common renal histopathology. Unlike most other studies, our aggressive immunosuppression appeared to minimize the different prognoses between LN classes II and IV. Male gender was the only independent risk factor of mortality.

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References

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Medullary sponge kidney associated with primary distal renal tubular acidosis and mutations of the H+-ATPase genes

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Abstract

Background. Medullary sponge kidney (MSK) is a rare congenital disease characterized by diffuse ectasia or dilation of precalyceal collecting tubules. Although its pathogenesis is unknown, the association with various congenital diseases suggests that it could be a developmental disorder. In addition to the typical clinical features of nephrocalcinosis and urolithiasis, patients with MSK show tubular

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Medullary sponge kidney (MSK) is a condition characterized by diffuse ectasia or dilation of precalyceal collecting tubules, with an estimated prevalence in the general population between 5/10 000 and 5/100 000. The clinical phenotype includes hypercalciuria, hypocitraturia, nephrocalcinosis and urolithiasis, tubular function defects of acidification and concentration, and a moderately increased risk of urinary tract infections. Exceptionally, chronic renal failure has also been reported [1]. Although it is usually a sporadic condition, familial cases with an autosomal dominant mode of transmission have also been described [2]. Different hypotheses about MSK pathogenesis have been considered. These include abnormal congenital development of renal tubules with secondary cystic dilations, collecting duct dilation secondary to obstruction by calcium salt and renal manifestation of a systemic connective tissue disorder or of primary hyperparathyroidism [1].

The hypothesis of MSK as a developmental disorder has been derived from several reports explaining its association with some malformative conditions, such as Beckwith–Wiedemann syndrome/hemihypertrophy, unilateral renal agenesis, unilateral or bilateral renal hypoplasia, and horseshoe kidney [1].

Distal renal tubular acidosis (dRTA) is a clinical condition characterized by a renal tubular defect causing abnormal H+ urinary excretion and failure to maintain a normal plasma HCO3− concentration [3]. Both primary and secondary forms are recognized: the former are inherited as Mendelian diseases, while the latter are secondary to a number of renal acquired diseases [4]. Both autosomal dominant and recessive forms of primary dRTA have been described. The autosomal dominant form is usually caused by mutations in the SLC4A1 gene encoding the basolateral Cl−/HCO3− exchanger. Autosomal recessive forms are associated with mutations of the ATP6V1B1 and ATP6V0A4 genes in patients with early or absent/late sensorineural hearing loss, respectively [5,6]. ATP6V1B1 and ATP6V0A4 encode for the B1 and a4 subunits of the apical H+-ATPase pump, respectively. The main symptoms of the disease in childhood are vomiting, failure to thrive and life-threatening acidosis. In older untreated patients, common clinical manifestations are nephrocalcinosis, recurrent renal calculi and osteomalacic bone disease [3].

Metabolic acidosis secondary to distal tubular defect in H+ excretion is frequently observed in patients with MSK. This is thought to arise as a consequence of distal tubular dysfunction associated with cystic changes of medullary collecting ducts and with nephrocalcinosis [2].

We describe here two patients with an as yet unreported association between MSK and primary dRTA with late sensorineural hearing loss confirmed by genetic analyses of ATP6V1B1 and ATP6V0A4 genes.

Case reports

Case 1

A 27-year-old man was referred to the Nephrology Department of the Ospedali Riuniti of Bergamo for haematuria and recurrent bilateral renal calculi. Past history revealed short stature and failure to thrive over the first years of life. At the age of 3 years, physical examination showed growth retardation with both weight and height below the third percentile, and rickets. At that time he had a bone age of 1.5 years. Laboratory investigations showed hyperchloaemic metabolic acidosis, hypokalaemia, polyuria with hypostenuria, high urine pH, hypercalciuria and nephrocalcinosis; a diagnosis of dRTA was established. However, when he was 5 years old, intravenous urography showed mild nephromegaly with bilateral brush-like appearance designating precalyceal dilated collecting tubules, in accordance with a diagnosis of MSK (Figure 1). During follow-up, despite potassium and magnesium citrate treatment, he underwent frequent episodes of ureteral and vesical nephrolithiasis requiring...
extracorporeal shock wave lithotripsy (ESWL). From the age of 20 years, he developed progressive bilateral sensorineural hearing loss. Molecular analysis showed the presence of a heterozygous missense mutation, c.1181G > A (p.Arg394Gln), of the ATP6V1B1 gene, while no mutations of the ATP6V0A4 gene were detected. Array-CGH analysis did not reveal any imbalance inside the two genes or in their flanking regions. We also performed molecular studies in other family members who were available; the father had died due to a car accident and never showed any symptom of dRTA, while the mother and the two brothers, who are all in good health, do not have the ATP6V1B1 missense change identified in the proband. We tested the presence of this variant on 100 controls and we have not found the same missense change in any of them. Analysis of the WT1 and RET genes was also performed and no alterations were found in this patient.

