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Copy number variation analysis in 138 families with steroid-resistant nephrotic syndrome identifies causal homozygous deletions in *PLCE1* and *NPHS2* in two families

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Abstract

Background—Steroid-resistant nephrotic syndrome (SRNS) is the second most common cause of kidney failure in children and adults under the age of 20 years. Previously, we were able to detect by exome sequencing (ES) a known monogenic cause of SRNS in 25–30% of affected families. However, ES falls short of detecting copy number variants (CNV). Therefore, we hypothesized that causal CNVs could be detected in a large SRNS cohort.

Methods—We performed genome-wide single nucleotide polymorphism (SNP)-based CNV analysis on a cohort of 138 SRNS families, in whom we previously did not identify a genetic cause through ES. We evaluated ES and CNV data for variants in 60 known SRNS genes and in 13 genes in which variants are known to cause a phenocopy of SRNS. We applied previously published, predefined criteria for CNV evaluation.

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Declarations

Ethics approval and consent This study was approved by the institutional review board of Boston Children's Hospital as well as institutional review boards of institutions where families were recruited. Before inclusion, written informed consent of each individual or their legal guardians was obtained.

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Results—We detected a novel CNV in two genes in 2 out of 138 families (1.5%). The 9,673 bp homozygous deletion in *PLCE1* and the 6,790 bp homozygous deletion in *NPHS2* were confirmed across the breakpoints by PCR and Sanger sequencing.

Conclusions—We confirmed that CNV analysis can identify the genetic cause in SRNS families that remained unsolved after ES. Though the rate of detected CNVs is minor, CNV analysis can be used when there are no other genetic causes identified. Causative CNVs are less common in SRNS than in other monogenic kidney diseases, such as congenital anomalies of the kidneys and urinary tract, where the detection rate was 5.3%.

Keywords

Steroid-Resistant Nephrotic Syndrome (SRNS); Copy Number Variation (CNV); Whole Exome Sequencing (WES); Exome Sequencing (ES); Monogenic disease causation

Introduction

Nephrotic syndrome (NS) in childhood is defined by proteinuria of > 40 mg/m^2 per hour, hypoalbuminemia, edema, and hyperlipidemia. It can cause severe complications, which include hypertension, infections, and thrombotic events. Children and young adults affected by NS can further be classified into two groups based on their response to steroid therapy. The first group includes 80% of patients and is considered steroid-sensitive, meaning they respond to steroid therapy [1]. The second group includes patients who are steroid-resistant. The lack of response to steroid treatment leads patients to overwhelmingly progress to chronic kidney disease (CKD) and kidney failure (KF), making steroid-resistant nephrotic syndrome (SRNS) the second-leading cause of KF in children and adults under the age of 20 years [2]. At this time, there is no effective therapy to control the relentless progression to KF.

So far, over 50 monogenic causes of SRNS have been identified [3]. Most of the genes follow a recessive mode of inheritance and, if mutated, primarily impact the glomerular podocyte and slit membrane [4]. Previously, we detected by exome sequencing (ES) a known monogenic cause in 25–30% of SRNS cases with onset before 25 years of age [5, 6]. Knowing the exact genetic cause behind a patient's SRNS greatly enhances the quality and precision of clinical management as well as pre- and post-transplant care. We showed that ES is very effective at uncovering causative variants in patients affected by SRNS, turning ES into a catalyst for achieving our goal of more personalized medical treatment for the condition. Consequently, ES should be considered in all individuals with SRNS diagnosed before the age of 25 years [5, 7].

Even though ES is a powerful tool for uncovering genetic causes of SRNS, it is an unreliable method for detecting copy number variants (CNV). This shortcoming is mostly due to the non-uniform nature of the targeted capture, rendering accurate calling of large genomic rearrangements challenging through ES [8].

However, considering the many advantages of ES, such as being more affordable than whole genome sequencing (WGS), many efforts are being made to circumvent this weakness,

resulting in the creation of CNV detection tools for ES, such as CoNIFER, cn.MOPS, CNVkit and ExomeCNV [9–12]. Alas, there is still a lack of recommended reference for ES-based CNV detection tools in medical applications. As of now, the accuracy of these tools is still highly susceptible to specific conditions and are as a whole subpar to CNV calling through WGS [8, 13].

