

**Identificazione e valutazione dell'impatto patogenetico di varianti introniche
profonde del gene *UROS* in pazienti affetti da porfiria eritropoietica congenita (CEP)**

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Disclosures

Company name

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Employee

Consultant

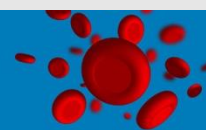
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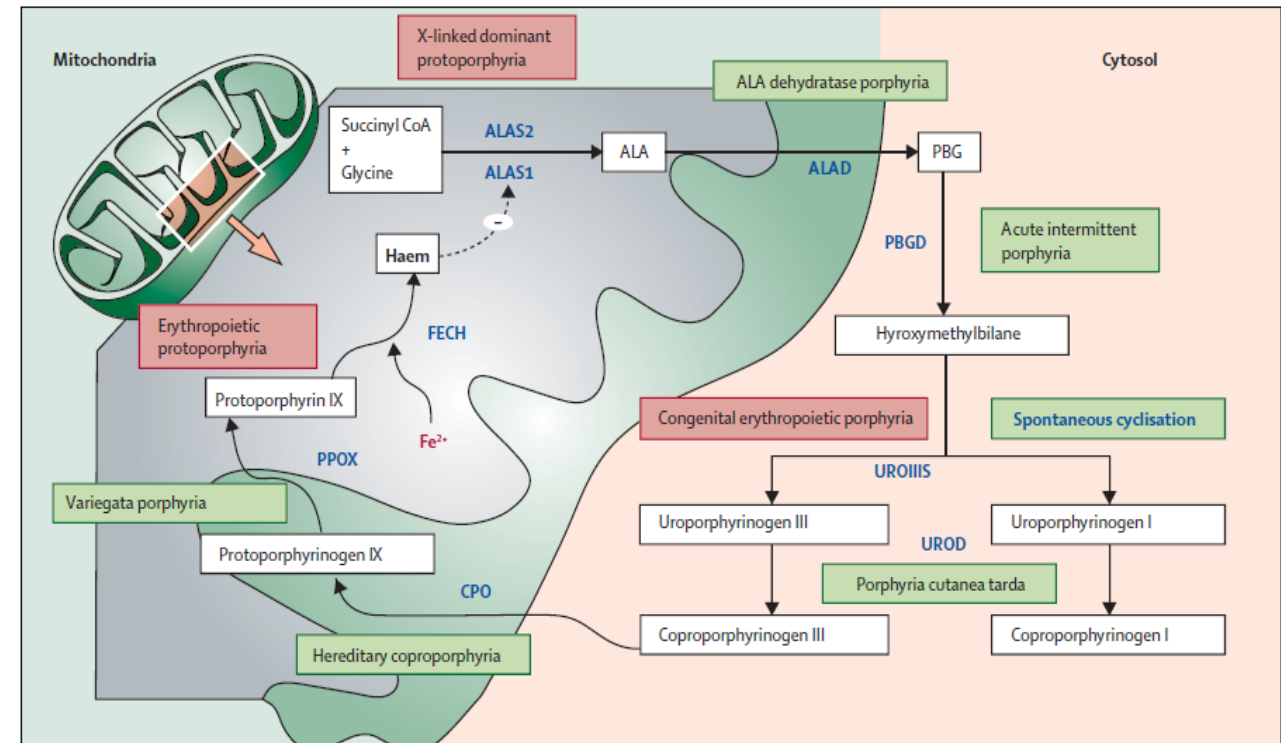
Other

**No conflicts of
interest to disclose**



Congenital Erythropoietic Porphyria

- Defect in heme biosynthesis
- Prevalence: <1 case per million (220–250 cases)
- Erythropoietic porphyria
- Chronic porphyria
- Variable onset



Congenital Erythropoietic Porphyria (CEP)

A highly heterogeneous clinical spectrum

More severe

Milder

Non-immune hydrops fetalis and potentially intrauterine death



- Non-immune hydrops fetalis
- Severe anemia
- Risk of intrauterine death

Severe childhood-onset disease characterized by hemolytic anemia and transfusion dependence



- Hemolytic anemia
- Transfusion dependence
- Photosensitivity (often variable)
- Cutaneous manifestations (e.g., blistering, scarring)