Case 2

This patient manifested anorexia, vomiting and failure to thrive at the age of 2 months. Laboratory investigations showed hyperchloreaemic metabolic acidosis and hypokalaemia. Renal US revealed bilateral nephrocalcinosis. dRTA was diagnosed and treatment with sodium bicarbonate was established. During follow-up, frequent urinary tract infections and renal lithiasis occurred. Intravenous urography performed at the age of 5 years showed typical MSK features (Figure 2). From 12 years of age, periodic follow-up was conducted at the Nephrology Department of the Ospedali Riuniti of Bergamo, and therapy with potassium citrate was established. At the age of 14 years, she manifested bilateral sensorineural hearing loss, which progressively worsened in the following years. Parents are non-consanguineous and family history is negative for short stature, renal diseases and urolithiasis. Analysis of the ATP6V0A4 gene coding region in the proband's DNA revealed the presence of two distinct mutations, a missense substitution, c.1571C>T (p.Pro524Leu) [7], and a 14 bp frameshift deletion c.414_417 +10delTGAGGTGGTCACGT. These were located on the two different ATP6V0A4 alleles, since each parent was found to be heterozygous for a single DNA change. Both WT1 and RET coding sequences were normal in the proband's DNA.

Materials and methods

Peripheral blood samples were obtained and genomic DNA was extracted by standard methods. ATP6V1B1, ATP6V0A4, WT1 and RET exons and intron–exon junctions were amplified by direct sequencing, using the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) in association with an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems). Primer sequences used for the ATP6V1B1 gene were as described by Karet et al. [8]; ATP6V0A4, WT1 and RET primers were designed on the basis of the published gene sequence and are available on request. The reference sequences used for mutation reporting are the following: NM_001692.3 for ATP6V1B1 gene, NM_020632.2 for ATP6V0A4 gene, NM_024426.3 for WT1 gene and NM_020754.4 for RET gene.

Array-CGH analysis was performed by using a 4 × 44K customized chip (Agilent Technologies, Santa Clara, CA, USA), specifically designed to investigate the ATP6V1B1 and ATP6V0A4 genes and 1 Mb segments flanking both sides of these genes. This high-density chip was designed with a custom software (http://array.chem.agilent.com/). The average spacing among probes, which were selected from those available in the Agilent database, was about 250 bp. Aliquots of 500 ng of patient DNAs and reference DNA (Promega Corporation, Madison, WI, USA) were double-digested with Rsal and AluI (Promega) for 2 h at 37 °C. After heat inactivation of the enzymes at 65 °C for 20 min, each digested sample was labelled by random priming (Agilent Technologies) for 2 h using Cy5-dUTP for patient DNAs and Cy3-dUTP for reference DNAs. Labelled products were purified, denaturated for 3 min at 95 °C, pre-annealing with 5 µg of Cot-1 DNA, and hybridized at 65 °C for 24 h with rotation. After post-hybridization washing steps, an image of the chip was acquired with the Agilent scanner and analysed using the Feature Extraction software 9.5. A graphical representation of the results was obtained with the CGH Analytics software 3.5. Informed consent for genetic investigations was obtained by all study participants.

Discussion

Chronic metabolic acidosis is a common metabolic alteration observed in MSK associated with hypercalciuria, hypocitraturia, nephrocalcinosis and renal calculi [9]. These findings are also present in primary dRTA, a genetic condition leading to metabolic acidosis [10]. Interestingly, primary classical dRTA and MSK were simultaneously present in the two cases reported here. In both patients, molecular analyses revealed mutations in one of the genes, ATP6V0A4 and ATP6V1B1, encoding the a4 and B1 subunits of the apical H+-ATPase pump [10]. Since its initial description, the diagnosis of MSK is performed by i.v. urography [1]. The collection of contrast medium in ectatic papillary ducts causes characteristic findings: presence of a brush in milder cases or linear striations (bouquets of papillae) in full-blown cases [2]. These features are essential for a diagnosis of MSK, and they were observed in both cases presented here, although they were less evident in case 2 due to technical difficulties in the execution of the urography.

Patient 1 was heterozygous for a monoallelic ATP6V1B1 missense mutation. This finding is in apparent contrast with
the autosomal recessive mode of transmission usually associated with dRTA related to ATP6V1B1 mutations. However, the same Arg394Gln change has been previously observed as a monoallelic mutation in two French unrelated probands affected by dRTA, one of whom also showed mild sensorineural hearing loss [6]. Gene regulatory elements may reside at a considerable distance from the transcription units on which they operate and, moreover, may be incorporated into the structure of neighbouring genes [11,12]. Both point mutations and genomic alterations of long distance regulatory sequences can interfere with the gene expression, thus leading to congenital diseases. In case 1, customized array experiments did not detect any genomic rearrangement of either ATP6V1B1 or ATP6V0A4. This finding excluded the presence of cryptic deletions or duplications that could alter the expression either of the ATP6V1B1 allele with a wild-type coding sequence or of ATP6V0A4.

Although, as previously stated by Vargas-Poussou et al. [6], we cannot exclude the presence of point mutations in regulatory elements, the recurrent observation of this specific ATP6V1B1 defect as a monoallelic mutation and its absence in all healthy family members investigated suggest that it might exert a negative dominant effect on the other allele, thus causing proton pump disruption.

