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Molecular study of genes associated with steroid-resistant nephrotic syndrome

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Abstract

Nephrotic syndrome (NS) is defined by heavy proteinuria, hypoalbuminemia, edema and hyperlipidemia. Recent advances in molecular genetics have identified single-gene causes of SRNS in more than 50 genes that encodes for structures of the glomerular filtration barrier (GFB). The aim of this study was the analysis of three variants previously identified by our group by whole exome sequencing (WES): the screening of c.459C>G (ACTN4) in 144 healthy controls and the validation by Sanger sequencing of the c.883_885dupTCT (ANLN) and c.3322A>C (LAMB2) variants.

Key words:

Steroid resistant nephrotic syndrome; variants; ACTN4, LAMB2, ANLN genes.

Introduction

Nephrotic syndrome (NS), one of the most common kidney conditions in childhood, is characterized by massive proteinuria, hypoalbuminemia, edema and hyperlipidemia. Approximately 20% of the children do not respond well to corticosteroid treatment and are classified as steroid-resistant NS (SRNS)¹. Recent advances in molecular genetics have identified single-gene causes of SRNS in more than 50 genes that encodes for the glomerular filtration barrier (GFB)².

Figure 1: Glomerular filtration barrier. The glomerular filtration barrier complex. Podocyte foot processes are shown in light beige. GBM = Glomerular filtration barrier².

Recently our group identified three novel heterozygous variants by WES in three different genes (Table 1). Therefore, the aims of this study were: 1) the screening of the ACTN4 variant in approximately 150 controls; 2) the validation by Sanger sequencing of the ANLN and LAMB2 variants.

Table 1: Variants analyzed in this study

Patients	Variant	Gene	AD/AR ^a	Analysis
P1	p.Phe153Leu	ACTN4	AD	Controls
P2	p.Ser295dup	ANLN	AD	Sanger
P3	p.Asn1108Hys	LAMB2	AR	Sanger

^aAD=autosomal dominant; AR=autosomal recessive.

Results and Discussion

The DNA was extracted by phenol:chloroform standard methods. Further PCR amplification using specific oligonucleotides and direct sequencing by Sanger were performed. Figure 2 and 3 illustrates the results.

Figure 2: A. P1's family heredogram. B. Electropherograms (Chromas 2.6.2) of ACTN4 variant validation. C. CLC sequence viewer alignment showing the C>G position. Only 10 controls are shown.

Figure 3: ANLN (A) and LAMB2 (B) variants identified by WES and analyzed by the IGV - 2.3.68 (above) and respective electropherograms (Chromas 2.6.2) (below).

Conclusions

The variant identified in ACTN4 gene was not identified (0%) in the 144 healthy controls screened and should be considered for further functional studies. The two variants identified in ANLN and LAMB2 genes were validated and should also be screened in controls.

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