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Submission to: Journal of Hypertension Title of Paper: Genetics of renovascular hypertension in children
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Abstract:	<p>Objective: In most cases of renovascular hypertension in children, the etiology is unclear. The aim of this study was to investigate genetic variation as a factor in the development of renovascular hypertension in children.</p> <p>Methods: In a cohort of 37 unrelated children from a single tertiary referral center, exome sequencing was performed. We assessed variants in recognized and suspected disease genes and searched for novel ones with a gene-based variant-burden analysis.</p> <p>Results: In the majority of patients, exome sequencing could not identify causative variants. We found a pathogenic variant in a recognized associated disease gene in five patients (three pathogenic variants in NF1, one in ELN and a deletion of chromosome 7q11.23, consistent with Williams Syndrome). In two other patients, (likely) pathogenic variants were found in putative renovascular hypertension genes (SMAD6 and GLA), with clinical implications for both. Ten additional patients carried variants of uncertain significance (VUS) in known (n=4) or putative (n=6) renovascular hypertension disease genes. Rare variant burden analysis yielded no further candidate genes.</p> <p>Conclusions: Genetic contributors, such as germline mutations in NF1, ELN, 7q11.23del were present in only 5 out of 37 (14%) children with renovascular hypertension. Twelve other children (32%) had potentially causal variants identified, including a pathogenic variant in SMAD6; a vasculopathy gene hitherto unknown to link with renovascular hypertension. Most importantly, our data show that exome sequencing can rarely identify the cause of renovascular hypertension in non-syndromic children. We suggest that non-genetic factors or somatic genetic variation will play a more important role.</p>

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All authors have signed this letter as confirmation that they have read and approved the paper, have met the criteria for authorship as established by the International Committee of Medical Journals Editors, believe that the paper represents honest work, and are able to verify the validity of the results reported.

Yours sincerely,

Detlef Bockenhauer
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Title: Genetics of renovascular hypertension in children

Running title: Genetics of renovascular hypertension

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Disclosures

None

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Abstract

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Results: In the majority of patients, exome sequencing could not identify causative variants. We found a pathogenic variant in a recognized associated disease gene in five patients (three pathogenic variants in *NF1*, one in *ELN* and a deletion of chromosome 7q11.23, consistent with Williams Syndrome). In two other patients, (likely) pathogenic variants were found in putative renovascular hypertension genes (*SMAD6* and *GLA*), with clinical implications for both. Ten additional patients carried variants of uncertain significance (VUS) in known (n=4) or putative (n=6) renovascular hypertension disease genes. Rare variant burden analysis yielded no further candidate genes.

Conclusions: Genetic contributors, such as germline mutations in *NF1*, *ELN*, 7q11.23del were present in only 5 out of 37 (14%) children with renovascular hypertension. Twelve other children (32%) had potentially causal variants identified, including a pathogenic variant in *SMAD6*; a vasculopathy gene hitherto unknown to link with renovascular hypertension. Most importantly, our data show that exome sequencing can rarely identify the cause of renovascular hypertension in non-syndromic children. We suggest that non-genetic factors or somatic genetic variation will play a more important role.

Keywords: Renal artery obstruction, neurofibromatosis 1, Williams syndrome, Fabry disease, whole exome sequencing

Introduction

Renovascular hypertension (RVH) is defined as high blood pressure due to renal arterial occlusive disease. Stroke, cardiac failure and chronic kidney disease are major complications of childhood RVH. Unfortunately, many pediatric cases are only recognized after onset of the first complications. It has an incidence of approximately 1.9 per million children per year and is responsible for 5-10% of secondary childhood hypertension.[1,2] The causes are usually different from adult RVH and include fibromuscular dysplasia, vasculitis, extrinsic compression of a renal artery, trauma, iatrogenic, congenital rubella and specific genetic diseases.[1] Radiological or surgical intervention to improve renal blood supply is performed in most for better blood pressure control, as pharmacological control alone, even with multiple agents, proves difficult.[2] Furthermore, in very difficult cases, nephrectomy might be the only option.[2] Timely diagnosis is important as the blood pressure is often seriously elevated, risking devastating complications, including stroke and cardiac failure.

Lack of understanding on the etiology is a major impediment for improvements in diagnostic and therapeutic approaches. Known causes include congenital rubella syndrome, vasculitis and genetic causes, but how these diseases trigger renal arterial occlusive disease is unclear. Moreover, such causes can only be identified in a minority of patients.

