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ORIGINAL ARTICLE

# Band-like calcification with simplified gyration and polymicrogyria: report of 10 new families and identification of five novel *OCLN* mutations

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**Band-like calcification with simplified gyration and polymicrogyria (BLC-PMG) is an extremely rare autosomal recessive disorder with distinctive clinical and neuroimaging findings. To date, only 17 patients from 9 unrelated families with BLC-PMG have been reported worldwide. Herein, we describe a series of 13 new patients derived from 10 unrelated Egyptian families. Patients presented at early life with the classic phenotype including severe microcephaly, failure to acquire developmental skills, growth failure and the distinguished calcification patterns involving the cortex, thalami, basal ganglia and pons. Additional features not reported before included calcification of the cerebellum (eight patients: 61.5%) and imperforate anus and undescended testis in a single patient. Molecular studies of the *OCLN* gene (NM_001205254) identified six distinct candidate mutations. Interestingly, the deletion mutation of the transmembrane domain in exons 3 and 4 (c.51-?_730-?del, p.Lys18_Glu243) was found in five unrelated families (50%), suggesting a founder mutation in our population. On the other hand, five novel truncating mutations (c.809delA (p.K270Rfs*62), c.858_861delTTAT (p.I286Mfs*45), c.1037+5G>C, c.1169C>G (p.S390*) and c.1180delG (p.E394Sfs*91)) were detected, each in one family. To our knowledge, this is the largest series of patients with BLC-PMG. Cerebellum calcification is an additional relevant finding in our series, thus expanding the neuroradiological phenotype of this syndrome.**
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## INTRODUCTION

Intracranial calcification is a heterogeneous sign in which the pattern of calcification together with the clinical phenotype promotes the diagnosis in many occasions.[1] Intracranial calcification, specifically involving the thalamus, has been seen in many syndromes.[2] It might occur because of induction of extensive microangiopathy with calcification as a result of exposure of the developing brain to a toxic level of interferon-α or because of direct endothelial disruption. The former is seen in intrauterine infection and Aicardi-Goutieres syndrome,[3–5] whereas the later results from mutations in junctional adhesion molecule 3 (*JAM3*)[6], occludin (*OCLN*)[7] or most recently the protocadherin 12 (*PCDH12*).[2]

Band-like calcification with simplified gyration and polymicrogyria (BLC-PMG, OMIM#251290) is a very rare autosomal recessive syndrome with only 17 reported patients derived from 9 unrelated families of various ethnic groups involving Turkey, Egypt, Mexico, United Kingdom, Saudi Arabia, Canada, Qatar and India.[7–10] It is a severe neurodevelopmental disorder that presents very early in life with progressive microcephaly, profound retardation, seizures, cortical visual impairment, feeding difficulties, growth arrest and spastic quadriparesis.[11–13]

The radiological pattern of BLC-PMG shows a generalized malformation of the cerebrum with very primitive sulcation simulating an hour-glass appearance to the cerebral hemispheres.[1] The gyration is abnormal with generalized polymicrogyria predominantly in the frontoparietal regions. Reduction of the white matter volume and hypoplasia of the cerebellum, brain stem and corpus callosum were reported in most of the patients. Calcifications appear usually symmetrical as a ribbon in the cortex, bilateral in the thalamus or basal ganglia, and may be central in the pons, which are considered a distinctive radiological phenotype that differentiates it from other cranial calcifications.[7–13]

Patent ductus arteriosus and cleft palate were recorded as extracranial manifestations in patients with BLC-PMG.[7] Further, chronic renal dysfunction, nephrogenic diabetes insipidus and liver calcification were described in diverse reports.[8–10]

BLC-PMG is caused by biallelic mutations in the occluding gene (*OCLN*), a tight junction protein that is expressed as an integral

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component in all epithelia and brain endothelia.[7] Previous studies revealed that tight junction proteins are essential for the cerebral blood vessel integrity early in fetal development and to maintain the blood–brain barrier during postnatal life.[7,14,15] Therefore, mutations in the *OCLN* gene disturb its vital functions in pre- and post-natal life resulting in cortical malformations.[7]

In the current study we report 13 new patients from 10 unrelated Egyptian families with BLC-PMG who exhibited additional clinical and imaging features. In addition, mutational analysis of the *OCLN* gene identified five novel mutations and a founder effect.

## MATERIALS AND METHODS

The study included 13 patients from 10 unrelated Egyptian families (nine male and four female patients). The patients were referred to Neurogenetics Clinic and Brain Malformations Clinic at National Research Centre, Cairo, Egypt, for diagnosis and genetic counseling. All patients were subjected to full medical history including prenatal, natal and postnatal histories with special emphasis on head circumference at birth, developmental history and detailed seizures nosology. Three-generation pedigree construction, complete general examination, full neurological assessment and basic anthropometric measurements (head circumference, height and weight) were conducted. Parents and available sibs were also examined. Neuroimaging analysis including both computed tomography (CT) and magnetic resonance image (MRI) was available for all patients. Other investigations as ophthalmological evaluation, electroencephalogram (EEG), visual evoked potential, brain stem auditory evoked potential, echocardiography, abdominal sonar specifically diverted to renal and hepatic assessment, blood chemistry, urine analysis, karyotyping, extended metabolic screening and lactate and pyruvate levels were also performed.