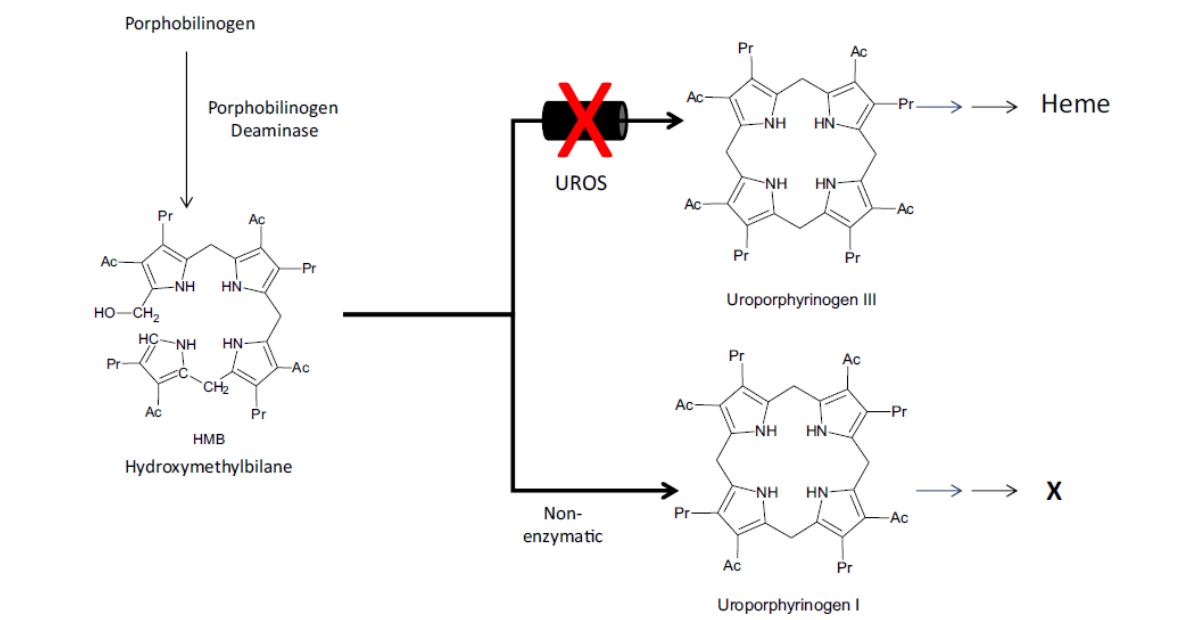
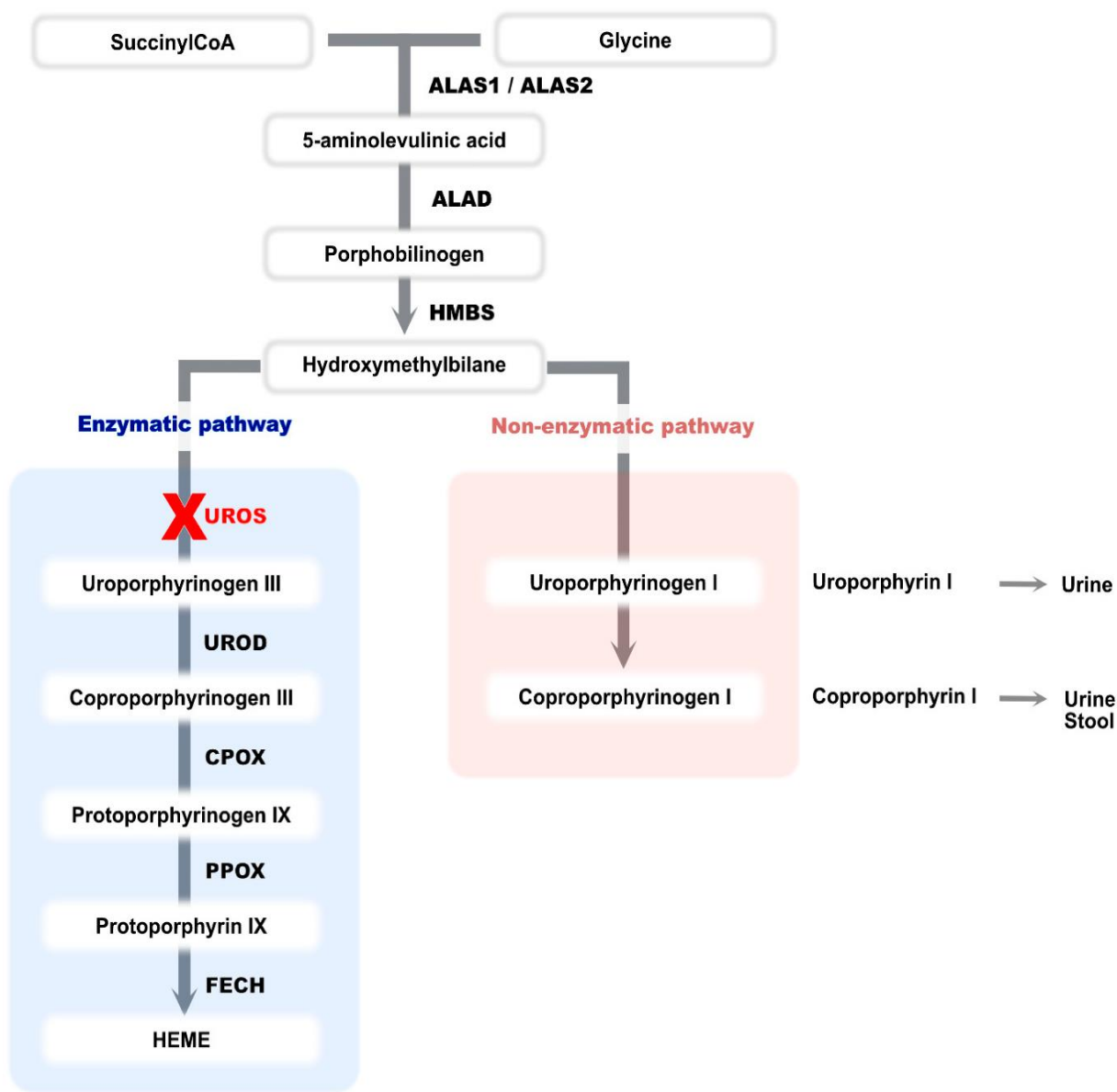
Milder, late-onset forms with predominant cutaneous manifestations in adulthood



