Phenotypic overlap between McKusick-Kaufman and Bardet-Biedl syndromes in two Egyptian families

Hisham Megahed[a], Samira Ismail[a], Mona S. Aglan[a], Samia A. Temtamy[a], Ahmed L Abulnasr[b], Engy A. Ashaat[a], Ghada A. Otaify[a], Rasha Elhossini[a], Sherif F. Abdel-Ghafar[c], Mohamed S. Abdel-Hamid[c]

Departments of [a]Clinical Genetics and [c]Medical Molecular Genetics, Center of Excellence of Human Genetics, Human Genetics and Genome Research Division, National Research Centre, [b]Department of Obstetrics and Gynecology, Faculty of Medicine, Cairo University, Cairo, Egypt

Correspondence to Mohamed S. Abdel-Hamid, Ph.D, Department of Medical Molecular Genetics, Human Genetics and Genome Research Division, National Research Centre, Tahrir Street, Dokki, Cairo, 12622, Egypt
Fax: +00 202 376 01877
e-mail: mohamadnrc@hotmail.com

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Background
McKusick-Kaufm an syndrome (MKKS) and Bardet-Biedl syndrome (BBS) are two conditions that can initially overlap in their clinical features.

Patients and methods
With time, additional features such as retinitis pigmentosa, obesity, learning disabilities, and progressive renal dysfunction can develop in both syndromes. Thus, clear phenotypic differentiation between BBS and MKKS is not feasible. Herein, we describe patients from two unrelated families who presented with clinical features suggestive of both MKKS and BBS. Molecular studies of the *MKKS* gene revealed a known pathogenic variant in exon 3, c.295T>C (p.C99R) in family 1. On the contrary, a novel likely pathogenic variant in exon 6 (c.1519G>T, p.E507*) was identified in family 2.

Results
Our results add more evidence that MKKS and BBS are genetically heterogeneous disorders with mutations resulting in a wide spectrum of phenotypically overlapping features.

Conclusion
We suggest that both MKKS and BBS be included as one entity in the ciliopathies group.

Keywords:
Bardet-Biedl syndrome, Egyptian families, McKusick-Kaufman syndrome, McKusick-Kaufman syndrome gene, phenotypic overlap

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Introduction

McKusick *et al.* (1964) described the McKusick-Kaufman syndrome (MKKS, OMIM# 236700) in two Amish sibships. This syndrome is characterized by hydrometrocolpos and polydactyly. In females, hydrometrocolpos is detected in 80–95% of cases, which may be owing to either vaginal atresia or imperforate hymen (David *et al.*, 1999). It is usually associated with urogenital sinus and gastrointestinal malformations. On the contrary, male patients usually have hypospadias, cryptorchidism, and prominent scrotal raphe (Slavotinek and Biesecker, 2000). Postaxial, less-common mesoaxial polydactyly or syndactyly is commonly found in 90% of MKKS cases. In addition, congenital heart disease in the form of VSD, AV canal defects, and hypoplastic left ventricle could be detected in 10–20% of MKKS cases (Robinow and Shaw, 1979).

Bardet-Biedl syndrome (BBS, OMIM# 209900) is a known combination of hypogenitalism, obesity, postaxial polydactyly, renal dysplasia, retinal degeneration, and mental impairment (Beales *et al.*, 1999). Classically, polydactyly is postaxial (63–81%) in patients with BBS, which may be associated with syndactyly and less commonly brachydactyly. Genital anomalies include vaginal atresia, hypoplastic uterus, and fallopian tubes in women (Green *et al.*, 1989). Anal atresia and Hirschsprung disease may also be present. Renal anomalies are present in 90% of cases but usually remain undiagnosed. Mental subnormality is present in 50% of cases, and also endocrine anomalies including obesity (90%) and diabetes mellitus (50%) may be present. Visual impairment in the form of retinitis pigmentosa with macular degeneration is pathognomonic of BBS but is usually delayed till the second or third decade of life.

Both syndromes are inherited in an autosomal recessive manner, although rare families with BBS showed evidence of a more complex inheritance (Katsanis *et al.*, 2001; Badano and Katsanis, 2002). Phenotypic overlap between these two syndromes has been observed. Therefore, patients presenting with MKKS may further develop retinitis pigmentosa and obesity and be reclassified as BBS. Moreover, hydrometrocolpos

the main distinguishing feature of MKKS has been reported in some patients with BBS (Stoetzel *et al.*, 2006; Billingsley *et al.*, 2010; Dulfer *et al.*, 2010).

