) variants. The patients with *WDR62* pathogenic variants had homozygous (three patients, 43%) or compound heterozygous (four patients, 57%) variants. Pathogenic variant types in our cohort were mostly nonsense, frameshift, and missense, while some splicing variants, a 2-exons deletion and a CNV were also identified (Table 1). Among the consanguineous patients, 26 (96%) carried a homozygous pathogenic variant, 1 (4%) a disease-causing *de novo* CNV, and none carried a compound heterozygous pathogenic variant. Among the non-consanguineous patients, nine (82%) carried

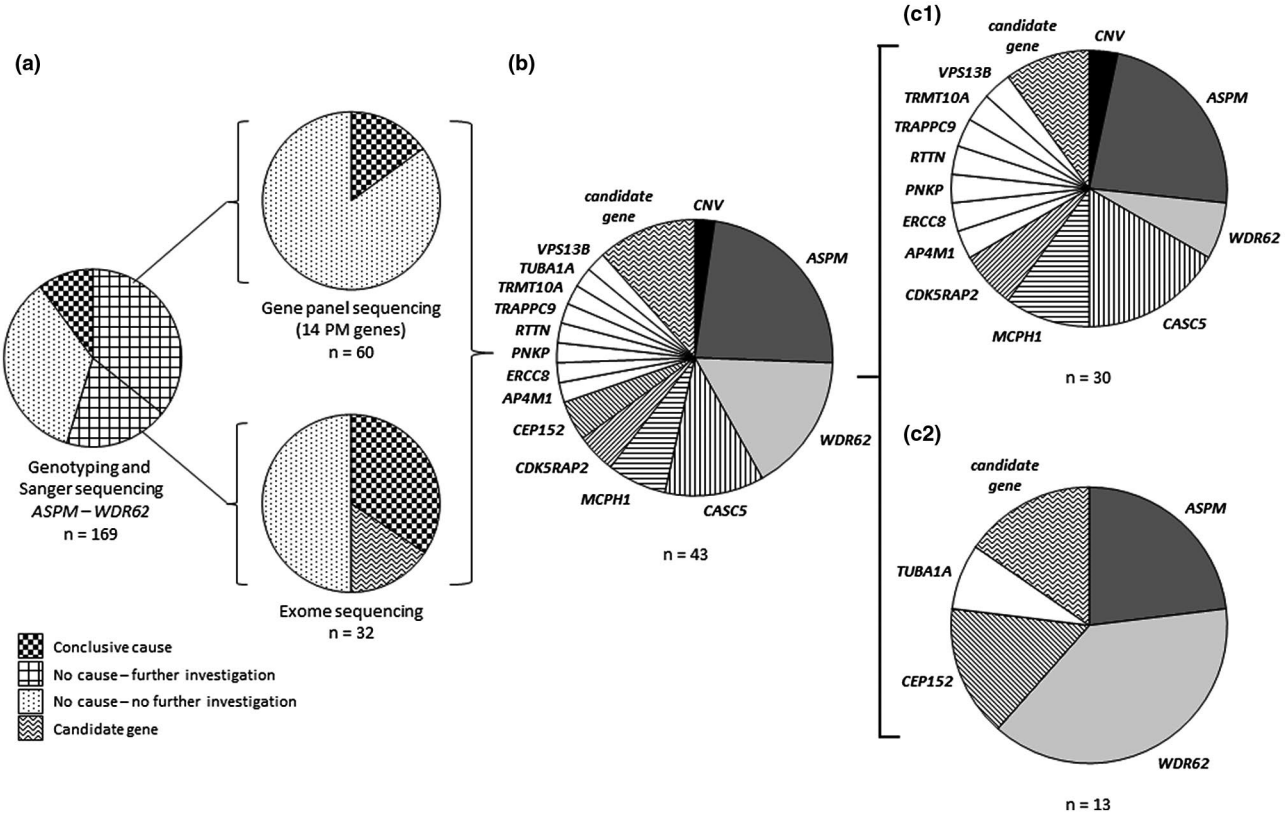

FIGURE 1 Study workflow and conclusive causes in PM patients. A. Diagnostic yield per technology, starting with genotyping and Sanger sequencing (*ASPM* and *WDR62*), followed by either gene panel (14 PM genes) or exome sequencing. B. Compilation of the conclusive causes identified with the different technologies. The more frequently involved genes in the overall cohort were *ASPM*, *WDR62*, *KNL1 (aka. CASC5)*, and *MCPH1*. C. Molecular diagnoses obtained in the consanguineous (C.1, n=30), and non-consanguineous (C.2, n=13) sub-cohorts, with *ASPM*, *KNL1*, and *MCPH1* most frequently involved in consanguineous, and *WDR62*, *ASPM*, and *CEP152* in non-consanguineous patients

a compound heterozygous pathogenic variant, one (9%) a homozygous variant, and one (9%) a de novo pathogenic variant in a gene following autosomal dominant inheritance. In the overall cohort, pathogenic variants in 36 of the 38 patients (95%) were thus identified in autosomal recessive genes.