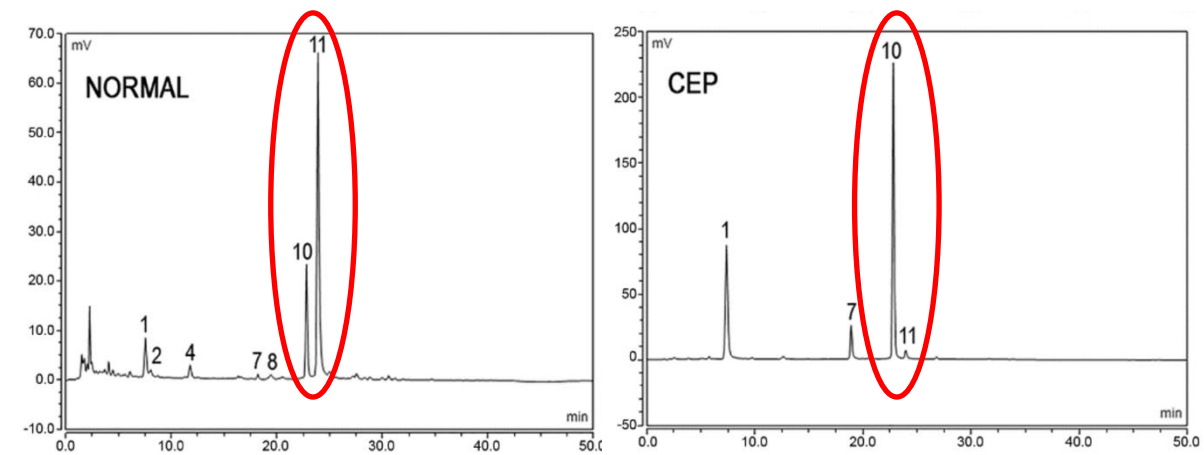
- Chronic blistering and scarring
- Photosensitivity
- Milder systemic involvement
- Later onset (adulthood)

The clinical presentation of CEP ranges from life-threatening prenatal forms to severe childhood disease and milder, late-onset cutaneous manifestations.

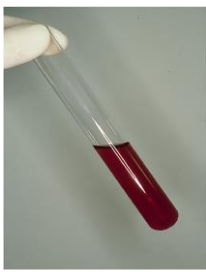
The phenotype is largely related to the residual activity of UROS and the specific genetic variants.



Accumulation of non-physiological **type I** porphyrin isomers



HPLC separation of porphyrin isomers in urine



1



3



KEY SYMPTOMS OF CONGENITAL ERYTHROPOIETIC PORPHYRIA (CEP)

Symptoms typically appear shortly after birth or in early infancy.

1 Severe Photosensitivity

- Skin blistering from sunlight (or strong artificial light).
- Causes immediate scarring.



2 Erythrodontia

- Hallmark reddish-brown teeth.
- Fluorescence under Wood's light (UV).



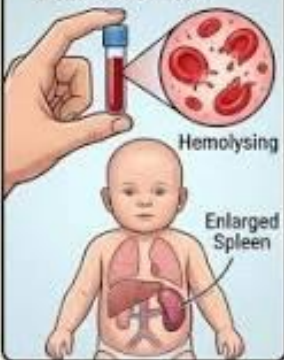
3 Hypertrichosis

- Excessive hair growth, often on face and arms.
- Protective body response?



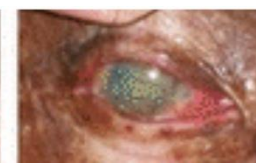
4 Hemolytic Anemia & Splenomegaly

- Hemolytic Anemia: Red blood cells break down.
- Enlarged Spleen (Splenomegaly).



5 Chronic Scarring

- Loss of cartilage (ears & nose).
- Fingertip loss or nail deformities.



● Eyes

Corneal scarring, Blepharitis, Conjunctivitis
Chronic ulcerative keratitis and Scleromalacia

2



5



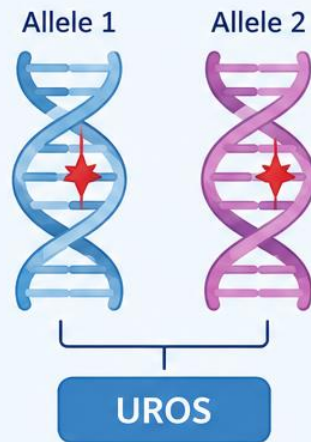
● Bones

Osteodystrophy (osteolysis and osteoporosis)
Mutilations in terminal digits
Fragility of the long bones and vertebrae
Fractures and shortened stature

Genetic causes of congenital erythropoietic porphyria (CEP)

Most cases

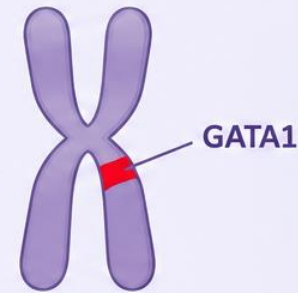
Biallelic variants of the *UROS* gene



Most cases are caused by biallelic variants of the *UROS* gene.

X-linked form

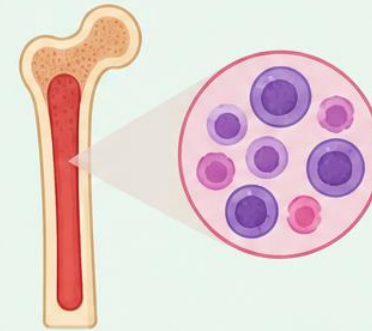
GATA1 variants



More rarely, X-linked forms associated with *GATA1* variants have been described.