### Mutational analysis

Genomic DNA was extracted from peripheral blood leukocytes of patients, their parents and available family members, after having a signed informed consent according to the Medical Ethics Committee of the National Research Centre. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The *OCLN* gene was amplified using eight pairs of primers designed using the PRIMER 3 INPUT SOFTWARE version 0.4.0 (Eric S. Lander, Chevy Chase, MD, USA). The coding regions and exon/intron boundaries of ~50 bp sequence were investigated to identify any splice site variation as well. Primers are available upon request from the corresponding author. The PCR products were purified using the ExoSap Clean-Up Kit (Fermentas, St Leon-Rot, Germany) and directly sequenced in both directions using the Big Dye Termination Kit (Applied Biosystems, Foster City, CA, USA) and analyzed on the ABI Prism 3500 Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions. The sequence data of the *OCLN* gene were compared with the reference genomic and complementary DNA sequence of the gene (NM_001205254.1). The identified novel mutations were further screened in 100 normal control subjects of Egyptian origin using Sanger sequencing.

## RESULTS

The detailed clinical, genetic and neuroradiological data of the 13 patients with BLC-PMG are summarized in Table 1.

### Clinical data

Our patients were derived from 10 independent Egyptian consanguineous families (Figure 1). Family history of similar condition was evident in three independent pedigrees (family 6, 7 and 10), whereas three families (2, 4 and 5) had histories of offspring with other disorders as anencephaly, mental retardation and absent kidney, respectively. Patients were nine males and four females, and their ages ranged from 4 months to 4 years. At birth, the head circumferences were deviated toward microcephaly and varied from −2.5 s.d. to −3.2 s.d. (Figure 2). No milestones or fine motor skills were acquired except in three patients who achieved some head control, social smile, visual tracking and could recognize parents and family members. Progressive microcephaly was recorded in all cases (ranged from −4 s.d. to −8.5 s.d.). Seizures occurred in 100% of cases, with an onset ranging from 1 to 4 months old with variable nosology including generalized tonic clonic, myoclonic, tonic and focal and were mostly intractable. Only three patients (23%) were fairly controlled on multiple therapies. Hypotonia was noted in the neonatal period, which progressed to spasticity with variable severity. Extracranial manifestations were identified in two families; patient 2 had high imperforate anus and genital anomalies including undescended testicles and hypoplastic scrotum, whereas patient 7 had atrial septal defect. None of our patients had renal involvement documented by both laboratory tests and sonography. Nevertheless, patient 5 had a history of a deceased sib, few minutes after birth, with bilateral absent kidneys and small head. The recorded microcephaly at birth might point to the similarity to our proband with suspicion.

### Neuroradiological data

Patients were diagnosed based on the specific neuroimaging findings in CT and MRI (Figure 3). Symmetrical subcortical band-like calcification and dense thalamic calcification in brain CT (that were also evident in most of the T1w-MRI) together with abnormal gyral pattern and areas of polymicrogyria (mainly frontoparietal) were characteristic and universal in all patients. Central pontine calcification was evident in all cases, except in patients 1 and 5 whose scans were done at the age of 7 and 30 days, respectively. Calcification in lentiform nucleus was found in 12 cases and appeared faint in 5 of them. Cerebellum calcification was prominent in eight patients (61.5%) and white matter calcification was in 10 cases (77%) that appeared either scattered or in linear pattern and was clearly dense in one case. Variable degrees of dilatation of lateral ventricles and thin corpus callosum were noted in almost all cases. Reduced white matter and cortical atrophic changes were detected in five and nine patients, respectively. Additional neuroimaging findings included cerebellar vermian hypoplasia in two patients (one of them had retrocerebellar cyst) and another two cases with cavum septum pellucidum.

### Molecular findings

Sequence analysis of the entire coding region of the *OCLN* gene (NM_001205254.1) in our 10 families revealed six distinct candidate pathogenic mutations, five of which were novel (Figure 4 and Table 1). The previously reported deletion mutation of the transmembrane domain in exons 3 and 4 (c.51-?_730-?del, p.Lys18_Glu243) was found in five unrelated families (50%). Each of the remaining five families carried a truncating *OCLN* mutation. The novel mutations were three frameshift mutations (c.809delA (p.K270Rfs*62) and c.858_861delTTAT (p.I286Mfs*45) in exon 4 and c.1180delG (p.E394Sfs*91) in exon 6), one splice site (c.1037+5G>C) and one nonsense mutation (c.1169C>G, p.S390*) in exon 6.

