ACIDOSI TUBULARE RENALE DISTALE: ANALISI CLINICO GENETICA

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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

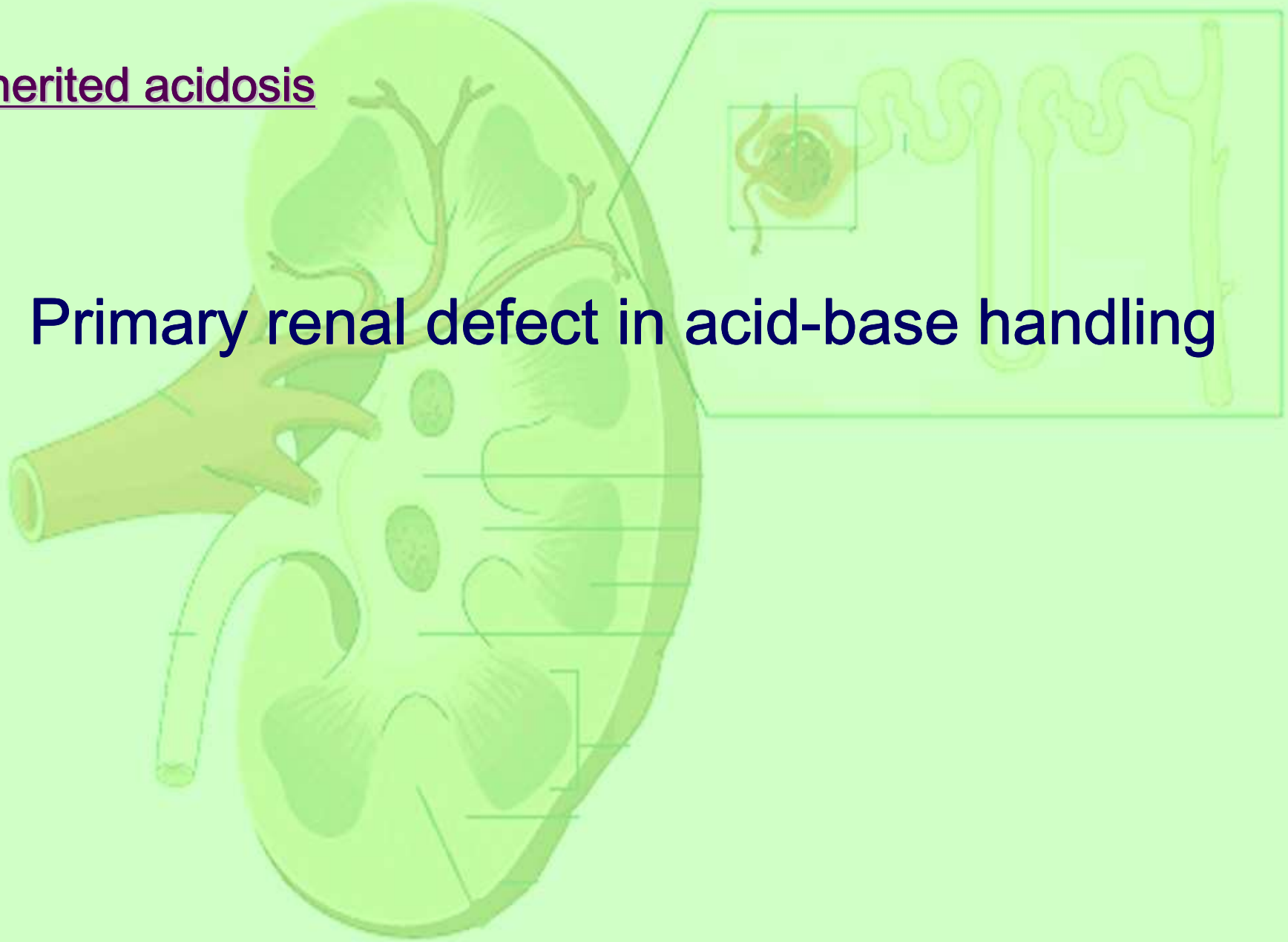
3) Inherited acidoses of non-renal origin

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



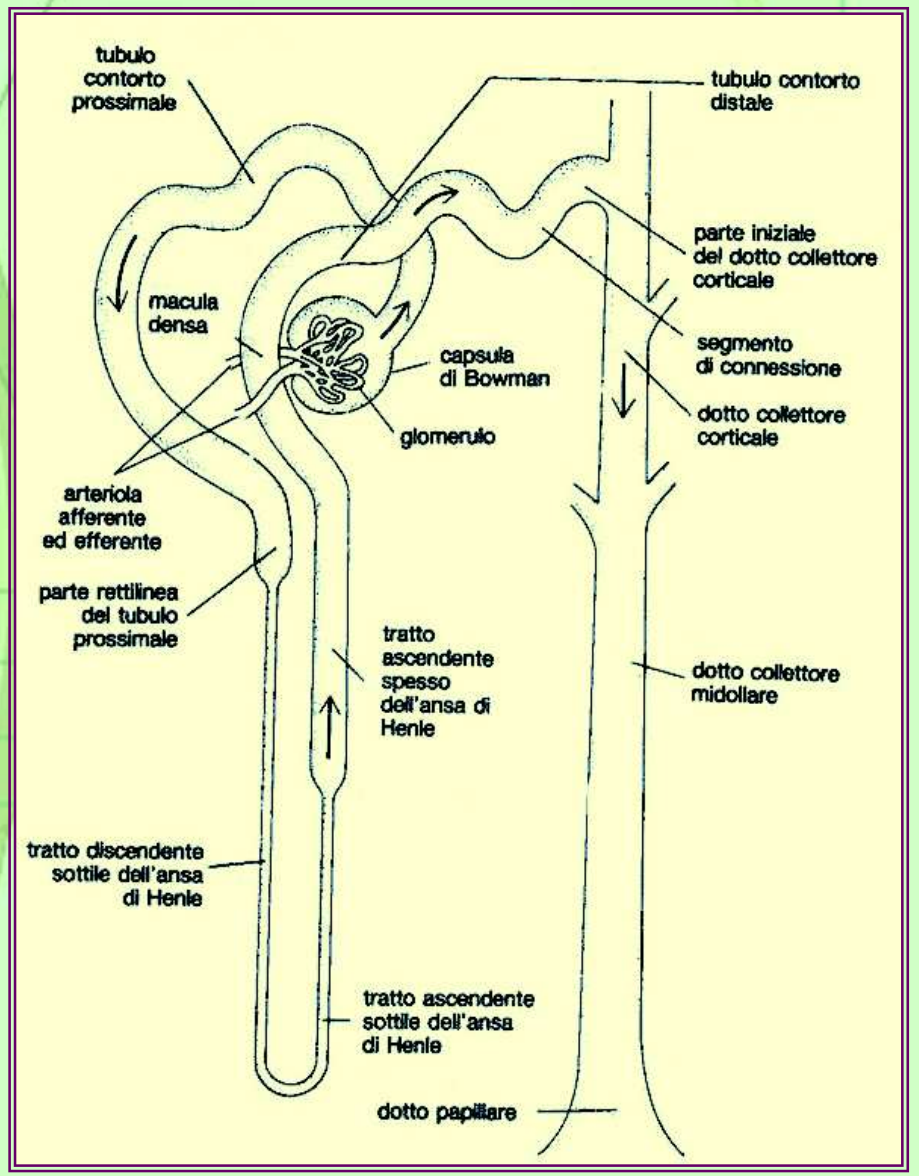
Primary renal defect in acid-base handling

Role of the renal tubule in acid-base regulation

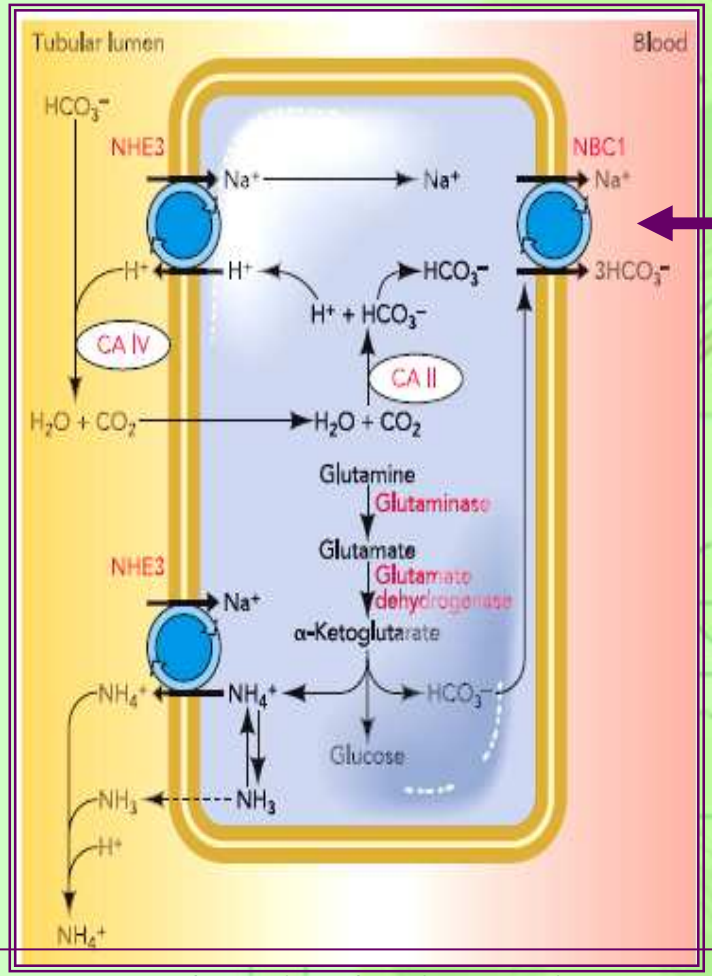
1) proximal reclamation of filtered bicarbonate

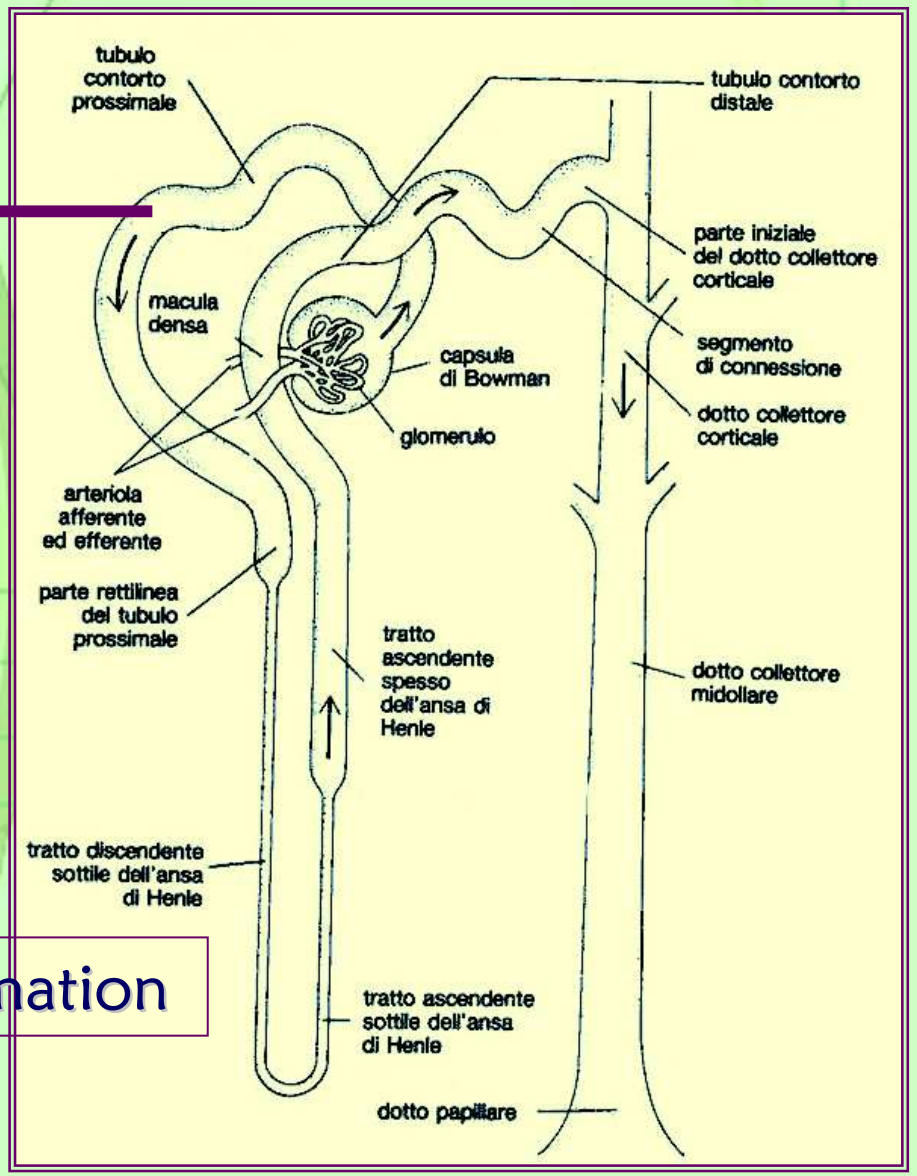
2) distal secretion of H^+, with phosphate buffers and ammonium