In the broader field of monogenic kidney disease, several large cohort studies have been conducted on pathogenic CNVs in congenital anomalies of the kidney and urinary tract (CAKUT) [14–17] and nephronophthisis [18]. However, there are very few publications on the role of CNV in SRNS [19, 20].

This prompted us to perform a genome-wide single nucleotide polymorphism (SNP)-based CNV analysis on a cohort of 138 families affected by SRNS, in whom we previously could not identify a genetic cause through ES. We hypothesized that causal CNVs could be detected in a large SRNS cohort, similar to the findings in CAKUT cohorts.

Material and methods

Human subjects

This study was approved by the institutional review board (IRB) of Boston Children's Hospital. From April 1998 to June 2016, patients were enrolled after obtaining informed consent. Inclusion criteria were the following: onset of symptoms before 25 years and a clinical diagnosis of SRNS (e.g. proteinuria, hypoalbuminemia, edema) or nephrotic range proteinuria with kidney histology of focal segmental glomerulosclerosis or diffuse mesangial sclerosis (Supplementary Table 2) [5]. Before December 2013, enrolled individuals were screened for variants in *WT1* and *NPHS2*. Those that screened positive were not included in this study [5].

Genotyping and CNV calling

As previously described by Wu et al. [14], genomic DNA was isolated from peripheral blood lymphocytes. SNP genotyping was performed on all cases using the Infinium Expanded Multi-Ethnic Genotyping Array (MEGA^{EX}; Illumina, San Diego, CA, USA). In brief, raw genotyping data were preprocessed with Illumina GenomeStudio software v2011 (cnvPartition algorithm) to obtain intensity data that included probe-level logR-ratio (LRR) and B allele frequency (BAF) values.

CNV calling was initially performed on hg19 assembly coordinates. The identified CNVs were then inspected manually using CLC Genomics Workbench software (CLC bio, Aarhus, Denmark) [21].

CNV analysis

CNV data were evaluated for 60 genes known to cause human SRNS and 13 genes known to cause a phenocopy of SRNS in humans, if mutated (Supplementary Table 1). After the identified CNVs were confirmed on the CLC Genomics Workbench software, breakpoint PCR was performed using original DNA samples from the respective individuals, following a previously described touchdown protocol [22].

The area of the predicted upstream breakpoint was narrowed down by performing PCR experiments using a series of overlapping primer pairs to provide continuous coverage of the upstream breakpoint area. The first PCR in the series without amplification indicated that the binding site of the reverse primer of the respective primer pair lies within the CNV. Next, the area of the downstream breakpoint was narrowed down by performing PCR experiments using primer pairs spanning across the CNV. The forward primer for all of these PCRs was the forward primer from the first non-amplifying PCR of the upstream breakpoint area. The reverse primers were positioned ~ 500 base pairs apart and around the predicted downstream breakpoint of the CNV. The first PCR in the series with amplification indicated that the reverse primer used in that reaction binds outside the CNV.

Subsequently, Sanger sequencing was performed to determine the exact position of the CNV breakpoints. Whenever parental DNA was available, segregation analysis was performed.

Results

Patient characteristics

A total of 294 individuals (236 affected, 58 reportedly unaffected) from 215 different families affected by SRNS were previously enrolled in our studies of exome sequencing in SRNS [5] and remained genetically unsolved after ES analysis. For 138 out of the 215 SRNS families, we maintained sufficient DNA samples to perform CNV analysis. We performed SNP microarray and CNV analysis in one affected individual for each family. Clinical characteristics of the 138 affected individuals are shown in Table 1.

Identification of a novel CNV in *PLCE1* and *NPHS2* in two families with SRNS

By performing genome-wide SNP-based CNV analysis, we identified in 2 out of 138 families (1.5%) a novel causal CNV in *PLCE1* and *NPHS2*.

The LogR-ratio and B allele frequency graphs for both CNVs that were detected by the Illumina GenomeStudio software are presented in Supplementary Fig. 1. Notably, no competing CNV attributable to a cause of the SRNS presentation was detected in any individual.

We confirmed a homozygous deletion of 9,673 bp in the *PLCE1* gene (NC_000010.10: g.96020851_96030523del or NC_000010.10:g.96020854_96030526del) and a homozygous deletion of 6,790 bp in the *NPHS2* gene (NC_000001.10:g.179519243_179526032del) by PCR and Sanger sequencing across the breakpoints (Fig. 1A and B). Details of the two CNVs and clinical features are summarized in Table 2.

