

## Funcional analysis of variants in NPHS2 promoter identified in Brazilian patients with Nephrotic Syndrome

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### Abstract

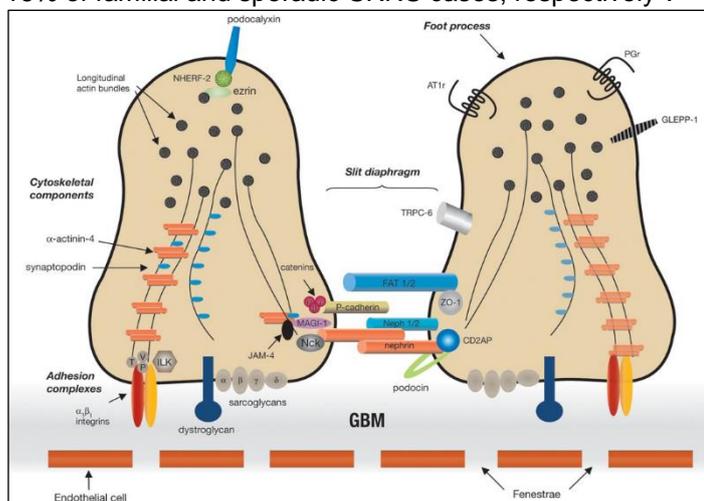
Nephrotic syndrome is defined by heavy proteinuria, hypoalbuminemia, edema and hyperlipidemia. Podocin (NPHS2) is an integral membrane protein of podocytes in the glomerular filtration barrier. The aim of this study was to verify if different variants, previously identified in the promoter region of NPHS2 gene in non-related SRNS patients, affect podocin expression.

### Key words:

Nephrotic Syndrome, NPHS2 gene, promoter variants

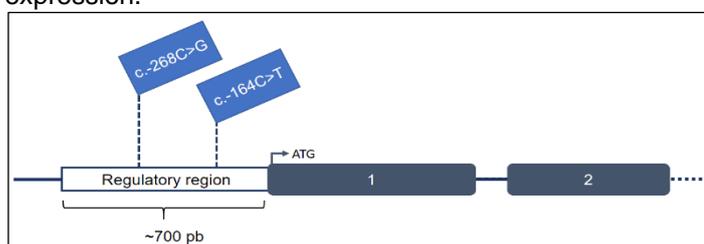
### Introduction

Nephrotic syndrome (NS), one of the most common kidney diseases in childhood, is characterized by massive proteinuria, hypoalbuminemia, edema and hyperlipidemia. Approximately 20% of the patients do not respond well to corticosteroid treatment and are classified as steroid-resistant NS (SRNS)<sup>1</sup>. NPHS2 gene encodes for podocin that is an integral membrane protein of podocytes in the glomerular filtration barrier (Figure 1). Variants in the NPHS2 gene are responsible for approximately 40% and 16% of familial and sporadic SRNS cases, respectively<sup>2</sup>.



**Figure 1.** Glomerular filtration barrier. The glomerular filtration barrier complex. GBM = Glomerular barrier membrane. Adapted from Michaud *et al* (2007).

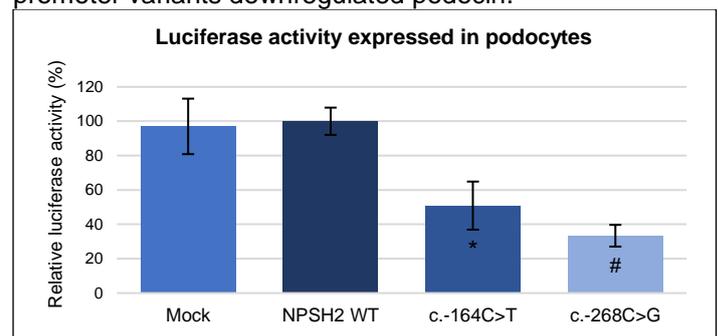
The aim of this study was to verify if different variants previously identified in the promoter region of NPHS2 gene (Figure 2) in non-related SRNS patients, affect podocin expression.



**Figure 2.** Illustration of the variants in the promoter region of NPHS2 gene that were focus of the study.

### Results and Discussion

The variants c.-164C>T and c.-268C>G showed - 49,1% and - 66,6% downregulation of luciferase (*Renilla sp*) gene expression, respectively, when compared to the wild-type NPHS2 promoter, when transfected in podocytes (Figure 3). Our results corroborate with previous results from Di Duca *et al* (2006)<sup>3</sup> study in which they also found that other promoter variants downregulated podocin.



**Figure 3.** Histogram results of relative luciferase activity of promoter NPHS2 variants c.-164C>T and c.-268C>G. The LightSwitch™ promoter reporter vector (*Active Motif Company*) was transfected in immortalized podocytes by FuGENE® HD transfection reagent (*Promega*) with the following constructs: empty vector (mock), wild-type NPHS2 (WT), and with the variants c.-164C>T and c.-268C>G. The data were analyzed and normalized using NPHS2 WT (Student's t-test, \* = P<0.006 and # = P<0.0003).

### Conclusions

Our results indicate that changes in podocin expression might interfere in the glomerular filtration slit and act in the background of the NS in patients carrying those variations.

### Acknowledgement

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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;25(9):1621–32.

<sup>2</sup>Karle SM, Uetz B, Ronner V, Glaeser L, Hildebrandt F, Fuchshuber A. Novel mutations in NPHS2 detected in both familial and sporadic steroid-resistant nephrotic syndrome. *J Am Soc Nephrol.* 2002 Feb;13(2):388–93.

<sup>3</sup>Di Duca M, Oleggini R, Sanna-Cherchi S, Pasquali L, Di Donato A, Parodi S, et al. Cis and trans regulatory elements in NPHS2 promoter: implications in proteinuria and progression of renal diseases. *Kidney Int.* 2006 Oct;70(7):1332–41.