Fakultät für Medizin

Technische Universität München

EXOME SEQUENCING IN HEREDITARY NEPHROPATHIES

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Vollständiger Abdruck der von der Fakultät für Medizin der Technischen Universität München zur Erlangung des akademischen Grades eines Doktors der Medizin (Dr. med.) genehmigten Dissertation.

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Die Dissertation wurde am 03.08.2020 bei der Technischen Universität München eingereicht und durch die Fakultät für Medizin am 16.02.2021 angenommen.

In Gedenken an meinen Vater, Dr. med. Hans Harald Riedhammer.

Everything is going to be fine in the end. If it's not fine it's not the end.

Oscar Wilde

ABSTRACT

Introduction: Hereditary kidney diseases affect about one in ten adults with chronic kidney disease (CKD) and about two-thirds of patients with CKD-onset under the age of 25 years. Hence, they pose a considerable burden of disease. All parts of the intricate organ that is the kidney and urinary tract can be altered and hereditary nephropathies are therefore clinically and genetically vastly heterogeneous. Exome sequencing (ES), that is, the analysis of the protein-coding regions of the human genome, is able to address this genetic heterogeneity. **Aim of this thesis:** Evaluation of ES in 260 index cases with a clinically presumed hereditary nephropathy with emphasis on the detection of phenocopies (clinical tentative diagnosis is different from genetic diagnosis), the prioritization of novel disease-associated genes ("candidate genes"), and the statistical analysis of the cohort to improve clinical decision-making. **Study design:** Cross-sectional study. **Methods:** ES in 260 genetically unsolved index cases recruited between October 2015 and February 2019. **Results:** 77 of 260 cases could be genetically solved (a diagnostic yield of 30%). In 12 of 77 solved cases (16%), a phenocopy was identified. In 8 of 260 cases (3%), a candidate gene could be prioritized. There were significant differences in Alport syndrome (AS) versus thin basement membrane nephropathy (TBMN), the two poles of disease severity of type-IV-collagen-related nephropathy: Diagnostic yield was significantly higher in AS than in TBMN (65% vs. 28%, $p = 0.01$). Median age at first manifestation was significantly lower in AS than in TBMN (5.5 years [3.0–9.0] vs. 16.0 years [5.0–32.3], $p = 0.001$). There were no extrarenal manifestations in TBMN cases, compared to 28% in AS cases ($p = 0.01$). A family history was less commonly reported in TBMN cases than in AS cases (39% vs. 78%, $p = 0.006$). For the total cohort, clinical predictors of a solved case were positive family history (odds ratio [OR] 6.61 [95% confidence interval 3.28–13.35], $p < 0.001$), an extrarenal manifestation (OR 3.21 [1.58–6.54], $p = 0.01$) and – with a borderline significance – younger age at first manifestation (OR 0.97 [0.93–1.00], $p = 0.048$). **Discussion:** This thesis shows the utility of ES in hereditary nephropathies by the identification of phenocopies, which have major implications for disease management and prognosis, and of novel disease-associated genes. Furthermore, the results of this thesis guide the genetic work-up of patients by presenting statistical evidence for predictors of a positive genetic result and for delineating the disease spectrum of type-IV-collagen-related nephropathy.

ACKNOWLEDGMENTS

I would like to thank my doctoral supervisor, PD Dr. Julia Hoefele, for her excellent support. Additionally, many thanks to Univ.-Prof. Dr. Thomas Meitinger, the head of the Institute of Human Genetics, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany, for filling me with enthusiasm for the field of human genetics.

My family has been a beacon of support – my mother, Margit Riedhammer; my sister, Anna-Katharina Hiemer; my brother-in-law, Daniel Hiemer; and my girlfriend, Johanna Greiner. All my love to them: I would not have completed this thesis without them.

I would further like to thank the medical technical assistants at the Institute of Human Genetics, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany, and at the Institute of Human Genetics and the Next-Generation Sequencing Core Facility at the Helmholtz Zentrum München – German Research Center for Environmental Health, Neuherberg, Germany. Further thanks to Dr. Matthias Braunisch, Dr. Bettina Lorenz-Depiereux, and Dr. Matias Wagner for proofreading of the manuscript.