On the other hand, as typically observed in autosomal recessive dRTA, patient 2 had biallelic ATP6V0A4 mutations, each inherited from one parent. One mutation is a previously reported missense change, p.Pro524Leu [6], and the other one is an as yet undescribed 14bp frameshift deletion, c.414_417 + 10delTGAGGTGGTCACGT. This variant causes the loss of a short sequence localized between exon 6 and intron 6, which includes the donor splice site. In accordance with previous findings on dRTA patients with ATP6V0A4 mutations, she developed mild sensorineural hearing loss during the second decade of life [13].

It is noteworthy that MSK was detected at the age of 3 and 5 years, respectively, in our patients, whereas this anomaly is usually diagnosed in adult patients presenting with repeated episodes of urinary tract infection, haematuria or renal calculi. Recently, Kasap et al. [14] described a case with similar clinical features, a 5-year-old girl, born to consanguineous parents, who presented with short stature and failure to thrive. Laboratory evaluation showed alkaline urine, hyperchloremic metabolic acidosis with normal anion gap and hypercalciuria; these findings were compatible with a diagnosis of dRTA, which was later confirmed by the ammonium-chloride-loading test. Bilateral medullary nephrocalcinosis was detected by abdominal ultrasound and intravenous urography showed typical features of MSK. The metabolic alterations and growth retardation improved with alkali therapy. No molecular studies were performed in this case and the authors concluded that dRTA was secondary to MSK. On the other hand, the girl showed severe staturo-ponderal growth deficit arising during the first years of life, which is typical of primary dRTA, and her parents were consanguineous, as frequently observed in families with rare genetic diseases with autosomal recessive inheritance. Therefore, it is possible that this girl was affected with primary genetic dRTA, followed by the development of MSK. The diagnosis of MSK occurred early, as observed in our cases, further supporting this possibility.

The concomitance of primary dRTA and MSK in these cases could represent a fortuitous association, determined by multiple distinct pathogenetic mechanisms, which altogether could have contributed to the development of a severe lithogenic nephropathy. Alternatively, mutations in the ATP6V1B1 and ATP6V0A4 genes, in addition to determining dRTA, might play a direct role in the development of MSK. Under this assumption, dysfunction of the proton pump would trigger ectasia and dilation of the collecting ducts.

Recent studies have shown that proton pumps are present in the embryonic kidney during early developmental phases [15]. Therefore, structural and/or functional alterations of the pump, possibly in association with other factors, might cause morphological disruption leading to the collecting duct anomalies observed in MSK. However, it is also possible that the final maturation of collecting ducts occurs postnatally in humans, as demonstrated for rabbits [16]. Chronic metabolic acidosis due to H^+-ATPase defects might prime a series of renal adaptive processes in early postnatal life. Metabolic acidosis determines expression changes in a large number of genes and proteins, thus affecting multiple metabolic and signalling processes, including those involved in cell proliferation and apoptosis [16]. So, in addition to well-established functional adaptive changes occurring in the collecting duct, metabolic acidosis could give rise to morphological changes, such as cell hypertrophy, hyperplasia and transdifferentiation, thus leading to nephron remodelling [17].

The frequent concomitance of MSK with malformative conditions or congenital syndromes supports the hypothesis that it might be a developmental disorder [18–21]. Congenital hemihypertrophy and Beckwith–Wiedemann syndrome, with or without Wilms tumour, are the conditions that have been reported most frequently in association with MSK [18]; since both are related to WT1 mutations, it has been suggested that this gene could also play a role in MSK development, although no clear demonstration of this hypothesis has been provided. In addition, in 2000, Diouf et al. found a RET proto-oncogene mutation in a patient affected by MEN-2a who presented with medullary thyroid cancer, hyperparathyroidism and MSK [22]. The authors pointed out that this could be a fortuitous association, or, alternatively, that there might be a causal relationship between the two conditions, considering the important role of RET in renal development [22,23].

Therefore, in order to verify whether pathologic variations of either WT1 or RET could account for the development of MSK, we undertook molecular analysis of these two genes in our patients. Since no mutations were identified, the possibility that they are involved in the pathogenesis of MSK in the two cases presented here is highly unlikely.

MSK has also been observed in association with autosomal dominant polycystic kidney disease (ADPKD), another inherited kidney disease. In addition to anecdotal reports, MSK has been observed in 15% of a series of 71 ADPKD cases with renal stones [24]. However, neither patient in this study had manifestations of ADPKD.
In conclusion, MSK can be considered a syndrome caused by multiple pathogenetic mechanisms, both genetic and environmental. Herein, we have provided evidence that the proton pump genes ATP6V0A4 and ATP6V1B1, responsible for primary dRTA, can also be associated with MSK. Interestingly, the H+-ATPase pump is expressed in the α-intercalated cells localized in the late distal tubule and in the cortical collecting duct, the same anatomical regions involved in MSK. Further studies on larger series will be important to confirm a causal relationship between mutations in these genes and MSK.

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