

## RESEARCH NOTE

# A novel nonsense mutation in *NPHS1*: is aortic stenosis associated with congenital nephropathy?

LARA GHARIBEH<sup>1</sup>, INAAM EL-RASSY<sup>1</sup>, AYMAN SOUBRA<sup>1</sup>, RAYA SAFA<sup>1</sup>, AKL FAHED<sup>1</sup>, RACHEL TANOS<sup>1</sup>,  
MARIAM ARABI<sup>2</sup>, ZAKARIA KAMBRIS<sup>3</sup>, FADI BITAR<sup>1,2\*</sup> and GEORGES NEMER<sup>1\*</sup>

<sup>1</sup>Department of Biochemistry and Molecular Genetics, <sup>2</sup>Department of Pediatrics and Adolescent Medicine, and  
<sup>3</sup>Department of Biology, American University of Beirut, P.O. Box 11-0236 Beirut, Lebanon

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Congenital nephrotic syndrome (CNS) is a life-threatening kidney disorder characterized clinically by massive proteinuria and oedema, starts shortly after birth. Mutations in the gene encoding nephrin (*NPHS1*), one of the essential proteins constituting the glomerular filtration barrier, have been linked to the Finnish-type of CNS. Lately, it was shown that this type is also encountered in many European countries. In the Middle-East, only two reports have shown mutations in *NPHS1* to be linked to CNS.

Here, we describe the first Lebanese familial Finnish-type nephropathy and unravel a novel mutation in *NPHS1*, leading to a truncated protein. In addition, we hypothesize that the heterozygous form of the mutation is linked to aortic stenosis, one of the most prevalent congenital heart disease (CHD), since two out of three individuals in the family who are heterozygous for this mutation have aortic stenosis.

Taken together with the recent findings in *NPHS1* knock-out mice which have severe coronary arteries malformations, the results presented here are an eye opener for CNS treating physicians who should have a complete cardiac assessment of their patients.

The term ‘nephrotic syndrome’ refers to any condition with excessive proteinuria, hypoalbuminaemia, and oedema (Fuchshuber *et al.* 1996; Avni *et al.* 2011). The glomerular capillary wall, which is composed of a basement membrane covered by endothelial cells on the inner part and visceral epithelial cells (podocytes) on the outer part, is responsible for plasma ultrafiltration during urine formation. Dysfunction of the glomerular barrier results in leakage of plasma proteins and the development of nephrotic syndromes (NS). Thus, one would suspect any abnormal expression and/or

function of the proteins involved in this filtration to be linked to NS.

NS is classified as congenital (CNS) when the onset starts *in utero* or during the first three months of life, infantile (INS) when it is manifested during the first year, and childhood when it occurs after one year (Benoit *et al.* 2010). Much progress has been achieved in understanding the genetic pathways involved in these NS in the recent years. Recessive mutations in *NPHS1* (OMIM 602716) which encodes the protein Nephrin has been shown to be the cause of most CNS that are also known as Finnish-type (CNF; OMIM 256300), because of their prevalence in Finland (1/8000). More than 165 mutations have been described since then, and the most prevalent ones are that affecting exons 2 (L41fsX90) and 26 (R1109X) referred to as Fin<sub>major</sub> and Fin<sub>minor</sub>, respectively (Fuchshuber *et al.* 1996; Bolk *et al.* 1999; Beltcheva *et al.* 2001). The human *nephrin* gene is mapped on 19q13.1: 29 exons span a 150 kbp region and encode a protein of 1241 amino acids. The Nephrin protein belongs to the immunoglobulin (Ig) family of adhesion molecules: it contains a transmembrane domain in addition to Ig and fibronectin domains (Lenkkeri *et al.* 1999; Ruotsalainen *et al.* 1999). Nephrin was shown to physically and functionally interact with other proteins, mainly NEPH1 (ortholog of the *Drosophila* Kirre) to promote the formation of the slit diaphragm, a key element in physiological waste removal from the blood.

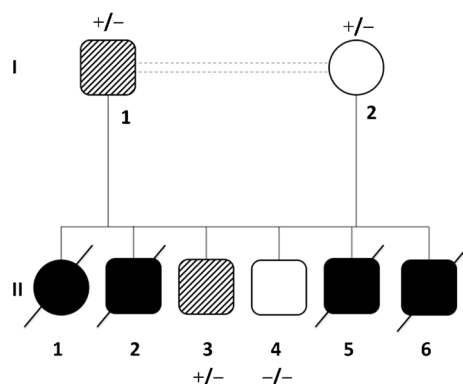
Here, we report the first familial case of a Finnish-type nephropathy in Lebanon. The parents are first-degree cousins, originating from a remote area in north-eastern Lebanon. The indexed patient II-3 (figure 1) was presented to the Pediatric Cardiology unit at the American University of Beirut Medical Center (AUBMC) with severe coarctation of the aorta (CoA), and aortic stenosis (AS) that necessitated surgery. Few years later his sister (II-6) was also referred to

\*For correspondence. E-mail: Fadi Bitar, fadi.bitar@aub.edu.lb;

Georges Nemer, georges.nemer@aub.edu.lb.