Genetic diseases that are known to associate with renovascular hypertension are neurofibromatosis type 1[3] (most common genetic cause), ELN-associated disease (elastin associated disease, including Williams-Beuren syndrome)[4,5], Alagille syndrome[6], tuberous sclerosis[7], Grange syndrome[8] and adenosine deaminase deficiency[9]. There is large variability in the severity of vascular disease in these genetic disorders, ranging from no vascular disease at all to involvement of all the major vessels in the arterial tree. One of the more severe forms is midaortic syndrome, a disease characterized by narrowing of the abdominal aorta, leading to reduced perfusion of the lower extremities, intestines and kidneys. Therefore, midaortic syndrome is almost invariably accompanied by renovascular hypertension.

Great Ormond Street Hospital is a large national and international referral center for renovascular hypertension and we therefore had access to a substantial number of children with this rare disorder. A recent study had characterized genetic contributors to the etiology of midaortic syndrome, reporting a genetic cause in almost half of children with midaortic syndrome.[10] We therefore hypothesized that there would also be a substantial genetic contribution to pediatric renovascular hypertension in general. We performed whole exome sequencing (WES) in our patient cohort, aiming to identify known causes as well as discover potentially novel ones.

Methods

Study design and participants

Ethical permission for the study was given by the London-Bloomsbury National Health Service Research Ethics Committee. Patients admitted to the pediatric nephrology ward of Great Ormond Street Hospital (UK) between the years 2009 and 2016 with evidence of renovascular hypertension were eligible for inclusion and asked to participate. Renovascular hypertension was defined as proven arterial narrowing on a digital subtraction angiogram. Informed consent was obtained from all participants before inclusion.

Whole exome sequencing and variant analysis

WES was performed as described previously.[11-13] Briefly, genomic DNA was isolated from blood lymphocytes and sent for exome sequencing (Perkin Elmer Genomics, Duluth GA, USA). Reads were aligned to the human genome assembly GRCh37/hg19. Variant calling format (VCF) files were uploaded to Ingenuity Variant Analysis (Qiagen, Aarhus, Denmark).

Variant filtering

We evaluated the WES data of all patients for possibly pathogenic variants in known and putative renovascular hypertension genes (Supplemental tables 1 and 2). A putative renovascular hypertension gene was defined as a gene that can cause large- or medium-sized arterial disease in humans when mutated. Ingenuity Variant Analysis (version 5.5.20190807) was used for variant filtering. We excluded all variants that were (1) located outside the known and putative renovascular hypertension genes, (2) had low call quality (Phred-scaled quality score <20), (3) were too frequent in any of the large population databases (GnomAD (2.0.1) or EVS (ESP6500SI-V2)) with the upper limit of the minor allele frequency (MAF) set between 0.005 and 0.1%, depending on mode of inheritance (for details see Supplemental Figure 1), (4) were in an intronic region (unless predicted to harbor a regulatory site or

be involved in splicing) or (5) did not fit the inheritance pattern of the respective gene (e.g. a mono-allelic variant in a gene causing recessive disease).

Additionally, we screened for evidence of Williams' Beuren syndrome by searching for unexpectedly long stretches of pseudo-homozygosity in the 7q11.23 region (GRCh37/hg19 chr7: 72,744,455 - 74,142,510).

Renovascular hypertension in children has been associated with congenital anomalies of the kidney and urinary tract (CAKUT).[14] Therefore, we also checked for pathogenic or likely pathogenic variants in known CAKUT genes (Supplemental table 3).

Variant classification with 2019 ACGS Guidelines

All remaining variants were scored according to the Association for Clinical Genomic Science Best Practice Guidelines for Variant Classification 2019 (<https://www.acgs.uk.com/quality/best-practice-guidelines/>, ratified 06-05-2019). This is a modification of the 2015 American College of Medical Genetics and Genomics criteria (ACMG criteria) and uses the same five variant classes: (1) pathogenic (probability of pathogenicity >99%), (2) likely pathogenic (probability >90%), (3) variant of uncertain significance (probability between 10% and 90%), (4) likely benign and (5) benign.[15]

Rare variant burden analysis

VCFs underwent quality control filtering using bcftools (v1.7) and were annotated with Variant Effect Predictor (VEP v98). Exome-wide rare variant analysis was carried out on a per-gene basis using TRAPD[16]. Only variants with a read depth > 10 were analyzed. The burden of rare (gnomAD_AF < 0.001) likely deleterious (high or moderate impact as determined by VEP) variants in cases compared to the publicly available gnomAD database (n= 125748), under both a dominant and recessive model, was calculated using a one-sided Fisher's exact test. Bonferroni corrected exome-wide significance level was calculated to be 3.1×10^{-6} (0.05/16149 genes).