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



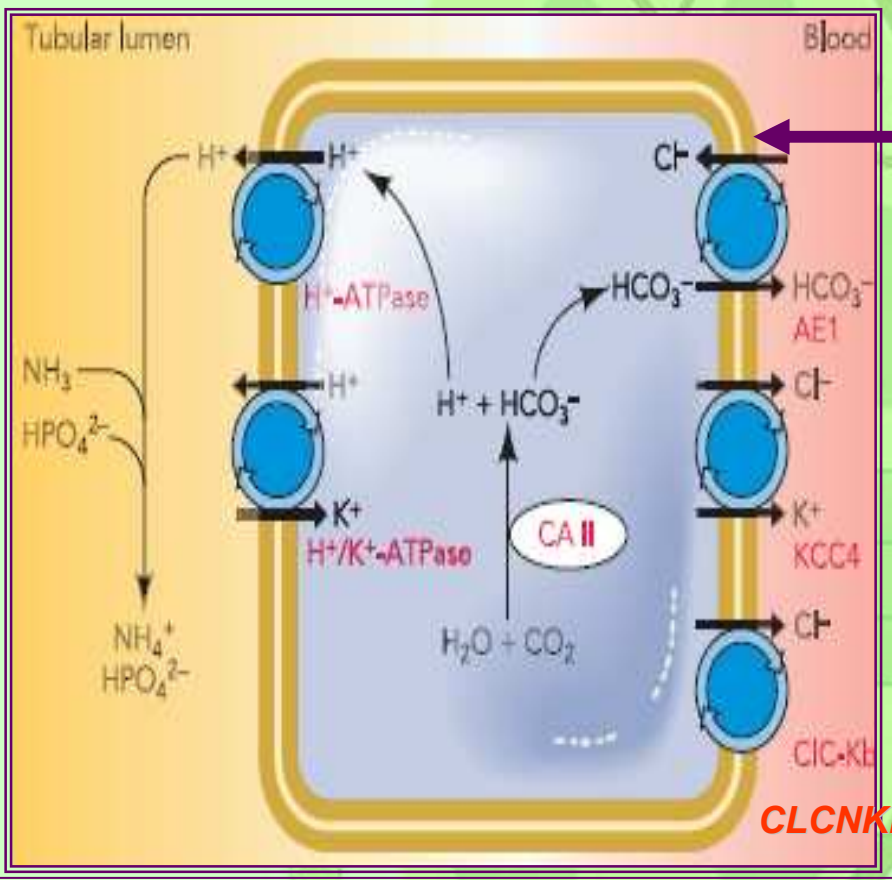
Control of acid-base homeostasis

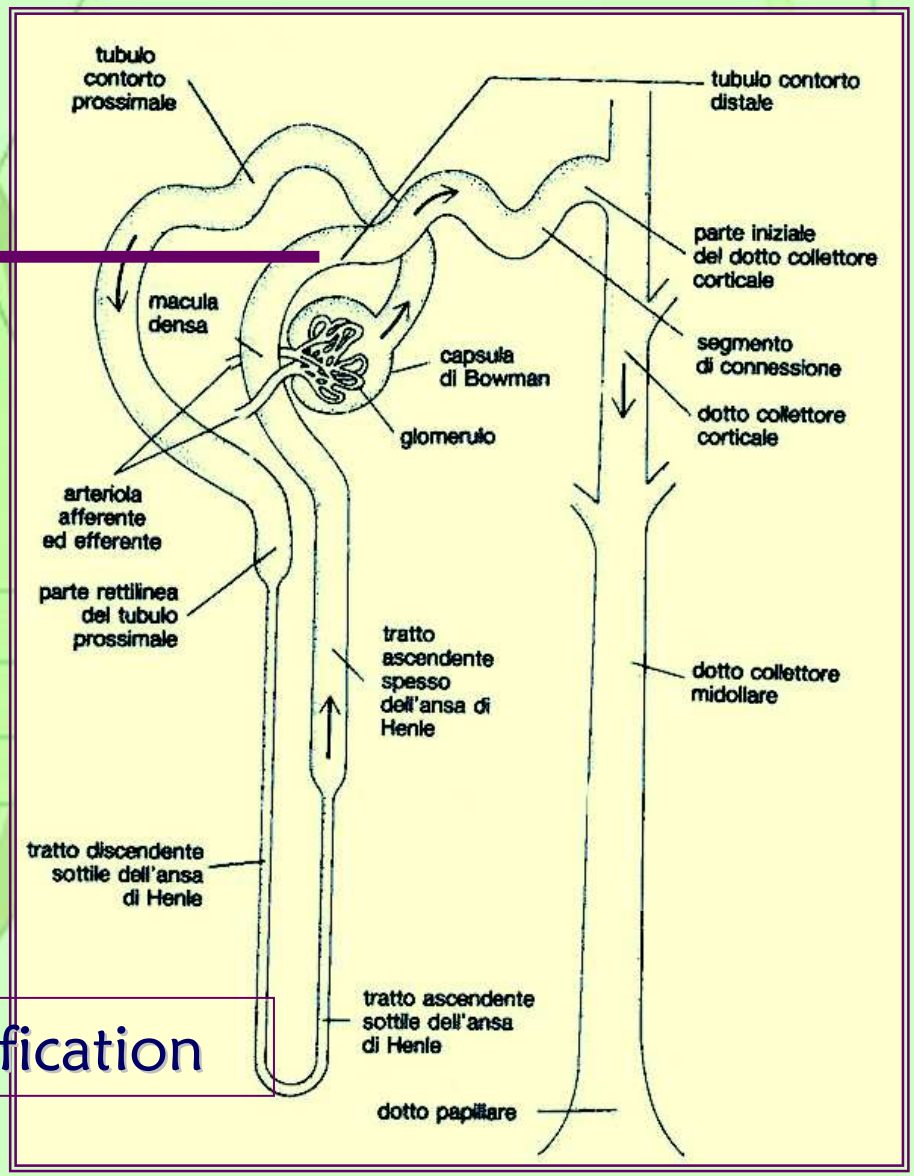




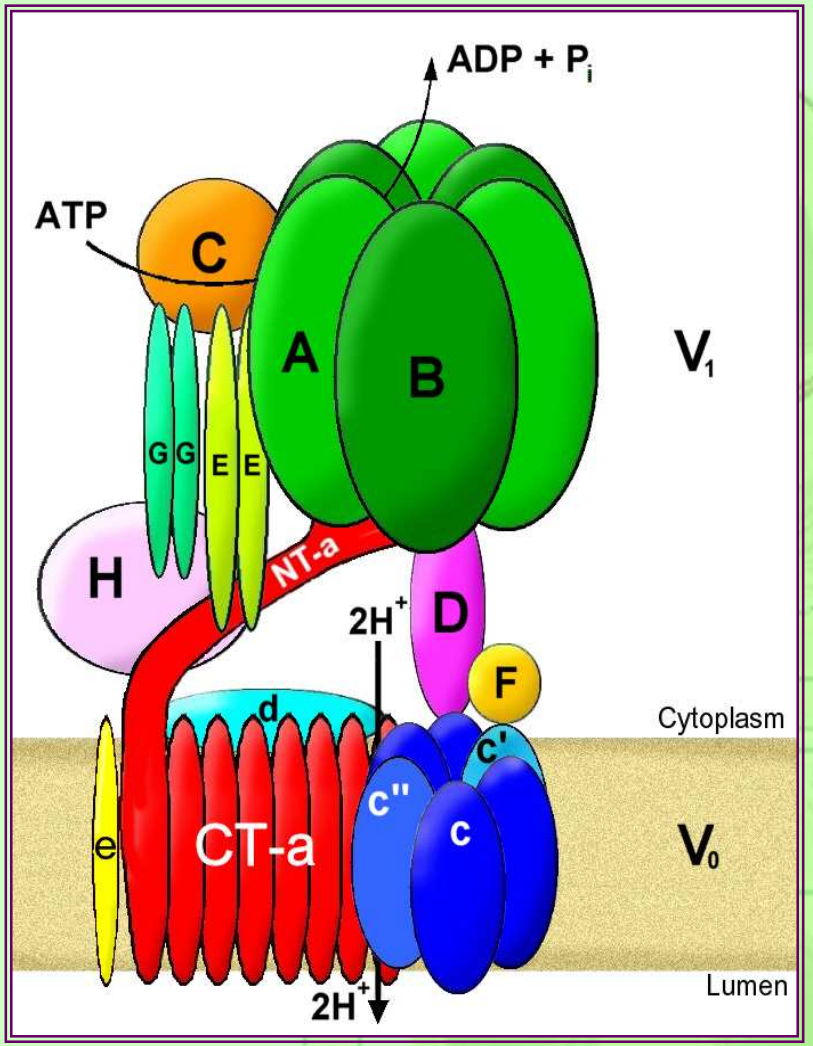
proximal tubule bicarbonate reclamation

Control of acid-base homeostasis





distal mechanisms of urinary acidification



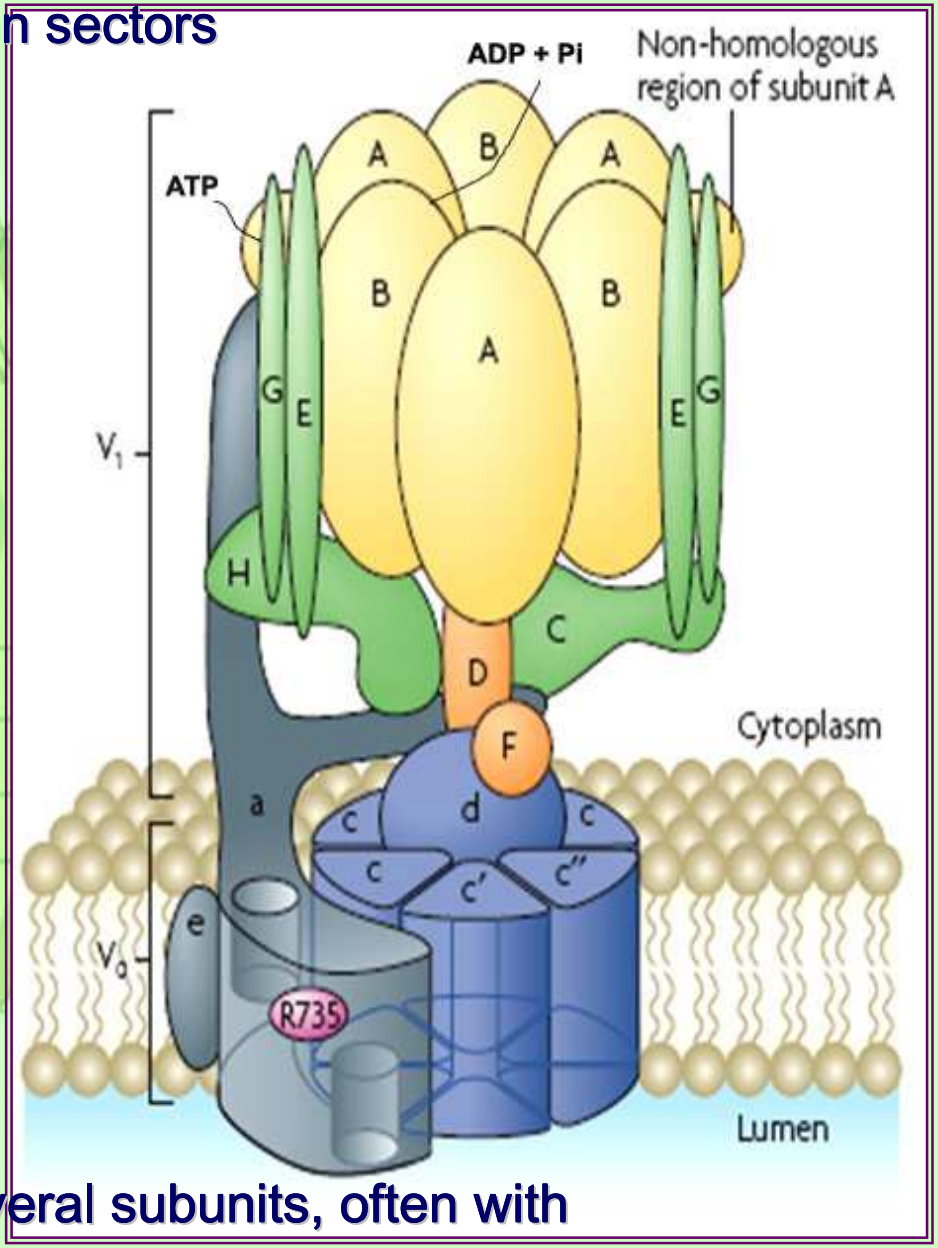
The V-ATPase belongs to the ubiquitous families of ATP-driven H^+-translocating ion pumps, which also include F- and P-ATPase families

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.



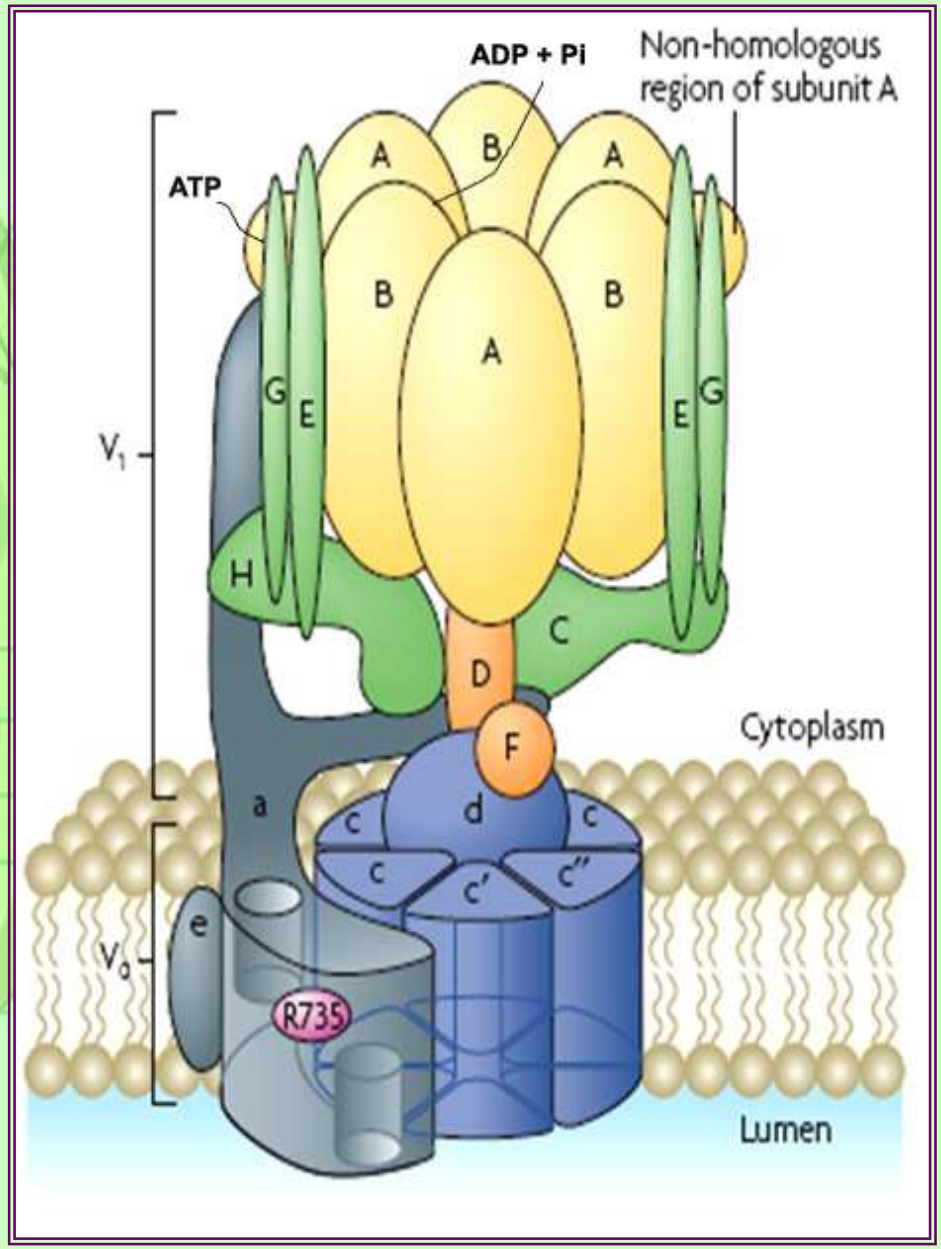
Both domains are assembled from several subunits, often with many isoforms