In 2000, the *MKKS* gene was identified in one Amish family and another non-Amish patient with clinical picture of MKKS (Stone *et al.*, 2000). BBS is caused by defects in several genes encoding proteins that control the primary ciliary formation and function. So far, mutations in 20 genes have been involved in BBS (Forsythe *et al.*, 2018). Mutations in one particular BBS chaperonin gene (*MKKS/BBS6*) seem to give rise to several phenotypically variable, yet similar syndromes (Katsanis *et al.*, 2000).

Herein, we report two unrelated Egyptian families; their affected members presented with overlapping features of MKKS and BBS syndromes. Mutations in *MKKS* gene were identified in both families, confirming the wide phenotypic variability associated with *MKKS* mutations and the marked phenotypic overlap between the two syndromes.

Patients and methods

Family 1

An Egyptian female patient (patient 1), 16 years old, presented to the Limb Malformations and Skeletal Dysplasia Clinic (LMSDC), at the National Research Centre (NRC), having obesity, limb anomalies, and hydrometrocolpos. She was born to first-cousin consanguineous parents, and there were no other similarly affected family members (Fig. 1). The pregnancy and delivery histories were unremarkable, but she was of large birth weight.

On examination, she had some dysmorphic features in the form of broad forehead, prominent supraorbital ridges, deep seated eyes, upturned nasal tip with full alae nasi, short philtrum, microstomia, retrognathia, and chubby cheeks (Fig. 2a and b). Orodental examination revealed a high-arched palate, prominent median palatine raphe, and deep bite with barrel-shaped malposed teeth. Anthropometric measurements were recorded as follows: height was 150 cm (−2.0 SD), weight was 75 kg (+1.95 SD), and her head circumference was on the mean for her age. She had mild mental retardation with learning disability, with an IQ of 54. Skeletal and limb examinations revealed postaxial polydactyly in both hands, an extra digit on the right side, and a small pedicle on the left hand with tapered fingers and crooked distal-phalanges. Her feet showed bilateral postaxial polydactyly with partial syndactyly between the second and third toes (Fig. 2c and d).

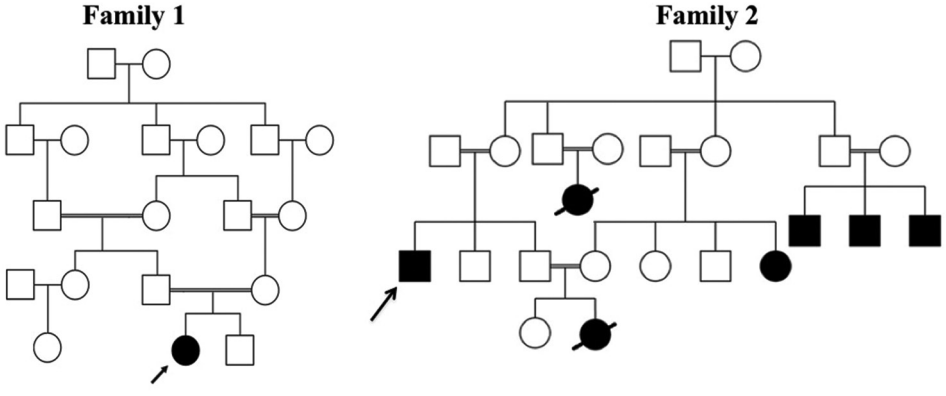
Genital examination showed single opening, and pubertal assessment showed well-developed breast (B3–4) with absent axillary and pubic hairs (A1 and P1, respectively). She had negative menarche at the time of assessment, which was explained later by pelvic ultrasound which showed normal ovaries and uterus with organized blood in the vagina (hematocolpos) owing to low vaginal obstruction.

Heart examination by echocardiography revealed trivial mitral regurge and tricuspid regurge. Abdominal ultrasound was normal apart from calculi gall bladder and gaseous distention. Eye examination by electroretinogram (ERG) and fundus examination revealed bilateral retinal dystrophy in the form of retinitis pigmentosa more on the right side.