Comparison of rare variant burden in RVH cases against a set of in-house controls (n=41) was conducted using rvtests to exclude sequencing artefacts.[17]

Results

Cohort description

37 patients with renovascular hypertension were included in the study. Median age at presentation was 5 years, 41% were female and most patients reported European ethnicity (Table 1). Midaortic syndrome was present in approximately half of the patients, an additional 19% had bilateral renal artery stenosis, 14% unilateral renal artery stenosis and 11% intrarenal stenoses.

Three patients had a genetic diagnosis before start of the study (pathogenic variants in *NF1* in two patients and in *ELN* in one patient). Additionally, three other patients had a clinically suspected diagnosis of neurofibromatosis type 1.

Identification of seven pathogenic or likely pathogenic variants in seven individuals

Variant filtering resulted in the identification of 27 candidate single nucleotide variants and small insertion-deletions (indels) in the known renovascular hypertension genes (Supplemental Figure 1). Using the 2019 ACGS criteria, four variants were classified as pathogenic, six as variants of unknown significance (VUS), fourteen as likely benign and four as benign (Supplemental Table 4). In the putative renovascular hypertension genes, a further 22 candidate single nucleotide variants (SNV) and small indels were identified (Supplemental Table 5). One variant was scored as pathogenic, one as likely pathogenic, ten as VUS, and ten as likely benign. Furthermore, in the analysis of the exome sequencing data in the 7q11.23 region, we observed (pseudo-)homozygosity for this region in one individual. The (pseudo-)homozygosity spanned the whole 1.4 million basepair Williams-Beuren syndrome region (containing 26 genes), suggesting a deletion of the region. In total we thus identified seven pathogenic or likely pathogenic DNA variants in seven different individuals in this cohort of 37 individuals with

renovascular hypertension (Figure 1). The individuals who carry these variants are listed in Table 1, including a summary of clinical and variant information.

Of the SNV classified as (likely) pathogenic, two variants were novel. These were the *SMAD6* variant c.433dupC and the *ELN* variant c.1228G>T leading to a frameshift and premature stop codon, respectively. The other four variants, all reported before to be disease-causing, included an SNV leading to a premature stop codon in *NF1*, the *NF1* p.K1444E missense variant in two unrelated patients and a missense variant in *GLA* (c.870G>C). [18-20]

No pathogenic or likely pathogenic variants were identified in any of the *CAKUT* genes (data not shown). Also, no rare variants were detected in *NF2*, *SPRED1*, *BRAF*, *KRAS*, *MAP2K1*, *RAF1*, *NRAS*, *PTPN11*, *RIT1*, *SOS1*, *GNAS*, *MSH2*, *MSH6*, *MLH1*, *PMS2* in those patients with a clinical suspicion of neurofibromatosis type 1 but without a detected variant in *NF1*.

We found variants of uncertain significance in ten further patients; variants and clinical summary are listed in Table 2.

Exome-wide rare variant burden analysis did not detect any novel genes enriched for rare variation

In an attempt to try and identify novel genes that might be enriched for rare likely deleterious variation in RVH, we compared the burden of qualifying variants (gnomad_AF < 0.001 and either protein truncating, splice-site, missense or inframe indel) on a per-gene basis in our cases to the publicly available gnomAD database. A total of ten genes reached exome-wide significance under the dominant (nine genes) and recessive (six genes) inheritance models. Although *NF1* did not reach exome-wide significance, some enrichment of rare variation under the dominant model was seen (p=0.004). However, when the rare variant burden in our cases was compared to a set of in-house controls who had been sequenced on the same platform, none of the ten genes remained significant.

Phenotype correlated with genotype in most individuals

Age at first presentation of the patients in which a genetic cause was identified varied between 8 months and 16 years (median 4 years). This age range corresponds with the age at inclusion. Of the five patients with a pathogenic variant in a known gene, all had bilateral renal stenosis, four had further involvement of other vessels, including three with some form of aortic and/or pulmonary artery stenosis; one had midaortic syndrome. Three patients with a pathogenic variant in *NF1* all had café-au-lait spots and a positive family history. One patient with an *ELN* mutation had more extensive vascular involvement as well as a history of pyloric stenosis and unilateral inguinal hernia. The patient with a pathogenic variant consistent with Williams-Beuren syndrome presented with hypertension as an incidental finding and a history of learning difficulties and nocturnal enuresis. The patient found to have a likely pathogenic variant in *GLA* presented with no reported history of Fabry disease. A full description of the clinical data of the patients in which a genetic cause or putative genetic cause was identified can be found in Supplemental Table 6.