V1 domain:

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

V0 domain:

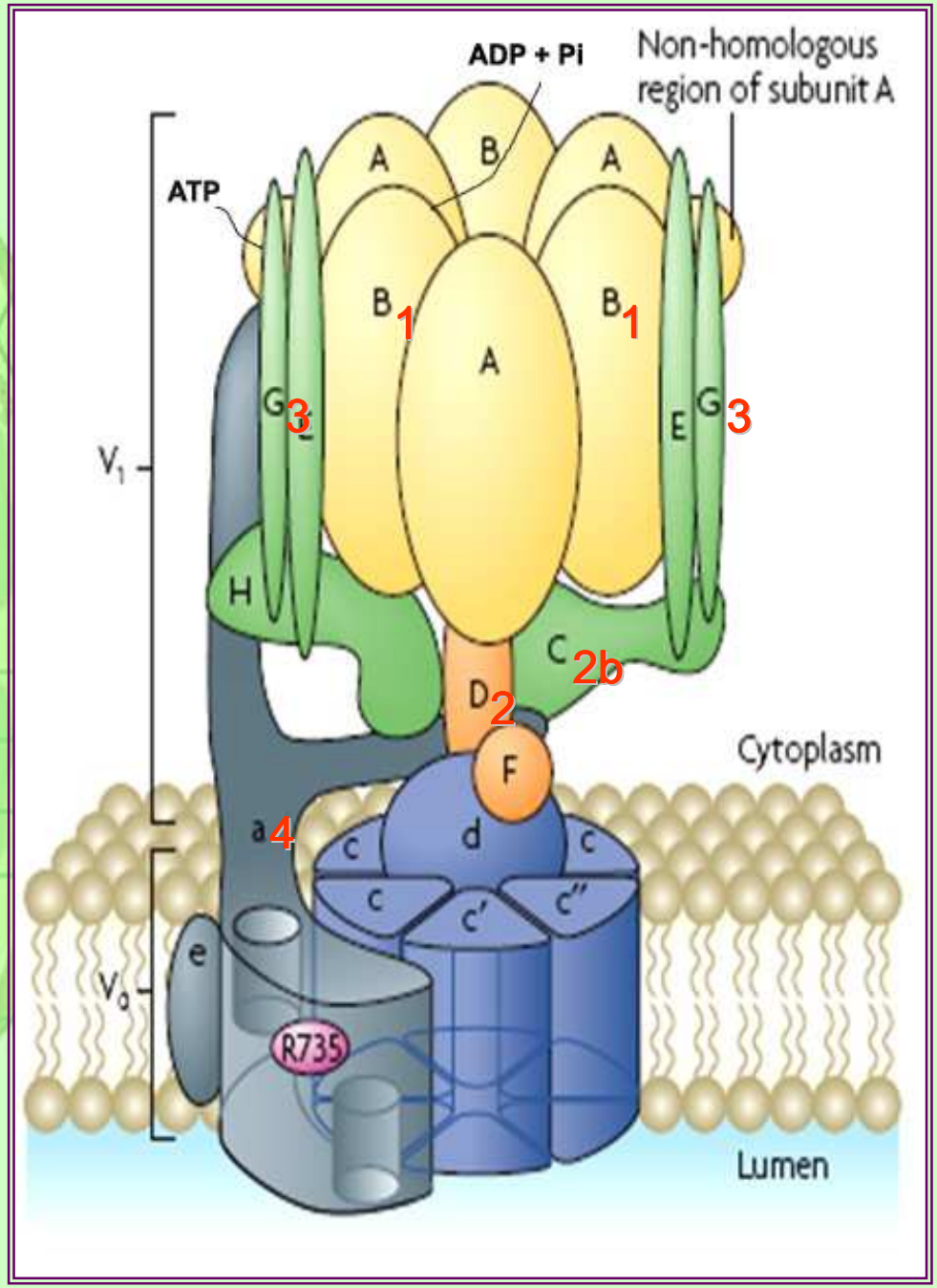
260 kDa composed of five subunits
in a possible complex of $a_1d_1c"_1(c, c')$

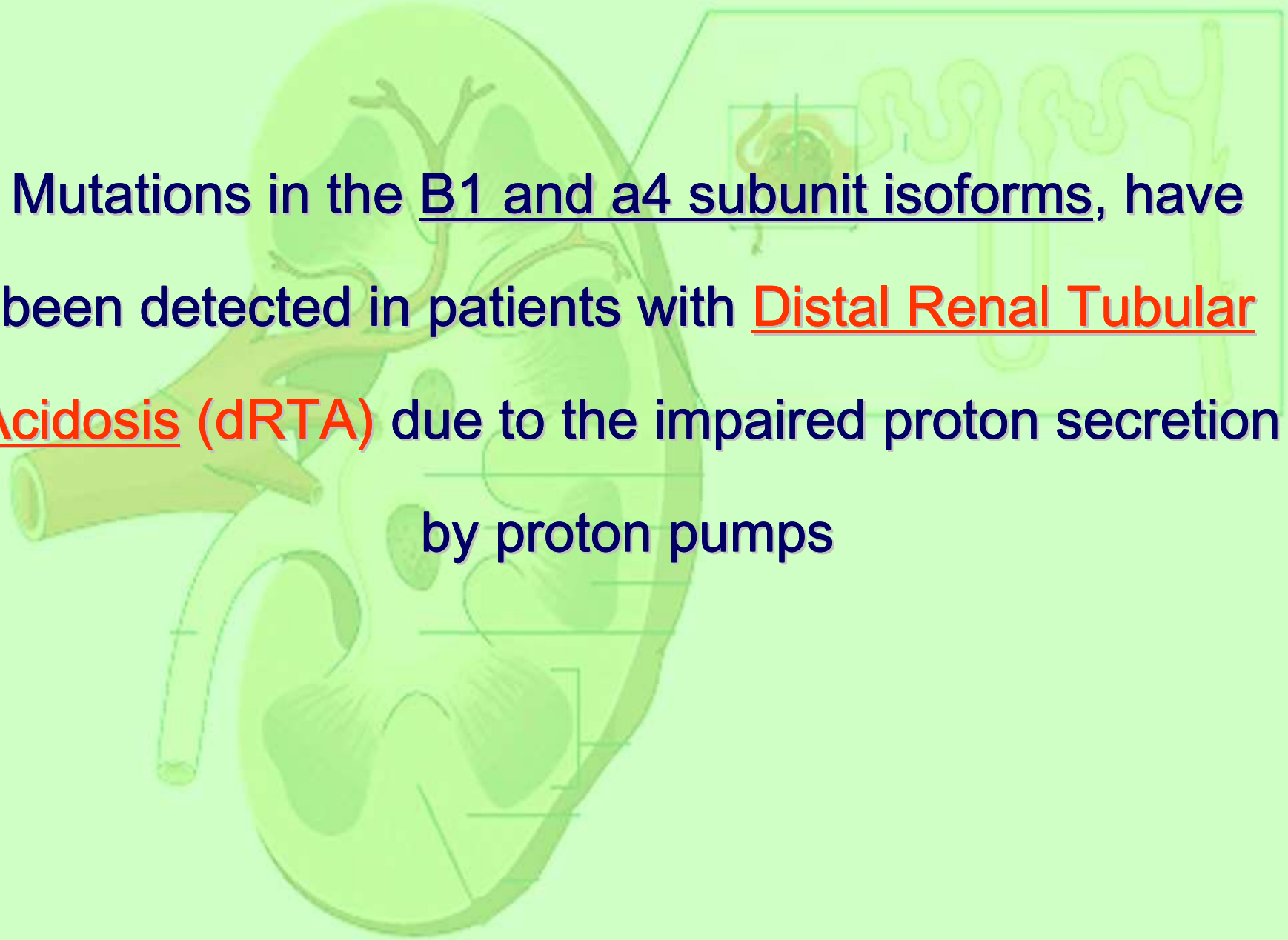


Mammals express a rich diversity of V-ATPase subunit isoforms

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 a4 subunit isoforms, have been detected in patients with Distal Renal Tubular Acidosis (dRTA) due to the impaired proton secretion by proton pumps

dRTA

PHYSIOLOGY -June 2007

Type of RTA	Subtype and Inheritance	Age at Presentation	Clinical Features	Protein	Gene(s)	OMIM
Distal (type 1)	Dominant	Older/adult	Mild/compensated metabolic acidosis Hypokalemia (variable) Hypercalciuria Hypocitraturia Nephrolithiasis Nephrocalcinosis Sometimes rickets/ osteomalacia Secondary erythrocytosis	AE1 encoding the basolateral Cl^-/HCO^-_3 exchanger	*SCL4A1*	179800
	Recessive	Childhood	Metabolic acidosis with hemolytic anemia Only reported in Southeast Asian populations	AE1	*SCL4A1*	602722
	Recessive with early onset hearing loss dRTA di tipo 1b	Infancy/childhood	Metabolic acidosis Early nephrocalcinosis Vomiting/dehydration Growth retardation Rickets Bilateral sensorineural hearing loss, from childhood	B1 subunit of H^+-ATPase	*ATP6V1B1*	267300
	Recessive with later onset hearing loss dRTA di tipo 1c	Infancy/childhood	As above, but later onset hearing loss in some (a few with normal hearing)	a4 subunit of H^+-ATPase	*ATP6V0A4*	602722

We clinically and genetically analyzed 30 families referred during this year to paediatric nephrologists and medical genetisists of Meyer Hospital with diagnosis of dRTA.

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STUDIO CLINICO-MOLECOLARE PER ACIDOSI TUBULARE DISTALE

Cognome | Nome

Data di nascita | Luogo di nascita

Residenza

Codice fiscale

Età alla diagnosi | Istituto di cura di provenienza

Medico referente

Padre (nome, cognome, luogo e data di nascita)

Madre (nome, cognome, luogo e data di nascita)

Anamnesi familiare

Albero genealogico allegato

Anamnesi personale

Esame obiettivo

Dati clinici	Alla diagnosi	Controllo	Controllo
altezza			
peso			
circonf cranio			
centile altezza			
centile peso			
centile circranio			
pressione arteriosa			
centile pa			
diuresi			
stipsi			
anoressia			
ipotonia			
rachitismo			
poliuria			
nefrocalcinosi			

Ipoacusia			
Altri			

Esami ematici

Esame	Alla diagnosi	Controllo	Controllo
azotemia			
creatininemia			
calcemia			
fosforemia			
fosfatasi alcalina			
sodiemia			
potassiemia			
cloremia			
pH ematico			
HCO3-			
BE			
gap anionico			
magnesiemia			
uricemia			
Altri			

Esami urinari

Esame	Alla diagnosi	Controllo	Controllo
creatininuria			
calciuria (spot)			
calciuria (24 h)			
sodio urinario			
potassio urinario			
cloro urinario			
pH urinario			
gap anionico			
magnesuria			
Altri			

Esami strumentali

Esame	Alla diagnosi	Controllo	Controllo
Ecografia renale			
CUM			
Rx scheletro			
Rx mano			
ECG			
Altri			

Terapia

Farmaco	Alla diagnosi	Controllo	Controllo
Bicarbonato Na			
Citrato K			
Vitamina D			
Calcio			
Altri			

Allegare consenso informato per analisi genetica

dRTA

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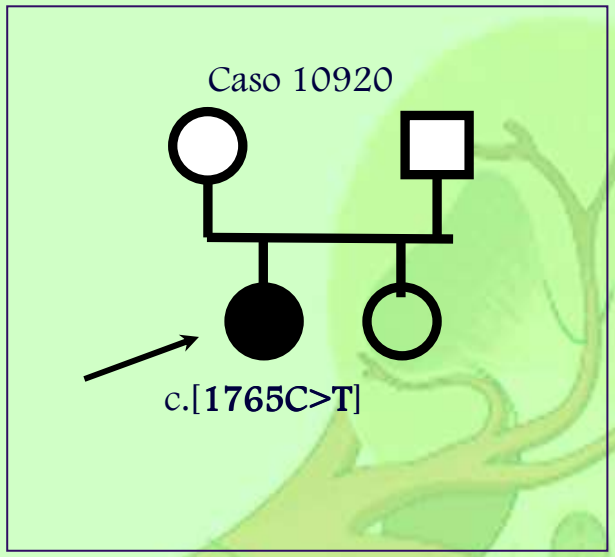
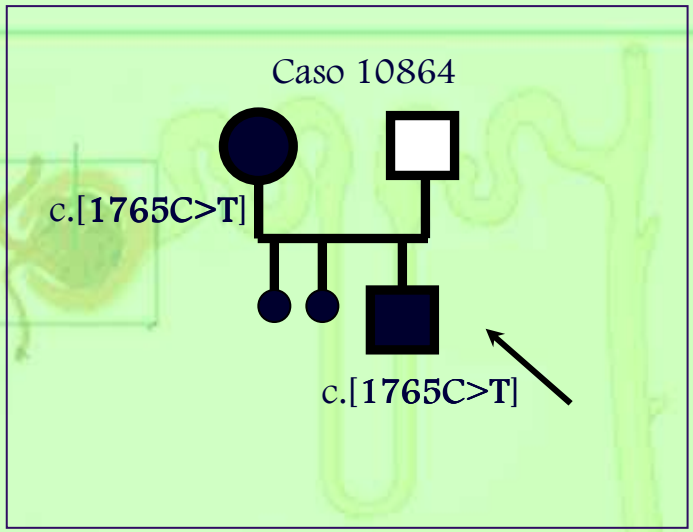
encoding the basolateral Cl^-/HCO^-_3 exchanger