3.2 | Novel pathogenic variants

Eleven pathogenic variants identified in this study were novel, including a CNV, a 2-exons deletion, four frameshift variants, one nonsense variant, two missense variants, and two splicing variants. They affected *CEP152*, *MCPH1*, *VPS13B*, and *WDR62* and are listed in Table 2.

A *de novo* deletion at 1q21.1 (arr 1q21.1(144757160x2, 144943150-146377870x1,146418803x2)dn) was considered pathogenic in one patient. Other CNVs in this region were already associated with a variable neurodevelopmental phenotype, frequently including microcephaly (Mefford et al., 2008).

A large homozygous intragenic deletion encompassing exons 42 and 43 of *VPS13B* was found in a patient with a subtle phenotype *a posteriori* consistent with Cohen syndrome (PM, fluctuating neutropenia, long eyelashes, truncal obesity, retinal dystrophy, joint hyperlaxity). Familial segregation and the exact position of the deletion were confirmed by PCR and Sanger sequencing (Figure S1).

A frameshift variant in *CEP152*, c.3249del p.(Val1084CysfsTer7) was found in one patient. Three novel variants of *MCPH1* were found, with splicing (c.322-1G>C p.?), frameshift (c.321dup p.(Arg108ThrfsTer2)) and missense (c.64G>A p.(Glu22Lys)) effects. Finally, five novel variants were found in *WDR62*, with frameshift (c.3469_3470del p.(Ala1157CysfsTer5) and c.3383_3401del p.(Ser1128TrpfsTer164)), nonsense (c.4345C>T p.(Gln1449Ter)), missense (c.1526C>T p.(Ser509Leu)), and splicing (c.1043+3A>G p.?) effects.

Both novel missense variants (*MCPH1* p.(Glu22Lys) and *WDR62* p.(Ser509Leu)) were classified as likely pathogenic variants (class 4) according to the ACMG guidelines, with two moderate and three supporting criteria. Both variants occurred in a functional domain of the protein (MCPH1 BRCT1 domain and WDR62 WD40 repeat domain) (*PM1 criterion*). MCPH1 BRCT1 domain is

TABLE 1 Genotype and phenotype in PM patients

Patient ID	Sex	Family	Ethnicity	Consanguinity	Gene #OMIM Number	RefSeq identifier	Transcript	Protein	Class	Phenotype	OFC at birth (SD)
#1	M		Morocco	same village	arr 1q21.1(144757160x2,144943150-146377870x1,146418803x2)dn				HC	PM	−2.5
#2	M		Turkey	yes	*AP4M1 #602296*	NM_004722.3	c.1012C>T/ c.1012C>T	p.(R338*)	HC	PM, progressive spasticity	−3.5
#3	M		Caucasian	no	*ASPM #605481*	NM_018136.4	c.2389C>T/ c.6686_6689del	p.(R797*)/ p.(R2229Tfs*10)	PF	PM, pyramidal syndrome	−6
#4	F	1 affected and terminated pregnancy	Turkey	yes	*ASPM #605481*	NM_018136.4	c.9841A>T/ c.9841A>T	p.(R3281*)	PF	PM	NA
#5	M	1 affected sister	Belgium	no	*ASPM #605481*	NM_018136.4	c.1932del/ c.8133_8136del	p.(F645Sfs*23)/ p.(K2712Lfs*16)	PF	PM	−3
#6	M	3 affected sibs	Turkey	yes	*ASPM #605481*	NM_018136.4	c.1366G>T/ c.1366G>T	p.(E456*)	PF	PM	NA
#7	M	1 affected sib	Turkey	yes	*ASPM #605481*	NM_018136.4	c.6513dup/ c.6513dup	p.(V2172Sfs*7)	PF	PM, closed fontanelles before birth	−2
#8	F		Morocco	yes	*ASPM #605481*	NM_018136.4	c.8700_8702 delinsCC/ c.8700_8702 delinsCC	p.(K2900Nfs*38)	PF	PM	−2
#9	M	1 uncle affected, 1 affected and terminated pregnancy	Turkey	yes	*ASPM #605481*	NM_018136.4	c.1631_1635del/ c.1631_1635del	p.(Y544Sfs*9)	PF	PM	NA
#10	F	1 affected brother	Morocco	yes	*ASPM #605481*	NM_018136.4	c.4195dup/ c.4195dup	p.(T1399Nfs*20)	PF	PM	−3.5
#11	F		Caucasian	no	*ASPM #605481*	NM_018136.4	c.4250_4251del/ c.5590_5591del	p.(Y1417*)/ p.(L1864Sfs*2)	PF	PM	−3.5