Discussion

Our study is the first to characterize the contribution of genetic factors in the etiology of pediatric renovascular hypertension. In our cohort of 37 unrelated children, we established or confirmed a genetic diagnosis in 5 patients (~14%). Additionally, we found a pathogenic variant in *SMAD6* and a likely pathogenic variant in *GLA* in two more patients; the two genes are known to cause arterial disease in humans when mutated. A further 10 patients had a variant of uncertain significance in a known or putative renovascular hypertension gene.

Interestingly, in three patients with a clinical suspicion of neurofibromatosis type 1, exome sequencing did not detect any mutation in *NF1*, nor in disease genes that can mimic aspects of neurofibromatosis type 1, i.e. *NF2*, *SPRED1*, *BRAF*, *KRAS*, *MAP2K1*, *RAF1*, *NRAS*, *PTPN11*, *RIT1*, *SOS1*, *GNAS*, *MSH2*, *MSH6*, *MLH1* and *PMS2*. Potentially, these patients had a variant in *NF1* that was not detected by exome sequencing (e.g. in a non-coding region), or were mosaic for a *NF1* mutation; alternatively, other genes might be causative.

Of further interest, two unrelated patients had the exact same pathogenic variant in *NF1* (p.K1444E). This might suggest that this variant is more often associated with renovascular hypertension than other pathogenic variants in *NF1*. This hypothesis is supported by a very recent report of a significantly increased prevalence of cardiovascular abnormalities in patients with this specific variant in *NF1*, when compared to patients with other variants.[18]

The finding of a pathogenic variant in *SMAD6* in a child with unilateral renal artery stenosis may extend the phenotype associated with pathogenic variants in this gene. Whether the observation of a cross-fused ectopic kidney in this child might also be a result of the variant is only speculative. The *SMAD6* protein is an inhibitory factor in the transforming growth factor beta (TGF β) pathway.[21] Dysfunction of any of the genes in this pathway cause aneurysms or stenosis of the large arterial vessels. Pathogenic variants in *SMAD6* have been repeatedly observed in patients with bicuspid aortic valve, supraaortic stenosis and aortic aneurysms.[22-24] Furthermore, a recent publication reports unilateral renal hypoplasia in a child with biallelic variants in *SMAD6*,[25] suggesting that this child

might have unnoticed renal artery disease. Our study suggests a possible link between *SMAD6* variants and renovascular hypertension.

The finding of a likely pathogenic variant in *GLA* in a girl who presented with bilateral renal artery stenosis at the age of 8 months was unexpected. Fabry disease, an X-linked disease caused by mutations in *GLA*, can result in severe vascular disease.[26] Males generally present earlier in life and with a more severe phenotype, but females can be affected as well. Accumulation of a glycolipid called globotriaosylceramide is believed to be the primary cause of symptoms, including for the stenosis of small arteries.[27] We consider it unlikely that a girl at this age would have sufficient glycolipid deposition around her intrarenal arteries to cause renovascular hypertension without apparent other symptoms of the disease. Therefore, the identification of this variant appears coincidental, yet with potentially significant clinical implications otherwise.

To identify novel causative genes for renovascular hypertension, we used a per-gene rare variant burden analysis. This initially identified ten genes significantly enriched for rare variation in our cohort. However, comparison to a group of in-house controls failed to validate the association, suggesting that sequencing artefacts and differences in variant calling accounted for the signal seen when using the gnomAD database as a control population.

Renovascular hypertension is a rare disease in children and a strength of our study is the relatively large size of our cohort. Since most national and international referrals to our center were for the expressed purpose of providing radiological or surgical intervention, it is however possible that our cohort is biased towards the more severe end of the spectrum. Another limitation is the lack of sequencing data for both parents in most cases, which would have allowed us to identify potentially causative *de novo* variants.

Interestingly, we show that finding a genetic diagnosis appears to be less common in our mixed cohort of renovascular hypertension than in a previously described cohort with midaortic syndrome.[10] A potential explanation for at least some of the observed difference is our more

stringent application of variant classification criteria. Establishing a correct genetic diagnosis is of paramount importance, as it can affect prognosis, treatment and reproductive counselling.[28] Therefore, classification criteria demand at least 90% certainty before assigning (likely) pathogenicity to an identified variant.[15] The difficulty of reaching this threshold is reflected in the large number of VUS in our study.