SLC4A1



22a

- 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*



17a

- Scarso accrescimento
- Calcolosi renale diagnosticata a 10 a
- Diagnosi di dRTA con ipercalciuria, ipocitraturia
- osteomalacia

dRTA

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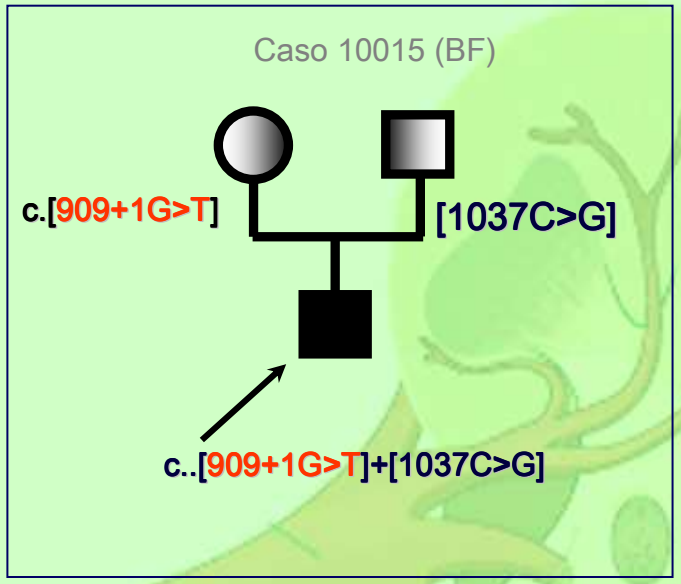
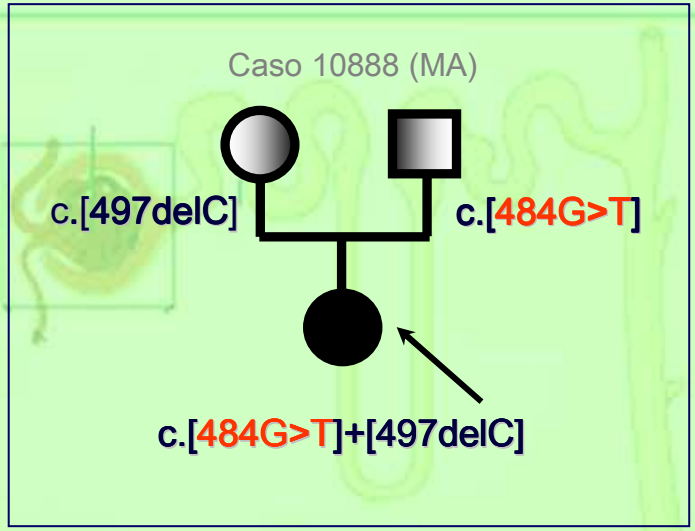
ATP6V1B1



36a3m

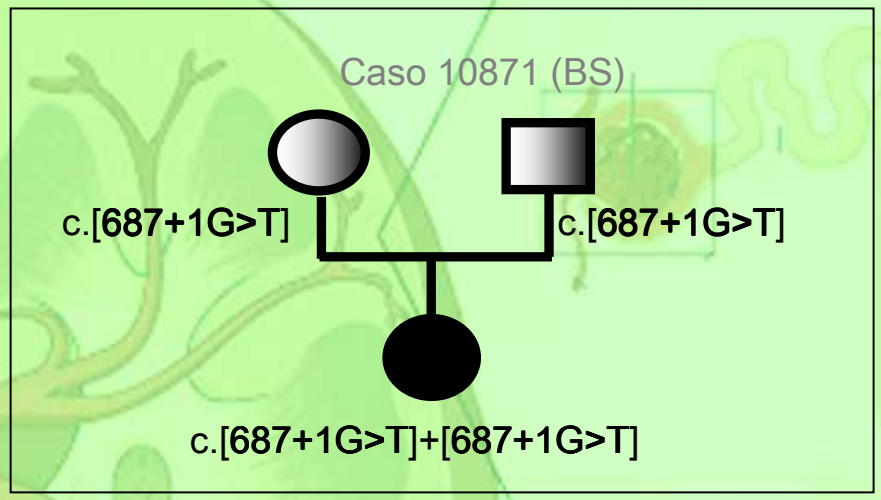
- Gravidanza: minaccia di aborto nel 1° trimestre
- 3° trimestre: tossicosi gravidica e parto prematuro al 7° mese
- Scarso accrescimento
- Sviluppo psicomotorio ritardato (prime parole 3 anni)
- 2a6m: diagnosi di acidosi tubulare renale distale con nefrocalcinosi
- 5 anni: sordità (protesi a 6 anni)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



12a

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

ATP6V1B1



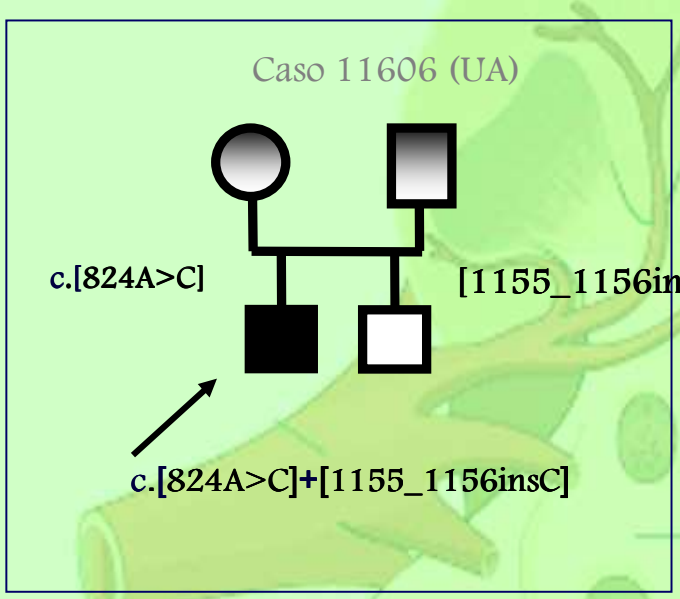
10a2m

•Dal 8° mese: anoressia e scarso accrescimento

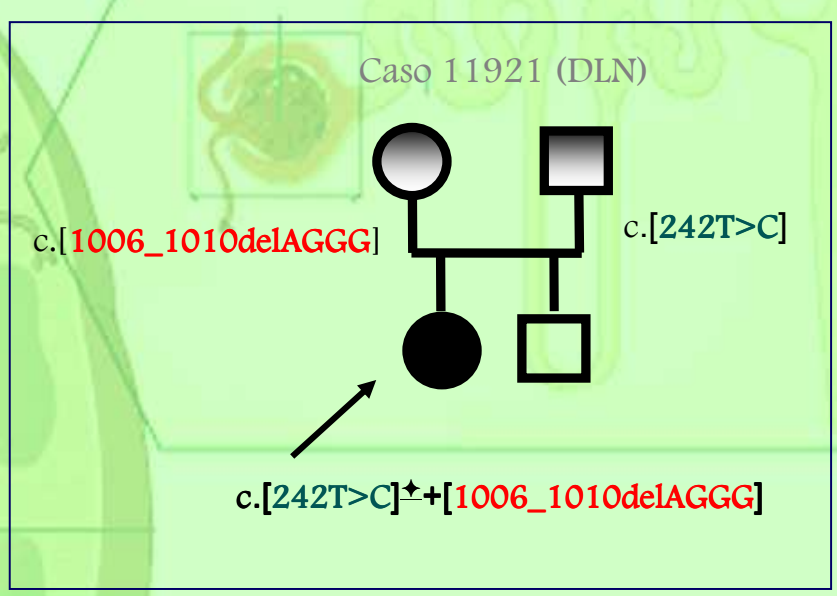
• 1a: Diagnosi di acidosi tubulare renale distale con nefrocalcinosi, disidratazione e diselettrolitemia

• 3a: sordità neurosensoriale (uso di protesi)

ATP6V1B1



2a

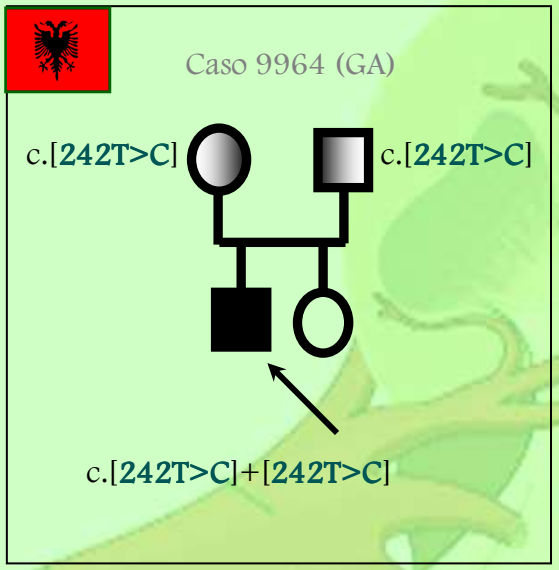


4m

- 11 mesi: Arresto dell'accrescimento
- 2 anni: Diagnosi di sordità neurosensoriale bilaterale grave
- Marcata ipercogenicità a livello delle piramidi renali

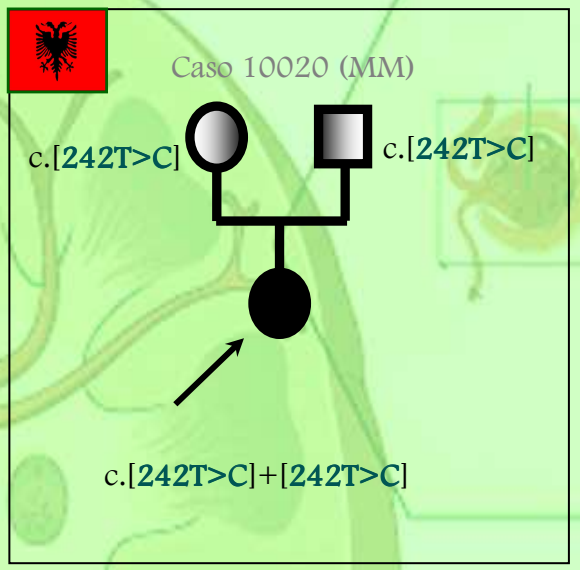
- 3 mesi: Arresto dell'accrescimento, nefrocalcinosi.
- Ipoacusia neurosensoriale
- Diagnosi di Acidosi Tubulare Renale distale

ATP6V1B1



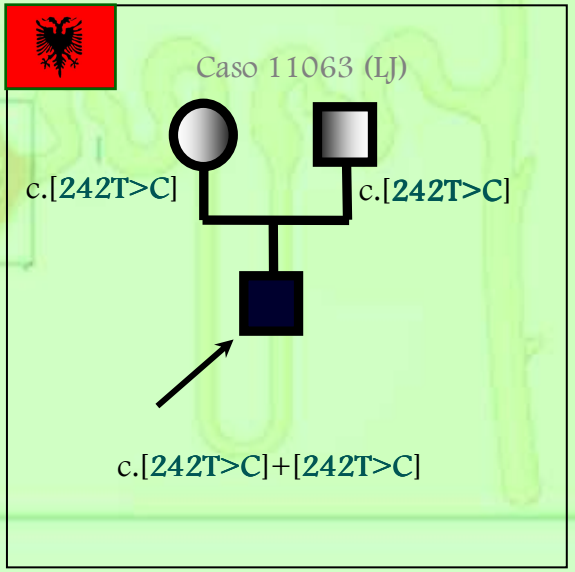
2a5m

- 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)



4a10m

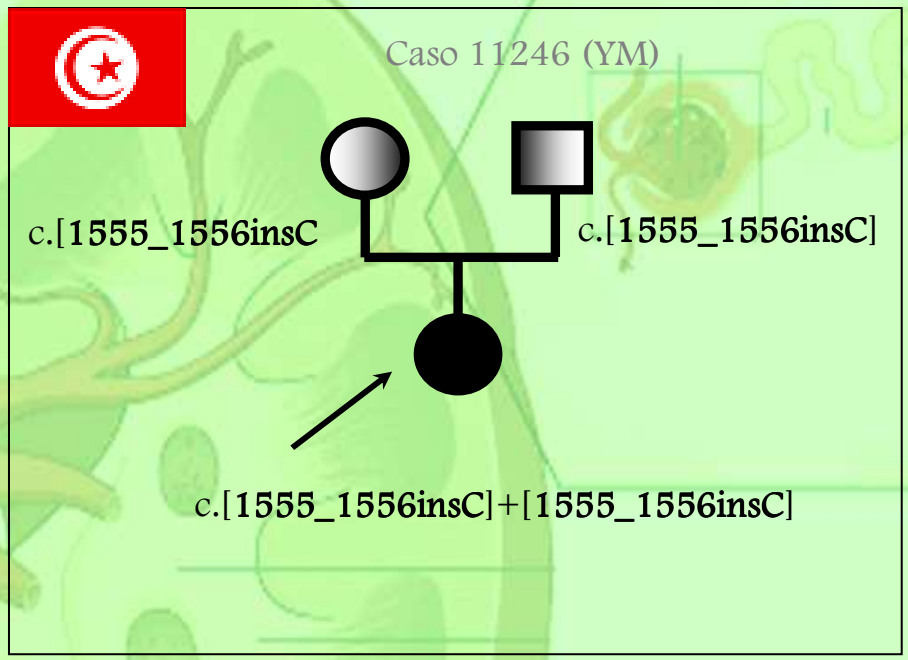
- Gravidanza: malformazione di forma e posizione del rene dx
- 3 mesi: scarso accrescimento
- 1a10m: Diagnosi di acidosi tubulare renale distale con nefrocalcinosi
- 2a1m: diagnosi di sordità neurosensoriale bilaterale (protesi)



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)