The mutations identified were homozygous in the patients and their respective parents were heterozygous confirming segregation of the disease. It is worth noting that all novel candidate mutations except for the c.858_861delTTAT (p.I286Mfs*45) were not found in the 1000 Genomes or ExAC databases. The p.I286Mfs*45 was present in ExAC with an allele frequency of 0.00002472. Moreover, they were not detected in 200 normal chromosomes of Egyptian origin (by Sanger sequencing) and were considered to be pathogenic by MutationTaster and Alamut.

## DISCUSSION

Herein, we present the clinical and imaging features of the largest series of patients with BLC-PMG. The relatively large number of

**Table 1 The clinical, neuroradiological and mutational data of the studied cases**

| | *Family 1* | *Family 2* | *Family 3* | *Family 4* | *Family 5* | *Family 6* | | | *Family 7* | | *Family 8* | *Family 9* | *Family 10* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Findings* | *Patient 1* | *Patient 2* | *Patient 3* | *Patient 4* | *Patient 5* | *Patient 6* | *Patient 7* | *Patient 8* | *Patient 9* | *Patient 10* | *Patient 11* | *Patient 12* | *Patient 13* |
| Age at examination | 13 m | 3.5/12 y | 1 y | 11 m | 3 y | 1 6/12 y | 4 m | 1 y | 2 4/12 y | 11 m | 4 y | 1 6/12 y | 10 m |
| Sex | M | M | M | M | M | F | F | M | F | M | M | M | F |
| Consanguinity | + | + | + | + | + | + | | + | + | + | + | + | + |
| Family history | – | Anencephaly | – | Sib with Micro-cephaly, MR | Sib with micro-cephaly and absent kidneys | Sibs | | Similarly affected deceased sib | Cousins | | – | – | Similarly affected deceased sib |
| OFC at birth in cm (s.d.) | 31 (–2.6 s.d.) | 31 (–2.6 s.d.) | 30.8 (–2.8 s.d.) | 31 (–2.6 s.d.) | 30.5 (–2.8 s.d.) | 30 (–2.5 s.d.) | 30 (–2.5 s.d.) | NA | 30 (2.5 s.d.) | 29.5 (–3.2 s.d.) | 31.2 (–2.5 s.d.) | 30.5 (2.7 s.d.) | 29 (–3.1 s.d.) |
| OFC at examination in cm (s.d.) | 38 (–5. s.d.) | 40 (–7.3 s.d.) | 37 (–6 s.d.) | 37 (–6.2 s.d.) | 39 (–8 s.d.) | 38.5(–5.3 s.d.) | 34.7(–4 s.d.) | 36.5 (–6.3) | 38 (–7.2 s.d.) | 36 (6.25 s.d.) | 42 (6.8 s.d.) | 37 (7.8 s.d.) | 32 cm (–8.5 s.d.) |
| Seizures onset/types | 4 m/focal myoclonic | 2 m/tonic, myoclonic | 1 m/GT, myoclonic | 3 m/Focal, GTC, myoclonic | 2 m/Focal, myoclonic | 3 m/focal, GTC, myoclonic | 4 m/Focal, GTC, myoclonic | 2 m/GTC | 2 m/Tonic, GTC, myoclonic | 1 m/Focal, myoclonic | 2 m/focal, myoclonic | 2 m/tonic, focal, myoclonic | 1 m/tonic with eye deviation, myoclonic |
| Seizures controlled | Fairly con-trolled/two drugs | Fairly con-trolled/two drugs | Uncontrolled/ three drugs | Uncontrolled/ four drugs | Fairly con-trolled/three drugs | Uncontrolled/three drugs | Uncontrolled/three drugs | Controlled/three drugs | Uncontrolled/ three drugs | Uncontrolled/ two drugs | Controlled/two drugs | Fairly controlled/ two drugs | Controlled/four drugs |
| Feeding difficulties | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Unexplained fever | – | – | + | + | + | + | + | + | + | + | Infrequent | + | – |