Lara Gharibeh and Inaam El-Rassy contributed equally to this work.

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**Figure 1.** Pedigree of the first Lebanese familial Finnish-type nephropathy. Black or dashed squares and circles indicate patients diagnosed with nephropathy or congenital aortic stenosis, respectively. Dashed lines indicate first-degree cousins, and lines through circles and squares refer to death. +/- refers to the heterozygous mutation Y439\*, and the -/- to the wild-type genotype on both alleles.

the AUBMC at seven weeks of age and was diagnosed as having a small atrial septal defect (ASD). However, the presence of ascites in her abdomen was indicative of a major malaise and her parents were advised to see a nephrologist. She died six months later, after being diagnosed in an outpatient clinic as having a congenital nephrotic syndrome of the Finnish type. Two years later a visit to the family in their remote area to recruit patient II-3 as part of our cohort of congenital heart disease patients unravelled that the couple had three children who died shortly after birth because of congenital nephropathy comparable to proband II-6. The father (I-1) has undergone a valve replacement surgery at the age of 40 due to severe aortic stenosis. Sanger sequencing of all coding exons of *NPHS1* (table 1) show that both parents and proband II-3 are heterozygous for a novel mutation

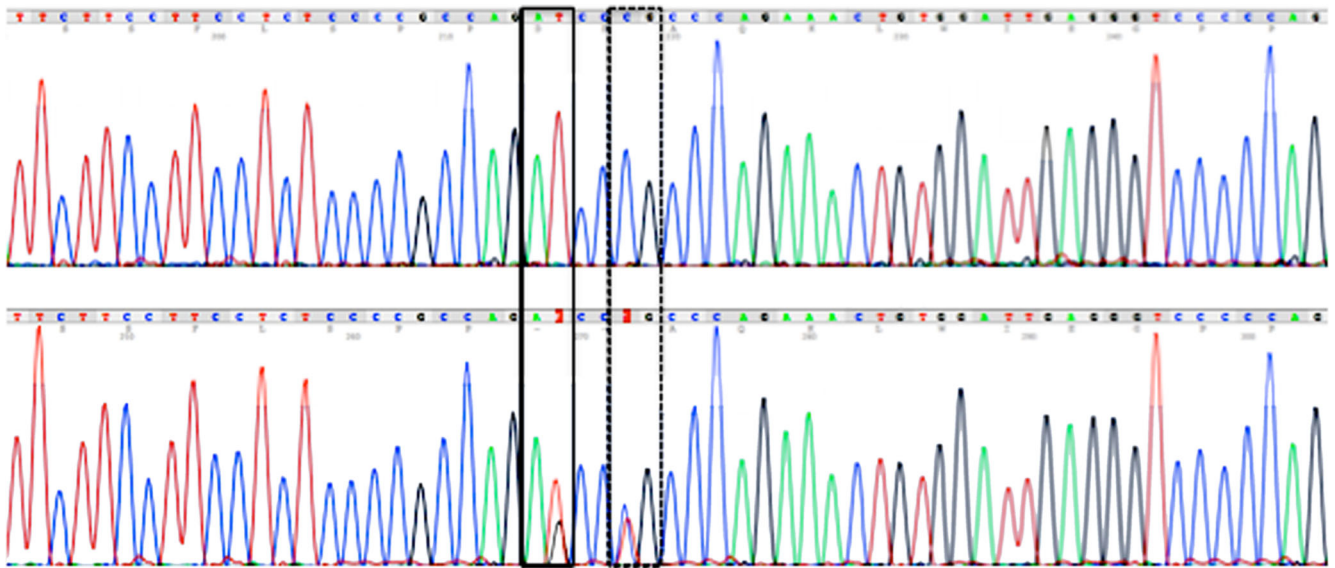
in exon 11 (c.1317 T>G), leading to a premature stop codon Y439\* and/or splicing since it is on the intron–exon junction (figure 2). Proband II-4 who is phenotypically normal has both wild-type alleles. This variant was not encountered in any of the 150 control healthy individuals, and was not found in any database including the exome sequencing project (ESP). We concluded that the homozygous form of this variant is responsible for the early neonatal death in this family, since the mutation leads to a truncated protein with only three Ig-like domains in concordance with the previously described mutations affecting *NPHS1* described so far, and causing the Finnish-type nephrotic syndrome.

This is the first report of a Finnish-type nephrotic syndrome in Lebanon, the third in the Middle-East region, and reflects the involvement of *NPHS1* mutations in this disease outside Finland and Europe (Schoeb *et al.* 2010; Ameli *et al.* 2013; Al-Hamed *et al.* 2013; Dincel *et al.* 2013). However, our results do point out to a potential role for *NPHS1* in aortic stenosis, a specific form of congenital heart disease, that could manifest either shortly after birth or during adulthood. Nearly one per cent of the population is born with aortic stenosis, which can be very mild and thus would not appear in childhood, or very severe, and necessitates surgery just after birth (Garg 2006; Laforest and Nemer 2012). The underlying genetic and molecular pathways that lead to the narrowing and malformation of this valve are however, still, poorly understood. Recent studies did show that mutations in genes encoding GATA5 or NOTCH1 could be disease causing in humans thus supporting the established mouse models whereby, inactivating one or the other gene would lead to aortic stenosis (Garg *et al.* 2005; Laforest *et al.* 2011; Padang *et al.* 2012).