Family 2

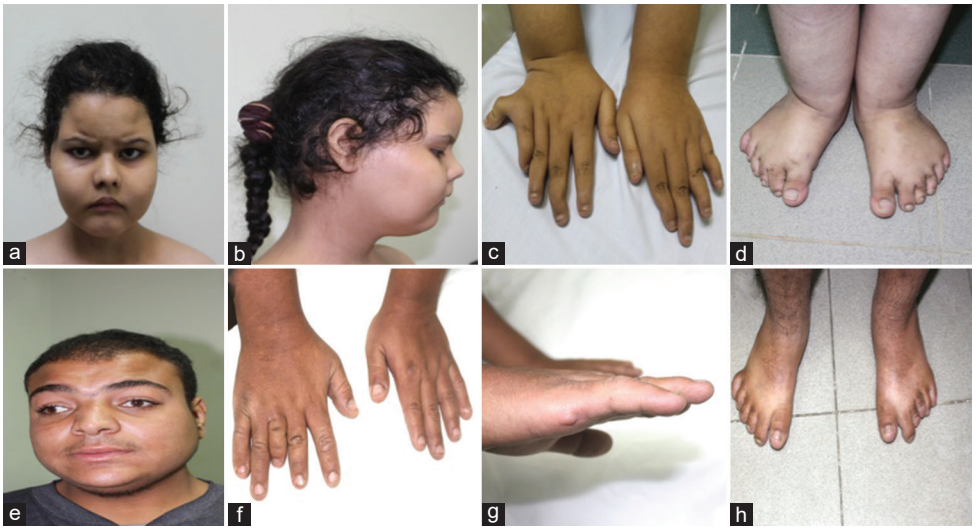
A first-cousin consanguineous couple presented to the outpatient clinic at the Clinical Genetics

Figure 1



Pedigrees of the two families.

Figure 2



(a and b) Face and profile of patient 1 showing deep set eyes, frontal bossing, short philtrum, and bow-shaped upper lip and retrognathia. (c) Hands showing postaxial polydactyly of right hand with swan-shaped appearance of fingers and hypoplastic nails. (d) Feet showing bilateral postaxial polydactyly, clinodactyly of second, third, and fourth toes of right foot. (e) Face of patient 2 showing apparent divergent squint, low anterior hair line, mild synophrys, and pear-shaped nose. (f) Postaxial minimus on the right hand. (g) Both hands showing polydactyly, probably mesoaxial, of the right hand with very short fourth digit and hypoplastic nails. (h) Both feet showing postaxial polydactyly of right foot and probable meso-axial polydactyly of left foot with brachydactyly of toes 2–6.

Department, NRC, with history of a previous child death; she was a female born at 28 weeks of gestation with birth weight of 1.7 kg. She had bilateral postaxial polydactyly, vaginal atresia, and urethral stenosis that was operated upon at 4 months of age but was complicated by intestinal adhesions and obstruction, leading to gangrenous loop. Vaginoplasty was done at 1 year of age, meanwhile, the patient did not acquire any motor or mental milestone skills, and died at 18 months old because of septicemia. Detailed analysis of pedigree revealed that there are multiple family members having postaxial polydactyly, obesity, diminution of vision, and mental subnormality, among them was a similarly affected female who died at 6 years owing to renal failure (Fig. 1).

One of the affected family members (paternal brother, patient 2) was called for examination. He was a 24-year-old offspring of a first-cousin couple. On general examination, he had high forehead, malar hypoplasia, apparent squint (Fig. 2e), and left accessory nipple. Examination of his hands showed postaxial minimus and probably mesoaxial polydactyly of the right hand with very short fourth digit and hypoplastic nails (Fig. 2f and g). Both feet showed postaxial polydactyly of right foot and probable mesoaxial polydactyly of left foot with brachydactyly of toes 2–6 (Fig. 2h).

He had neonatal history of bilateral undescended testes that were seen by pelvic ultrasound in the inguinal canal and were descended later. At the time of examination, he had lipomastia, normal axillary, and pubic hair, and his penile length was 8 cm (5th percentile). Orodental examination revealed high-arched palate with mal-posed teeth. His height was 160 cm (−2.2 SD), his weight was 88 kg (+2.2 SD), and the head circumference was on the mean for his age. He had poor scholastic achievements and learning disabilities, with an IQ of 65; however, his brain MRI result was normal. Echocardiography finding was normal. Fasting blood glucose was 75 mg/dl, and thyroid profile was normal. ERG and fundus examination revealed bilateral retinal dystrophy in the form of retinitis pigmentosa in both eyes.

