

## ASSOCIATION STUDY OF NEPHROTIC SYNDROME IN CHILDREN WITH NEW VARIANTS OF NPFS2 GENE.

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### Abstract

This project aimed to screen four variants (SNVs) which were previously identified in the promoter region of the gene NPFS2 in children with nephrotic syndrome, in controls without renal disease. Therefore, we used two different methods after DNA extraction: polymerase chain reaction (PCR) with subsequent sequencing by Sanger method and real time PCR with TaqMan allelic discrimination kit. Only one of the controls presented the c.-268C>G SNV in heterozygosis confirming the rarity of these SNVs .

### Key words:

*Nephrotic Syndrome, NPFS2, Single Nucleotid Variants (SNVs)*

### Introduction

Nephrotic Syndrome is a kidney disease characterized by massive proteinuria, hypoalbuminemia, edema and hyperlipidemia<sup>1</sup>. The disease is usually classified according to the age and on the basis of the response to standard steroid treatment<sup>2</sup>. The aim of this study was to screen four variants (SNVs): c.-53C>T c.-164C>T, c.-196C>G, c.-268C>G, previously identified on the promoter region of NPFS2 that codifies podocin, structural protein found in podocytes, the visceral cells of glomerular filtration barrier. The number of controls analyzed were, respectively: 126, 223, 228 and 218. All variants were analyzed by PCR and sequencing in approximately 130 controls. In addition, the variants c.-164C>T, c.-196C>G and c.-268C>G were genotyped in 100 more controls, by real time PCR with TaqMan® allelic discrimination kits (Applied Biosystems, Foster City, CA – USA)<sup>3</sup>.

### Results and Discussion

The results are presented in Table I.

**TABLE I.** SNVs genotype of controls analyzed

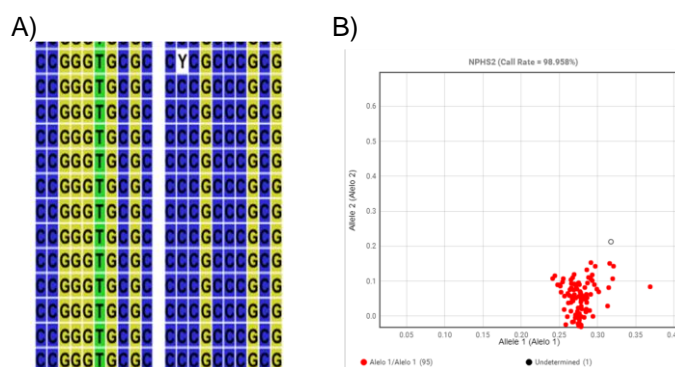
	Controls		Previous <sup>2</sup>		Total
<b>rs575516772</b> c.-268C>G MAF <0.01	217 C/C	1 C/G	278 C/C		495 C/C 1 C/G
<b>rs542360647</b> c.-196C>G MAF <0.01	228 C/C		277 C/C	1 C/G	505 C/C 1 C/G
<b>rs553068590</b> c.-164C>T MAF <0.01	223 C/C		278 C/C		501 C/C
<b>rs750269028</b> c.-53C>T * MAF	126 C/C		278 C/C		404 C/C

<sup>1</sup>Data obtained in ensembl data base [www.ensembl.org](http://www.ensembl.org).

<sup>2</sup>Controls screened previous at this Project.

\*Data not found.

Only one control presented the allele c.-268G in heterozygosis.



**FIGURE 1. Illustration of result analysis of c.-164C>T variant.** A) Alignment created by CLC sequence viewer 7.0 after sequencing. The position of the variant is represented with the letter Y on the image and the allele C found in 12 controls is shown. B) Graphic created by Thermofisher Cloud software analysis. The red spots show the resulting alleles (in this case, allele C) from real time PCR with specific probes for c.-164C and c.-164T alleles.

### Conclusions

After all molecular analyses only one control presented the SNV c.-268C>G in the heterozygous state, confirming the rarity of the SNVs.

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<sup>1</sup>Benoit G, Machuca E, Antignac C: Hereditary nephrotic syndrome: a systematic approach for genetic testing and a review of associated podocyte gene mutations. *Pediatr Nephrol* 2010 Sep;25:1621–32.

<sup>2</sup>ISKDC: The primary nephrotic syndrome in children. Identification of patients with minimal change nephrotic syndrome from initial response to prednisone. A report of the International Study of Kidney Disease in Children. *J Pediatr* 1981 Apr;98:561–4.

<sup>3</sup>Heid CA, Stevens J, Livak KJ, William PM. Real time quantitative PCR. *Genome Res.*1996; 6(10): 986 – 994.