Future perspectives

Our data do not support routine exome sequencing in children with renovascular hypertension. Apart from the scientific benefit of genetic screening, a genetic diagnosis might have important clinical implications by providing an explanation for the disease and informing treatment and genetic counselling.[29] However, these benefits should be weighed against the cost of screening and the potential of incidental findings. In our study, the genetic diagnosis was clinically suspected in four of the five patients in which we found a pathogenic variant in a recognized associated disease gene. The fifth patient had symptoms that might have suggested the clinical diagnosis (Williams-Beuren syndrome). Our data thus suggest that genetic testing is most likely to identify a causative variant only in patients with a clinical suspicion of an underlying inherited disorder.

Nevertheless, from a scientific point of view, studies like the current are of great importance. They might provide novel leads for genes and pathways involved in renovascular hypertension, as shown by our finding of a pathogenic variant in *SMAD6*. Many questions remain on the pathophysiology and genotype-phenotype-treatment relationships of this disease. Exome or genome sequencing of novel large cohorts and pooled analyses of such cohorts might help to answer these questions.

A potentially important aspect to be investigated in future studies concerns the role of somatic or mosaic mutations, which may be missed when using DNA from peripheral leukocytes. The fact that exome sequencing could not reveal an etiological diagnosis in most patients, suggests that factors such as these play a more important role. The absence of a genetic diagnosis in three patients with a clinical suspicion of *NF1* in our study further supports this hypothesis. Somatic and mosaic mutations

are of particular potential relevance for patients with unilateral or otherwise very localized disease. Sequencing of DNA extracted from affected tissue obtained during surgical interventions would be ideal to address this important question.

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Supplemental Table 2: putative renovascular hypertension disease genes

Supplemental Table 3: known CAKUT genes

Supplemental Table 4: list of identified rare variants in known renovascular hypertension disease genes

Supplemental Table 5: list of identified rare variants in putative renovascular hypertension disease genes

Supplemental Table 6: phenotypes of patients with pathogenic variant, likely pathogenic variant or variant of uncertain significance