Weigth at birth (SD)	Length at birth (SD)	Age at last evaluation	OFC (SD)	Weigth (SD)	Heigth (SD)	Epilepsy	ID evaluation	Reference if previously reported	Reference if variant previously reported	MRI findings
−3	−3	17y	−2.5	−1	−1.5	No	SON-IQ 67 (5y6m) (SON-R)			No abnormality
−2.5	−2.5	5y2m	−5	−2.5	−3.5	Generalized, tonic-clonic, and atonic. From age 10m. Controlled with VPA and LTG.	severe ID (BSID-III 5m at age 95m; no words at age 8y)	Duerinckx et al. (2017)		ACC, enlarged lateral ventricles
+1	−2	3y	−7	median	median	No	DQ 42 (3y11m) (Brunet-Lezine revised scale)	Passemard et al., 2009 (patient #10); Duerinckx et al. (2020) (panel #17)		Simplified pattern posterior, ACC, enlarged left ventricle, focal parietal cortical dysplasia
NA	NA	9y	−10	−1.5	−0.5	No	NA	Tunca et al. (2006); Désir et al. (2006) (family A)		NA
−0.5	median	9y6m	−6.5	−1	−0.5	No	IQ 64 (5y10) (WPPSI-R)	c.1932del Létard et al. (2018) (patient #2)	c.8133_8136del Tan et al. (2014)	NA
NA	NA	20y	−8	−1.5	−1	No	IQ 30 (20y) (Terman-Merrill)	Jamieson et al. (2000); Bond et al. (2003); Duerinckx et al. (2020) (panel #15)		NA
+1	median	NA	NA	NA	NA	No	NA	Létard et al. (2018) (patient #16); Duerinckx et al. (2020) (panel #1)		NA
NA	2	15y	−3.5	+1.5	−1	Combined Generalized and Focal (first tonic-clonic sz, later non-motor focal and atonic sz). From age 14y. Refractory - daily sz and nearly all AED tried.	moderate ID (IQ evaluated around 50)	Létard et al. (2018) (patient #22)		No abnormality
NA	NA	10y	−8	−0.5	−0.5	No	moderate to severe ID	Tunca et al. (2006); Désir et al. (2006) (family B)		No abnormality
+0.5	median	14y	−5.5	−1.5	−1	Generalized, tonic-clonic. From age 3y. Never treated.	IQ 50 (6y) (Mc Carthy)	Désir et al. (2008); Duerinckx et al. (2020) (panel #12)		Simplified pattern
−0.5	−1	4y6m	−6.5	−1	−0.5	No	SON-IQ 50 (5y) (SON-R)	Létard et al. (2018) (patient #9); Duerinckx et al. (2020) (panel #16)		Simplified pattern

(Continues)

TABLE 1 (Continued)

Patient ID	Sex	Family	Ethnicity	Consanguinity	Gene #OMIM Number	RefSeq identifier	Transcript	Protein	Class	Phenotype	OFC at birth (SD)
#12	M		Portugal	same village	*ASPM #605481*	NM_018136.4	c.7782_7783del/ c.7782_7783del	p.(K2595Sfs*6)	PF	PM	−2.5
#13	M		Morocco	yes	*KNL1 #609173*	NM_170589.4	c.6123G>A/ c.6123G>A	p.(M2041I)	PF	PM, short stature	NA
#14	M	1 affected brother	Morocco	yes	*KNL1 #609173*	NM_170589.4	c.6123G>A/ c.6123G>A	p.(M2041I)	PF	PM, short stature	−2.5
#15	F		Morocco	yes	*KNL1 #609173*	NM_170589.4	c.6123G>A/ c.6123G>A	p.(M2041I)	PF	PM	−3.5
#16	M	3 affected sibs	Morocco	yes	*KNL1 #609173*	NM_170589.4	c.6123G>A/ c.6123G>A	p.(M2041I)	PF	PM, short stature	−2.5
#17	F	1 affected sister	Morocco	yes	*KNL1 #609173*	NM_170589.4	c.6123G>A/ c.6123G>A	p.(M2041I)	PF	severe PM, short stature	NA
#18	F		Morocco	yes	*CDK5RAP2 #608201*	NM_018249.5	c.1376del/ c.1376del	p.(N459Ifs*7)	PF	PM	−2
#19	F	1 affected sister	Morocco	yes	*CDK5RAP2 #608201*	NM_018249.5	c.1376del/ c.1376del	p.(N459Ifs*7)	PF	PM, *café au lait* spots	−2
#20	M	1 affected sister	Belgium	no	*CEP152 #613529*	NM_001194998.1	c.2878T>C/ c.2959C>T	p.(W960R)/ p.(R987*)	HC	PM	−2
#21	M		Caucasian	no	*CEP152 #613529*	NM_001194998.1	c.2878T>C/ c.3249del	p.(W960R)/ p.(V1084Cfs*7)	HC	PM	−3
#22	F		Turkey	yes	*ERCC8 #609412*	NM_000082.3	c.295_297delinsTG/ c.295_297delinsTG	p.(R99Cfs*26)	PF	Cockayne syndrome	−1
#23	F		Belgium	yes	*MCPH1 #607117*	NM_024596.3	c.322-1G>C/ c.322-1G>C	p.?	PF	PM	−3
#24	M		Morocco	yes	*MCPH1 #607117*	NM_024596.3	c.321dup/ c.321dup	p.(R108Tfs*2)	PF	PM	−2.5
#25	F		Turkey	yes	*MCPH1 #607117*	NM_024596.3	c.64G>A/ c.64G>A	p.(E22K)	HC	PM	−5