| Motor milestones | – | Head support | – | – | – | – | – | – | – | – | – | Head support | Mild head support |
| Cognitive function | – | Smile, follow object | – | – | – | – | | – | – | – | – | Smile, fairly and follow object | Fairly follow object |
| Length in cm (s.d.) | 72 (–1.8 s.d.) | 86 (1.5 s.d.) | 69 (–2.7 s.d.) | 66 (–3.82 s.d.) | 82 (–3 s.d.) | 71 (–2.5 s.d.) | 60 (–0.8 s.d.) | 65 (–4.6 s.d.) | 82 (–1.5 s.d.) | 70 (–1.5 s.d.) | 95 (–1.5 s.d.) | 77 (–1.5 s.d.) | 67 (–1.6 s.d.) |
| Weight in kg (s.d.) | 7.2 (–3 s.d.) | 9.6 (–3 s.d.) | 6 (–4 s.d.) | 6.9 (–3.2 s.d.) | 9 (–3.5 s.d.) | 7 (–3.5 s.d.) | 5 (Mean) | 6.5 (–4 s.d.) | 8.8 (–3.8 s.d.) | 5 (–4.7 s.d.) | 12 (–2.4 s.d.)/ | 10.2 (–1.2 s.d.) | 6 (3.3 s.d.) |
| Hearing impairment | – | – | – | + | – | – | – | – | – | NA | Mild | – | – |
| Nystagmus/ impairment of vision | +/+ | +/– | + | +/+ | +/+ | +/– | +/– | +/+ | +/+ | +/? | +/– | +/– | +/– |
| Strabismus | – | – | + | – | + | – | – | – | + | – | – | – | – |
| Spasticity | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hyper-reflexia | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Dystonic back/limbs | –/+ | –/– | –/+ | +/+ | +/+ | +/+ | –/+ | –/+ | +/+ | +/+ | +/+ | –/+ | –/+ |
| Extracranial manifestations | – | High imperfo-rate anus, undescended testicles, hypoplasitc scrotum | – | – | – | – | ASD | – | – | – | – | – | – |
| *Neuroimaging findings* | | | | | | | | | | | | | |
| Patterns of calcifications | | | | | | | | | | | | | |
| Subcortical | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Basal ganglia | + | +Faint | +Faint | + | + | + | + | +Faint | +Faint | + | – | +Faint | + |
| Thalami | + | + | + | + | + | + | + | + | + | + | + | + | + |
| White matter | – | + | +Dense | + | – | – | + | + | + | + | + | + | + |
| Pons | – | + | + | + | – | + | + | + | + | + | + | + | + |
| Cerebellum | – | +Faint | + | + | – | + | + | + | + | – | – | – | +Faint |
| Other neuroimaging | | | | | | | | | | | | | |
| PMG | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thin CC | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Dilated lateral V | + | +Asymmetric | +Mild | + | + | + | + | +Mild | + | – | +Mild | + | + |
| WM loss | – | + | – | + | + | – | + | – | + | – | – | – | – |
| Brain atrophy | – | + | + | + | + | – | + | + | + | – | – | + | +Mild |
| Other | – | – | – | – | CVH | CVH—retrocerebel-lar cyst | – | – | – | – | – | Cavum SP | Cavum SP |
| Deceased/age (Y) | Alive | +/5 y | Alive | +/1.2/12y | +/3.3/12 | +/1.7/12 | Alive | Alive | Alive | +/1 y | Alive | Alive | Alive |
| *OCLN* mutation | **c.809delA (p.K270R*62)** | **c.1037 +5G>C (IVS5 +5G>C)** | p.Lys18_Glu243 | p.Lys18_Glu243 | **c.1180 delG (p.E394Sfs*91)** | **c.858_861delTTAT (p.I286Mfs*45)** | **c.858_861delTTAT (I286Mfs*45)** | **c.858_861delTTAT (p.I286Mfs*45)** | **c.1169C>G (p.S390*)** | **c.1169C>G (p.S390*)** | p.Lys18_Glu243 | p.Lys18_Glu243 | p.Lys18_Glu243 |