As compared to CAKUT, in which diagnostic CNVs are mostly constituted by known genomic disorders or large CNVs containing multiple coding genes [14, 16], here the contribution of genomic disorders is probably negligible, but small CNVs in known SRNS genes can contribute to the disease etiology.

There was no competing variant detected through ES analysis that may otherwise explain the cause of SRNS in individual A4214_21. However, individual B1391_21 also harbors a

homozygous missense variant (c.2810G > A; p.Arg937Gln) in the SRNS gene *GAPVD1* [22].

Discussion

We performed genome-wide SNP-based CNV analysis on a cohort of 138 families affected by SRNS, in whom we previously did not identify a genetic cause through ES [5]. We detected a novel CNV in two genes in two families (2/138 families, 1.5%).

We surveyed the genomic variants database ClinVar looking for reported CNVs in cases of syndromic and non-syndromic cases of SRNS, congenital NS, infantile NS and focal segmental glomerulosclerosis. We found a pathogenic homozygous deletion of 1,623 bp in the *SMARCAL1* gene and one likely pathogenic inversion of 110 kbp including the same gene in two cases of Schimke immuno-osseous dysplasia. The search also showed three pathogenic deletions of over 20 kbp which include parts of the *WT1* gene in cases of 11p partial monosomy syndrome. We also found two CNVs, a duplication and a deletion, in the SRNS gene *INF2*, that were however of uncertain clinical significance. A survey of the DECIPHER [25, 26] and ISCA [27] databases did not yield any additional results.

We also conducted a literature search and to date, there are two publications that mention CNVs in the context of SRNS [19, 20]. Nagano et al. present seven cases of CKD caused by a CNV, two of which presented with Alport syndrome and have a CNV in the X-linked 'SRNS phenocopy gene' *COL4A5* [20]. Nakanishi et al. present a case of infantile nephrotic syndrome (INS) with a single nucleotide variant (SNV) in the *COQ6* gene and a CNV in the same gene [19]. The heterozygous SNV in *COQ6* was detected through next generation sequencing (NGS), but still left the case genetically unsolved, since *COQ6* follows an autosomal recessive (AR) mode of inheritance [28]. Through NGS-data-based CNV analysis, i.e. pair analysis, and custom array comparative genomic hybridization the authors detected a heterozygous deletion of exons 1 and 2 in *COQ6*, thereby confirming the genetic diagnosis. This subsequently allowed the child to receive treatment with coenzyme Q10 and achieve complete remission [28].

In our CNV analysis, we only considered homozygous deletions in known SRNS genes, as almost 80% of known SRNS genes follow an AR mode of inheritance. We only included families in whom no pathogenic variant was found in any of the known SRNS genes during ES analysis. With our study, we show that causative CNVs can be detected by genome-wide SNP-based CNV analysis in families with SRNS and that disease-causing CNVs are a much rarer cause of SRNS (1.5% of unsolved SRNS cases) than SNVs (11–30% of SRNS cases) [5, 6, 29].

Further considering the autosomal recessive etiology of SRNS, a growing body of evidence in the field of AR disorders shows an increased genetic diagnostic rate for CNVs in individuals in whom previously only a heterozygous single nucleotide variant (SNV) was found in an AR gene [30–32]. It seems that a promising approach to further increase the genetic diagnostic rate in SRNS through CNV analysis, is to examine for heterozygous

CNVs in patients in whom a heterozygous SNV in a known AR SRNS gene was previously detected by ES [19].

In our study, individual B1391_21, in whom we detected a CNV in *NPHS2*, also harbors a competing potentially causative homozygous missense variant (c.2810G > A; p.Arg937Gln) in the SRNS candidate gene *GAPVD1* [22]. Sanger sequencing of parental DNA confirmed segregation of the SNV in *GAPVD1* [22] and our segregation analysis through gel electrophoresis showed that the CNV in *NPHS2* was also inherited.

Hermle et al. identified and functionally characterized the *GAPVD1* gene as a potential novel and rare monogenic cause of SRNS [22]. To map the interacting domains on *GAPVD1*, they performed truncation mapping by co-IP and found that the two functional domains RasGAP and VPS9 of *GAPVD1* were precipitated by full-length nephrin, whereas the large interjacent domain of *GAPVD1* (aa 458–1355) was not. Hermle et al. observed that each one of the functional domains of *GAPVD1* seems to be sufficient for interaction with nephrin. The SNV (p.Arg937Gln) in individual B1391_21 is located within the gene's interjacent domain (aa 458–1355). However, with subsequent experiments, Hermle et al. showed that this SNV in *GAPVD1* does affect the interaction with nephrin and alters RAB5-binding, thus potentially causing nephrotic syndrome.