Weigth at birth (SD)	Length at birth (SD)	Age at last evaluation	OFC (SD)	Weigth (SD)	Heigth (SD)	Epilepsy	ID evaluation	Reference if previously reported	Reference if variant previously reported	MRI findings
−1	−1	16y	−4	−2.5	−3.5	No	NA		Létard et al. (2018)	Suspicion of dysplasia
NA	NA	19y	−7	−2	−3.5	No	moderate to severe ID	Duerinckx et al. (2020) panel #60)	Genin et al. (2012)	NA
median	−0.5	9y	−7	+1	−0.5	No	severe ID (speaks several words, cognitive developmental index 18m at age 3y10m Mc Carthy developmental scale	Genin et al. (2012) (patient #S1)		No abnormality
−1	−2	5y	−6	median	−1	No	severe ID (began to speak at 45y, at 12y not able to wash herself, recognizes some letters, count to 5)	Genin et al. (2012) (patient #Y1)		Small cerebellum hemispheres
+0.5	NA	18y	−8	−3	−2.5	No	IQ 35 (Terman-Merill)	Jamieson et al. (1999); Genin et al. (2012) (patient #E1)		Simplified pattern
NA	NA	19y	−8	−2	−3.5	Generalized, tonic-clonic. From age 24y. Controlled with LEV, TPM, and CBZ.	mild to moderate ID	Duerinckx et al. (2020) (exome #28)	Genin et al. (2012)	Slight cerebral and cerebellar atrophy, ACC, colpocephaly
median	median	4m	−4	−2	−1.5	No	borderline	Duerinckx et al. (2020) (panel #59)		Mild under–development of frontal lobe
−2	−1	12y	−5.5	−1	−1.5	No	moderate ID		Duerinckx et al. (2020)	Extreme microcephaly
median	median	22y	−6.5	−1.5	−1	No	IQ 60 (Mc Carthy)	Duerinckx et al. (2020) (exome #21)	c.2959C>T Guernsey et al. (2010)	NA
−1	−1.5	3y6m	−4	NA	+1	No	IQ 87		c.2878T>C Duerinckx et al. (2020)	No abnormality
−0.5	−0.5	11y	−6	−3	−6	Generalized, tonic-clonic. From age 22m. Controlled with VPA.	severe ID	Duerinckx et al. (2020) (exome #3)	Rump et al. (2016)	NA
median	−1	13y	−5.5	+0.5	−1	No	severe ID			Enlarged ventricles, delayed myelinization
−1	−3	15y	−9	−2.5	−4.5	No	IQ<42 (Terman-Merill)			Simplified pattern
−2	−3	6y	−6	−1	−0.5	No	IQ 63 (WPPSI-R)			Simplified pattern, multiple grey matter ectopias, cyst of the Rathke pouch

(Continues)

TABLE 1 (Continued)

Patient ID	Sex	Family	Ethnicity	Consanguinity	Gene #OMIM Number	RefSeq identifier	Transcript	Protein	Class	Phenotype	OFC at birth (SD)
#26	F		Turkey	yes	*PNKP #605610*	NM_007254.3	c.1253_1269dup/ c.1253_1269dup	p.(T424Gfs*49)	PF	PM	median
#27	F		Morocco	yes	*RTTN #610436*	NM_173630.3	c.2953A>G/ c.2953A>G	p.(R985G)	PF	PM, short stature, spastic quadriparesia	−4.5
#28	F	1 affected brother	Morocco	yes	*TRAPPC9 #611966*	NM_031466.7	c.533T>C/ c.533T>C	p.(L178P)	HC	PM, hyperkinesia	NA
#29	F	2 affected sibs	Morocco	yes	*TRMT10A #616013*	NM_152292.4	c.379C>T/ c.379C>T	p.(R127*)	HC	PM, young onset diabetes, short stature	NA
#30	M		Belgium	no	*TUBA1A #602529*	NM_006009.3	c.5G>A/ wildtype	p.(R2H)	PF	PM, axial hypotonia, visual abnormalities	−1
#31	M	1 affected brother	Belgium	yes	*VPS13B #607817*		del 8128bp (ex42-43)		HC	PM, fluctuant neutropenia, truncal obesity, retinal dystrophy, joints hyperlaxity	−2
#32	M		Caucasian	no	*WDR62 #613583*	NM_001083961.1	c.1531G>A/ c.4345C>T	p.(D511N)/ p.(Q1449*)	HC	PM	−2
#33	F		Belgium	no	*WDR62 #613583*	NM_001083961.1	c.1531G>A/ c.3469_3470del	p.(D511N)/ p.(A1157Cfs*5)	PF	PM	−2
#34	M		Turkey	yes	*WDR62 #613583*	NM_001083961.1	c.1526C>T/ c.1526C>T	p.(S509L)	HC	PM	NA
#35	M		Belgium	no	*WDR62 #613583*	NM_001083961.1	c.1043+3A>G/ c.1043+3A>G	p.?	PF	PM	+0.5