Acquired form

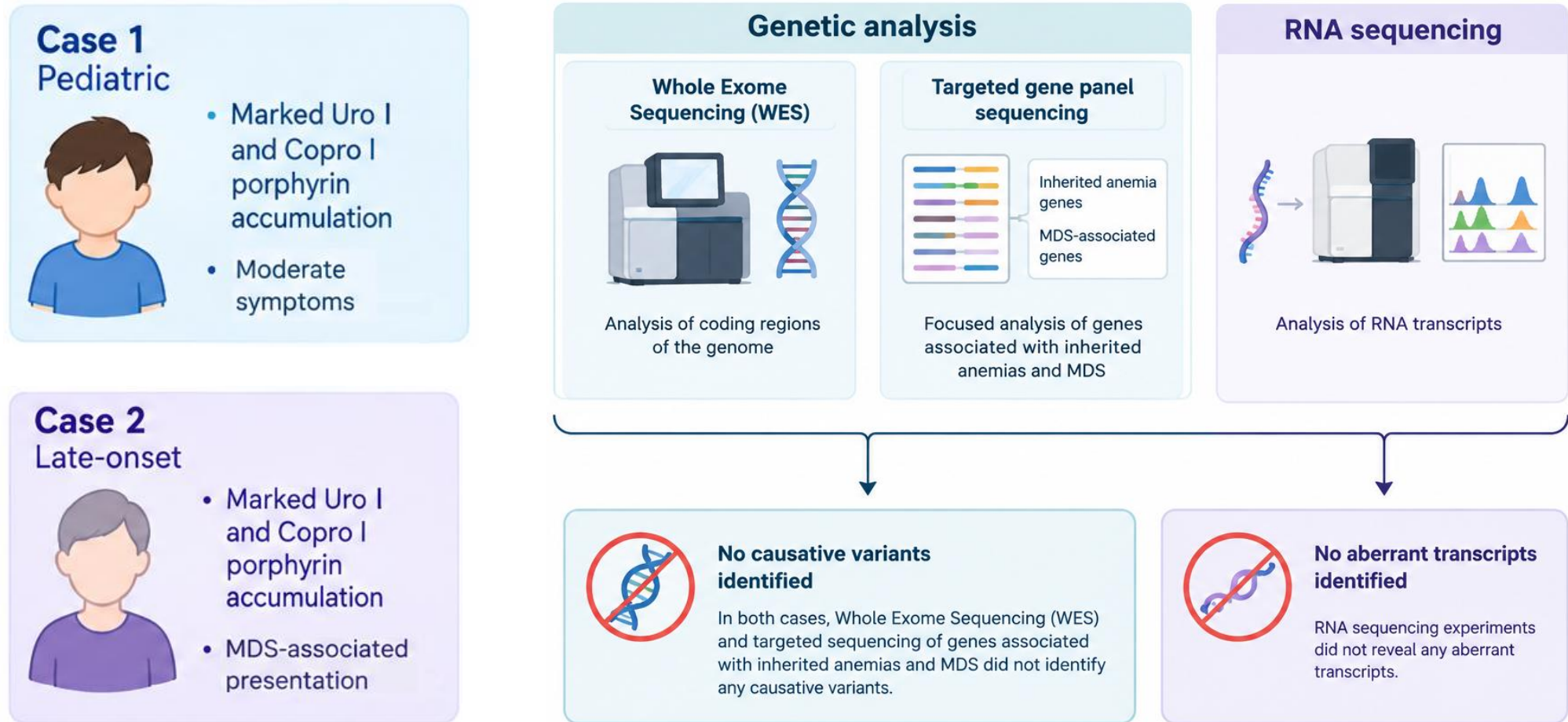
Myelodysplastic syndromes (MDS)



Acquired forms associated with myelodysplastic syndromes (MDS) have been described.

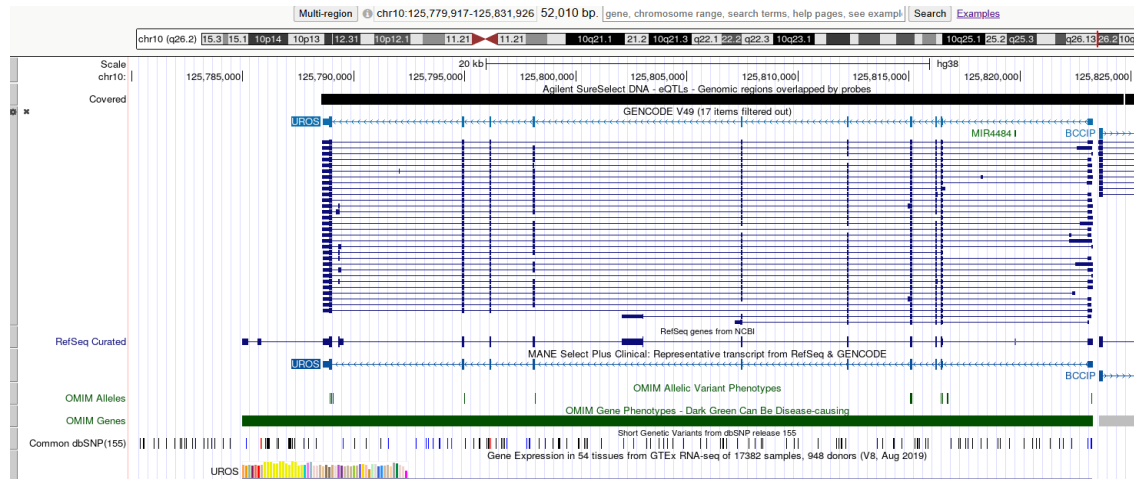
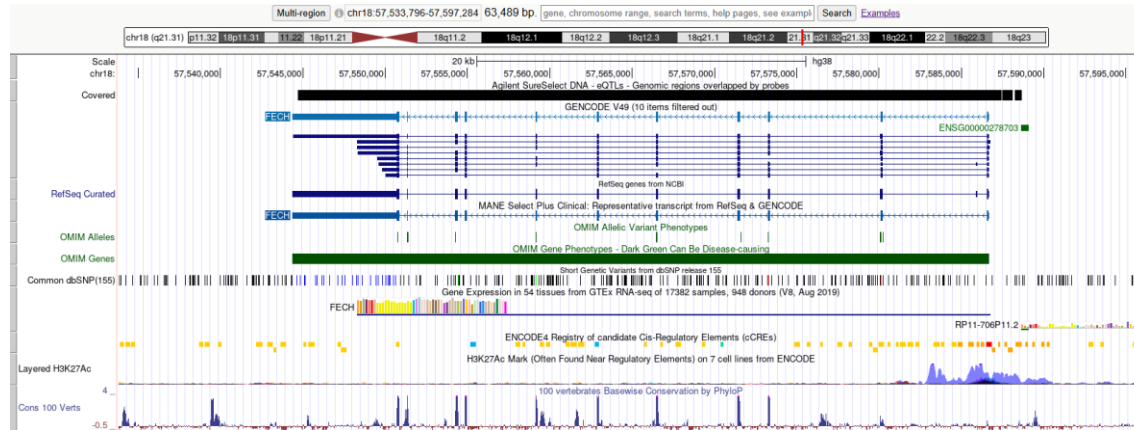
AIM and Patients