Abbreviations: ASD, atrial septal defect; CC, corpus callosum; CVH, cerebral vermian hypoplasia; GT, generalized tonic; GTC, generalized tonic–clonic; m, month; N, nucleus; NA, not available; OFC, occipitofrontal head circumference; PMG, polymicrogyria; SP, septum pellucidum; V, ventricle; WM, white matter; y, year.
Novel mutations are in bold.

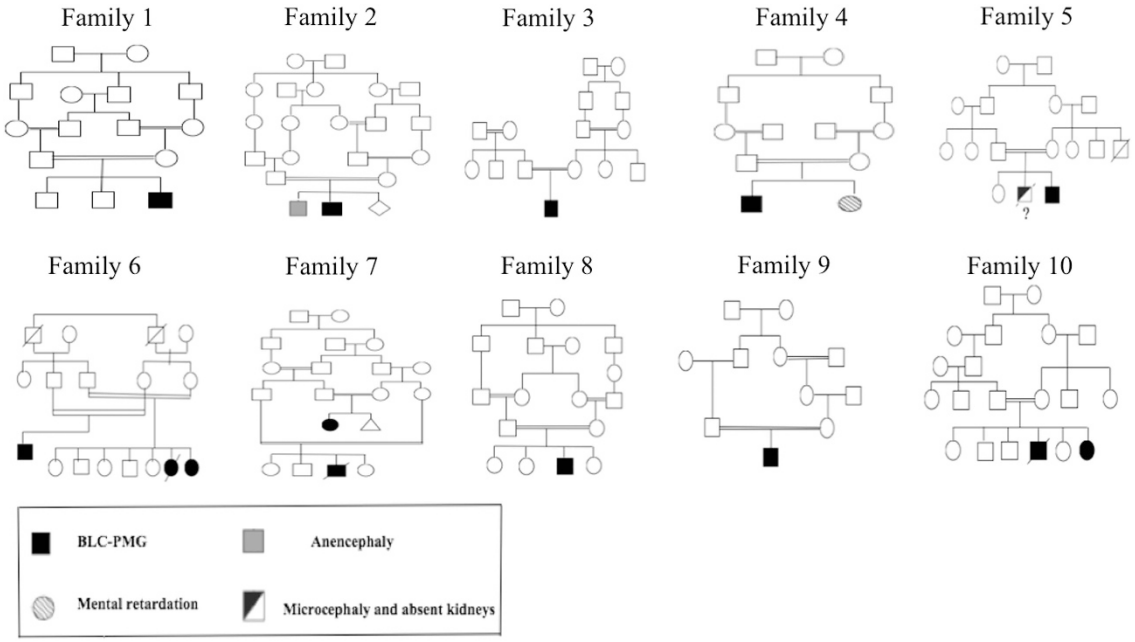


**Figure 1** Pedigrees of the 10 studied families.

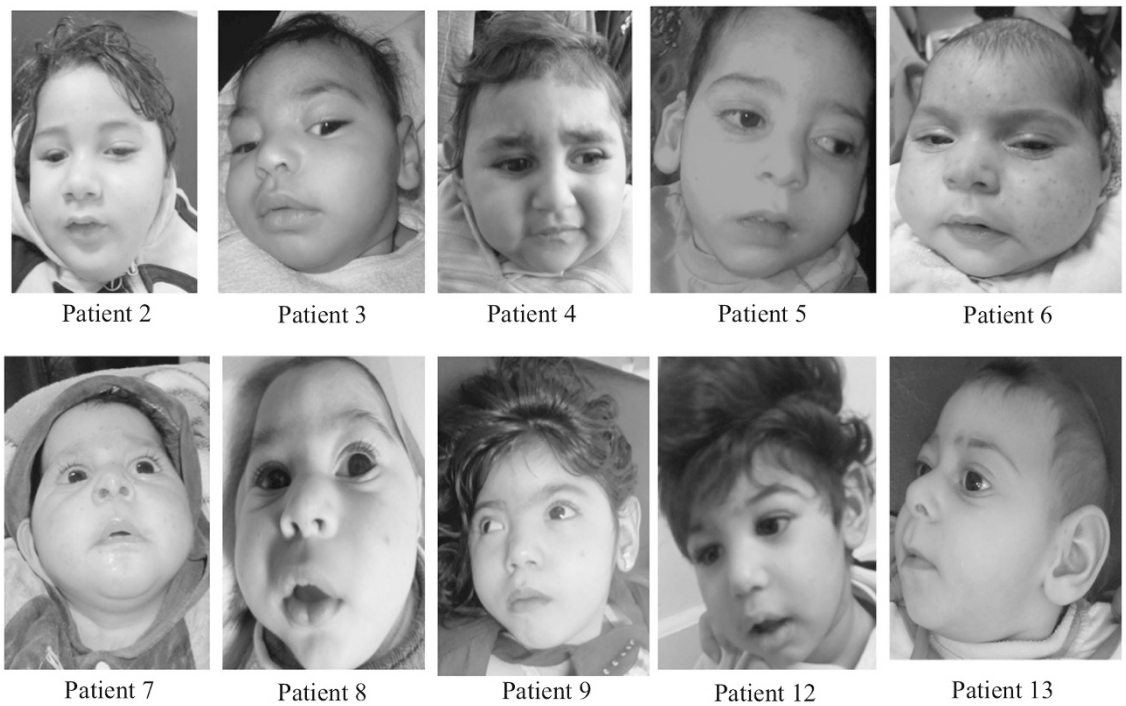


**Figure 2** Facial photos of the patients. Note the microcephaly, bitemporal hollowing, prominent ears in all patients and similarity of some facial features as prominent nose, long philtrum with prominent pillars, pointed chin and strabismus. A full color version of this figure is available at the Journal of Human Genetics journal online.

BLC-PMG families in our population (10 families from the current study and a previous one described in O'Driscoll series)[7] versus eight families from various ethnic groups could be partially explained by the high rate of consanguineous marriages reported in our population[15] as all our patients were offspring of consanguineous Egyptian parents. They all presented with progressive microcephaly, profound retardation, failure to thrive, early-onset seizures and spasticity that are considered the fundamental clinical criteria in reported cases.[7–13] Clinical data of our patients in comparison to those described in literature are presented in Table 2.

The characteristic calcification patterns including cortical band, thalami, basal ganglia and central pons were predominant in our patients. Moreover, pontine calcification was significant in all patients except in patients 1 and 5 who performed brain CT at the age of 7 and