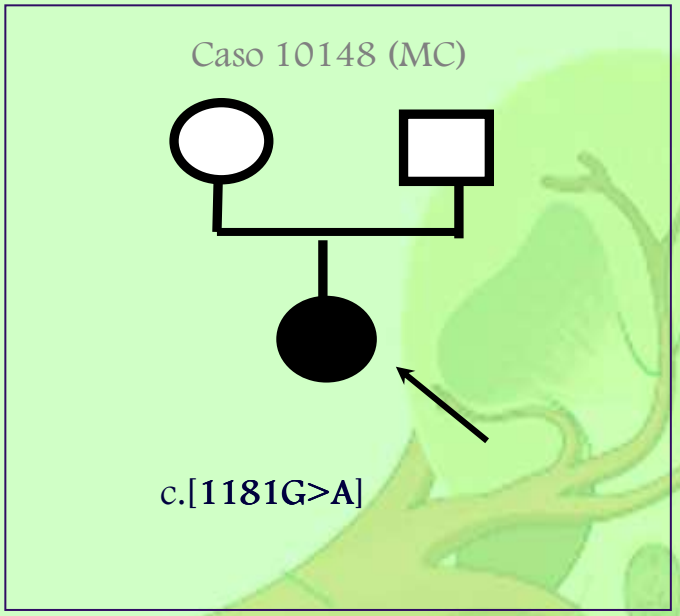
ATP6V1B1



3m

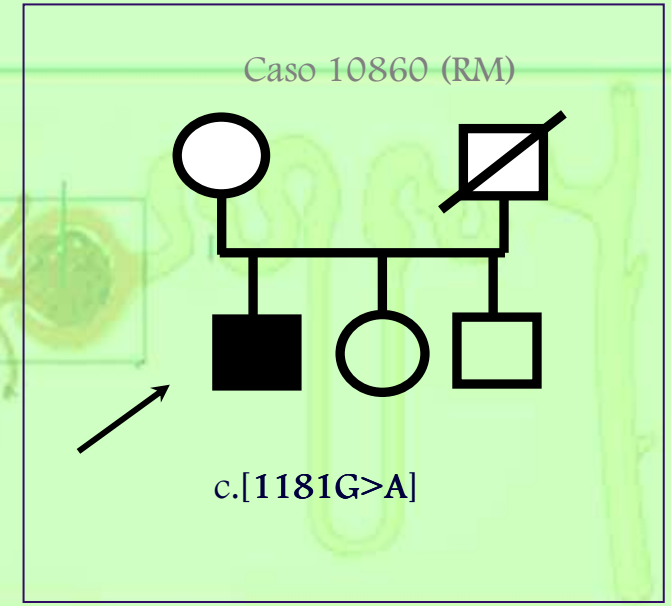
- Gravidanza: riferita nella norma
- 3 mesi: scarso accrescimento
- diagnosi di acidosi tubulare renale distale con nefrocalcinosi
- lieve rachitismo
- sordità neurosensoriale bilaterale

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; 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
- 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

Journal of the American Society of Nephrology

Clinical characteristics of dRTA patients with *ATP6V1B1* mutations[a]

Patient	Age	Origin	Age at Diagnosis	Gender	Consanguineous	SNHL (Age at Diagnosis)	Blood pH	HCO_3^-	K^+	Urine pH	Nephrocalcinosis	Mutation		
												DNA	Protein	Characteristic
1-1	22 yr	Algeria	3 wk	F	Yes	Yes[b]	ND	10	2.9	7.2	Yes	1149–1155insC	I386fsX441[d]	Homozygous
4-1	4 yr	Algeria	1 mo	F	Yes	Yes (3 yr) severe first degree	ND	18	3.5	8	Yes	IVS2-1 G>C	Loss of splice acceptor site	Homozygous
6-1	7 yr	France	3 wk	F	No	Yes (7 yr) mild	7.28	14.4	2.4	7.3	Yes	1181G>A	R394Q	Heterozygous
8-1	4 yr	France	5 mo	F	No		7.30	12	3.2	7.3	Yes	1181G>A	R394Q	Heterozygous
18-1	12 yr	Algeria	6 mo	M	Yes	Yes[b]	7.16	12.9	1.7	6.9	Yes	1149–1155insC	I386fsX441[d]	Homozygous
20-1	13 yr	Tunisia	5 mo	F	Yes	Yes (3 yr) severe first degree	7.29	17	2.3	8	Yes	IVS2-1 G>C	Loss of splice acceptor site	Homozygous
28-1	9 mo	Algeria	6 m	F	No	Yes (2 yr) moderate second degree	7.24	13	3.3	Inappropriate	Yes	IVS2-1 G>C /1149–1155insC	Loss of splice acceptor site	Compound
													I385fsX441[d]	Heterozygous
36-1	9 mo	Algeria	5 mo	F	Yes	No[c]	7.15	9	2.6	7.1	Yes	1149–1155insC	I386fsX441[d]	Homozygous
37-1	15 mo	Tunisia	4 mo	F	Yes	No	7.24	10	2.8	7.5	Yes	1149–1155insC	I386fsX441[d]	Homozygous
39-1	15 mo	Algeria	ND	F	No	Yes (1 yr) severe second degree	ND	16	4.7	Inappropriate	Yes	1037C>G	P346R[e]	Compound
												1149–1155insC	I386fsX441[d]	Heterozygous

[a]Nucleotides numbered according to the sequence in GenBank NM_001692.
[b]Data concerning SNHL were not available, but onset probably was early given abnormal language and cognitive development.
[c]Father's cousin with dRTA and SNHL.
[d]Mutation previously described (3).
[e]Mutation previously described (5).

Rosa Vargas-Poussou et al: *J Am Soc Nephrol* 17: 1437–1443, 2006.

dRTA

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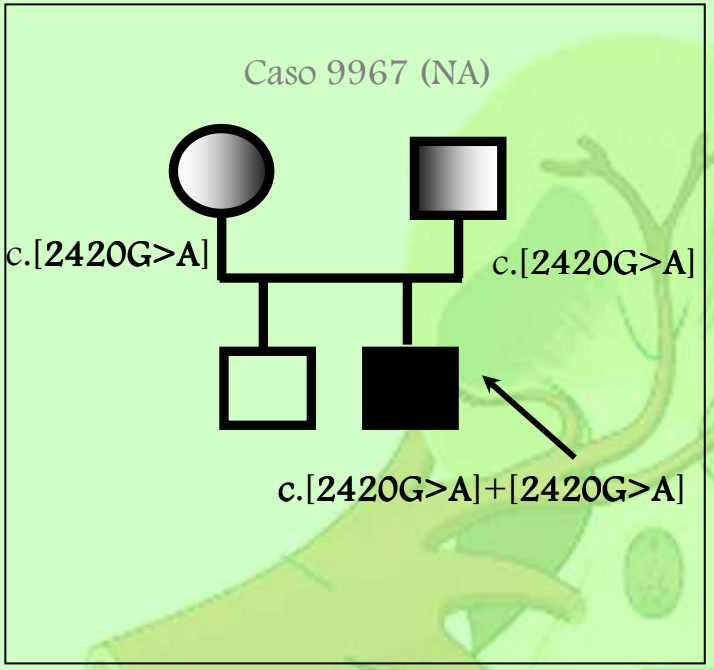
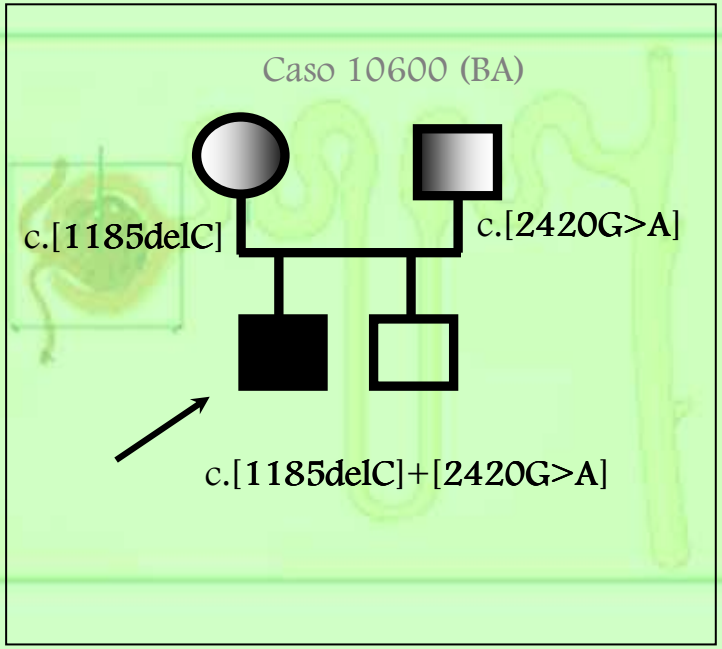
ATP6V0A4





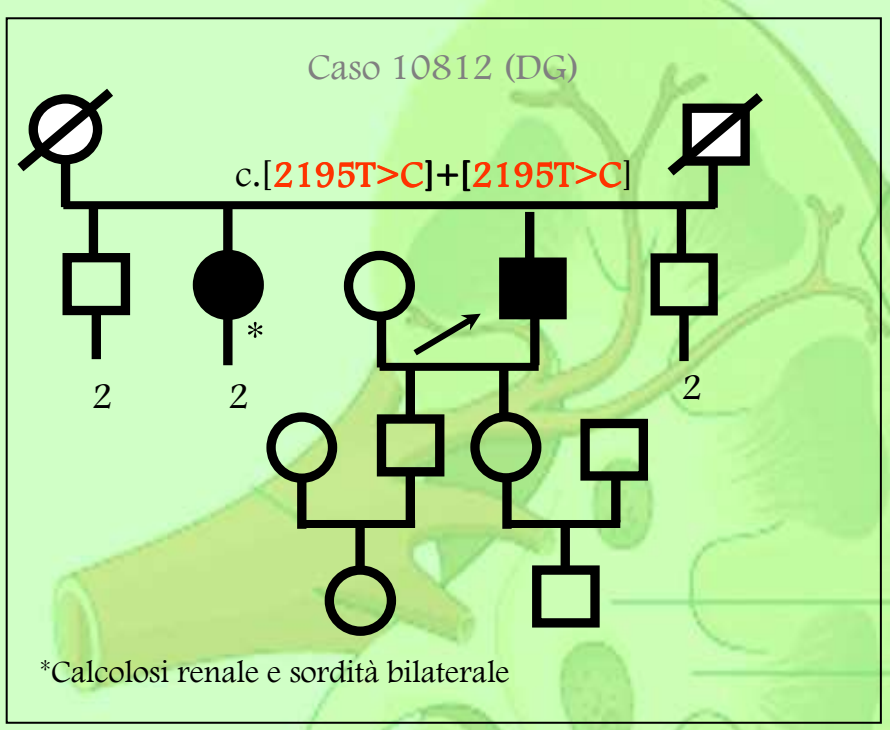
2a6m

- Gravidanza regolare
- 1 mese: grave ritardo di crescita.
- Diagnosi di acidosi tubulare renale distale e sordità neurosensoriale bilaterale (protesi)

25a

- Acidosi tubulare renale distale
- Nefrocalcinosi
- 17 anni: ipoacusia neurosensoriale bilaterale

ATP6V0A4



55a11m

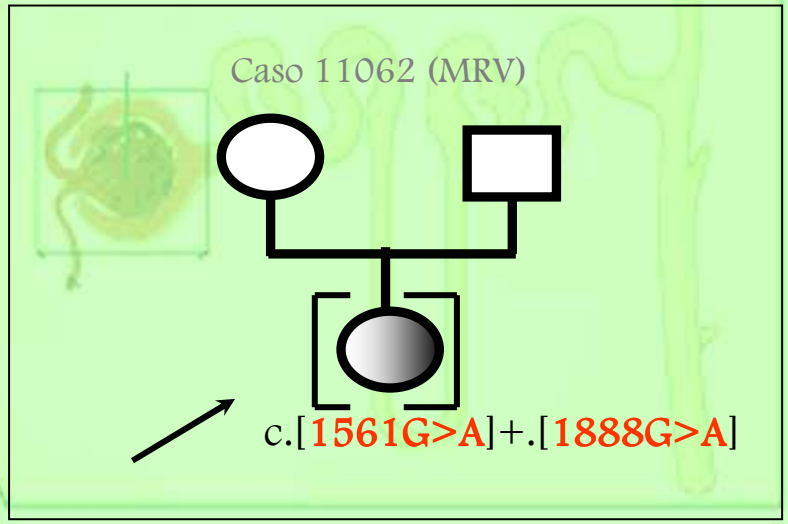
•25-55anni: numerosi episodi di coliche renali con emissione di calcoli

•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 Strptococchi di tipo D

•Rene dx pielonefrite



4a

•Origine colombiana (bambina adottata)

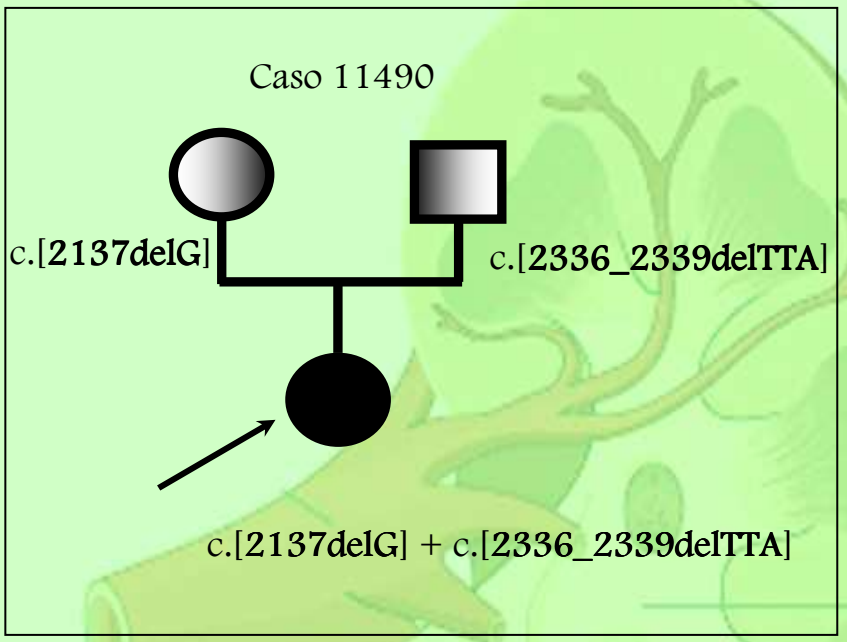
•Deficit di crescita (<<3° centile)

