ACIDOSI TUBULARE RENALE DISTALE:
ANALISI CLINICO GENETICA

SABRINA GIGLIO
Genetica Medica, Firenze
Causes of acidosis

1) Inherited acidoses of renal origin
   primary failure of the kidney to secrete acid or reclaim bicarbonate, or
   secondary due to defects in handling of other electrolytes

2) Acquired acidoses of renal origin
   most commonly seen as a result of impaired renal function

3) Inherited acidoses of non-renal origin
   with the excess production of acid elsewhere in the body due to an
   inherited metabolic defect

4) Acquired acidoses of non-renal origin
   e.g., lactic acidosis as a result of poor tissue oxygenation
Inherited acidosis

Primary renal defect in acid-base handling
Role of the renal tubule in acid-base regulation

1) proximal reclamation of filtered bicarbonate

2) distal secretion of $\text{H}^+$, with phosphate buffers and ammonium phosphate buffers and ammonium

Renal tubular acidosis (RTA) arises from either a failure of proximal mechanisms of bicarbonate conservation or of distal acid secretion.
Control of acid-base homeostasis

proximal tubule bicarbonate reclamation
Control of acid-base homeostasis

distal mechanisms of urinary acidification
The V-ATPase belongs to the ubiquitous families of ATP-driven H\(^+\)-translocating ion pumps, which also include F- and P-ATPase families. V-ATPases serve multiple cellular functions and are crucial for lysosomal function, synaptic transmission, bone resorption, inner ear endolymph pH regulation, and systemic acid–base homeostasis by participating in renal acid excretion.
H⁺-ATPases are composed of two main sectors

- **cytosolic V1 domain**
- **membrane-bound V0 domain**

ATP binds to the V1 domain, and its hydrolysis provides the energy to pump protons across the cell membrane-embedded V0 domain.

Both domains are assembled from several subunits, often with many isoforms.
**V1 domain:**

640 kDa composed of subunits A–H in a reported $A_3B_3C_1D_1E_1F_1G_2H_1$ stoichiometry

**V0 domain:**

260 kDa composed of five subunits in a possible complex of $a_1d_1c''_1(c, c')$
Mammals express a rich diversity of V-ATPase subunit isoforms

Most of these isoforms are expressed in different tissues:

a4, B1, C2b, d2 and G3 are highly expressed in kidney and epididymis
Mutations in the B1 and α4 subunit isoforms, have been detected in patients with Distal Renal Tubular Acidosis (dRTA) due to the impaired proton secretion by proton pumps.
<table>
<thead>
<tr>
<th>Type of RTA</th>
<th>Subtype and Inheritance</th>
<th>Age at Presentation</th>
<th>Clinical Features</th>
<th>Protein</th>
<th>Gene(s)</th>
<th>OMIM</th>
</tr>
</thead>
</table>
| Distal (type 1)   | Dominant                 | Older/adult         | Mild/compensated metabolic acidosis
Hypokalemia (variable)
Hypercalciuria
Hypocitraturia
Nephrolithiasis
Nephrocalcinosis
Sometimes rickets/
osteomalacia
Secondary erythrocytosis | AE1     | SCL4A1   | 179800 |
| Recessive         | Childhood                |                     | Metabolic acidosis with hemolytic anemia
Only reported in Southeast Asian populations | AE1     | SCL4A1   | 602722 |
| Recessive with early onset hearing loss | Infancy/childhood |                     | Metabolic acidosis
Early nephrocalcinosis
Vomiting/dehydration
Growth retardation
Rickets
Bilateral sensorineural hearing loss,
from childhood | B1 subunit of ATP6V1B1 | ATP6V1B1  | 267300 |
| dRTA di tipo 1b   |                          |                     |                                                                                   |         |         |       |
| Recessive with later onset hearing loss | Infancy/childhood |                     | As above, but later onset hearing loss in some
(a few with normal hearing) | a4 subunit of ATP6V0A4 | ATP6V0A4  | 602722 |
| dRTA di tipo 1c   |                          |                     |                                                                                   |         |         |       |
We clinically and genetically analyzed 30 families referred during this year to paediatric nephrologists and medical geneticists of Meyer Hospital with diagnosis of dRTA.
## Exam i ematologi

<table>
<thead>
<tr>
<th>Esame</th>
<th>Alla diagnosi</th>
<th>Controllo</th>
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## Exam i urinari

