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Biologie Moléculaire Appliquée aux Maladies Cardiovasculaires, aux Néphropathies Héréditaires et à la Pharmacogénomique

GENETIC SCREENING FOR RESISTANT NEPHROTIC SYNDROME IN A TUNISIAN POPULATION

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BACKGROUND

Nephrotic syndrome (NS) is one of the most common kidney diseases seen in childhood. It is a clinically heterogeneous disease characterized by different histological variants and genetic determinants. Several genes have been involved in the genetic forms of NS occurring in children including NPHS1, NPHS2, WT1, PLCE1, and LAMB2.

Aims: Our aim was to identify causative mutations in these genes among 15 children with NS manifesting with various ages of onset.

PATIENTS AND METHODS

Study population:

- ✓ 15 children diagnosed with sporadic or familial NS, from 15 unrelated families, admitted to the pediatric departments of university hospitals of Sahloul, Farhat Hached in Sousse and Fattouma Bourguiba in Monastir.
- ✓ The diagnosis of NS was based on the criteria established by the International Study of Kidney Disease in Children «ISKDC».

Methods:

- ✓ **Direct exon sequencing:** NPHS1, NPHS2 and WT genes.
- ✓ The age of onset, clinical symptoms, renal and extra-renal signs, and renal biopsy (RB) findings were the primary criteria used to select the gene for sequencing.
- ✓ **Bioinformatics software (HSF, PolyPhen, SIFT, PROVEAN):** to analyze the potential impact of the identified mutations on the protein.

RESULTS AND DISCUSSION

Cilinical Characteristics of the study population:

- ✓ The age of our patients ranged from 40 days to 13 years, with an average of 57.96 months:

•4 patients were ≤ 3 months old, 26.6%: Congenital Nephrotic Syndrome
•2 patients were ≤ 1 year old, 13.4%: Infantile Nephrotic Syndrome
•9 patients were > 1 year old, 60%: Juvenile Nephrotic Syndrome

- ✓ sex ratio of 1.142 (7 female/ 8 male).
- ✓ Renal biopsy showed:
- 4 patients : minimal change disease (MC),
- 2 patients: MCD progressing to focal segmental glomerulosclerosis (FSGS),
- 3 patients: FSGS,
- 1 patient: membranoproliferative glomerulonephritis (MPGN)
- 5 patients, No RB.

Genetics Characteristics of the study population:

- ✓ The sequencing of the NPHS1, NPHS2 and WT1 genes allowed us to identify various intronic and exonic mutations

Table 1: The identified variations after the sequencing of the NPHS1, NPHS2, and WT1 genes

Variations	NPHS1 gene	NPHS2 gene	WT1 gene
Exonique	c.349G>A; c.791C>G; 1040G>A; c.1151T>C; c.1320C>T; c.2289C>T; c.2553A>G; c.3230A>G; c.3315G>A	c.59C>T ; c.102A>G; c.954C>T ; c.1038A>G	c.198G>T; c.330C>T ; c.594C>T ; c.1107A>G
Intronique	c.274+33C>A;c.397+61A>G; c.608+34A>C;c.1012+44G>A; c.1170+2T>C; c.1170+4A>G ; c.2927+24G>C; c.3287-67G>A; c.3286+36C>T; c.3287-54C>T;c.3481+45C>T	c.*157G>A; c.*54G>C; c.-116C>T; c.-52C>G; c.-196C>G	c.872+82G>T /c.872+85G>C; c.950+85A>G; c.1250-32C>A; c.1433-49T>C

Table 1: Effect of intronic and exonic variations affecting the NPHS1, NPHS2 and WT1 gene evaluated by the HSFinder, SIFT, PROVEAN and PolyPhen tool

Variations	HSFinder	SIFT	PROVEAN	PolyPhen
c.1170+4A>G	Abolition of a donor Splice site de WT1	-	-	-
c.397+61A>G	Alteration of a putative branch box sequence			-
c.608+34C>A		-	-	-
c.274+33C>A	Creation of a putative branch box sequence	-	-	-
c.791C>G	Abolition of Exonic Splicing Enhancers (ESE)	-	-	-
c.1040G>A				
c.59C>T		D	N	B
c.198G>T		T	N	B
c.2289C>T		T	N	B
c.2553A>G		T	N	B
c.3230A>G	Creation of Exonic Splicing Silencers (ESS)	D	N	B
c.3315G>A		T	N	B
102A>G		T	N	B
c.954C>T		T	N	B
c.2927+24G>C	Creation of Intronic Splicing Enhancers (ISE)	-	-	-
c.3481+45C>T		-	-	-
c.3230A>G	Activation of an exonic cryptic acceptor site	-	-	
c.3315G>A		-	-	-
c.349G>A	-	T	N	-
c.1151T>C	-	T	N	B
c.1320C>T	-	T	N	B
c.1038A>G	-	T	N	B
c.330C>T	-	T	N	B

D: Damaging; T: Tolerated; B: Benign; N: Neutral

- ✓ The HSFinder tool revealed the involvement of 17 variations in the splicing-altering phenomena across the three studied genes, including splice site, branch point, exonic and intronic splicing enhancers (ESE/ISE), and exonic/intronic splicing silencers (ESS/ISS) sites. while the other tools showed only neutral, tolerated or benign effects.

CONCLUSION

Congenital and infantile NS, in our Tunisian population, can be explained by identified mutations in the NPHS1, NPHS2 or WT1 genes.

These mutations molecular effects described by HSF, SIFT, PROVEAN, Regulome DB, and PolyPhen did not reveal a clear pathogenicity, but the coexistence of multiple variations could explain the observed phenotypic differences.