The CNV that we detected in *NPHS2* fully deletes exons 6, 7, and 8, which make up a large part of the SPFH protein domain. The age of onset of nephrotic syndrome in individual B1391_21 was 18 months. Deleterious variants in exons 6 to 8 have been shown to be causative of SRNS [2], contributing to *NPHS2* being the most frequently mutated gene in individuals with onset of SRNS between the ages of 1 and 18 years [2]. We conclude that the CNV in *NPHS2* is more likely causative of SRNS in individual B1391_21, than the SNV in *GAPVD1*.

We showed that CNV analysis performed in SRNS families in whom a genetic cause was not determined through ES, leads to an increase in the genetic diagnostic rate. We recommend performing genome-wide SNP-based CNV analysis in families affected by SRNS as an extension to genetic testing beyond ES, especially when no sequencing variants have been found in any known SRNS gene, but a high suspicion of genetic disease causation still remains due to the clinical presentation, such as in cases of CNS or INS and in families with multiple affected individuals.

Conclusions

Genome-wide SNP-based CNV analysis can identify CNVs in a large SRNS cohort, which remained genetically unsolved after ES analysis. We identified a novel homozygous causal deletion in *PLCE1* and *NPHS2* in two out of 138 SRNS families (1.5%).

However, the CNV detection rate is lower than in other monogenic kidney diseases, such as congenital anomalies of the kidneys and urinary tract, where the detection rate was 5.3% [14].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

Depersonalized data that this study is based on are available from the corresponding author upon request.

References

1. Barnett HL, Edelmann CM, Greifer I (1981) Primary nephrotic syndrome in children: Clinical significance of histopathologic variants of minimal change and of diffuse mesangial hypercellularity. A report of the international study of kidney disease in children. Kidney Int 20:765–771. 10.1038/ki.1981.209 [PubMed: 7334749]
2. Leonard MB, Donaldson LA, Ho M, Geary DF (2003) A prospective cohort study of incident maintenance dialysis in children: an NAPRTC study. Kidney Int 63:744–755. 10.1046/j.1523-1755.2003.00788.x [PubMed: 12631143]
3. Connaughton DM, Kennedy C, Shril S et al. (2019) Monogenic causes of chronic kidney disease in adults. Kidney Int 95:914–928. 10.1016/j.kint.2018.10.031 [PubMed: 30773290]
4. Wiggins RC (2007) The spectrum of podocytopathies: A unifying view of glomerular diseases. Kidney Int 71:1205–1214 [PubMed: 17410103]
5. Warejko JK, Tan W, Daga A et al. (2018) Whole exome sequencing of patients with steroid-resistant nephrotic syndrome. Clin J Am Soc Nephrol 13:53–62. 10.2215/CJN.04120417 [PubMed: 29127259]
6. Sadowski CE, Lovric S, Ashraf S et al. (2015) A Single-Gene Cause in 29.5% of Cases of Steroid-Resistant Nephrotic Syndrome. J Am Soc Nephrol 26:1279–1289. 10.1681/ASN.2014050489 [PubMed: 25349199]
7. Mann N, Braun DA, Amann K et al. (2019) Whole-Exome sequencing enables a precision medicine approach for kidney transplant recipients. J Am Soc Nephrol 30:201–215. 10.1681/ASN.2018060575 [PubMed: 30655312]
8. Zhao L, Liu H, Yuan X et al. (2020) Comparative study of whole exome sequencing-based copy number variation detection tools. BMC Bioinformatics 21:97. 10.1186/s12859-020-3421-1 [PubMed: 32138645]
9. Krumm N, Sudmant PH, Ko A et al. (2012) Copy number variation detection and genotyping from exome sequence data. Genome Res 22:1525–1532. 10.1101/gr.138115.112 [PubMed: 22585873]
10. Klambauer G, Schwarzbauer K, Mayr A et al. (2012) Cn.MOPS: Mixture of Poissons for discovering copy number variations in next-generation sequencing data with a low false discovery rate. Nucleic Acids Res 40:e69. 10.1093/nar/gks003 [PubMed: 22302147]
11. Talevich E, Shain AH, Botton T, Bastian BC (2016) CNVkit: Genome-Wide Copy Number Detection and Visualization from Targeted DNA Sequencing. PLoS Comput Biol 12:e1004873. 10.1371/journal.pcbi.1004873 [PubMed: 27100738]