Thank you to Kayla Friedman and Malcolm Morgan of the Centre for Sustainable Development, University of Cambridge, United Kingdom, for producing the Microsoft Word thesis template used to produce this document.

Finally, I thank all referring clinicians and collaboration partners and, last but not least, the index patients and their legal guardians for participating in this study.

TABLE OF CONTENTS

GLOSSARY 9

LIST OF ABBREVIATIONS AND ACRONYMS 18

ONLINE RESOURCES 23

1 INTRODUCTION 25

1.1 HEREDITARY NEPHROPATHIES 26

1.1.1 Autosomal dominant tubulointerstitial kidney disease (ADTKD) 27

1.1.2 Alport syndrome (AS) 31

1.1.3 Congenital anomalies of the kidney and urinary tract (CAKUT) 35

1.1.4 Ciliopathies 40

1.1.5 Hereditary focal segmental glomerulosclerosis and steroid-resistant nephrotic syndrome (FSGS/SRNS) 47

1.1.6 VACTERL/VATER association 54

1.1.7 Other hereditary nephropathies 54

1.1.7.1 Tubulopathies 54

1.1.7.2 Inherited metabolic disorders 55

1.1.7.3 Mitochondrial disorders 56

1.1.7.4 Atypical hemolytic uremic syndrome (complement-mediated hemolytic uremic syndrome) 56

1.2 EXOME SEQUENCING 58

1.2.1 Exome sequencing in general, its utility and limitations 58

1.2.2 Exome sequencing in hereditary nephropathies 63

1.2.3 Comprehensive genetic testing for the detection of phenocopies in hereditary nephropathies 66

1.3 GENOMICS IN HEREDITARY NEPHROPATHIES – OUTLOOK 67

1.4 GENETICS OF NON-MONOGENIC KIDNEY DISEASES 67

2 AIM OF THIS THESIS 70

3 PATIENT COHORT, MATERIAL, AND METHODS 71

3.1 PATIENT COHORT 71

3.1.1 Patient recruitment and consent 71

3.1.2 Phenotype ascertainment 73

3.2 SAMPLE PROCESSING, POLYMERASE CHAIN REACTION, AND SANGER SEQUENCING 77

3.2.1 DNA isolation 77

3.2.1.1 Manual DNA isolation 77
3.2.1.2 Automated DNA isolation 78
3.2.2 DNA quantification and quality check 78
3.2.3 Polymerase chain reaction 79
3.2.3.1 Basic method 79
3.2.3.2 Materials and procedure 79
3.2.3.3 Agarose gel electrophoresis 80
3.2.3.4 PCR purification 81
3.2.4 Sanger sequencing 81
3.2.4.1 Basic method 81
3.2.4.2 Materials and procedure 82
3.2.4.3 Sequencing 83
3.3 EXOME SEQUENCING 84
3.3.1 Method 84
3.3.2 Variant interpretation 85
3.3.3 Algorithm to identify variants in novel potentially disease-associated genes 89
3.4 STATISTICAL ANALYSIS AND GRAPHICAL VISUALIZATION 92
4 RESULTS 93
4.1 EXOME SEQUENCING IN A COHORT OF 260 GENETICALLY UNSOLVED INDEX CASES WITH A CLINICALLY PRESUMED HEREDITARY NEPHROPATHY 93
4.1.1 Cohort description 93
4.1.2 Diagnosis of a hereditary kidney disease by exome sequencing 101
4.2 DIFFERENCES IN TYPE-IV-COLLAGEN-RELATED NEPHROPATHY CLASSIFIED AS ALPORT SYNDROME VERSUS THIN BASEMENT MEMBRANE NEPHROPATHY 104
4.3 DETECTION OF PHENOCOPIES AND RECATEGORIZATION OF DISEASE BY EXOME SEQUENCING 104
4.3.1 Description of detected phenocopies 104
4.3.2 Phenocopy detection rate of targeted NGS panels 111
4.4 EXOME SEQUENCING TO PRIORITIZE NOVEL HEREDITARY-KIDNEY-DISEASE-ASSOCIATED GENES 111
4.5 PREDICTORS OF A SOLVED CASE 115
5 DISCUSSION 116