Characterization of the genetic background in two clinically distinct cases



Methods

- A custom target panel covering the promoter region and all intronic sequences of porphyria-associated genes was designed using Agilent SureDesign and sequenced on the Illumina MiSeq platform.



- Bioinformatic analysis was performed using VarSome Clinical software for sequence alignment, variant calling, and variant interpretation.
- Rare deep intronic variants were selected for further analysis using in silico splicing prediction tools, including SpliceAI and Human Splicing Finder.
- Qualitative mRNA analysis was performed, followed by sequencing of aberrantly spliced transcripts.

Results

Multi sample analysis from 1392, 1393, 1394 (hg38) 

									
☐ ...	Variant	Variant Type	Gene Symbol		Germline Class	Germline Rules	HGVS		
☒	chr10:125791981 C⇒A	SNV	UROS		Likely benign	BP4 Strong PM2 Support	NM_000375.3:c.6...		
☐	chr10:125792948 G⇒A	SNV	UROS		Likely benign	BP4 Strong PM2 Support	NM_000375.3:c.6...		
☐	chr10:125806628 T⇒C	SNV	UROS		Benign	BA1 BP4 Strong	NM_000375.3:c.3...		
☐	chr10:125820556 T⇒C	SNV	UROS		Benign	BA1 BP4 Strong	NM_000375.3:c.-...		
☐	chr10:125793226 T⇒A	SNV	UROS		Benign	BA1 BP4 Strong	NM_000375.3:c.6...		

HGVS Coding	Transcript Position	Ov...	Phenotypes	Inheritance	Function	Zygosity	Phasing Group	Frequency
c.660+2899G>T	intron 9 of 9 position...	UROS	1	AR	3u in nc	● ● ●	- - -	0.000006577
c.660+1932C>T	intron 9 of 9 position...	UROS	1	AR	3u in	● ● ●	- - -	0.00009191
c.394+785A>G	intron 6 of 9 position...	UROS	1	AR	in	● ● ●	- - -	0.3019
c.-27+2473A>G	intron 1 of 9 position...	UROS	1	AR	Phenotypes	● ● ●	- - -	0.3045
c.660+1654A>T	intron 9 of 9 position...	UROS	1	AR	3f 3u in	● ● ●	- - -	0.3102
c.694G>C	exon 10 of 10 positio...	UROS	1	AR	c in nc ...	● ● ●	- - -	0.3241
c.-26-1970_-26-1968dup	intron 1 of 9 before ...	UROS	1	AR	in	● ● ●	- - -	0.7073
c.394+150A>G	intron 6 of 9 position...	UROS	1	AR	3u in	● ● ●	- - -	0.7671
c.394+820G>A	intron 6 of 9 position...	UROS	1	AR	in	● ● ●	- - -	0.7727
c.-26-1117_-26-1116dup	intron 1 of 9 before ...	UROS	1	AR	in	● ● ●	- - -	0.7745

Analysis approach UROS(NM_000375.3):

- Case1: following trio analysis, variants were **filtered for coverage >20×** and **allele frequency >30%**, then prioritized based on population frequency (<0,0005), zygosity, and inheritance pattern.
- Case 2: following single analysis, variants were **filtered for coverage >20×** then prioritized based on population frequency (<0,0005) and zygosity.
- All identified variants were classified as **Likely Benign**.

c.[660+1932C>T;660+2899G>T]

c.395-1591G>A

c.[660+1932C>T;660+2899G>T]



Pangolin scores: ⓘ

Variant	Transcript	Δ type	Δ score ⓘ	position ⓘ
NM_000375.3 (UROS):c.660+1932C>T ⇒ 10:125792948 G > A UCSC, gnomAD, GeneBe, AlphaGenome	UROS (ENSG00000188690.17 / ENST00000368797.10 / NM_000375.3) protein coding MANE Select transcript (minus strand) intron variant OMIM, GTEx, gnomAD, ClinGen, Ensembl, Decipher, GeneCards	Splice Loss	0.00	-369 bp
		Splice Gain	0.00	-12 bp