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## Exam i strumentali

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<td>Ecografia reale</td>
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<td>C Ultrasound</td>
<td></td>
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## Terapia

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Allegare consenso informato per analisi genetica
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<td>Infancy/childhood</td>
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<td>B1 subunit</td>
<td>ATP6V1B1</td>
<td>267300</td>
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<td>ATP6V0A4</td>
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*Encoding the basolateral Cl-/HCO₃⁻ exchanger*
• Scarso accrescimento

• Calcolosi renale diagnosticata a 10 a

• Diagnosi di dRTA con ipercalciuria, ipocitraturia, osteomalacia

• Normale crescita staturo-ponderale

• Sviluppo psicomotorio nella norma

• 10 a: diagnosi di dRTA con iperecogenicità delle regioni midollari senza sicure immagini di calcoli

• 20 anni: lieve nefrocalcinosi

• MUTAZIONE DE NOVO

Caso 10920

22a

SLC4A1

c.[1765C>T]

Caso 10864

17a

c.[1765C>T]
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<td>a4 subunit of ATP6V0A4</td>
<td>ATP6V0A4</td>
<td>602722</td>
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</tbody>
</table>
ATP6V1B1

36a3m

• Gravidanza: minaccia di aborto nel 1° trimestre
• 3° trimestre: tossicosi gravidica e parto prematuro al 7° mese
• Scarso accrescimento
• Sviluppo psicomotorio lievemente ritardato (prime parole 3 anni)
• 2a6m: diagnosi di acidosi tubulare renale distale con nefrocalcinosi
• 5 anni: sordità (protesi a 6 anni)
• Oggi: RM (lieve)

12a

• Scarso accrescimento
• Sviluppo psicomotorio lievemente ritardato
• 4aa diagnosi di acidosi tubulare renale distale con nefrocalcinosi
• sordità ???
ATP6V1B1

Caso 10871 (BS)

c.[687+1G>T] c.[687+1G>T] c.[687+1G>T]

c.[687+1G>T]+[687+1G>T]

10a2m

• Dal 8° mese: anoressia e scarso accrescimento

• 1a: Diagnosi di acidosi tubulare renale distale con nefrocalcinosi, disidratazione e diselettrolitemia

• 3a: sordità neurosensoriale (uso di protesi)
• 11 mesi: Arresto dell'accrescimento
• 2 anni: Diagnosi di sordità neurosensoriale bilaterale grave
• Marcata ipercogenicità a livello delle piramidi renali

• 3 mesi: Arresto dell'accrescimento, nefrocalcinosi.
• Ipoacusia neurosensoriale
• Diagnosi di Acidosi Tubulare Renale distale
ATP6V1B1

Caso 9964 (GA)
c.[242T>C]+[242T>C]

2a5m
- 2° gravidanza; decorso regolare
- 2 mesi: rallentamento crescita e poi arresto
- Ricovero: acidosi metabolica cronica
- Diagnosi di acidosi tubulare renale distale
- 10m: diagnosi di sordità neurosensoriale bilaterale (protesi)

Caso 10020 (MM)
c.[242T>C]+[242T>C]

4a10m
- Gravidanza: malformazione di forma e posizione del rene dx
- 3 mesi: scarso accrescimento
- 1a10m: Diagnosi di acidosi tubulare renale distale con nefrocalcinosi
- 2a1m: diagnosi di sordità neurosensoriale bilaterale (protesi)

Caso 11063 (IJ)
c.[242T>C]+[242T>C]

3a1m
- Gravidanza: riferita nella norma
- 4 mesi: scarso accrescimento
- 1a+6m: Diagnosi di acidosi tubulare renale distale con nefrocalcinosi
- 2a1m: bassa statura, osteodistrofia e rachitismo, ritardo psicomotorio
- 3a: sordità neurosensoriale bilaterale (protesi)
ATP6V1B1

- Gravidanza: riferita nella norma
- 3 mesi: scarso accrescimento
- diagnosi di acidosi tubulare renale distale con nefrocalcinosi
- lieve rachitismo
- sordità neurosensoriale bilaterale