Methods

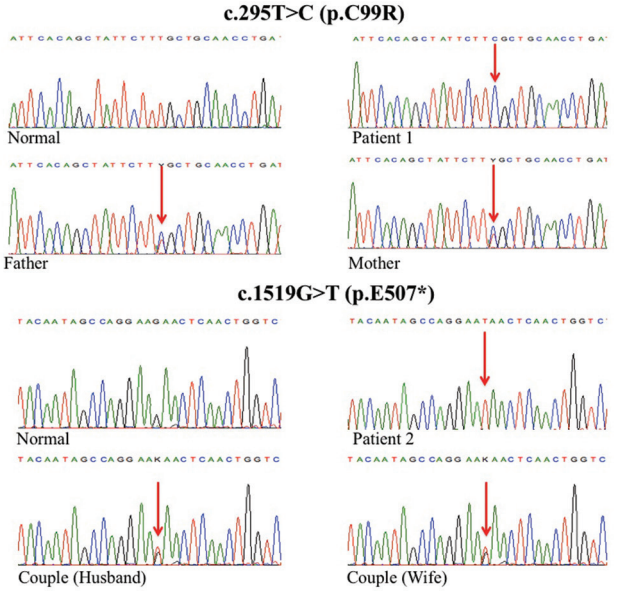
Genetic testing was performed for the patient and parents of family 1 and for the couple and affected paternal brother of family 2. Genomic DNA was extracted from peripheral blood lymphocytes after having a signed informed consent according to the guidelines of the Medical Research Ethics Committee of the NRC. DNA was extracted using Qiagen Blood DNA Kit (Qiagen, Germany). The entire coding region of the *MKKS* gene (exons 3–6) was amplified using specific primers designed by Primer3 SOFTWARE. The coding region and exon/intron boundaries of ~50 bp sequence were investigated to identify any splice site variants as well. Primers are available upon request from the corresponding author. Our standard PCR cycling conditions were initial denaturation at 96°C for 5 min; 30 cycles of denaturation at 96°C for 30 s; annealing at 62°C for 30 s; extension at 72°C for 30 min; and a final extension at 72°C for 5 min.

PCR products were purified using Exo-SAP PCR Clean-up kit (Fermentas, Braunschweig, Germany) and sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) and analyzed on the ABI Prism 3500 Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions. The sequence data of *MKKS* gene were compared with the reference genomic and cDNA sequence of the gene (NM_018848.3). The variants identified were inspected in dbSNP141, gnomAD, Exome Variant Server, and 1000 Genomes databases. Furthermore, the effect of mutations was predicted using MutationTaster, PolyPhen2, and SIFT software.

Results

Mutational analysis of the *MKKS* gene identified one pathogenic and one likely pathogenic variants in the two families (Fig 3). The patient of family 1 had a known missense variant in exon 3 of the gene, c.295T>C (p.C99R). On the contrary, a new nonsense variant in exon 6 (c. 1519G > T, p.E507*) was identified in the examined affected member of family 2, and the counseled couple proved to be carriers. Mutations segregated perfectly with the phenotype in the two families as were found in the homozygous form in patients and in the heterozygous state in their respective parents. The novel nonsense variant was not reported before in the dbSNP, 1000G, and gnomAD and was predicted to be disease causing by various bioinformatics tools.

Figure 3



Portion of the sequencing electropherograms showing the two *MKKS* variants identified in our patients. The arrow indicates the site of mutation. MKKS, McKusick-Kaufman syndrome.

Discussion

The association of hydrometrocolpos and polydactyly may cause diagnostic and prognostic dilemma between MKKS and BBS prenatally and during early infancy. Beyond infancy, the clinical progression can differentiate between the two conditions. Fath *et al.* (2005) claimed that patients with MKKS remain free, whereas patients with BBS develop late-onset complications. The same authors generated a knock-out mouse MKKS (-/-) that did not demonstrate neither genital anomalies nor polydactyly; however, other features coinciding with BBS in the form of retinitis pigmentosa and obesity were present. They explained such observation that the phenotype of the MKKS (-/-) mice closely resembles the phenotype of other mouse models of BBS Bbs2 (-/-) and Bbs4 (-/-). They also suggested that the complete absence of MKKS leads to BBS, whereas the MKS phenotype is likely to be owing to specific mutations.