Weigth at birth (SD)	Length at birth (SD)	Age at last evaluation	OFC (SD)	Weigth (SD)	Heigth (SD)	Epilepsy	ID evaluation	Reference if previously reported	Reference if variant previously reported	MRI findings
NA	NA	4y	−7.5	NA	NA	DEE from age 3m. Refractory. Under LEV and VPA.	NA		Shen et al. (2010)	NA
−2	−3	5y	−11	−5	−5	No	DQ 30 (dvpmt age 6m at age 1y9m), no speech		Grandone et al. (2016)	Micro-lissencephaly, ACC
NA	NA	13y	−4	−1	−2	Generalized, tonic-clonic. From age 8y. Controlled with VPA.	severe ID, no speech	Duerinckx et al. (2018)		Atrophy of the corpus callosum and cerebellum, abnormal signals in the supratentorial white matter
NA	NA	26y	−4	NA	−3	Generalized, absences. From age 10y. Controlled with VPA.	ID	Igoillo-Esteve et al. (2013); Duerinckx et al. (2020) (exome #6)		No abnormality
−0.5	median	4y6m	−4	−2.5	−2.5	Generalized, tonic and atonic. From age 23m. Controlled with LTG and TPM.	severe ID	Duerinckx et al.,l., 2020 (exome #24)	Gardner et al.,l., 2018	Partial ACC, partial agenesis of the vermis, occipital white matter abnormalities, neuronal migration abnormalities
−2.5	−3	15y5m	−4	−1	−4	No	severe ID, no speech			No abnormality
−2	−0.5	26y	−5	−2	+0.5	Generalized, tonic-clonic. From age 2y. Refractory, under VPA and CMZ +gamma-globulins.	severe ID		c.1531G>A Nicholas et al. (2010)	Lissencephaly, pachygyria
−1	NA	6y	−4	+1	−0.5	No	NA	Ruaud et al. (in preparation)	c.1531G>A Nicholas et al. (2010)	Lissencephaly, pachygyria
NA	NA	13y	−5	−0.5	−1	No	moderate ID (IQ 65 in Turkey)	Ruaud et al. (in preparation)		No abnormality
NA	NA	13y	−2.5	+1.5	+1.5	Combined Generalized and Focal, tonic-clonic sz, and non-motor focal sz. From age 12y. Partially controlled with 3 AED's.	borderline			No abnormality

(Continues)

TABLE 1 (Continued)

Patient ID	Sex	Family	Ethnicity	Consanguinity	Gene #OMIM Number	RefSeq identifier	Transcript	Protein	Class	Phenotype	OFC at birth (SD)
#36	M		Belgium	no	*WDR62 #613583*	NM_001083961.1	c.1043+3A>G/ c.3383_3401del	p.?/ p.(S1128Wfs*164)	PF	severe PM, hearing deficiency	−3.5
#37	F	1 affected sister	Belgium	no	*WDR62 #613583*	NM_001083961.1	c.1521G>A/ c.2788C>T	p.(L507=)/ p.(Q930*)	PF	PM	−2
#38	F		Belgium	yes	*WDR62 #613583*	NM_001083961.1	c.3936dup/ c.3936dup	p.(V1313Rfs*18)	PF	PM	−2.5

Note: The variant descriptions follow HGVS recommendations. Variant class: PF, pathogenic found; HC, high candidate. OFC z-scores (SD, standard deviation) according to reference curves (Rollins et al., 2010). Height, length, and weight z-scores (SD) according to the World Health Organization reference curves. Sz, seizures. AED, anti-epileptic drugs: CBZ, Clobazam; CMZ, Carbamazepine; LEV, Levetiracetam; LTG, Lamotrigine; OXC, Oxcarbazepine; TPM, Topiramate; VPA, Valproate. DEE, developmental and epileptic encephalopathy. ID, intellectual deficiency. MRI, magnetic resonance imaging. ACC, agenesis of the corpus callosum.

important for the centrosomal localization of the protein (Pulvers et al., 2015). WDR62 WD40 repeat domain is important for protein–protein interactions, and almost half of *WDR62* pathogenic variants are located in the WD40 protein domains (Ruaud et al., submitted). Both *MCPH1* and *WDR62* variants were absent from the GnomAD database (*PM2 criterion*) and were predicted to be disease causing by Mutation Taster algorithm (http://www.mutationtaster.org) (*PP3 criterion*). In both *MCPH1* and *WDR62* genes, missense variants are a common mechanism of disease (*PP2 criterion*). Both families were consanguineous, and family histories were specific for a disease with a single genetic etiology (*PP4 criterion*).

3.3 | Clinical characteristics in patients with identified variants: high incidence of epilepsy

The 38 patients with an identified molecular cause of PM are reported in Table 1, along with detailed clinical information. The median age at the last evaluation was 12 years (mean: 12.0, range: 0.3–28). The OFC at birth and at last evaluation is represented in Figure 2, showing the progression of microcephaly over time in affected children in terms of SDs below the norm in all patients (mean OFC at birth, −2.5 SD; mean OFC at last evaluation, −5.5 SD; *p*-value from Student's paired *t* test <.001). Short stature, defined as a height at last evaluation lower than 2 SD below the age and sex-related mean, was present in 12 patients. Intellectual deficiency was always present, ranging from mild to severe.

Epilepsy was noted in 13 patients (34%). The type of epilepsy was generalized, with tonic-clonic seizures in most cases (11 patients), and combined generalized and focal in 2 patients. The age at onset ranged between 3 months and 24 years. Severity was highly variable, from controlled with one AED to refractory. The presence of epilepsy was not evenly distributed among the different genes. In the 10 patients with *ASPM* pathogenic variants, only 2 (20%) had epilepsy. In seven patients with *WDR62* pathogenic variants, four (57%) had epilepsy. The other patients with epilepsy had pathogenic variants in *AP4M1*, *KNL1* (1 out of 5 patients), *ERCC8*, *PNKP*, *TRAPPC9*, *TRMT10A*, and *TUBA1A*.