12. Sathirapongsasuti JF, Lee H, Horst BAJ et al. (2011) Exome sequencing-based copy-number variation and loss of heterozygosity detection: ExomeCNV. Bioinformatics 27:2648–2654. 10.1093/bioinformatics/btr462 [PubMed: 21828086]
13. Tan R, Wang Y, Kleinstein SE et al. (2014) An Evaluation of Copy Number Variation Detection Tools from Whole-Exome Sequencing Data. Hum Mutat 35:899–907. 10.1002/humu.22537 [PubMed: 24599517]
14. Wu C-HW, Lim TY, Wang C et al. (2022) Copy Number Variation Analysis Facilitates Identification of Genetic Causation in Patients with Congenital Anomalies of the Kidney and Urinary Tract. Eur Urol Open Sci 44:106–112. 10.1016/J.EUROS.2022.08.004 [PubMed: 36185583]
15. Sanna-Cherchi S, Kiryluk K, Burgess KE et al. (2012) Copy-number disorders are a common cause of congenital kidney malformations. Am J Hum Genet 91:987–997. 10.1016/j.ajhg.2012.10.007 [PubMed: 23159250]
16. Verbitsky M, Westland R, Perez A et al. (2019) The copy number variation landscape of congenital anomalies of the kidney and urinary tract. Nat Genet 51:117–127. 10.1038/s41588-018-0281-y [PubMed: 30578417]
17. Westland R, Verbitsky M, Vukojevic K et al. (2015) Copy number variation analysis identifies novel CAKUT candidate genes in children with a solitary functioning kidney. Kidney Int 88:1402–1410. 10.1038/ki.2015.239 [PubMed: 26352300]
18. Snoek R, van Setten J, Keating BJ et al. (2018) NPHP1 (Nephrocystin-1) gene deletions cause adult-onset ESRD. J Am Soc Nephrol 29:1772–1779. 10.1681/ASN.2017111200 [PubMed: 29654215]
19. Nakanishi K, Okamoto T, Nozu K et al. (2019) Pair analysis and custom array CGH can detect a small copy number variation in COQ6 gene. Clin Exp Nephrol 23:669–675. 10.1007/s10157-018-1682-z [PubMed: 30584653]
20. Nagano C, Nozu K, Morisada N et al. (2018) Detection of copy number variations by pair analysis using next-generation sequencing data in inherited kidney diseases. Clin Exp Nephrol 22:881–888. 10.1007/s10157-018-1534-x [PubMed: 29372472]
21. Gee HY, Otto EA, Hurd TW et al. (2014) Whole-exome resequencing distinguishes cystic kidney diseases from phenocopies in renal ciliopathies. Kidney Int 85:880–887. 10.1038/ki.2013.450 [PubMed: 24257694]
22. Hermle T, Schneider R, Schapiro D et al. (2018) GAPVD1 and ANKFY1 mutations implicate RAB5 regulation in nephrotic syndrome. J Am Soc Nephrol 29:2123–2138. 10.1681/ASN.2017121312 [PubMed: 29959197]
23. Hinkes B, Wiggins RC, Gbadegesin R et al. (2006) Positional cloning uncovers mutations in PLCE1 responsible for a nephrotic syndrome variant that may be reversible. Nat Genet 38:1397–1405. 10.1038/ng1918 [PubMed: 17086182]
24. Boute N, Gribouval O, Roselli S et al. (2000) NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. Nat Genet 24:349–354. 10.1038/74166 [PubMed: 10742096]
25. Swaminathan GJ, Bragin E, Chatzimichali EA et al. (2012) DECI-PHER: web-based, community resource for clinical interpretation of rare variants in developmental disorders. Hum Mol Genet 21:R37–R44. 10.1093/hmg/dds362 [PubMed: 22962312]
26. Firth HV, Richards SM, Bevan AP et al. (2009) DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. Am J Hum Genet 84:524–533. 10.1016/j.ajhg.2009.03.010 [PubMed: 19344873]
27. Miller DT, Adam MP, Aradhya S et al. (2010) Consensus Statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies. Am J Hum Genet 86:749–764. 10.1016/j.ajhg.2010.04.006 [PubMed: 20466091]
28. Heeringa SF, Chernin G, Chaki M et al. (2011) COQ6 mutations in human patients produce nephrotic syndrome with sensorineural deafness. J Clin Invest 121:2013–2024. 10.1172/JCI45693 [PubMed: 21540551]