•Nefrocalcinosi

• No ipoacusia

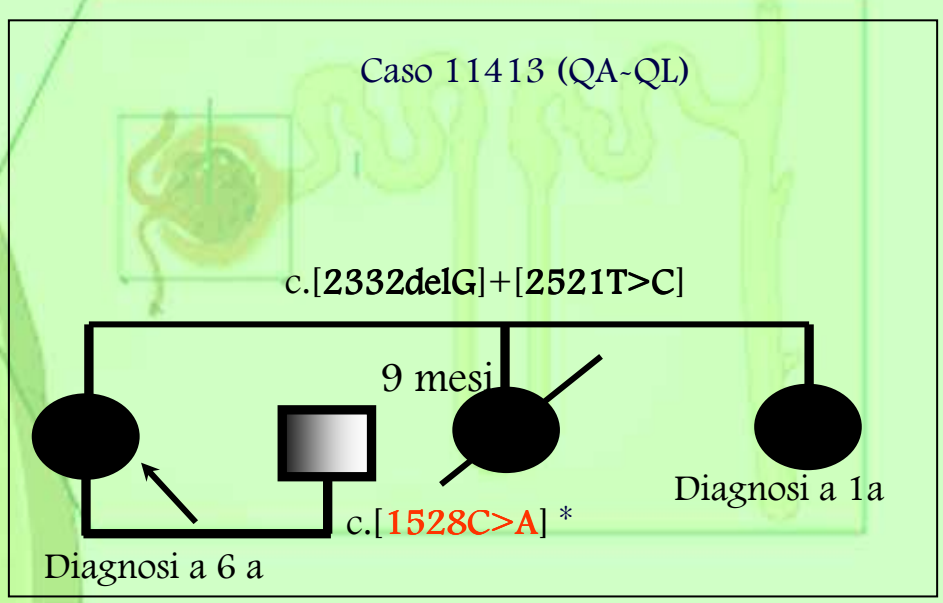
•Acidosi e infezioni ricorrenti delle vie urinarie

ATP6V0A4



9m

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

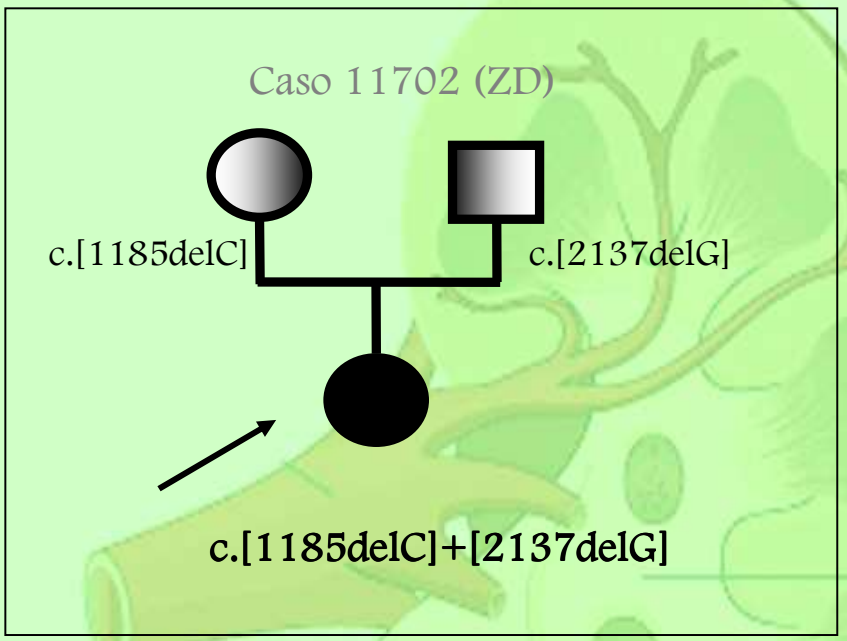


38a 26a

Acidosi Tubulare Renale Distale

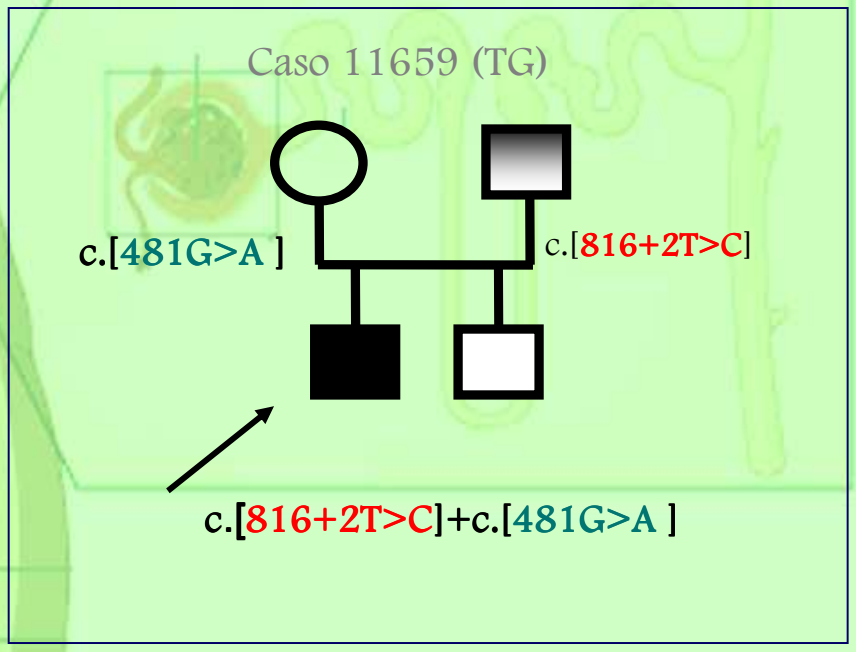
- Nefrocalcinosi
- Lieve ipoacusia
- *Il marito non presenta caratteristiche cliniche di acidosi, ha una mutazione de novo con predizione benigna

ATP6V0A4



1a6m

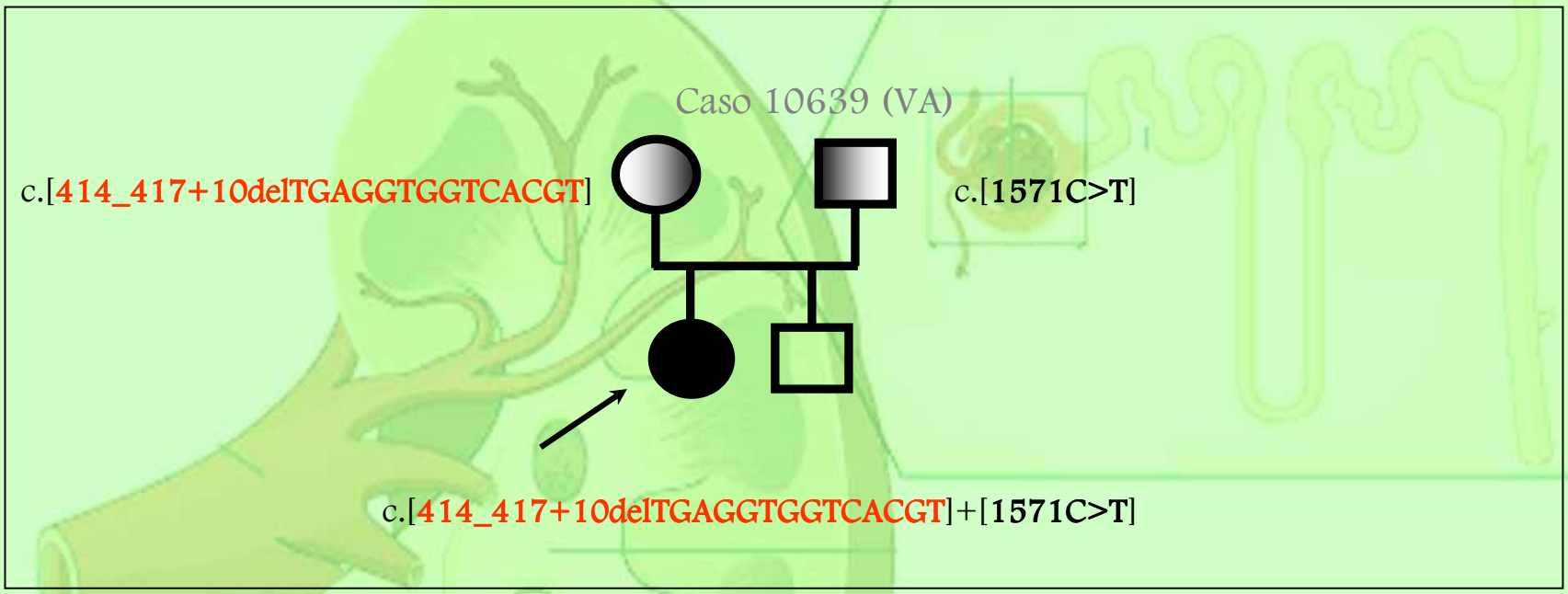
- 2 mesi: ricovero per scarso accrescimento, segni di sottoidratazione, acidosi metabolica e nefrocalcinosi. Diagnosi di acidosi tubulare renale distale
- NON presenta sordità neurosensoriale
- Genitori con calcoli renali



7a6m

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

ATP6V0A4



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

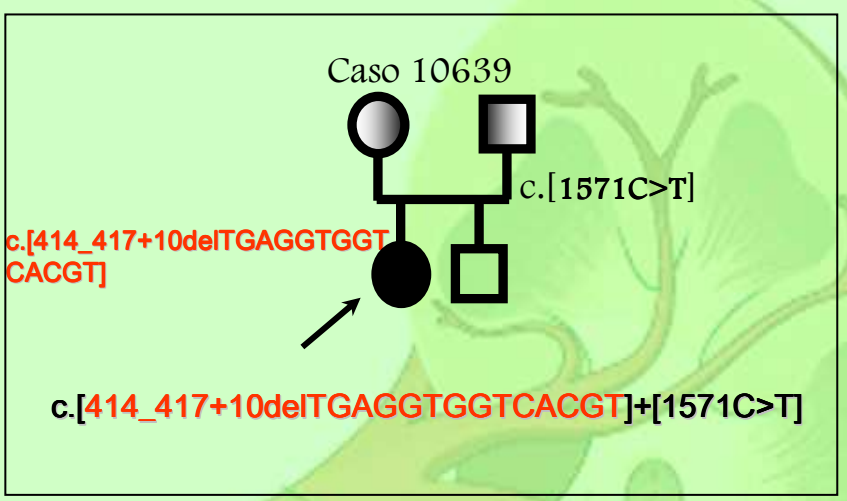
- In tutti sono state riscontrate mutazioni patogeniche, otto delle quali mai riportate in letteratura

- 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.

ATP6V0A4



18a4m

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

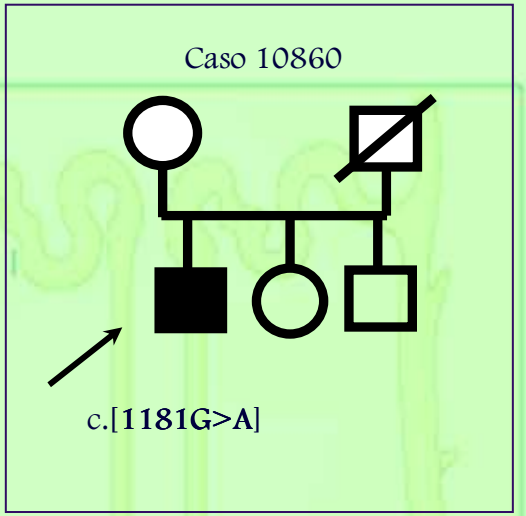
• 5 anni: Diagnosi di RENE A SPUGNA MIDOLLARE BILATERALE

•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

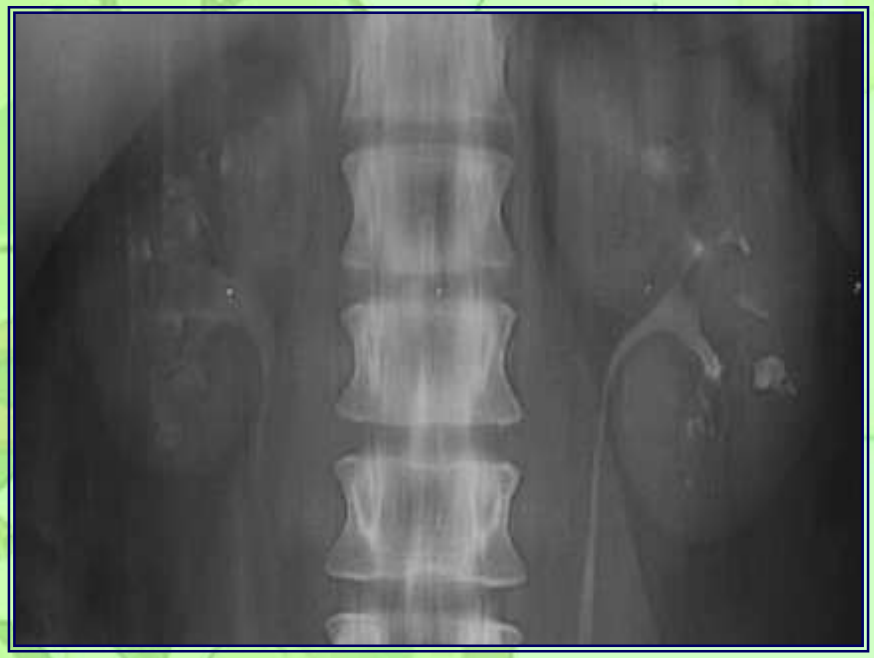
•28 anni: ripetute ESWL a sin

•34 anni: nefrocalcinosi; calcificazioni papillari diffuse; macroematuria ogni 2-3 mesi. Sordità neurosensoriale bilaterale.