Brain MRI findings included disorders of neuronal migration with simplified gyral pattern, lissencephaly/pachygyria, and focal cortical dysplasia, agenesis of the corpus callosum, enlarged ventricles, delayed myelinization, white matter abnormalities, and hypoplastic cerebellum hemispheres or vermis.

3.4 | Candidate genes

In 4 of the 21 patients in whom exome sequencing did not reveal pathogenic variants in known PM genes, we identified qualifying variants in novel candidate genes. These variants

Weigth at birth (SD)	Length at birth (SD)	Age at last evaluation	OFC (SD)	Weigth (SD)	Heigth (SD)	Epilepsy	ID evaluation	Reference if previously reported	Reference if variant previously reported	MRI findings
−3	−4	28y	−6	median	−3	Generalized, tonic-clonic sz, and absences. From adolescence. Under TPM and OXC.	NA			No abnormality
median	+1	5y6	−3	+1.5	+3	Generalized, absences. From age 4y. Controlled with VPA.	moderate ID	Duerinckx et al. (2020) (panel #14); Ruaud et al. (in preparation)		Simplified pattern
median	NA	9y	−8	−2	−1.5	No	severe ID (first words at 4y, DQ 60 6y	Nicholas et al. (2010) (patient #PC12); Ruaud et al. (in preparation)		Simplified pattern, pachygyria, lissencephaly

and their main pathogenicity characteristics are listed in Table S3, and the alignments are shown in Figure S2.

A homozygous missense variant in *IGF2BP3* (OMIM #608359, NM_006547.2, c.922A>G p.(Thr308Ala)) was found in a consanguineous Iranian patient with PM and short stature, and in his affected sister. The variant that occurred in exon 8 of 15 was absent from GnomAD (Karczewski et al., 2020) and was predicted to be deleterious with a CADD score (Kircher et al., 2014) of 26.1. Threonine 308 is highly conserved among species, UCSC alignments of 100 vertebrates (https://genome.ucsc.edu/) showed the presence of a Threonine in all species at this position. *IGF2BP3* was predicted to be extremely intolerant to variation by GnomAD (z score=2.12) (Karczewski et al., 2020). Sanger sequencing confirmed homozygosity of the mutation in the two probands and heterozygosity in both parents. The Thr308 is located in the middle of the second K homology domain of the protein. K homology domains are important for RNA binding and include nucleic acid recognition motifs (Valverde et al., 2008). IGF2BP3 binds to the 5'UTR of the insulin-like growth factor 2 (IGF2) leader 3 mRNA. Mouse *imp3* was identified as an ortholog of human *IGF2BP3*. Imp3 expression level in mouse brain was the highest from E10 till E18 during the period of neuroepithelial cells proliferation. P19 cells transfected with flag-tagged imp3 failed to differentiate into neurons in response to retinoic acid and remained undifferentiated neural progenitor cells. A partial rescue was observed with igf2 (Mori et al., 2001). Furthermore, *IGF2BP3* is a stress granule-related protein that was predicted to bind to Zika Virus RNA (https://www.biorxiv.org/content/10.1101/412577v1.full.pdf). *IGF2BP3* was also shown to be upregulated in patients with a neurodevelopmental phenotype and pathogenic variants in *HNRPNR* genes (Duijkers et al., 2019). *HNRNPR* genes encode proteins involved in the spliceosome C complex. An alteration of *HNRNPR* seems to affect stress granules disassembly after exposure to oxidative stress (Duijkers et al., 2019). *IGF2BP3* pathogenic variants could thus lead to a microcephaly phenotype either by altering the timing of the switch from proliferative to neurogenic divisions or through a higher sensitivity of neural progenitors to oxidative stress and increased apoptosis.

Compound heterozygous variants in *DNAH2* (OMIM #603333) were discovered in PM probands from two different families, originating from Turkey and Russia, and in one proband of an in-house hydrocephalus cohort (NM_020877.2, c.730C>T, p.(Arg244Trp)/c.5732G>C, p.(Gly1911Ala); c.1786C>T, p.(Arg596Ter)/c.3236A>G, p.(Asp1079Gly); c.1033C>A, p.(Pro345Thr)/c.11374G>A, p.(Val3792Ile)). These six variants all had an allelic frequency lower than 0.05% in GnomAD (Lek et al., 2016), and were predicted to be deleterious with a CADD score (Kircher et al., 2014) ranging from 12.22 to 28.9. *DNAH2* encodes a heavy chain of axonal dynein. Axonal dynein heavy chains are multisubunit microtubule-dependent motor ATPase complexes providing the driving force for ciliary and flagellar motility (Chapelin et al., 1997). *DNAH2* biallelic variants were recently