29. Tan W, Lovric S, Ashraf S et al. (2018) Analysis of 24 genes reveals a monogenic cause in 11.1% of cases with steroid-resistant nephrotic syndrome at a single center. Pediatr Nephrol 33:305–314. 10.1007/s00467-017-3801-6 [PubMed: 28921387]

30. Av D, Ghosh R, Yuan B et al. (2019) Copy number variant and runs of homozygosity detection by microarrays enabled more precise molecular diagnoses in 11,020 clinical exome cases. Genome Med 11:30. 10.1186/s13073-019-0639-5 [PubMed: 31101064]

31. Boone PM, Campbell IM, Baggett BC et al. (2013) Deletions of recessive disease genes: CNV contribution to carrier states and disease-causing alleles. Genome Res 23:1383–1394. 10.1101/gr.156075.113 [PubMed: 23685542]

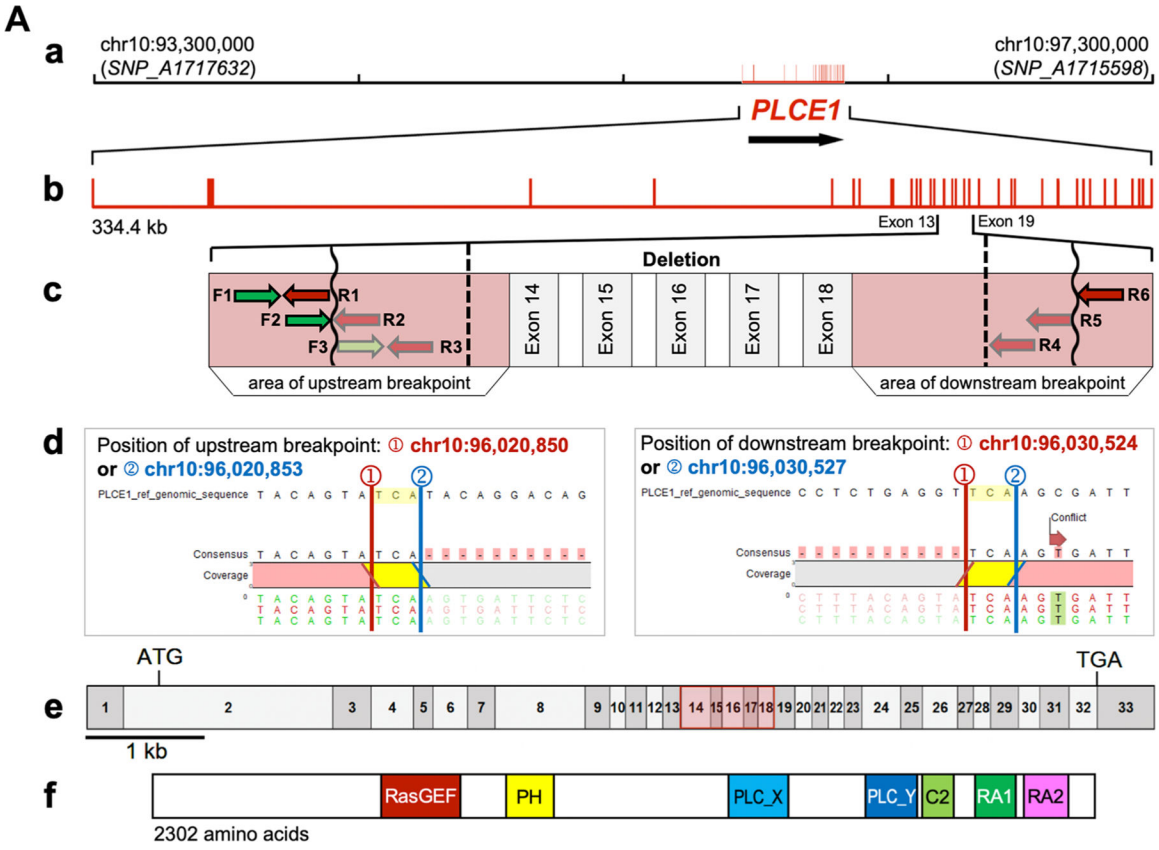
32. Lalani SR, Liu P, Rosenfeld JA et al. (2016) Recurrent Muscle Weakness with Rhabdomyolysis, Metabolic Crises, and Cardiac Arrhythmia Due to Bi-allelic TANGO2 Mutations. Am J Hum Genet 98:347–357. 10.1016/j.ajhg.2015.12.008 [PubMed: 26805781]