Caso 11246 (YM)

c.[1555_1556insC]+[1555_1556insC]
ATP6V1B1

6a5m
- **Gravidanza**: minaccia di aborto alla 22ma e 32ma settimana
- **Dal 4° mese**: anoressia e scarso accrescimento
- **8m**: Diagnosi di acidosi tubulare renale distale con nefrocalcinosi, disidratazione e diselettrolitemia
- **Ultimo audiogramma**: nella norma
- **Genitori non portatori** (confermata la paternità)

27a1m
- **1 anno**: ricovero per scarso accrescimento; ipopotassemia; ipospadia.
- **3 anni**: ritardo di crescita; grave acidosi; diagnosi di **RENE A SPUGNA MIDOLLARE BILATERALE** con calcificazioni e nefromegalia bilaterale lieve.
- **14 anni**: correzione del valgismo e scoliosi
- **17 anni**: calcolosi renale bilaterale e numerosi calcoli vescicali mobili: asportazione. **Litrotissia** dei calcoli dell’uretere dx e pielocalicotomia dx
- **18 anni**: ripetute ESWL a sin
- **24 anni**: nefrocalcinosi; calcificazioni papillari diffuse; macroematuria ogni 2-3 mesi.
- **RM lieve**
- **Familiari**: NO MUTAZIONE
Lo studio mediante array-CGH non ha messo in evidenza microriarrangiamenti a carico dell’allele “normale” e delle regioni circostanti nei nostri due pazienti.

Clinical characteristics of dRTA patients with ATP6V1B1 mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Origin</th>
<th>Age at Diagnosis</th>
<th>Gender</th>
<th>Consentaneous</th>
<th>SNHL (Age at Diagnosis)</th>
<th>Blood pH</th>
<th>HCO₃⁻</th>
<th>K⁺</th>
<th>Urine pH</th>
<th>Nephrocalcinosis</th>
<th>DNA</th>
<th>Protein</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>22 yr</td>
<td>Algeria</td>
<td>3 wk</td>
<td>F</td>
<td>Yes</td>
<td>Yes³</td>
<td>ND</td>
<td>10</td>
<td>2.9</td>
<td>7.2</td>
<td>Yes</td>
<td>1149-1155insC</td>
<td>P368fsX441⁴</td>
<td>Homozygous</td>
</tr>
<tr>
<td>2-1</td>
<td>4 yr</td>
<td>Algeria</td>
<td>1 mo</td>
<td>F</td>
<td>Yes</td>
<td>Yes (3 yr severe first diagnosis)</td>
<td>ND</td>
<td>18</td>
<td>3.5</td>
<td>8</td>
<td>No</td>
<td>IVS2-1 C&gt;G</td>
<td>R394Q</td>
<td>Homozygous</td>
</tr>
<tr>
<td>6-1</td>
<td>7 yr</td>
<td>France</td>
<td>3 wk</td>
<td>F</td>
<td>No</td>
<td>Yes (7 yr mild)</td>
<td>7.26</td>
<td>14.4</td>
<td>0.4</td>
<td>7.3</td>
<td>Yes</td>
<td>1161C&gt;A</td>
<td>R394Q</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>8-1</td>
<td>4 yr</td>
<td>France</td>
<td>5 mo</td>
<td>F</td>
<td>No</td>
<td>No</td>
<td>7.20</td>
<td>12</td>
<td>3.2</td>
<td>7.3</td>
<td>Yes</td>
<td>1161C&gt;A</td>
<td>R394Q</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>15-1</td>
<td>12 yr</td>
<td>Algeria</td>
<td>6 mo</td>
<td>M</td>
<td>Yes</td>
<td>Yes²</td>
<td>7.16</td>
<td>12.9</td>
<td>1.7</td>
<td>6.9</td>
<td>Yes</td>
<td>1149-1155insC</td>
<td>P368fsX441⁴</td>
<td>Homozygous</td>
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<tr>
<td>20-1</td>
<td>13 yr</td>
<td>Tunisia</td>
<td>5 mo</td>
<td>F</td>
<td>Yes</td>
<td>Yes (3 yr severe first diagnosis)</td>
<td>7.29</td>
<td>17</td>
<td>2.3</td>
<td>8</td>
<td>Yes</td>
<td>IVS2-1 G&gt;C</td>
<td>R366fsX441⁴</td>
<td>Homozygous</td>
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<tr>
<td>28-1</td>
<td>9 mo</td>
<td>Algeria</td>
<td>6 m</td>
<td>F</td>
<td>No</td>
<td>Yes (2 yr moderate second degree)</td>
<td>7.24</td>
<td>13</td>
<td>3.3</td>
<td>Inappropriate</td>
<td>Yes</td>
<td>IVS2-1 C&gt;C /1149-1155insC</td>
<td>Loss of splice acceptor site</td>
<td>Compound</td>
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<tr>
<td>36-1</td>
<td>9 mo</td>
<td>Algeria</td>
<td>5 mo</td>
<td>F</td>
<td>Yes</td>
<td>No</td>
<td>7.15</td>
<td>9</td>
<td>2.6</td>
<td>7.1</td>
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<td>37-1</td>
<td>15 mo</td>
<td>Tunisia</td>
<td>4 mo</td>
<td>F</td>
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<td>No</td>
<td>7.21</td>
<td>10</td>
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<td>15 mo</td>
<td>Algeria</td>
<td>ND</td>
<td>F</td>
<td>No</td>
<td>Yes (1 yr severe second degree)</td>
<td>ND</td>
<td>16</td>
<td>4.7</td>
<td>Inappropriate</td>
<td>Yes</td>
<td>1037C&gt;G</td>
<td>P368fsX441⁴</td>
<td>Homozygous</td>
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</tbody>
</table>