•RM lieve

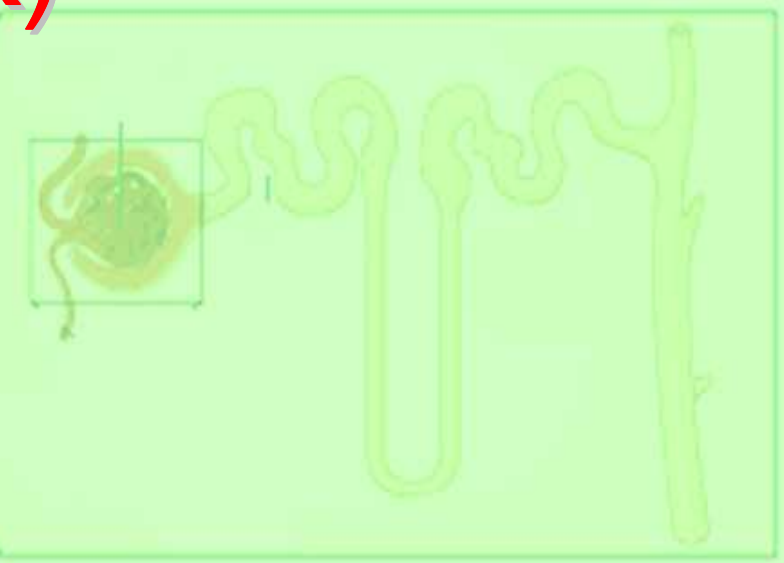
•Familiari: NO MUTAZIONE

Medullary sponge kidney (MSK)



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

(MSK)

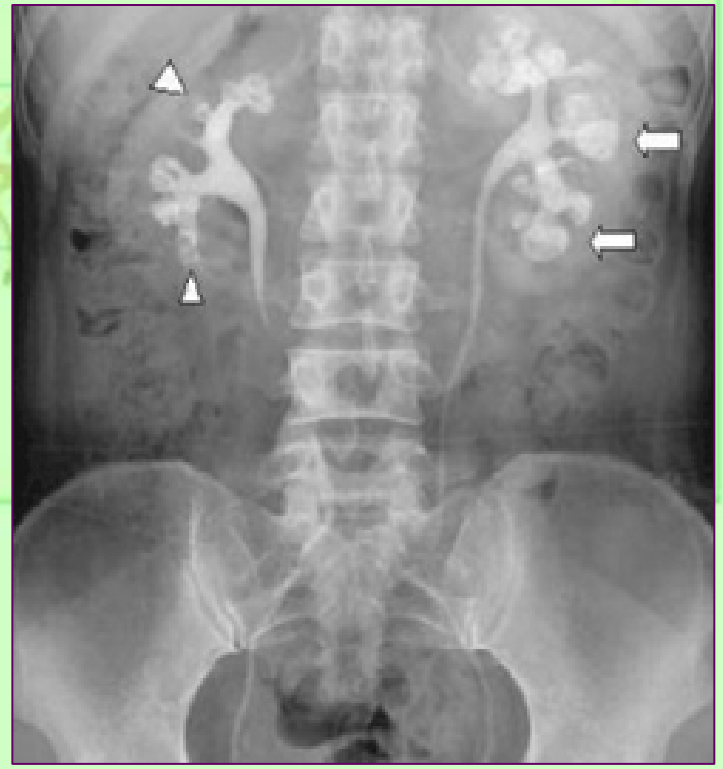


Clinical phenotype:

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

Exceptionally, chronic renal failure

(MSK)

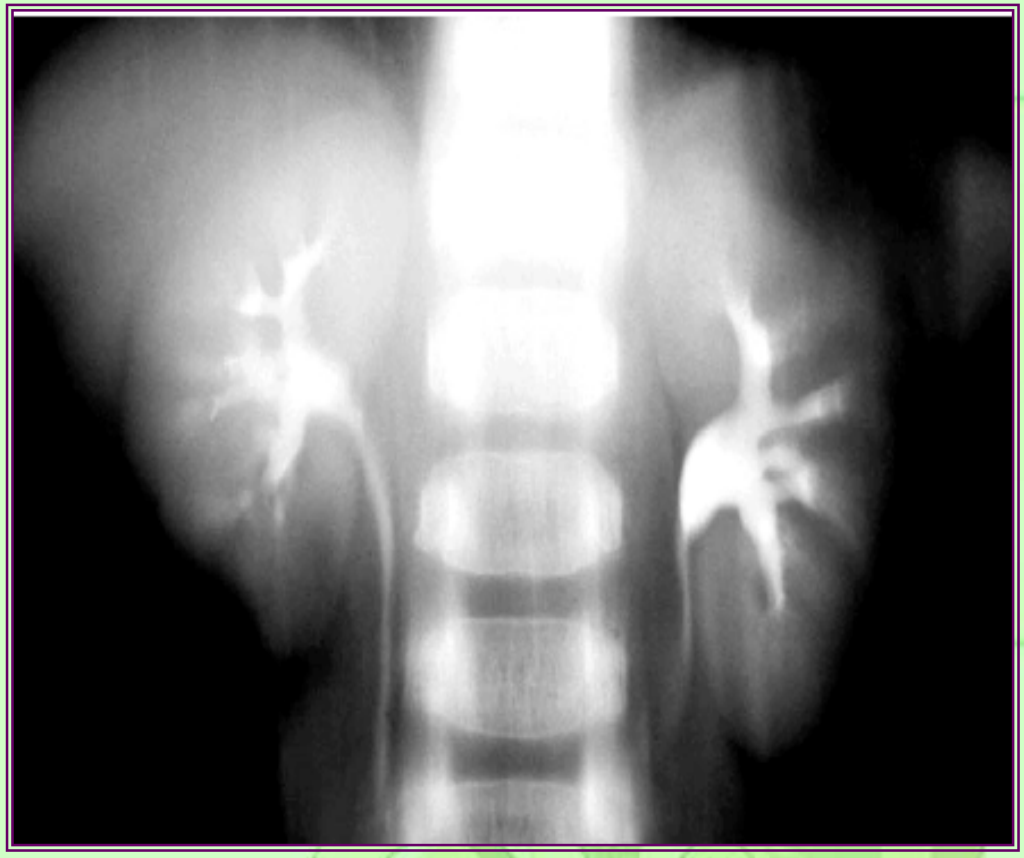


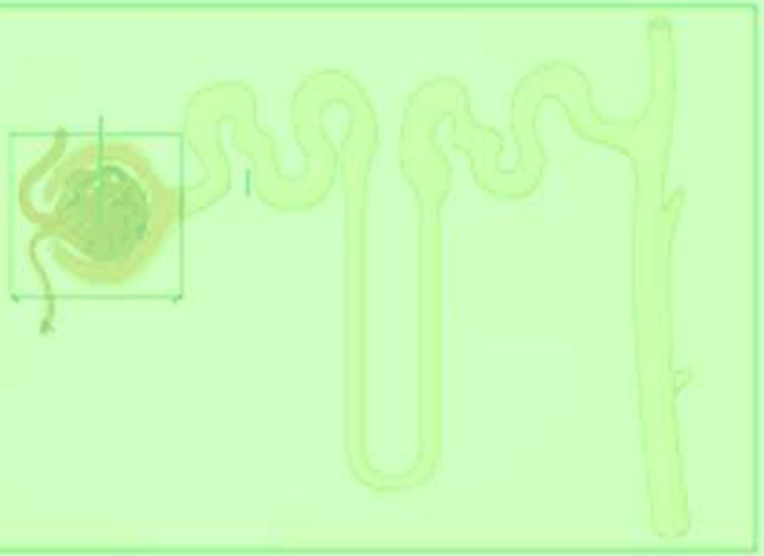
Diagnosis is radiogragraphic:

ectatic papillary ducts give the apparance 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

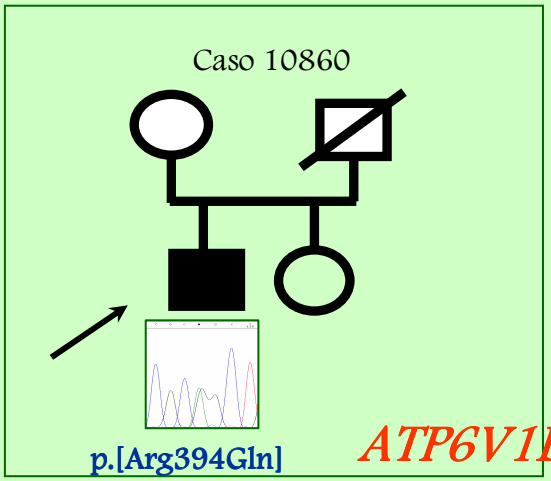
Case 1



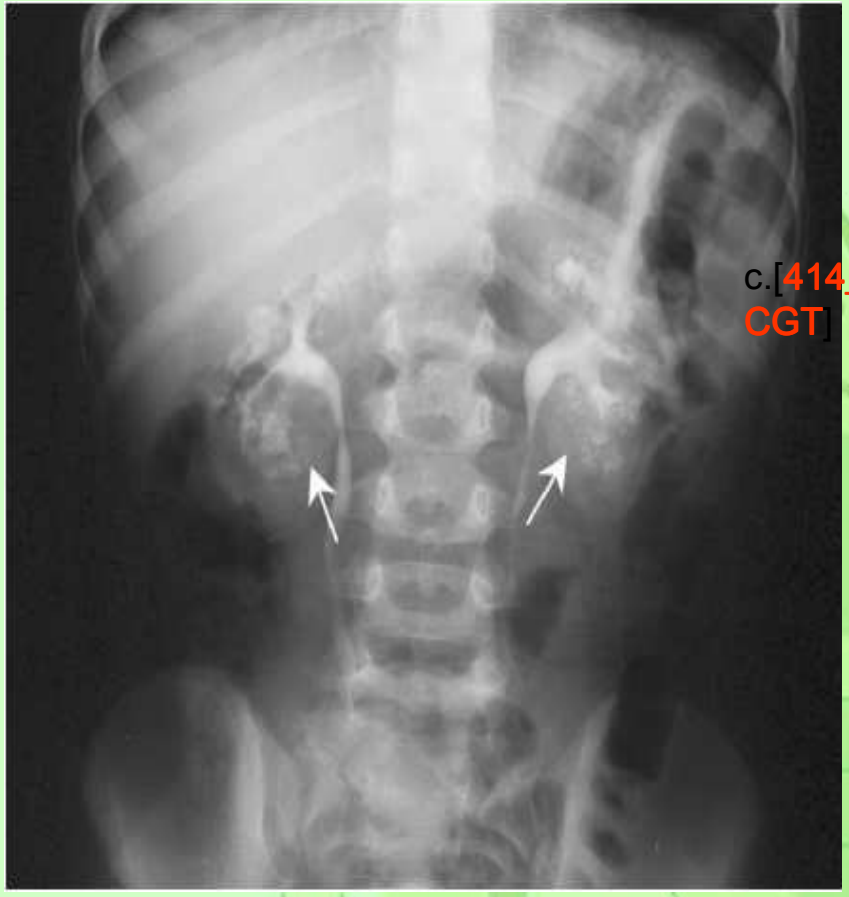


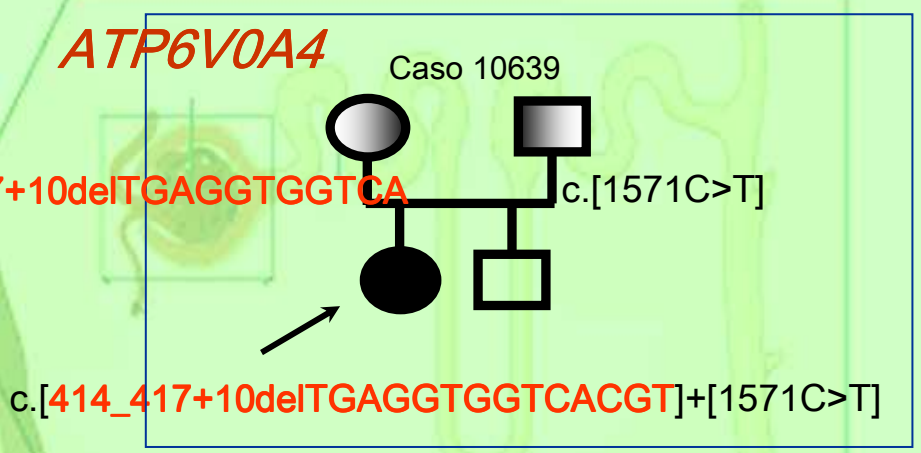
Molecular analysis showed the presence of a heterozygous missense mutation, c.1181G>A (p.Arg394Gln), of the *ATP6V1B1* gene; no mutations of the *ATP6V0A4* gene were detected

Array-CGH analysis did not reveal any imbalance inside the two genes or in their flanking regions



Case 2





Analysis of the *ATP6V0A4* gene coding region revealed the presence of two distinct mutations, a missense substitution, c.1571C>T (p.Pro524Leu) and a 14 bp 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

Eur J Pediatr (2006) 165: 648–651
DOI 10.1007/s00431-006-0125-0

SHORT REPORT

Belde Kasap · Alper Soylu · Oğuz Ören ·
Mehmet Türkmen · Salih Kavukçu

Medullary sponge kidney associated with distal renal tubular acidosis in a 5-year-old girl

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.

Eur J Pediatr (2006) 165: 648–651
DOI 10.1007/s00431-006-0125-0

SHORT REPORT

Belde Kasap · Alper Soylu · Oğuz Ören ·
Mehmet Türkmen · Salih Kavukçu

Medullary sponge kidney associated with distal renal tubular acidosis in a 5-year-old girl

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.