5.1 Diagnostic yield in index patients with a clinically presumed hereditary nephropathy examined by exome sequencing 116
5.2 Type-IV-collagen-related nephropathy – Alport syndrome vs. thin basement membrane nephropathy 119
5.3 Identifying phenocopies by exome sequencing and the clinical implications of the recategorization of disease 122
5.4 Novel hereditary-kidney-disease-associated genes 126
5.5 Whom to test? Predictors of a positive genetic test 129
5.6 Limitations 131
5.7 Conclusion and outlook 132
6 REFERENCES 135
7 APPENDICES 174
7.1 List of tables 174
7.2 List of figures 175
7.3 Publications pertinent to the thesis 177
7.4 Further publications 177
7.5 Presentations at conferences 180
7.6 URL/DOI for Online Supplementary Table (featuring all index cases with detailed phenotypic and genotypic information) 182

Glossary

Adapted from Strachan & Read, 2018, if not specifically indicated.

3’ end	End of a DNA/RNA strand at the third carbon of the sugar-ring of a nucleic acid
5’ end	End of a DNA/RNA strand at the fifth carbon of the sugar-ring of a nucleic acid
Allele	Different types of the same gene
Allele frequency	Frequency of an allele at a certain locus in a population
Allelic heterogeneity	Different variants in the same gene leading to the same phenotype in different individuals
Amino acid	Molecules that constitute proteins (there are 20 proteinogenic amino acids in the standard genetic code)
Amplification	Increase in the amount of a DNA sequence (e.g., by PCR)
Annealing	Formation of a double strand out of single-stranded nucleic acids
Association	Non-random occurrence of two observable features of an individual (e.g., phenotype)
Autosome	Chromosomes that are not sex chromosomes (i.e., chromosomes 1–22).
Balanced translocation	Chromosomal rearrangement among nonhomologous chromosomes in which there is no loss of genetic material
Base complementarity	Association of two bases on opposite strands of double-stranded nucleic acids (A with T [in DNA] or U [in RNA], G with C)

Base pair	Unit of length of double-stranded nucleic acids (pairing of a purine base with a pyrimidine base by a hydrogen bond)
Biallelic	See *in trans*
cDNA	Complementary DNA; DNA synthesized from RNA by reverse transcriptase
Chromosome	Structure comprised of nucleic acids and proteins containing genetic information (genes)
Codon	A triplet of nucleotides in RNA translated into an amino acid (or start/stop signal for translation)
Common variant	Variant with an allele frequency > 5.0% in a population
Compound heterozygote	An individual with two different alleles at the same locus
Congenital	A phenotype existing at birth
Consanguinity	When mating partners have a known shared ancestor (e.g., first cousins)
Conserved sequence	A DNA (or amino acid) sequence that is the same in multiple species
Copy number variant	Deletions and duplications of genomic regions larger than 50 base pairs (newer definition) or 1 kilobase pairs (older definition; Feuk et al., 2006; Nowakowska, 2017)
CRISPR-Cas	Clustered regularly interspaced short palindromic repeats- (CRISPR-) associated – part of the adaptive immune system of prokaryotes, utilized for genome editing
Cytogenetics	A branch of genetics engaged in the study of chromosomes