Pangolin scores: ⓘ

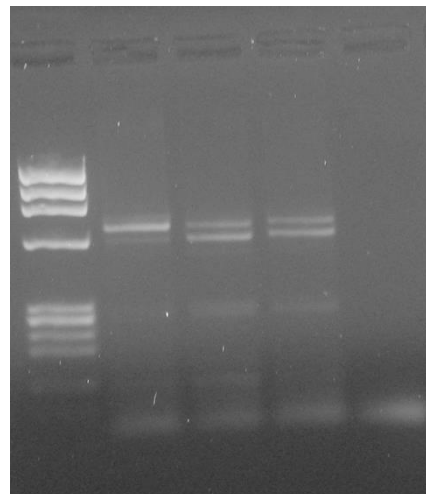
Variant	Transcript	Δ type	Δ score ⓘ	position ⓘ
NM_000375.3 (UROS):c.660+2899G>T ⇒ 10:125791981 C > A UCSC, gnomAD, GeneBe, AlphaGenome	UROS (ENSG00000188690.17 / ENST00000368797.10 / NM_000375.3) protein coding MANE Select transcript (minus strand) intron variant OMIM, GTEx, gnomAD, ClinGen, Ensembl, Decipher, GeneCards	Splice Loss	0.00	81 bp
		Splice Gain	0.23	6 bp

c.660+1932C>T			
Sequences	Variation	Signal	Interpretation
CTGGCCTTG>CTGGTCTTG	41.39>68.53 (65.57%)	New Donor splice site	Activation of a cryptic Donor site. Potential alteration of splicing (cryptic exon activation)
CTGGCCTTG>CTGGTCTTG	41.39>68.53 (65.57%)	New Donor splice site	Activation of a cryptic Donor site. Potential alteration of splicing

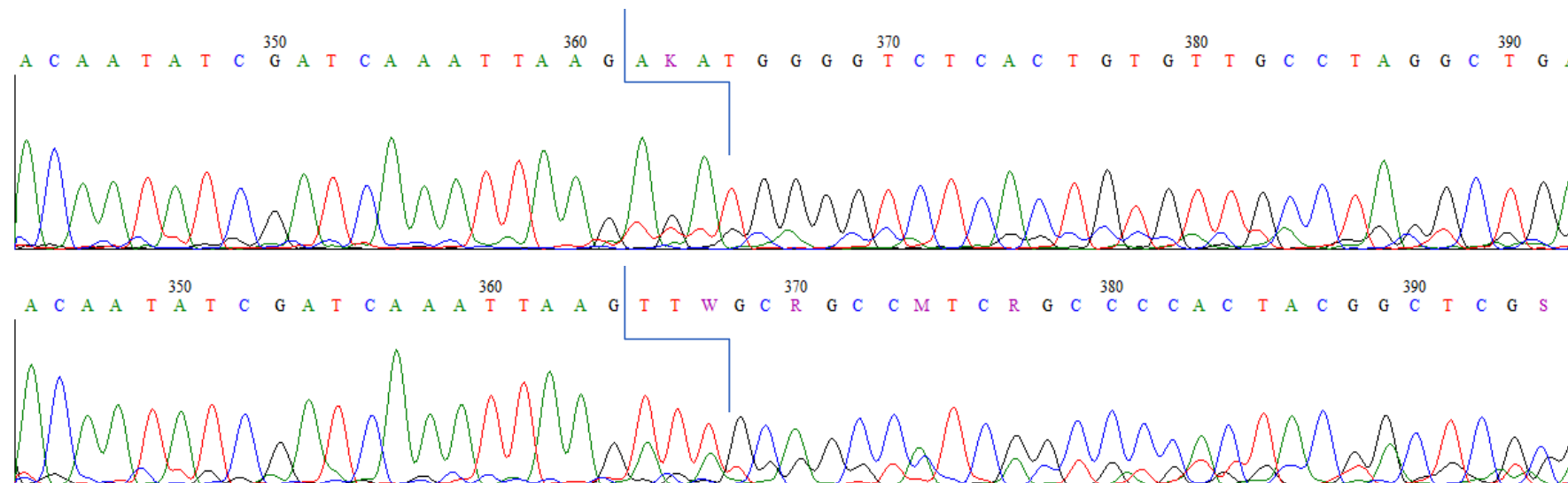
c.660+2899G>T			
Sequences	Variation	Signal	Interpretation
TAGGGATTA>TAGGTATTA	51.78>78.92 (52.41%)	New Donor splice site	Activation of a cryptic Donor site. Potential alteration of splicing (cryptic exon activation)
TAGGGATTA>TAGGTATTA	51.78>78.92 (52.41%)	New Donor splice site	Activation of a cryptic Donor site. Potential alteration of splicing

ccccctgcctcttactacagtgattgattaccctgcctgcc
agtagttaacacctctgccattgctgaggaggagaaagtg
acagagctggCcttgctcacagtgactatggactgcttgg
gggccacgaaggcactgtgtgtgggttgtagttgtccctc
gacctgggacttggcgctggatagtgaggtgctttcaag
gttcacagaggacacagtcctagcccgtgagttggtagtt
aggcttttctgtctcagctcagcctcagatttgctactgtcc
tcctcccaggagtcctgtagggcccactgtttgtacag
cattgcacaac.....
.....ggggcagcatcatgtgagcattcattgacaca
ggagtgaaggctcctgtgaaaaagctgctgtttcaccaa
actgttgatacccctgctctaggactacagtcattgtttgcc
attgctgtgttcccagtcaggaggagtttcttttttcaaatt
tttaaatagctttattgagatgtaattcacaaccataaaa
tttactttttatgattttaatatgtaaacttaaaaattttttt
ttatttttttagAGATGGGGTCTCACTGTGTTGCC
TAGGCTGATCTCAAACCTGGCCTCAAGTA
ATCTTCCCACCTTGGCCTCCCAAATgtaggGa
ttacaggtgtg