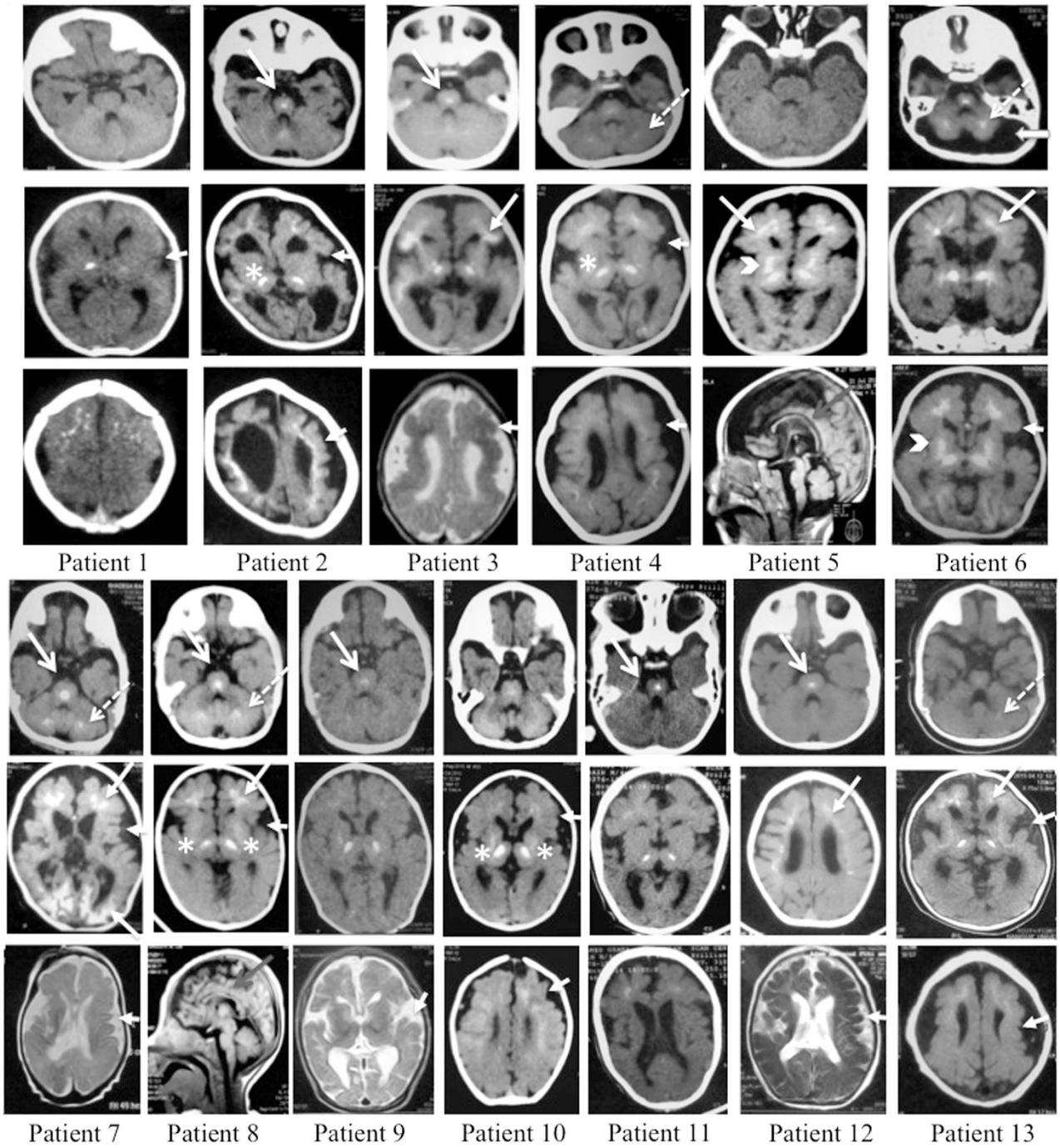


**Figure 3** Neuroimaging findings (CT and MRI) in our 13 patients. Note: abnormal gyration of the cerebrum with polymicrogyria predominantly frontoparietal and primitive sulcation simulating an hour-glass appearance to the cerebral hemispheres (short arrow). Calcifications are either subcortical band-like (closed head arrow), bilateral thalamic (asterisk), basal ganglia specifically lentiform nucleus (arrow head), pontine (open head arrow) and bilateral in cerebellum (dashed arrow). Thin corpus callosum (red arrow), retrocerebellar cyst (filled arrow) and note generally brain volume. A, axial; C, coronal; S, sagittal. A full color version of this figure is available at the Journal of Human Genetics journal online.

30 days, respectively. We believe that pontine calcification probably appears after the neonatal period, which is in accordance with Elsaid *et al.*[9] whose patient developed pontine calcification at 18 months.

Livingston *et al.*[1] mentioned that cerebellar calcification has not been seen to date in reported patients with BLC-PMG. Interestingly, cerebellum calcification was detected in eight of our patients (61.5%), which varied from mild to clearly prominent. This expands the radiological features of this disorder and points to the importance of enumerating cerebellar calcification as an additional site of calcification in this syndrome. In general, we think that follow-up brain CT scanning could be of help in characterizing the extent of calcification and its progression and in better understanding of the pathogenic sites in this disorder.