The mean age for BBS clinical diagnosis is 9 years; however, it can be suspected earlier at 2–3 years, as obesity develops at younger age, and ERG changes often occur before 5 years of age. Accordingly, it is also recommended to follow-up patients diagnosed with MKKS during their first 5 years of life (David *et al.*, 1999; Schaefer *et al.*, 2011).

Despite the genetic heterogeneity of BBS and the rapidly growing number of causative genes, MKKS is only caused by mutations in the *MKKS* gene. So far, only three mutations have been described in patients with MKKS. Of them, a missense mutation c.250C>T (p.H84Y) was reported in the Old Order Amish family (Stone *et al.*, 2000). This mutation was found in cis with another missense variant (c.724G>T, p.A242S), and the frequency of the allele carrying the two variants was estimated to be around 2% in the Amish population, although it is extremely rare in other populations. In addition, two compound heterozygous mutations (c.1225_1226delGG, G409Rfs*5, and c.110A>G, p.Y37C) have been described in a non-Amish family with MKKS. On the contrary, *MKKS* gene mutations have been reported in many families with BBS from various ethnic groups. Overall, mutations in *MKKS* gene account for only 4–11% of patients with BBS. Surprisingly, *MKKS* mutations have been also described in two sibs from an Indian family with retinitis pigmentosa and polydactyly without other findings of MKKS or BBS (Hulleman *et al.*, 2016). Such data have broadened the phenotypic heterogeneity associated with *MKKS* gene.

To date, ~30 different *MKKS* gene mutations have been reported in the literature in patients with MKKS and BBS (Katsanis *et al.*, 2000; Beales *et al.*, 2001;

Slavotinek *et al.*, 2002; Chetta *et al.*, 2011; Schaefer *et al.*, 2011; Hulleman *et al.*, 2016; Qi *et al.*, 2017). Majority of these mutations are missense and are distributed across the coding exons of the gene with a notable clustering in exon 3. The clustering of mutations in exon 3 is mainly owing to its large size rather than being a hot-spot exon of mutations. In view of all reported mutations, there were no common or recurrent mutations, except for c.110A>G (p.Y37C), which was reported in three families (Katsanis *et al.*, 2000; Stone *et al.*, 2000; Schaefer *et al.*, 2011), and c. 280_281delTT (p.F94Hfs*10) and c. 429_430delCT (p.F144*), which were described in two families each (Katsanis *et al.*, 2000; Slavotinek and Biesecker, 2000).

In the BBS syndrome, chaperonin complex consist of three chaperonin-like BBS proteins (MMKS-BBS6, BBS10, and BBS12), suggesting that this phenotype may be 'chaperonin-like specific' (Billingsley *et al.*, 2010). Mutations in *MMKS/BBS6* have also been identified in patients with MKKS. These syndromes are generally less severe than BBS. However, mutations in one particular BBS chaperonin gene *MKKS/BBS6* seem to give rise to several phenotypically variable syndromes. Thus, it is obvious that mutations in genes encoding for BBS proteins do not always cause BBS but may cause syndromes that phenotypically overlap with BBS (Schaefer *et al.*, 2011).

By molecular analysis, we identified two different mutations in the *MKKS* gene. The patient of family 1 had a homozygous missense mutation in exon 3, c.295T>C (p.C99R). This mutation affects a highly conserved amino acid residue in the equatorial domain of the protein and thus predicted to disrupt protein function. Interestingly, this mutation has been described before in a Portuguese patient with BBS (Deveault *et al.*, 2011). Furthermore, we identified a new nonsense mutation (c. 1519G > T, p. E507*) in family 2. This mutation is expected to result in early protein truncation at position 507, and the mutant protein will lack 63 highly conserved amino acids residues at the C-terminal.

Conclusion

In conclusion, BBS and MKKS are two conditions that can initially overlap in their clinical features. With time, additional features such as retinitis pigmentosa, obesity, learning disabilities, and progressive renal dysfunction can develop. Thus, clear phenotypic differentiation between BBS and MKKS is difficult. The two new families presented in this report add more evidence that MKKS and BBS are genetically heterogeneous disorders, with mutations resulting in a wide spectrum of phenotypic features. As the 'two' syndromes are primary ciliary disorders and that it is difficult on both clinical and molecular bases to differentiate between them, we suggest that patients with this phenotype be considered as one entity, the BBS/MKKS spectrum.

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Conflicts of interest
There are no conflicts of interest.

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