Web resources

33. ClinVar. National Center for Biotechnology Information, U.S. National Library of Medicine Accessed July 1, 2023. http://www.ncbi.nlm.nih.gov/clinvar

34. DECIPHER. Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources Accessed July 1, 2023. https://decipher.sanger.ac.uk/

35. ISCA. International Standards for Cytogenomic Arrays Accessed July 1, 2023. https://www.clinicalgenome.org/



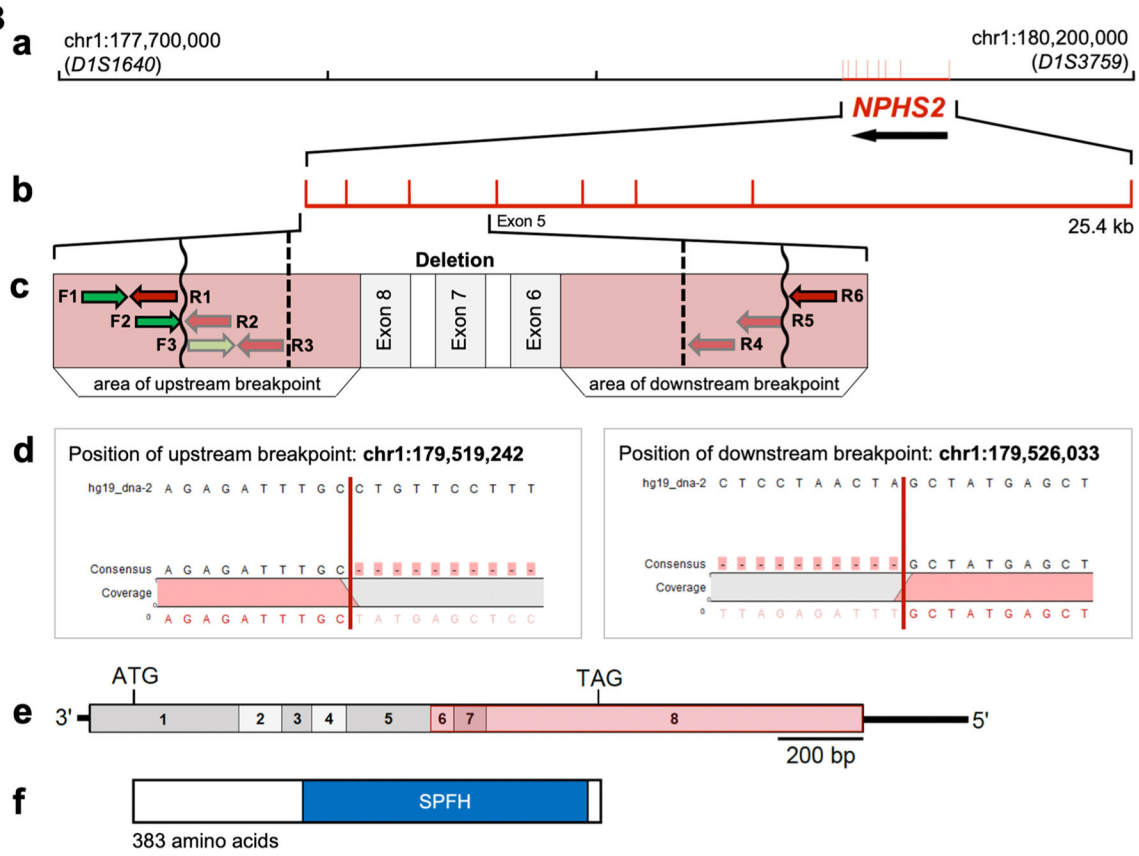


Fig. 1.