M Pt Mother Father



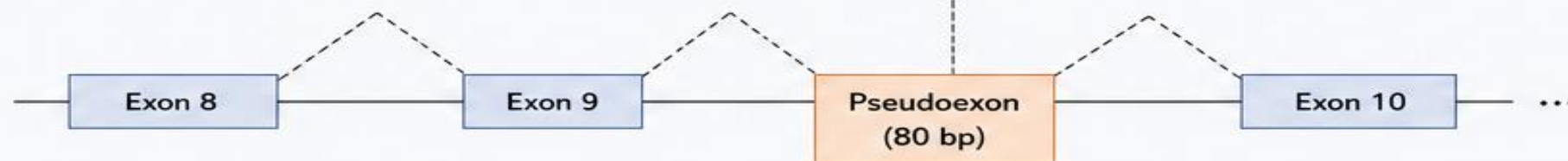
799bp
719bp

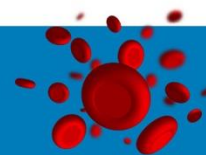
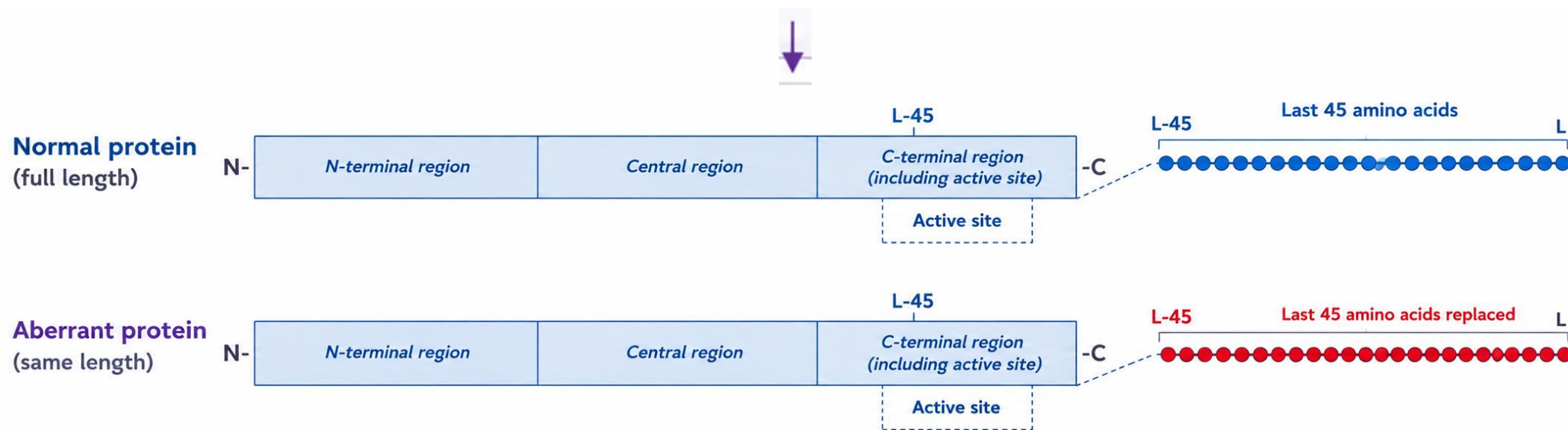


Genomic DNA



Pre-mRNA





c.395-1591G>A



Pangolin scores: ?

Variant	Transcript	Δ type	Δ score ?	position ?
UROS(NM_000375.3):c.395-1591G>A ⇒ 10:125799736 C> T UCSC , gnomAD , GeneBe , AlphaGenome	UROS (ENSG00000188690.17 / ENST00000368797.10 / NM_000375.3) protein coding MANE Select transcript (minus strand) intron variant OMIM, GTEx, gnomAD, ClinGen, Ensembl, Decipher, GeneCards	Splice Loss	0.06	27 bp
		Splice Gain	0.00	-3 bp



c.395-1591G>A

No Signal

Interpretation

No significant impact on splicing signals.