Of note, polymicrogyria appeared to be generalized in most of the cases with predominance of the frontoparietal regions and notable abnormal brain sulcation. Thin corpus callosum, white matter loss and

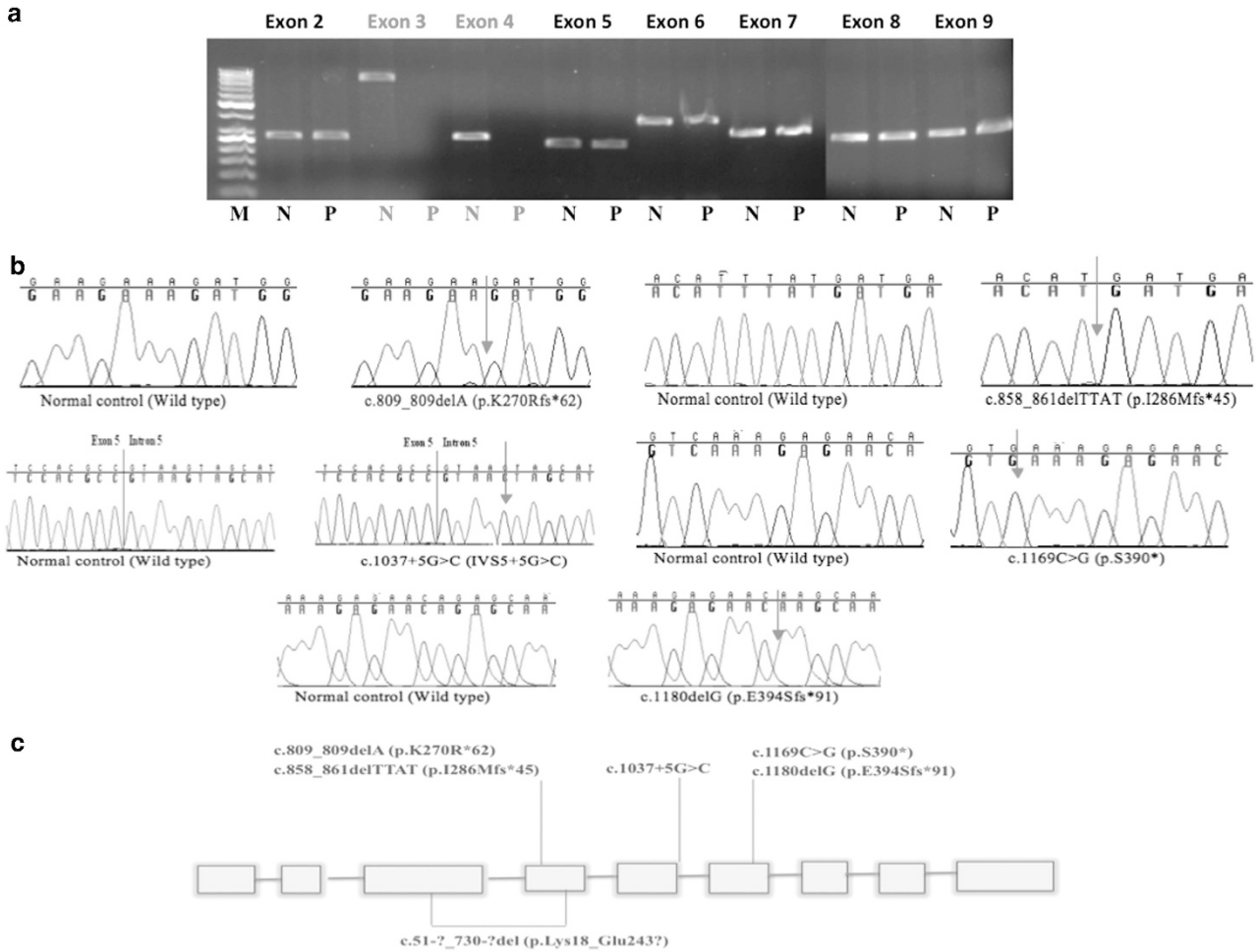


**Figure 4** (**a**) Agarose gel (2%) illustrating the detection of the deletion mutation of exons 3 and 4 of the *OCLN* gene in our study, c.51_730del (p.Lys18_Glu243). N, amplification of the eight coding exons in a normal control sample. P, amplification of the eight coding exons in one of the patients with this deletion mutation. M, 50 bp DNA Ladder (Fermentas). (**b**) Portions of the sequencing electropherograms showing the five novel *OCLN* mutations identified in our studied cases. The arrow indicates the site of mutation. (**c**) Schematic diagram of the *OCLN* gene showing all mutations identified in the current study and their location. A full color version of this figure is available at the Journal of Human Genetics journal online.