In the case of *NPHS1*, there were no direct links between mutations in this gene and aortic stenosis. Two previous studies in 1963 (Fournier *et al.* 1963) and 2000 (Grech *et al.*

**Table 1.** Oligonucleotides used for PCR and sequencing of all exons of *NPHS1*.

Exon number	Size	Sequence forward	Sequence reverse
1–2	601	GAAAAGCAGGTGGCAGAGAC	GTCATCGCTGAGGTACAGG
3–4	582	GGGACCCTGCTAGAGGTAAG	TGGTCAGGAACACACACCC
5	252	CCACCCTGAGGACTTCCAG	ACCTAGCCCAAGCTTCATGC
6–7	588	GGTGACATCCCCACCTCTTC	ATTCTGGAGACAGGGACAGG
8	358	GCTGAAGGTGAGGGCAAAC	CCAGTAGGCATAATTTGGGG
9	304	CTCAGCATCTGTCTCCAGTCC	GACCCACCCTCCCTATC
10–11	518	GGACTGGGATGGACTCAGG	TCCAAGATTGCCCAAGATTC
12	349	ACCCAGTGGGCAGGGTAG	CCAGGGTTCGCTGGGTC
13–14	656	AGACTGGGCGGAGCCTG	CATTGCAGCCTGAGCGAC
15–16	523	TTAACCCCTGAACCTGTGCC	AGCTCCCAACAATGAGGAGAC
17	279	TAAGACATCCCTCCACCTG	CCCAAGGAACTCACAGTCAAG
18–19	589	ACAGAAGGGACAATTTGGGC	CCTTCTCTGCAGGGACTCAG
20	319	TGGATGCATAGATGATTCCAAG	TTTCAGAAACATGGGCAGC
21–22	541	GGGAAAACCTGGACAGAATC	CAGTAACTCATCATAAAAGGGGAATAG
23	227	ATGAATCTAATAGGCTTAAGAAGAGG	CAGAGGAGGTAGGGTCAGAGAC
24–26	631	TCAGGGCTACACTTTCTCGG	ATCAACCTGATGCTAACGGC
27–28	465	CAGGTTGATCATTGCCCTTC	GCTGGCCCTAACTAATAACAAGC
29	294	TTAAGCAGGGGCATGTATCC	GGCCAGGCTGTAATGAG



**Figure 2.** *NPHS1* sequencing results. Representative chromatograms for probands II-4 (upper panel) and II-3 (lower panel) show the variant c.1317 T>G at the beginning of exon 11, that leads to the p.Y439\* protein (lined rectangle). The dotted rectangle shows the c.1320 C>T variant that segregates with the c.1317 T>G variant, but has no effect on the amino acid composition of the protein.

2000) did, however, show that a couple of patients with the Finnish-type nephrotic syndrome have, in addition, either aortic or pulmonary stenosis. In addition, a couple of congenital nephrotic syndrome cases were also associated with cardiac defects, mainly pulmonary stenosis and septal defects (Frishberg *et al.* 2002; Sonmez *et al.* 2008; Malaki *et al.* 2011). The underlying mutations were in the *NPHS2* gene that encodes the Podocin protein, thus suggesting that septation and valve formation in the cardiac outflow tract could be regulated by *NPHS1/NPHS2*. Our results did show that the heterozygous form of the mutation is potentially responsible for the aortic stenosis phenotype encountered in both father and son. The mother (I-2) is healthy, despite being genotypically positive, but there are no echocardiographs to support the phenotype. Whether or not the phenotype is due to the mutation thus, needs, much more investigation starting from the ontology of the protein in aortic valve precursor cells. The accumulating evidence for a role of *NPHS1* in aortic valve formation could be better appreciated now, with the recent implication of *NPHS1* in the formation of coronary arteries in mice (Wagner *et al.* 2011). Wagner *et al.* (2011) showed that Nephtrin is expressed in the proepicardial cells, which migrate to form the coronary arteries, and that mice with deletion of both alleles of *nephtrin* have abnormal epicardial morphology and reduced number of coronary vessels (Wagner *et al.* 2011). Although there was no thorough analysis of the expression of Nephtrin in endocardial and/or endothelial cells in the heart, previous studies showed expression in pancreatic islet endothelial cells. Taken together, we hypothesize that the expression in endothelial precursor aortic valve cells is crucial for the complete formation and functionality of the valves. Though the process of coronary

artery formation is independent of aortic valve formation, both involve an epithelial to mesenchymal transformation.

Our result suggests the need of a thorough analysis of individuals with heterozygous mutations in *NPHS1* and a full cardiac assessment of patients with congenital nephrotic syndrome of the Finnish type for better follow-up and clinical management.

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