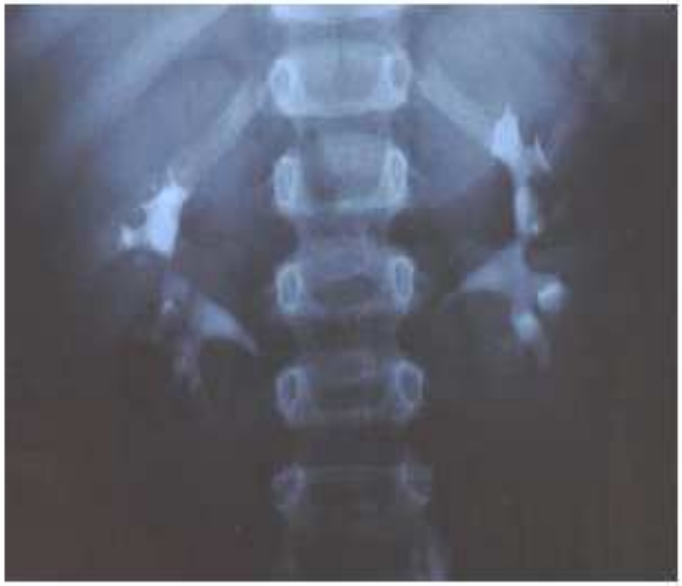


Fig. 2 Intravenous urography showing accumulation of contrast in papillary cysts giving papillae the appearance of "bouquet of flowers"

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

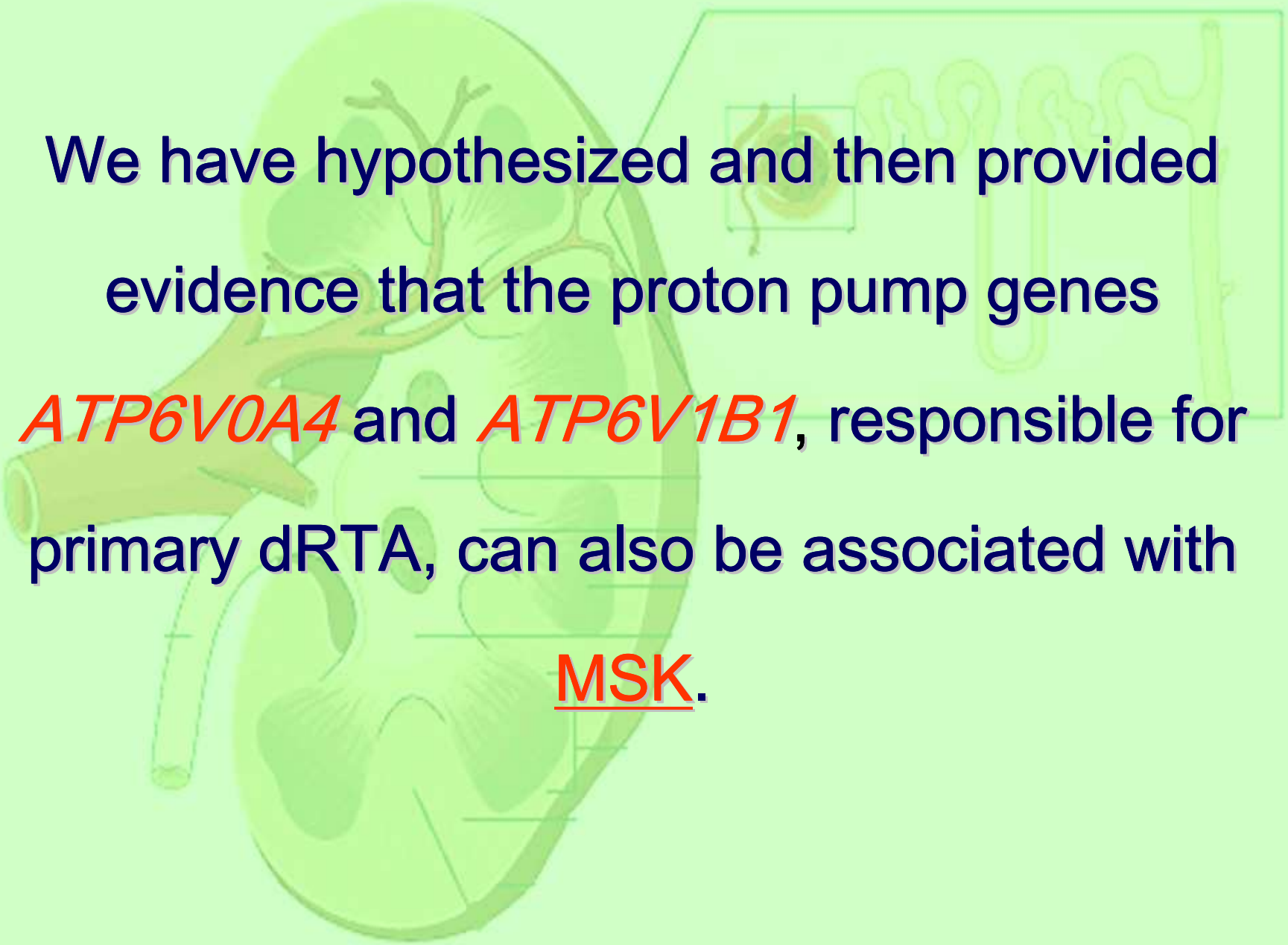
(Gambaro et al, 2006- Kidney International 69, 663-670)

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.

Diouf B et al. (2000) Nephrol Dial Transplant 15: 2062–2066

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.

Nephrol Dial Transplant (2009) 1 of 5
doi: 10.1093/ndt/gfp160

NDT
Nephrology Dialysis Transplantation

Original Article

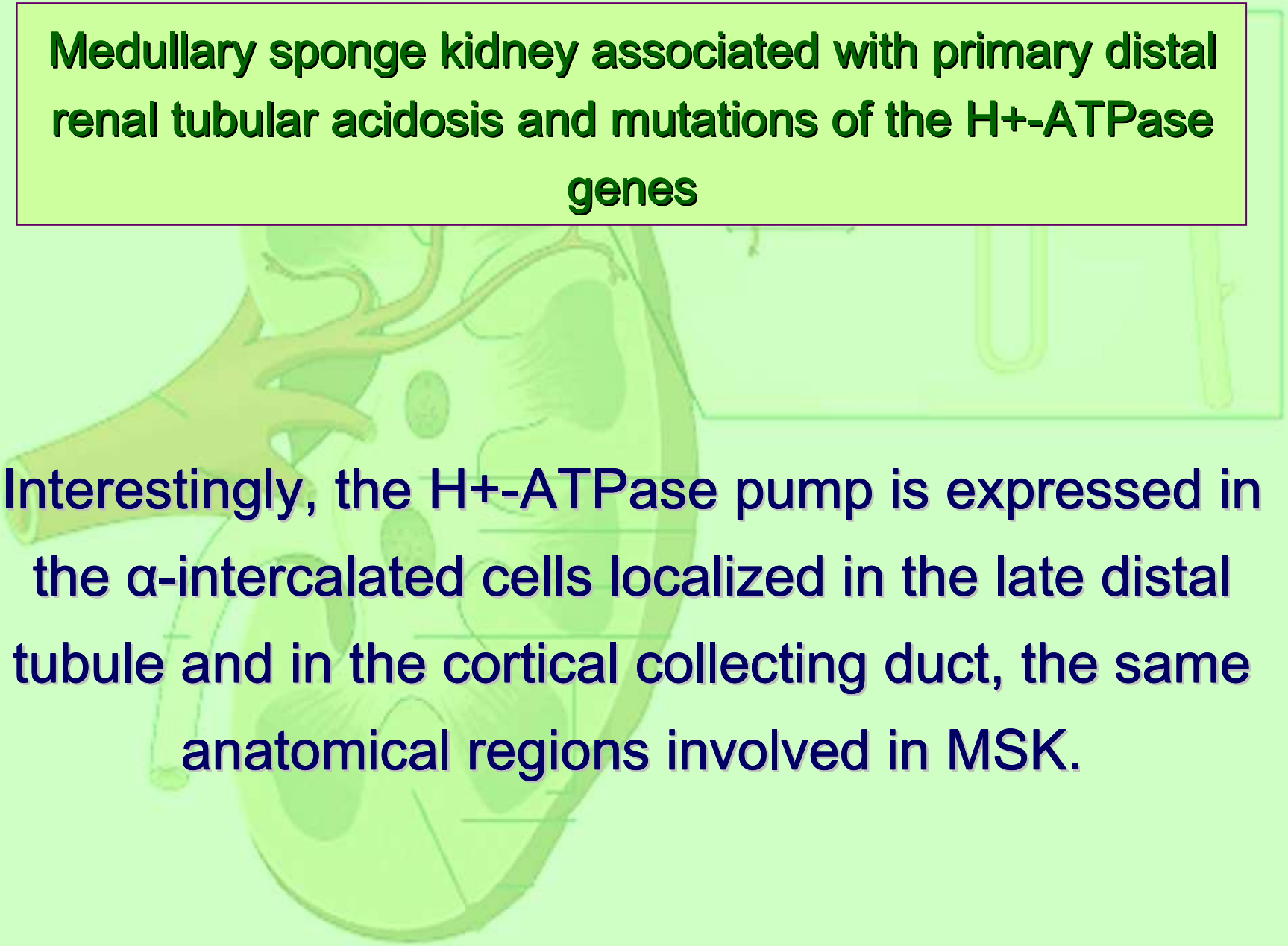
Medullary sponge kidney associated with primary distal renal tubular acidosis and mutations of the H^+-ATPase genes

Ilaria Carboni[1], Elena Andreucci[1,2], Maria R. Caruso[3], Roberto Ciccone[4], Orsetta Zuffardi[4], Maurizio Genuardi[1,2], Ivana Pela[5] and Sabrina Giglio[1,2]

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.

RINGRAZIAMENTI

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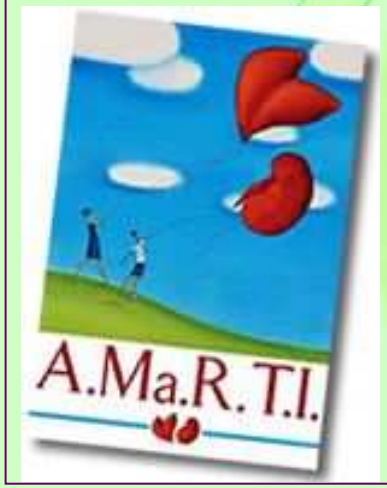
UNIVERSITA' DI FIRENZE

ALDESIA PROVENZANO

Associazione contro le
Malattie Renali
della Toscana per l' Infanzia
Sig.ra PATRIZIA TOFANI



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AOU MEYER- Dip PEDIATRIA
UNIVERSITA' DI FIRENZE

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

Elena Andreucci MD[1], Benedetta Bianchi MD[2], Ilaria Carboni[1], Giancarlo Lavoratti MD[3], Marzia Mortilla MD[4], Claudio Fonda MD[4], Minna Bigozzi MD[2], Maurizio Genuardi MD[1,5],Sabrina Giglio MD[1,5]& Ivana Pela MD[3]

Pediatric Nephrology, published July 2009

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

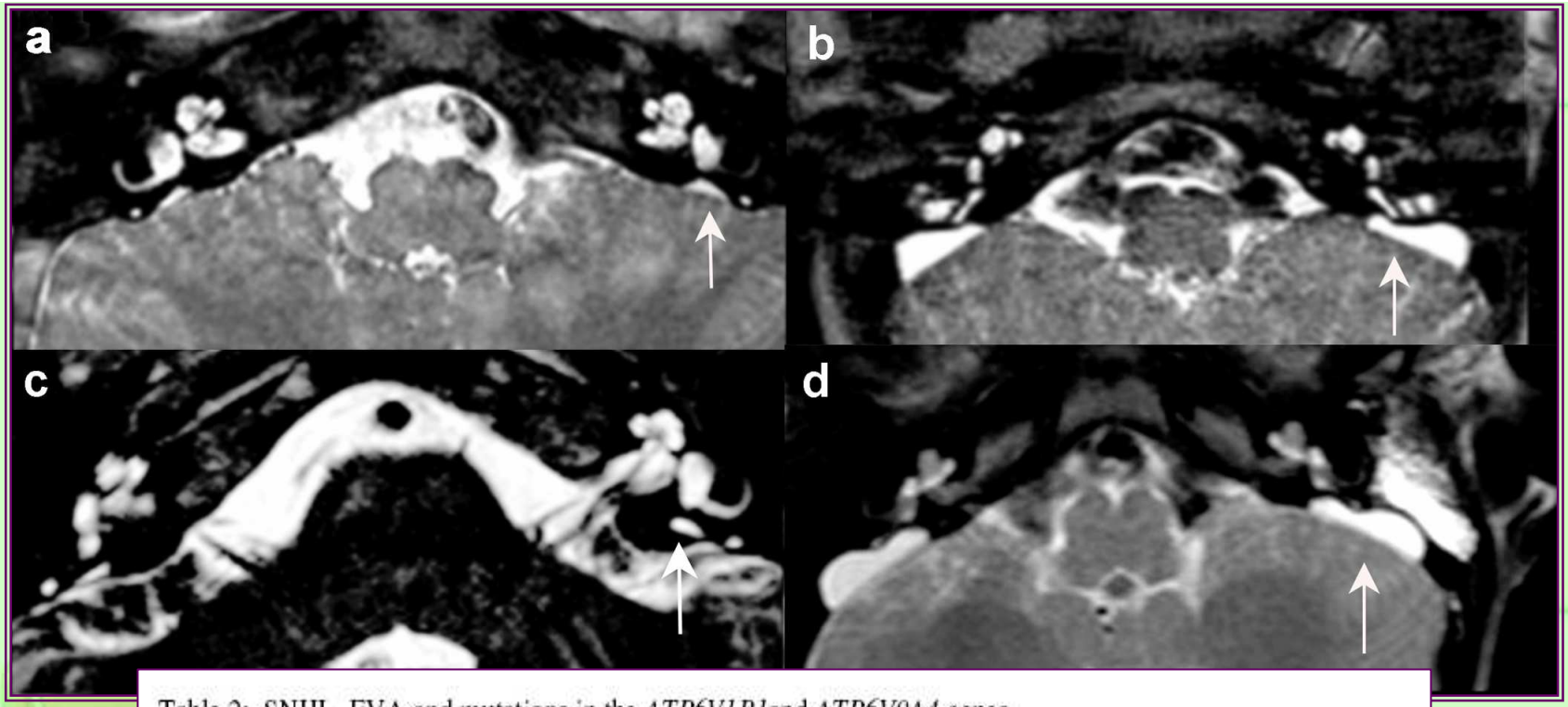

Table 2: SNHL, EVA and mutations in the *ATP6V1B1* and *ATP6V0A4* genes

Patients Sex	SNHL	EVA	*ATP6V1B1* gene mutation	*ATP6V0A4 gene* mutation
Case 1 M	Early	Mild (MRI at 2 y)	c.[242T>C]+[242T>C] (p.Leu81Pro)	
Case 2 F	Early	Evident (MRI at 22 m)	c.[242T>C]+[242T>C] (p.Leu81Pro)	
Case 3 M	Late (at 17 y)	Absent (MRI at 24 y)		c.[1185delC]+[2420G>A] (deletion c.1185del C and missense mutation p.Arg807Gln)
Case 4 M	Early	Evident (MRI at 3 y)		c.[2420G>A]+[2420G>A] (p.Arg807Gln)

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
Yeasts (S. cerevisiae)

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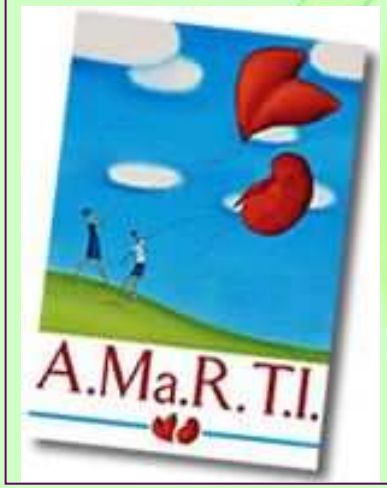

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