**Table 2 Comparison between the clinical features of our patients and all BLC-PMG patients reported in the literature**

| *Findings* | *This study* N = *13* | *Published*[7–12] N = *17* | *Total* N = *30* |
|---|---|---|---|
| Sex | 9M/4F | 7M/10F | 16M/14F |
| Consanguinity | 13/13 | 14/17 | 27/30 |
| Microcephaly at birth | 13/13 | 12/15 | 25/28 |
| Microcephaly on presentation | 13/13 | 17/17 | 30/30 |
| Seizures | 13/13 | 17/17 | 30/30 |
| Feeding difficulties | 13/13 | 9/13 | 22/26 |
| Growth retardation | 12/13 | 6/11 | 18/24 |
| No acquired motor or cognitive skills | 10/13 | 15/17 | 25/30 |
| Hearing impairment | 2/12 | 4/6 | 6/18 |
| Impaired vision | 5/11 | 12/14 | 17/25 |
| Spasticity/hyper-reflexia | 13/13 | 14/15 | 27/28 |
| Cerebellar calcifications | 8/13 | 0/17 | 8/30 |
| Other system involvement | 2/13 CHD (1), imperforate anus (1) | 4/11 CHD (2), liver calcif (1), hernia (1) | 6/24 |
| Renal involvement | 0/13 | 8/17 | 8/30 |

Abbreviations: BLC-PMG, band-like calcification with simplified gyration and polymicrogyria; CHD, congenital hear disease; F, female; M, male.

dysplastic ventricles were striking in our patients and were in accordance with previous reports.[7–13] In addition, progressive reduction in cerebrum volume was clear in patients subjected to successive scans confirming the observation of LeBlanc *et al.*[8] and Elsaid *et al.*[9] in their patients. It is worthy to say that there were no clinicoradiological correlations detected among our patients as some were able to acquire few skills and had stabilization of seizures activity without remarkable difference in their neuroimaging findings.

The *Ocln* knockout mouse has chronic gastritis, thinning of compact bone, abnormal salivary striatal duct and atrophy of the seminiferous ducts of the testes with sparing of the Sertoli cells.[16] Although none of the previously reported patients showed any of these manifestations, patient 2 had high imperforate anus and undescended testicles. We think that these unreported extracranial anomalies could be associated with this syndrome.

So far, eight distinct *OCLN* mutations have been described in the literature. All are protein-truncating but one is missense mutation (Supplementary Table S1). Mutational analysis of the *OCLN* gene in our cohort of BLC-PMG patients revealed six different candidate pathogenic mutations distributed across the *OCLN* gene. The deletion of the transmembrane domain spanning exons 3 and 4 (p.Lys18_Glu243) was found in five unrelated families (50%). This mutation

was previously reported in an Egyptian family as well as in two other families from Turkey and Mexico.[7] Interestingly, all patients from the five families described here were also homozygous for a rare missense variant in exon 9 (c.1512G>C, p.K504N) that has an allele frequency of <0.0001, suggesting that they might share a similar haplotype.

Therefore, we think that the p.Lys18_Glu243 mutation is a founder mutation in our population being described in six unrelated families from different governorates of Egypt. In view of this possible founder effect, the carrier rate of this common deletion mutation of exons 3 and 4 might be high and this could be behind this large number of patients with this mutation. However, future study detecting the carrier status of this mutation is warranted to confirm this assumption.

In the current study, we also identified three novel small deletion mutations, c.809delA and c.858_861delTTAT in exon 4 and c.1180delG in exon 6. In addition to a novel nonsense mutation detected in exon 6 (p.S390*) in the two cousins of family 7. These four mutations are protein-truncating and are predicted to result in nonsense-mediated mRNA decay by MutationTaster. Moreover, a novel homozygous mutation in the donor splice site of exon 5 (c.1037+5G>C) was detected in Patient 2. This splice site mutation is predicted to result in a splicing error leading to exon-skipping. Interestingly, another mutation (c.1037+5G>A) in the same position was reported before in a Saudi patient.[7]

Renal problem as dysplasia due to calcification or impairment was reported in the literature three times in patients with BLC-PMG.[8–10] In 2011, LeBlanc[8] reported a deletion/rearrangement mutation in exon 9 of the *OCLN* gene in two patients with BLC and renal dysplasia. Authors suggested that the loss of peripheral *OCLN* in their patients due to the interruption of exon 9 might explain the unusual renal involvement. However, subsequent reports have showed that renal involvement could be considered a consistent feature of BLC-PMG and is not specifically related to particular mutations.[9,10] Interestingly, renal involvement was not a manifestation in any of our 13 patients at the time of examination. Nevertheless, one of our patients (patient 5) had a deceased newborn with renal aplasia and microcephaly at birth. Unfortunately, we could not confirm the possibility of similarity to his sib due to the absent detailed clinical and radiological information or DNA for genetic testing but this might give another evidence that renal anomalies should be considered among the extracranial manifestations of BLC-PMG.

It is intriguing to find another genetic disorder in families 2 and 4, anencephaly and microcephaly and mental retardation, respectively. We could not explain this frequency of associated other developmental disorders. This could be referred to the increased incidence of genetic diseases among highly consanguineous populations as Egypt.[17]

In conclusion, our patients were found to exhibit additional features including cerebellar calcifications, imperforate anus and undescended testis. In addition, our data support that pontine calcification could develop with age. Further, we identified five novel mutations, thus raising the number of *OCLN* mutations identified so far to 13 disease-causing mutations. Thus, our findings expand the *OCLN* mutational spectrum and the neuroradiological phenotype of BLC-PMG.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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