Marginal notes:

°Nucleotides numbered according to the sequence in GenBank NM_001692.

³Data concerning SNHL were not available, but onset probably was early given abnormal language and cognitive development.

²Father’s cousin with dRTA and SNHL.

⁴Mutation previously described (3).

⁵Mutation previously described (5).

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Gravidanza regolare

1 mese: grave ritardo di crescita.

Diagnosi di acidosi tubulare renale distale

sordità neurosensoriale bilaterale (protesi)

Acidosi tubulare renale distale

Nefrocalcinosi

17 anni: ipoacusia neurosensoriale bilaterale
ATP6V0A4

55a11m

- 25-55 anni: numerosi episodi di coliche renali con emissione di calcoli
- 45 anni: protesi per sordità bilaterale
- 49 anni: paralisi degli arti sup ed inf dovuta ad ipokaliemia
- Acidosi e infezioni ricorrenti delle vie urinarie da Streptococchi di tipo D
- Rene dx pielonefrite

Caso 10812 (DG)

- Origine colombiana (bambina adottata)
- Deficit di crescita (<<3° centile)
- Nefrocalcinosi
- No ipoacusia
- Acidosi e infezioni ricorrenti delle vie urinarie

Caso 11062 (MRV)

- c.[2195T>C]+[2195T>C]
- c.[1561G>A]+[1888G>A]

*Calcolosi renale e sordità bilaterale
**ATP6V0A4**

64

38a

Acidosi Tubulare Renale Distale

- Ritardo di crescita
- Diagnosi di acidosi tubulare renale distale
- No ipoacusia

26a

- Nefrocalcinosi
- Lieve ipoacusia

*Il marito non presenta caratteristiche cliniche di acidosi, ha una mutazione de novo con predizione benigna*
**Caso 11702 (ZD)**

- **c.[1185delC]**
- **c.[2137delG]**
- **c.[1185delC]+[2137delG]**

- **2 mesi:** ricovero per scarso accrescimento, segni di sottoidratazione, acidosi metabolica e nefrocalcinosi. Diagnosi di acidosi tubulare renale distale
- **NON presenta sordità neurosensoriale**
- Genitori con calcoli renali

**Caso 11659 (TG)**

- **c.[481G>A]**
- **c.[816+2T>C]**
- **c.[816+2T>C]+c.[481G>A]**

- **2 mesi:** Diagnosi di acidosi tubulare renale distale
- **Presenza di Nefrocalcinosi**
- **NON presenta sordità neurosensoriale**

**ATP6V0A4**
2 mesi: ricovero per dispepsia, scarso accrescimento, acidosi plasmatica e nefrocalcinosi. Diagnosi di acidosi tubulare renale distale.

5 anni: Diagnosi di RENE A SPUGNA MIDOLLARE BILATERALE

12 anni: peggioramento del quadro di nefrocalcinosi

Infezioni persistenti delle vie urinarie da Proteus

17a 5m: sordità neurosensoriale bilaterale

18a 4m

Caso 10639 (VA)

c.[414_417+10delTGAGTGTCACGT] c.[1571C>T]

c.[414_417+10delTGAGTGTCACGT]+[1571C>T]
In tutti sono state riscontrate mutazioni patogeniche, otto delle quali mai riportate in letteratura.