TABLE 2 Novel pathogenic variants identified in our cohort

Patient ID	Gene #OMIM	Gene variant description	Transcript variant description	Predicted effect on protein	Protein effect	Ethnicity
#1	Copy Number Variant	arr 1q21.1(144757160x2,144943150-146377870x1,146418803x2)dn				Morocco
#21	*CEP152 #613529*	NG_027518.1:g.60148del	NM_001194998.1:c.3249del	NP_001181927.1:p.(Val1084CysfsTer7)	frameshift	Caucasian
#23	*MCPH1 #607117*	NG_016619.1:g.34456G>C	NM_024596.3:c.322-1G>C	NP_078872.2:p.?	splicing effect	Belgium
#24	*MCPH1 #607117*	NG_016619.1:g.29995dup	NM_024596.3:c.321dup	NP_078872.2:p.(Arg108ThrfsTer2)	frameshift	Morocco
#25	*MCPH1 #607117*	NG_016619.1:g.7729G>A	NM_024596.3:c.64G>A	NP_078872.2:p.(Glu22Lys)	missense	Turkey
#31	*VPS13B #607817*	del 8128bp (ex42-43)		p.?	deletion	Belgium
#32	*WDR62 #613583*	NG_028101.1:g.54921C>T	NM_001083961.1:c.4345C>T	NP_001077430.1:p.(Gln1449Ter)	nonsense	Caucasian
#33	*WDR62 #613583*	NG_028101.1:g.53282_53283del	NM_001083961.1:c.3469_3470del	NP_001077430.1:p.(Ala1157CysfsTer5)	frameshift	Belgium
#34	*WDR62 #613583*	NG_028101.1:g.33337C>T	NM_001083961.1:c.1526C>T	NP_001077430.1:p.(Ser509Leu)	missense	Turkey
#35, #36	*WDR62 #613583*	NG_028101.1:g.21839A>G	NM_001083961.1:c.1043+3A>G	NP_001077430.1:p.?	splicing effect	Belgium
#36	*WDR62 #613583*	NG_028101.1:g.53115_53133del	NM_001083961.1:c.3383_3401del	NP_001077430.1:p.(Ser1128TrpfsTer164)	frameshift	Belgium

Note: The variants are named according to the HGVS nomenclature recommendations, with RefSeq identifiers.

FIGURE 2 OFC progression. OFC at birth (left) and at last evaluation (right) in the PM patients with a molecular diagnosis. OFCs are represented as Z-scores (SD, standard deviation) in growth reference curves (Rollins et al., 2010). The nine patients with missing values are not represented. ***p-value from Student's paired t test <001

associated with multiple morphological abnormalities of the sperm flagella (Li et al., 2019), *dnah2* homozygous knockout mice from the International Mouse Phenotyping Consortium (https://www.mousephenotype.org) showed male infertility, and several studies in animal models and in humans have already established a link between ciliary defects and hydrocephalus (Kousi & Katsanis, 2016). However, the mouse knockdown of another axonal dynein gene, *Left–right dynein*, resulted in abnormal segregation of sister chromatids, suggesting that axonal dynein may play a role in mitotic spindles positioning for cell division (Arai et al., 2015; Armakolas & Klar, 2007), providing a mechanistic link with microcephaly.

Finally, a *de novo* variant in a novel gene was identified in one of our probands with extreme PM. We queried GeneMatcher and got connected with two additional, unrelated probands from other countries with the exact same variant, and a strikingly similar phenotype (further studies are in progress).

4 | DISCUSSION

We report on a large PM patient cohort, with a total of 169 unrelated PM patients. Our cohort is mostly non-consanguineous

(76%), with 40 PM patients (24%) being consanguineous. Most studies published so far reported only consanguineous families (Darvish et al., 2010; Gul et al., 2006; Roberts et al., 2002; Sajid Hussain et al., 2013; Shaheen et al., 2019), or were restricted to a small number of patients (Boonsawat et al., 2019; Rump et al., 2016).

Our diagnostic workflow consisted of successive steps from routine chromosome analysis to gene panel or exome sequencing, and allowed for the identification of a genetic cause in 38 patients. The diagnostic rate was much higher in consanguineous (67%) than in non-consanguineous patients (9%). This could be explained by different factors. First, consanguinity itself increases the chance for autosomal recessive, genetic disorders. The portion of non-genetic causes is thus expected to be higher in non-consanguineous, singleton cases. Furthermore, consanguineous families were larger in our sample, so there was often more than one affected child (see Table 1), further increasing the likelihood of a genetic cause, and facilitating variant filtration. Third, genetic heterogeneity could be even larger in non-consanguineous populations. The two previously published microcephaly cohorts in non-consanguineous populations, indeed, showed a higher diversity of PM genes involved. Fourth, our strategy of only sequencing the proband in most cases did not allow us to systematically identify *de novo* dominant variants in novel candidate genes. And last, there might be a higher proportion of digenic or oligogenic causes in non-consanguineous populations (Duerinckx et al., 2020).

Among the 38 patients in whom a molecular diagnosis was identified, 11 novel pathogenic variants were identified. In the overall cohort, *ASPM* was the most frequently mutated gene, followed by *WDR62*, as described in previous studies. In non-consanguineous patients, however, *WDR62* was the most prevalent gene. After *ASPM* and *WDR62*, the genes harboring most pathogenic variants in our cohort were *KNL1* and *MCPH1*. *KNL1* is over-represented in the consanguineous subgroup of our cohort as compared to other reports. This may reflect a patient recruitment bias, as all *KNL1* patients originated from the Rif region in Morocco, or better awareness of the importance of this gene, which was historically included early in our gene panel.