Supplemental Figure 1: overview of variant filtering strategy

References

1. Peco-Antic A, Stajic N, Krstic Z, Bogdanovic R, Milosevski-Lomic G, Dukic M, et al. Associated extrarenal vascular diseases may complicate the treatment and outcome of renovascular hypertension. *Acta paediatrica* 2016; 105:e35-41.
2. Tullus K, Brennan E, Hamilton G, Lord R, McLaren CA, Marks SD, et al. Renovascular hypertension in children. *Lancet* 2008; 371:1453-1463.
3. Duan L, Feng K, Tong A, Liang Z. Renal artery stenosis due to neurofibromatosis type 1: case report and literature review. *Eur J Med Res* 2014; 19:17.
4. Rose C, Wessel A, Pankau R, Partsch CJ, Bursch J. Anomalies of the abdominal aorta in Williams-Beuren syndrome--another cause of arterial hypertension. *European journal of pediatrics* 2001; 160:655-658.
5. Metcalfe K, Rucka AK, Smoot L, Hofstadler G, Tuzler G, McKeown P, et al. Elastin: mutational spectrum in supravalvular aortic stenosis. *European journal of human genetics : EJHG* 2000; 8:955-963.
6. Kamath BM, Spinner NB, Emerick KM, Chudley AE, Booth C, Piccoli DA, et al. Vascular anomalies in Alagille syndrome: a significant cause of morbidity and mortality. *Circulation* 2004; 109:1354-1358.
7. Salerno AE, Marsenic O, Meyers KE, Kaplan BS, Hellinger JC. Vascular involvement in tuberous sclerosis. *Pediatric nephrology* 2010; 25:1555-1561.
8. Guo DC, Duan XY, Regalado ES, Mellor-Crummey L, Kwartler CS, Kim D, et al. Loss-of-Function Mutations in YY1AP1 Lead to Grange Syndrome and a Fibromuscular Dysplasia-Like Vascular Disease. *American journal of human genetics* 2017; 100:21-30.
9. Nanthapaisal S, Murphy C, Omoyinmi E, Hong Y, Standing A, Berg S, et al. Deficiency of Adenosine Deaminase Type 2: A Description of Phenotype and Genotype in Fifteen Cases. *Arthritis Rheumatol* 2016; 68:2314-2322.
10. Warejko JK, Schueler M, Vivante A, Tan WZ, Daga A, Lawson JA, et al. Whole Exome Sequencing Reveals a Monogenic Cause of Disease in approximate to 43% of 35 Families With Midaortic Syndrome. *Hypertension* 2018; 71:691-699.
11. Enerback S, Nilsson D, Edwards N, Heglind M, Alkanderi S, Ashton E, et al. Acidosis and Deafness in Patients with Recessive Mutations in FOXL1. *Journal of the American Society of Nephrology : JASN* 2018; 29:1041-1048.
12. Cabezas OR, Flanagan SE, Stanescu H, Garcia-Martinez E, Caswell R, Lango-Allen H, et al. Polycystic Kidney Disease with Hyperinsulinemic Hypoglycemia Caused by a Promoter Mutation in Phosphomannomutase 2. *Journal of the American Society of Nephrology : JASN* 2017; 28:2529-2539.
13. Reichold M, Klootwijk ED, Reinders J, Otto EA, Milani M, Broeker C, et al. Glycine Amidinotransferase (GATM), Renal Fanconi Syndrome, and Kidney Failure. *Journal of the American Society of Nephrology : JASN* 2018; 29:1849-1858.
14. Kari JA, Roebuck DJ, Tullus K. Renal artery stenosis in association with congenital anomalies of the kidney and urinary tract. *Saudi Med J* 2014; 35:1264-1266.
15. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in medicine : official journal of the American College of Medical Genetics* 2015; 17:405-424.
16. Guo MH, Plummer L, Chan YM, Hirschhorn JN, Lippincott MF. Burden Testing of Rare Variants Identified through Exome Sequencing via Publicly Available Control Data. *American journal of human genetics* 2018; 103:522-534.
17. Zhan X, Hu Y, Li B, Abecasis GR, Liu DJ. RVTESTS: an efficient and comprehensive tool for rare variant association analysis using sequence data. *Bioinformatics* 2016; 32:1423-1426.
18. Koczkowska M, Callens T, Chen Y, Gomes A, Hicks AD, Sharp A, et al. Clinical spectrum of individuals with pathogenic NF1 missense variants affecting p.Met1149, p.Arg1276, and p.Lys1423: genotype-phenotype study in neurofibromatosis type 1. *Human Mutation* 2019.

19. Fokkema IFAC, Taschner PEM, Schaafsma GCP, Celli J, Laros JFJ, den Dunnen JT. LOVD v.2.0: the next generation in gene variant databases. *Human Mutation* 2011; 32:557-563.
20. Colon C, Ortolano S, Melcon-Crespo C, Alvarez JV, Lopez-Suarez OE, Couce ML, et al. Newborn screening for Fabry disease in the north-west of Spain. *European journal of pediatrics* 2017; 176:1075-1081.
21. Miyazawa K, Miyazono K. Regulation of TGF-beta Family Signaling by Inhibitory Smads. *Cold Spring Harb Perspect Biol* 2017; 9.
22. Gillis E, Kumar AA, Luyckx I, Preuss C, Cannaeerts E, van de Beek G, et al. Candidate Gene Resequencing in a Large Bicuspid Aortic Valve-Associated Thoracic Aortic Aneurysm Cohort: SMAD6 as an Important Contributor. *Front Physiol* 2017; 8:400.
23. Tan HL, Glen E, Topf A, Hall D, O'Sullivan JJ, Sneddon L, et al. Nonsynonymous variants in the SMAD6 gene predispose to congenital cardiovascular malformation. *Human mutation* 2012; 33:720-727.
24. Luyckx I, MacCarrick G, Kempers M, Meester J, Geryl C, Rombouts O, et al. Confirmation of the role of pathogenic SMAD6 variants in bicuspid aortic valve-related aortopathy. *European journal of human genetics : EJHG* 2019; 27:1044-1053.
25. Kloth K, Bierhals T, Johannsen J, Harms FL, Juusola J, Johnson MC, et al. Biallelic variants in SMAD6 are associated with a complex cardiovascular phenotype. *Human genetics* 2019; 138:625-634.
26. Chan B, Adam DN. A Review of Fabry Disease. *Skin Therapy Lett* 2018; 23:4-6.
27. Chimenti C, Morgante E, Tanzilli G, Mangieri E, Critelli G, Gaudio C, et al. Angina in fabry disease reflects coronary small vessel disease. *Circ Heart Fail* 2008; 1:161-169.
28. Ashton E, Bockenhauer D. Diagnosis of uncertain significance: can next generation sequencing replace the clinician? *Kidney international* 2019; in press.
29. Bockenhauer D, Medlar AJ, Ashton E, Kleta R, Lench N. Genetic testing in renal disease. *Pediatric nephrology* 2012; 27:873-883.