In 3 pazienti albanesi affetti da dRTA è stata riscontrata in assetto omozigote la stessa mutazione Leu81Pro nel gene ATP6V1B1; tale evidenza può suggerire per questa variante un effetto fondatore.

In due soggetti affetti da dRTA tipica era presente la variante monoallelica Arg394Gln nel gene ATP6V1B1, già descritta in altri due casi in letteratura, allo stato di eterozigosi semplice.

Un paziente con sordità neurosensoriale grave ad insorgenza precoce con, in assetto omozigote, la variante Arg807Gln nel gene ATP6V0A4, solitamente correlato ad un’insorgenza più tardiva della sordità; il solo altro caso riportato in letteratura presentava un quadro clinico sovrapponibile al paziente da noi analizzato.
**ATP6VOA4**

- **18a4m**
  - 2 mesi: ricovero per dispepsia, scarso accrescimento, acidosi plasmatica e nefrocalcinosi. Diagnosi di acidosi tubulare renale distale
  - 5 anni: Diagnosi di RENE A SPUGNA MIDOLLARE BILATERALE
  - 12 anni: peggioramento del quadro di nefrocalcinosi
  - Infezioni persistenti delle vie urinarie da Proteus
  - 17a 5m: sordità neurosensoriale bilaterale

**ATP6V1B1**

- **27a1m**
  - 1 anno: ricovero per scarso accrescimento; ipopotassiemia, ipospadia.
  - 3 anni: ritardo di crescita; grave acidosi; diagnosi di RENE A SPUGNA MIDOLLARE BILATERALE con calcificazioni e nefromegalia bilaterale lieve.
  - 14 anni: correzione del valgismo e scoliosi
  - 27 anni: calcolosi renale bilaterale e numerosi calcoli vescicali mobili: asportazione. Litrotissia dei calcoli dell’uretere dx e pielocalicotomia dx
  - 28 anni: ripetute ESWL a sin
  - 34 anni: nefrocalcinosi; calcificazioni papillari diffuse; macroematuria ogni 2-3 mesi. Sordità neurosensoriale bilaterale.
  - RM lieve
  - Familiari: NO MUTAZIONE

**Caso 10639**

- c.[414_417+10delTGAGGTGGTCACGT]
- c.[1571C>T]

**Caso 10860**

- c.[1181G>A]
Medullary sponge kidney (MSK)

Rare congenital disease characterized by diffuse ectasy or dilatation of precalyceal collecting tubules
Clinical phenotype:

- hypercalciuria
- hypocitraturia
- nephrocalcinosis
- urolithiasis
- tubular function defects of acidification and concentration
- moderately increased risk of urinary tract infections

Exceptionally, chronic renal failure
Diagnosis is radiographic:

ectatic papillary ducts give the appearance of a brush (in the mildest cases) or linear striation, or bouquets of papillae, when is seen dilation of the collecting ducts

Although it is usually a sporadic condition, familial cases with an autosomal dominant transmission have also been described.
Molecular analysis showed the presence of a heterozygous missense mutation, c.1181G>A (p.Arg394Gln), of the ATP6V1B1 gene; 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.
Analysis of the \textit{ATP6V0A4} gene coding region revealed the presence of two distinct mutations, a missense substitution, c.1571C>T (p.Pro524Leu) and a 14 bp deletion, c.414_417+10delTGAGGTGGTCACGT.
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, hematuria or renal calculi.

This paper 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:
- alkaline urine
- hyperchloremic metabolic acidosis with normal anion gap
- hypercalciuria
Findings compatible with dRTA

Bilateral medullary nephrocalcinosis was detected by abdominal ultrasound and intravenous urography showed typical features of MSK.
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.
The concomitance of MSK with malformative conditions or congenital syndromes, supports the hypothesis that it might be a developmental disorder.

Congenital hemihypertrophy and Beckwith-Wiedemann syndrome, with or without Wilms tumor, are the conditions that have been reported in MSK (Gambaro et al, 2006- Kidney International 69, 663-670).