While our custom-capture gene panel was limited to 14 genes, it covered the most prevalent ones and identified the majority of PM patients with a coding mutation. Indeed, exome sequencing of panel-negative patients did not reveal another prevalent gene, that is, a gene found mutated in a significant subset of patients. Most of the other genes found by whole-exome sequencing were very heterogeneous and identified in a single patient.

We provide detailed clinical information about the 38 patients reported with a molecular diagnosis, including birth term, OFC, weight, and length at birth and at last evaluation, epilepsy phenotype, intellectual deficiency evaluation, and brain MRI findings. This clinical information is crucial to improve genetic counseling in families. Indeed, phenotypic comparison between patients is needed to identify the molecular causes of PM in new families and to refine the prognosis.

We show a deceleration pattern of the OFC as already observed in previous studies (Boonsawat et al., 2019; Létard et al., 2018; Nasser et al., 2020; Shaheen et al., 2019), suggesting pre- and postnatal roles for PM genes. We also observed an overlap between PM and primordial dwarfism, with several of our PM patients presenting with short stature, as already described (Shaheen et al., 2019; Verloes et al., 1993). We confirmed the presence of associated features previously reported in the literature for specific genes: short stature in patients with *KNL1* pathogenic variants (Genin et al., 2012; Saadi et al., 2016), developmental, and epileptic encephalopathy with early-onset refractory seizures in patients with *PNKP* pathogenic variants (Shen et al., 2010), short stature, and profound microcephaly with very abnormal gyration in patients with *RTTN* pathogenic variants (Cavallin et al., 2018; Grandone et al., 2016; Shamseldin, et al., 2015; Stouffs et al., 2018), agenesis of the corpus callosum and of the cerebellar vermis in patients with *TUBA1A* pathogenic variants (Gardner et al., 2018; Hebebrand et al., 2019; Romaniello et al., 2018). We previously reported that *AP4M1* pathogenic variants are associated with progressive spasticity and short stature (Duerinckx et al., 2017), and that *TRAPPC9* pathogenic variants are associated with severe intellectual deficiency and abnormalities of the corpus callosum (Duerinckx et al., 2018).

We noted that epilepsy was much more prevalent in *WDR62* patients than in *ASPM* patients. Epilepsy in *WDR62* patients is generally considered as consistent with *WDR62* mutations being often associated with brain malformations (Bhat et al., 2011). In our cohort, however, we did not observe systematic brain malformations on MRI in *WDR62* patients with epilepsy.

Among the exome patients remaining without a molecular diagnosis, we identified some candidate genes for PM, including *IGF2BP3* and *DNAH2*. Finding additional PM patients harboring variants in the same genes, and functional testing, will be required for further proof of pathogenicity.

In 16 individuals, exome sequencing did not reveal disease-causing variants. This could be explained by non-genetic causes, non-coding, for example, intronic variants, or non-Mendelian modes of inheritance. We, indeed, showed some evidence for oligogenic inheritance in PM, in particular, digenic inheritance among centrosomal genes, for example, double heterozygosity for *CEP135* and *WDR62* coding variants (Duerinckx et al., 2020).

Finally, our study shows the efficiency of a multi-steps diagnostic workflow in PM. Based on our observations and on the genetic heterogeneity of PM (Boonsawat et al., 2019; Rump et al., 2016; Shaheen et al., 2019), we suggest the

following diagnostic steps in all new patients with PM. First, microarray should be performed to exclude the presence of deleterious CNVs. Second, a gene panel (either captured or preferably exome-based) should be analyzed, focusing first on *ASPM* and *WDR62*, then on a larger neurodevelopmental gene panel, and eventually extended to the whole exome, which could lead to the discovery of novel candidate genes. If the budget is very tight, a relatively small gene panel (14 genes) will find the diagnosis in the majority of patients with a coding mutation.

In conclusion, we report a very large PM patient cohort, provide detailed clinical information on all patients with a molecular diagnosis and widen the spectrum of known pathogenic variants in PM genes. Epilepsy was a frequently associated feature. Our findings will help to better manage PM patients, accelerate molecular diagnoses, and provide more detailed information for genetic counseling.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

MA designed the study. SD analyzed the high throughput sequencing and clinical data. IP and MA supervised the whole study. JD, CB, VJ, and JS contributed to genetic and bioinformatic analyses. CP provided expert technical support. JD, IM, YT, BB, BC, WC, FGD, AD, KD, AJ, KK, DL, BL, MM, SM, GM, MCN, TS, RVC, JVDE, NVDA, HVE, OV, HV, CV, SW, SP, AV, AA, ND, and PVB recruited the patients and assessed their phenotypes. All co-authors contributed to writing the paper.

ETHICAL COMPLIANCE

All procedures complied with the ethical guidelines of Hôpital Erasme—Université Libre de Bruxelles, whose Ethics Committee approved our study under reference P2016/199 (Ethics Committee Erasme Hospital, OMO21). Informed consent was obtained from the patients' representatives.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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