	Number	Proportion
<i>Sex</i>		
Male	22	59%
Female	15	41%
<i>Age</i>		
Below 2 years	9	24%
2-6 years	16	43%
Above 6 years	12	32%
<i>Type of renovascular hypertension</i>		
Midaortic syndrome	20	54%
Bilateral renal artery stenosis	7	19%
Unilateral artery stenosis	5	14%
Segmental or intrarenal stenoses	4	11%
Graft for aortic aneurysmatic disease	1	3%
<i>Clinical etiological diagnosis at inclusion</i>		
Vasculitis	2	5%
Fibromuscular dysplasia	3	8%
Neurofibromatosis type 1	6	16%
Pathogenic variants in ELN	1	3%
No clear clinical etiological diagnosis	25	68%
<i>Involvement of other vessels</i>		
Yes	23	62%
None noticed	14	38%

Table 1. Baseline characteristics of the cohort.

Patient	Gene/locus	Zygoty	Variant class	Effect on protein	Clinical diagnosis before sequencing	Clinical summary
<i>Variants in known renovascular hypertension genes</i>						
1	<i>NF1</i>	Het*	Pathogenic	p.K1444E	Neurofibromatosis	Bilateral RAS‡, café-au-lait spots, optic pathway glioma, bilateral high frequency hearing loss
2	<i>NF1</i>	Het	Pathogenic	p.K1444E	Neurofibromatosis	Bilateral RAS, café-au-lait spots, valvular pulmonary artery stenosis
3	<i>NF1</i>	Het	Pathogenic	p.Q452*	Neurofibromatosis	MAS ⁺ with bilateral RAS, café-au-lait spots, scoliosis, bone changes
4	<i>ELN</i>	Het	Pathogenic	p.G415*	ELN-associated disease	Bilateral RAS, stenosis supra valvular aorta, brachiocephalic artery, pulmonary artery and unilateral carotid artery, pyloric stenosis, inguinal hernia.

5	7q11.23del	Het	Pathogenic	-	-	Bilateral RAS, intra-renal stenosis, midaortic syndrome, learning difficulties.
<i>Variants in putative renovascular hypertension genes</i>						
6	GLA	Het	Likely pathogenic	p.M290I	-	Bilateral RAS
7	SMAD6	Het	Pathogenic	p.L145fs*158	-	Unilateral RAS, crossed fused ectopic kidney

Table 2. Summary of pathogenic and likely pathogenic variants. Patients in which a pathogenic variant was identified, including a summary of the variant information and the clinical information. *Het, heterozygous; † MAS, midaortic syndrome; ‡ RAS, renal artery stenosis. ACGS scoring criteria applied for each variant are listed in Supplemental table 4 and 5.

Patient	Gene/locus	Zygoty	Variant class	Predicted effect	
				on protein	Clinical summary
<i>Variants in known renovascular hypertension genes</i>					
8	<i>TSC1</i>	Het	VUS	p.P231L	Bilateral RAS, MAS
9	<i>TSC1</i>	Het	VUS	p.D1136E	Bilateral RAS, major aneurysms aorta
10	<i>TSC2</i>	Het	VUS	Promoter/5'UTR	Bilateral RAS, MAS, cerebrovascular accident
11	<i>ELN</i>	Het	VUS	p.G764R	Unilateral RAS
6	<i>NOTCH2</i>	Het	VUS	Promoter/5'UTR	Bilateral RAS
5	<i>JAG1</i>	Het	VUS	p.R1042H	Bilateral RAS, intra-renal stenosis, midaortic syndrome, learning difficulties.
<i>Variants in putative renovascular hypertension genes</i>					
12	<i>PRKG1</i>	Het	VUS	5'UTR	Bilateral RAS, MAS
13	<i>FBN1</i>	Het	VUS	p.E2102Q	Bilateral RAS, MAS, transient facial palsy
14	<i>RNF213</i>	Het	VUS	p.P243L	Bilateral RAS, history of meningitis