Since both are related to WT1 mutations, it has been suggested that this gene could also play role in MSK development, although no clear demonstration of this hypothesis has been provided.
A *RET* proto-oncogene gene mutation in a patient affected by MEN-2a: presented medullary thyroid cancer, hyperparathyroidism and MSK.

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.
We have hypothesized and then provided evidence that the proton pump genes ATP6V0A4 and ATP6V1B1, responsible for primary dRTA, can also be associated with MSK.
Medullary sponge kidney associated with primary distal renal tubular acidosis and mutations of the H⁺-ATPase genes

Ilaria Carboni¹, Elena Andreucci¹,², Maria R. Caruso³, Roberto Ciccone⁴, Orsetta Zuffardi⁴, Maurizio Genuardi¹,², Ivana Pela⁵ and Sabrina Giglio¹,²

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

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.
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IVANA PELA
SNHL and dRTA

No mechanism has yet been proposed to account for the variability of SNHL in terms of both severity and age at onset, depending on the gene affected.

Variability in SNHL is observed both for genes and for missense and nonsense mutations.

The maintenance of acidic conditions in the endolymphatic sac seems to be important for cell integrity in the inner ear.

This function is fulfilled in part by vacuolar type H⁺-ATPases.
Inner ear abnormalities in four patients with dRTA and SNHL: clinical and genetic heterogeneity

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Enlarged vestibular aqueduct (EVA) was described in patients with recessive dRTA and SNHL and recently this abnormality has been associated with mutations in the ATP6V1B1 gene
In our study, we evaluated the presence of inner ear abnormalities in four patients affected by dRTA and SNHL, characterized by molecular analysis.

Two patients affected by severe dRTA with early onset SNHL showed the same mutation in the *ATP6V1B1* gene and bilateral EVA with a different degree of severity.

The other two presented similar clinical manifestations of dRTA and different mutations in the *ATP6V0A4* gene:

- one patient, showing EVA, developed an early SNHL
- the other one the SNHL appeared in the second decade of life and the vestibular aqueduct was normal
Table 2: SNHL, EVA and mutations in the $ATP6V1B1$ and $ATP6V0A4$ genes

<table>
<thead>
<tr>
<th>Patients</th>
<th>SNHL</th>
<th>EVA</th>
<th>$ATP6V1B1$ gene mutation</th>
<th>$ATP6V0A4$ gene mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1 M</td>
<td>Early</td>
<td>Mild</td>
<td>c.[242T&gt;C]+[242T&gt;C]</td>
<td>c.[1185delC]+[2420G&gt;A] (deletion c.1185del C and missense mutation p.Arg807Gln)</td>
</tr>
<tr>
<td>Case 2 F</td>
<td>Early</td>
<td>Evident</td>
<td>c.[242T&gt;C]+[242T&gt;C]</td>
<td></td>
</tr>
<tr>
<td>Case 3 M</td>
<td>Late (at 17 y)</td>
<td>Absent</td>
<td></td>
<td>c.[2420G&gt;A]+[2420G&gt;A] (p.Arg807Gln)</td>
</tr>
<tr>
<td>Case 4 M</td>
<td>Early</td>
<td>Evident</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(MRI at 2 y), (MRI at 22 m), (MRI at 24 y), (MRI at 3 y)
In our study we have confirmed that **EVA** may be associated with dRTA due to *ATP6V1B1* gene mutations, although the severity could be of variable degree.

We’ve demonstrated for the first time that **EVA** can be observed also in patients with *ATP6V0A4* gene mutations, perhaps with a relationship between type of mutation, precocity and severity of the SNHL and morphological abnormalities of inner ear.

It is evident that, also in the absence of a large vestibular aqueduct, there is no relationship between the severity of dRTA and precocity of SNHL.
Our study confirms the association of EVA and mutations in the *ATP6V1B1* gene and demonstrates that mutations in *ATP6V0A4* gene can also be associated with EVA probably only when the SNHL has an early onset.
dRTA PROJECT: WORK PROGRAM

Task 1
IDENTIFICATION OF MUTATIONS IN FURTHER dRTA PATIENTS

Task 2.
MUTAGENESIS AND CLONING OF THE ATPase SUBUNIT GENES

To obtain cDNA for mutated and wild type ATPase subunits
Expression vector assembly

Task 3.
CELL MODELS FOR FUNCTIONAL STUDIES

Human NIH3T3 fibroblasts
Human renal tubular cells
Yeast (S. cerevisiae)
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