BRANCH POINT: AGCTGAG

ESS: GCTGAGCT

M PT CTRL

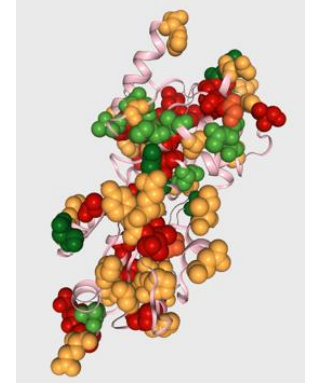


cagccttaacatcccattggcctgttggggctgaagata
aatcccaggcattaagcatattccaaaagggaataact
cctttgtctttggccttttgaagtgggctagtttcaggaca
tgtagttgtagatctctcctcaccagcaaaggaggactca
gccaggacatttcaattttctcctttctgctgacagggtg
gtgccttttcagtgaagtcagctGagctgggtggacattt
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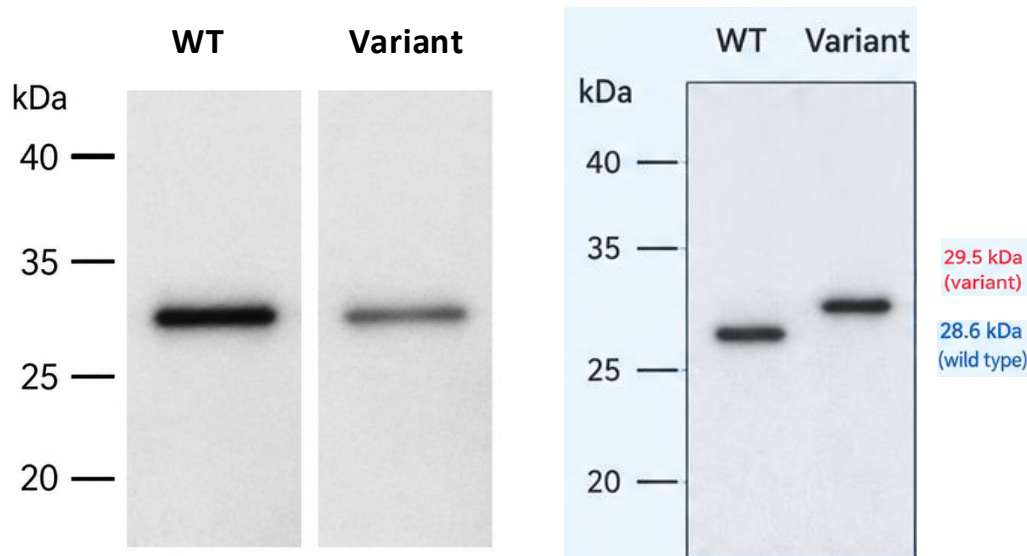
No qualitative differences were observed, **although quantitative** differences may be present. However, the **absence of informative polymorphic markers** prevented us from confirming the monoallelic origin of the analyzed transcript.

Work in progress

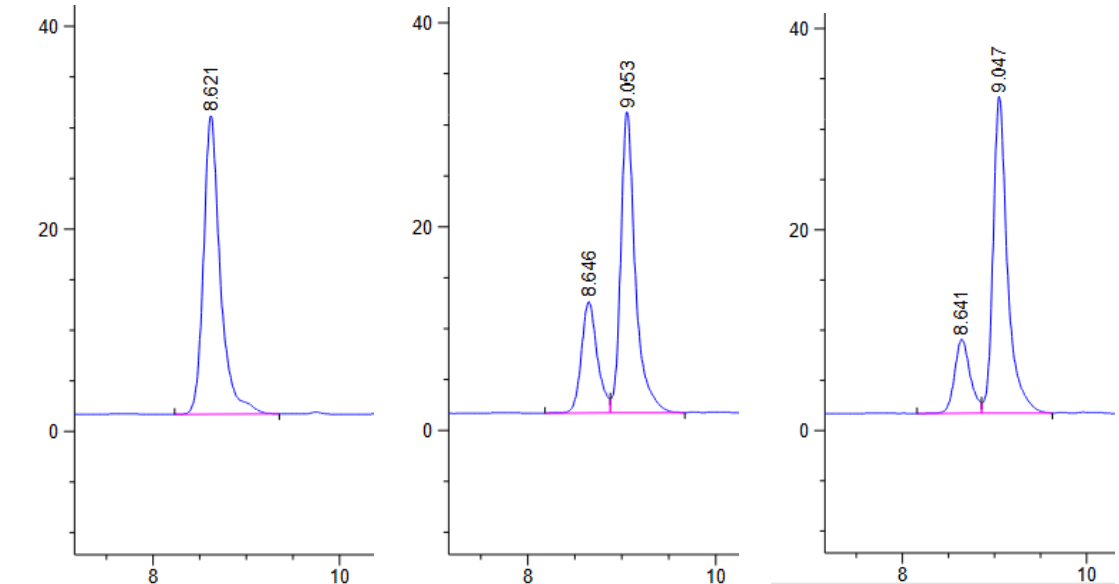
Optimization of **Western Blotting** and **UROS enzymatic activity** assays to assess the presence of an abnormal protein and reduced enzymatic activity



Expected changes in **UROS protein expression** and **apparent molecular weight**, including shift from 28.6 kDa to 29.5 kDa

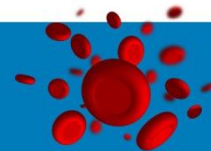


HPLC separation of Uroporphyrin I (**URO I**) and Uroporphyrin III (**URO III**) from RBC lysates



Conclusions

- Our findings **confirm the marked genetic heterogeneity** of the genes associated with porphyria.
- **Functional studies play a crucial role in establishing the pathogenicity** of variants of uncertain significance (VUS) identified by NGS.
- Overall, this study underscores the importance of incorporating functional validation into routine genetic testing to improve **the clinical interpretation of genomic findings** and enable more accurate, clinically actionable diagnoses in patients with porphyria, particularly given that predicted effects on protein function may not always correspond to the actual biological consequences.
- **RNA sequencing cannot** reliably be used to **detect low-abundance abnormal mRNA transcripts** or to identify **subtle differences in transcript abundance**.





Fondazione IRCCS Ca' Granda
Ospedale Maggiore Policlinico

Sistema Socio Sanitario



Regione
Lombardia



European
Reference
Network

Hematological Diseases
(ERN EuroBloodNet)

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