15	<i>RNF213</i>	Het	VUS	p.R2196K	Unilateral RAS, supraventricular tachycardia
16	<i>OBSCN</i>	Het	VUS	p.M3024I	Bilateral RAS, MAS, left ventricular failure
6	<i>PHACTR1</i>	Het	VUS	3'UTR	Bilateral RAS
		Compound		p.L182M,	
17	<i>RNF213</i>	het.?	VUS	p.I258N	Unilateral RAS, vasculitic rash

Table 3. Summary of variants of uncertain significance. Patients in which a variant of uncertain significance was identified, including a summary of the variant information and the clinical information. Note that all variants have a MAF below the relevant threshold (detailed in Supplemental Figure 1). None of the patients had a clinical diagnosis before sequencing. *Het, heterozygous; † MAS, midaortic syndrome; ‡ RAS, renal artery stenosis; VUS, variant of uncertain significance. ACGS scoring criteria applied for each variant are listed in Supplemental table 4 and 5.

Figure legends

Figure 1: Patients in which a variant of the indicated class was identified. Individuals are counted only once, and only in the highest category. Thus, an individual with both a pathogenic variant and a variant of uncertain significance would be counted once as an individual with a pathogenic variant. A) Visualisation of the number of variants in each class for the known renovascular hypertension genes (i.e. upper part of Tables 1 and 2). B) Visualisation of the number of variants in each class for the known AND putative renovascular hypertension genes (i.e. lower part of Tables 1 and 2). *VUS*, variant of uncertain significance.



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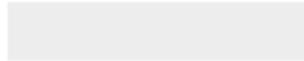
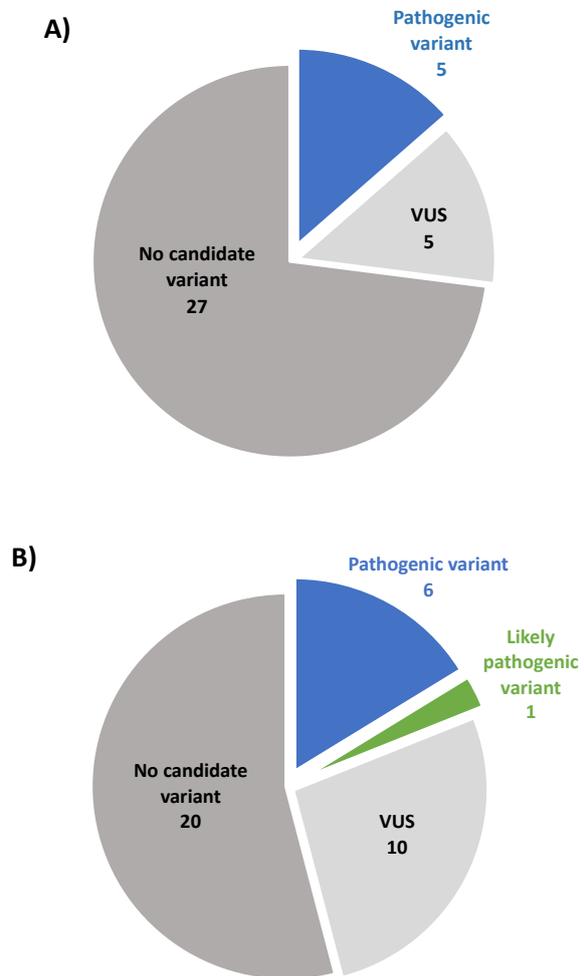


Figure 1. Known (A) – and known and putative (B) – genetic causes of renovascular hypertension in our cohort



Abbreviations definition list

ACGS	Association for Clinical Genomic Science
CAKUT	Congenital anomalies of the urinary tract
MAF	Minor allele frequency
RVH	Renovascular hypertension
SNV	Single nucleotide variant
VUS	Variant of uncertain significance
WES	Whole exome sequencing

Title: Genetics of renovascular hypertension in children

Condensed abstract

To investigate the etiology of renovascular hypertension in children, we performed exome sequencing in a cohort of 37 unrelated children. We found a pathogenic variant in a recognized associated disease gene in five patients (3x *NF1*, 1x *ELN* and 1x deletion of chromosome 7q11.23). Additionally, twelve children had potentially causal variants identified, including a pathogenic variant in *SMAD6*; a gene hitherto unknown to link with renovascular hypertension. Most importantly, our data show that exome sequencing can rarely identify the cause of renovascular hypertension in non-syndromic children. Other factors such as somatic genetic variation might play a more important role.