A CNV detected and confirmed in *PLCE1* in individual A4314_21. (a) The *PLCE1* critical genetic region extends over a 4.0-Mb interval between flanking markers *SNP_A1717632* and *SNP_A1715598*. The arrow indicates the location and transcriptional direction of the *PLCE1* gene [23]. (b) The *PLCE1* gene measures 334.4 kb and extends over 33 exons (vertical hatches). GenBank accession number NM_016341.3. (c) Primer positioning for the PCR experiments. To narrow down the area of the deletion's upstream and downstream breakpoint, we performed PCR experiments around the breakpoint positions predicted by the CNV analysis software Illumina GenomeStudio. These hypothetical positions are represented by the dashed lines. After having narrowed down the area of the upstream breakpoint through PCRs, we narrowed down the area of the downstream breakpoint by running PCR experiments using primers spanning across the deletion. Primers that turned out to still lie within the deletion are represented by pale arrows and the squiggly lines show the location of the new, more precise breakpoint positions, that were determined by the PCR experiments. FP = forward primer, shown as green arrows; RP = reverse primer, shown as red arrows. (d) Sanger sequencing across the upstream and downstream breakpoint of the deletion determined its exact size. The CNV found in this individual's *PLCE1* gene is a homozygous exonic deletion of 9,673 bp. However, since both the up- and downstream breakpoint are framed by the nucleotides TCA (highlighted in yellow), their position cannot be defined unambiguously. The deletion extends from chr10:96,020,850 to chr10:96,030,524 if TCA is deleted at the upstream breakpoint (see ①, red lines) or from chr10:96,020,853 to chr10:96,030,527 (see ②, blue lines) if TCA is deleted at the downstream breakpoint.

It is impossible to determine exactly whether TCA is deleted at the up- or downstream breakpoint of the deletion. (e) Exon structure of human *PLCE1* cDNA showing positions of the start codon (ATG) at nt + 1 and stop codon (TGA). Exon size, which ranged from 62 to 1570 bp, is shown to scale. The exons deleted by the CNV are shaded in red. (f) Positions of putative protein domains, in relation to the encoding exon position in e, **B** CNV detected and confirmed in *NPHS2* in individual B1391_21. (a) The *NPHS2* critical genetic region extends over a 2.5-Mb interval between flanking markers *D1S1640* and *D1S3759*. The arrow indicates the location and transcriptional direction of the *NPHS2* gene [24]. (b) The *NPHS2* gene measures 25.4 kb and extends over 8 exons (vertical hatches). GenBank accession number NM_014625.4. (c) Primer positioning for the PCR experiments, see section c from the caption of Fig. 1A. (d) Sanger sequencing across the upstream and downstream breakpoint of the deletion determined its exact size and location. The CNV found in this individual's *NPHS2* gene is a homozygous exonic deletion of 6,790 bp, chr1:179,519,242 to chr1:179,526,033. (e) Exon structure of human *NPHS2* cDNA showing positions of the start codon (ATG) and stop codon (TAG). Exon size, which ranged from 56 to 911 bp, is shown to scale. The exons deleted by the CNV are shaded in red. (f) Position of the putative protein domain, in relation to the encoding exon position in e

Table 1

Clinical characteristics of the 138 individuals (from 138 families) with SRNS who underwent CNV analysis

Parameter	Result, *n* (%)
Gender	
Female	65 (47)
Male	70 (51)
Unknown	3 (2)
Total	138 (100)
Extrarenal manifestations	
Yes	44 (32)
No	85 (62)
Unknown	9 (6)
Total	138 (100)
Consanguinity (reported)	
Yes	32 (23)
No	103 (75)
Unknown	3 (2)
Total	138 (100)
Age of onset	
≤ 3 months	9 (6.5)
≤ 12 months	29 (21)
1 to 5 years	43 (31)
> 5 years	48 (35)
Unknown	9 (6.5)
Total	138 (100)

Table 2

Details of the two CNVs in *PLCE1* and *NPHS2* identified in our cohort

Individual ID	Exact SRNS phenotype	Extrarenal phenotype	CNV position (hg19)	CNV length (bp)	Copy number	Genes involved	ACMG
A4314_21	INS	NR	chr10:96,020,850-chr10:96,030,524 or chr10:96,020,853- chr10:96,030,527	9,673	1	*PLCE1*	P
B1391_21	SRNS	NR	chr1:179,519,242- chr1:179,526,033	6,790	1	*NPHS2*	P

ACMG, American College of Medical Genetics; CNV, copy number variation; hg19, human genome assembly 19; INS, infantile nephrotic syndrome; NR, none reported; P, pathogenic; SRNS, steroid-resistant nephrotic syndrome