De novo variant	A variant identified in an individual but not in the biological parents of this individual
Denaturation	Disassembly of double strands of nucleic acids into single strands (also, the destruction of protein structure)
Diploid	Two copies of every chromosome are present (normal state of human somatic cells)
DNA polymerase	Enzymes that elongate DNA at the 3’ end
Dominant	A trait manifesting in heterozygotes
Dominant negative effect	Heterozygous variant leading to an altered protein, which affects the function of the non-altered protein
Epigenetic	Inherited phenotype not caused by an altered DNA sequence
ExAC	Exome Aggregation Consortium (database of exome-sequencing data of 60,706 unrelated individuals; Lek et al., 2016)
Exome	The entirety of exons of the genome
Exon	Parts of a gene present in spliced RNA
Expressivity	Variable range of a phenotype caused by a particular genotype
Frameshift variant	A variant leading to an alteration of the reading frame of mRNA (due to insertion/deletion of nucleotides by a number which cannot be divided by three)
Gene	Section of DNA transcribed into RNA (mRNA or functional noncoding RNA)
Gene expression	Expression of the gene product (i.e., protein, functional noncoding RNA)

Gene knockout	Abrogation of expression of a certain gene in a cell/organism (e.g., mouse)
Genetic redundancy	The loss of one gene product can be compensated by the gene product of a different gene
Genotype	List of alleles of a certain individual at one or more loci
Genotype-phenotype correlation	Prediction of a phenotype from a given genotype
gnomAD	Genome Aggregation Database (database of 125,748 exome and 15,708 genome sequences from unrelated individuals [v.2.1.1]; Karczewski et al., 2020)
Haploid	One copy of every chromosome is present (e.g., in sperm and egg cells)
Haploinsufficiency	If there is a loss of one gene product (e.g., a heterozygous nonsense variant), in a haploinsufficiency locus, a phenotype will occur
Haplotype	Cluster of linked alleles on a single chromosome (in linkage disequilibrium)
Hardy-Weinberg principle	Relation of allele and genotype frequencies in a population
Hemizygous	One copy of DNA/a gene in a diploid cell (e.g., X-chromosomal genes in males)
Heteroplasmy	Degree of mosaicism of a mitochondrial DNA variant
Heterozygote	An individual who has two different alleles at a certain locus
Homoplasmy	The entirety of mitochondrial DNA carries a variant
Homozygote	The same alleles at a certain locus

Hypomorphic variant	A variant leading to a mild phenotype (altered gene product has a residual function/expression; Wilkie, 1994)
Imputation	Inference of genotypes that are not sequenced from reference genotype panels of known haplotypes (used in genome-wide association studies)
In cis	Variants in a gene, which are *in cis*, are on the same chromosome (= monoallelic; https://varnomen.hgvs.org/recommendations/DNA/variant/alleles/)
Indel	**In**sertion or **del**etion of bases
In trans	Variants in a gene, which are *in trans*, are on different chromosomes (= biallelic; https://varnomen.hgvs.org/recommendations/DNA/variant/alleles/)
Intron	Parts of a DNA sequence removed by the splicing of RNA
Karyotype	The set of chromosomes of a cell or an individual
Linkage disequilibrium	Alleles at linked loci with limited recombination
Locus	Defined position of a gene/DNA sequence within the genome
Locus heterogeneity	A phenotype/disease is caused by variants in different genes/loci
Loss-of-function variant	A variant leading to a loss of gene product function (e.g., a nonsense variant)
Mendelian	A single-gene/CNV cause for a given disease inheritable by Mendelian laws (i.e., a monogenic disease)

Messenger RNA	RNA transcribed from a certain DNA sequence with introns removed used as a template for protein translation
Missense variant	Variant leading to an exchange of amino acids in the respective protein
Monoallelic	See *in cis*
Monosomy	The condition of having a single copy of a chromosome (e.g., monosomy X)
Mosaic	Different cell lines in one individual
Motif	A distinct sequence, typically in a protein, important for structural/functional properties
Multifactorial	A disease/phenotype caused by a combination of several factors (genetic, epigenetic, environmental)
Mutation	The event of a DNA sequence change (also used to describe the result of the change, e.g., missense mutation)
Next-generation sequencing	Described in detail in Section 1.2
Noncoding RNA	RNA not translated into a protein
Nonsense variant	A variant leading to a premature termination codon
Nonsense-mediated mRNA decay	Pathway of mRNA surveillance degrading transcripts with premature termination codons (e.g., due to a nonsense variant). Nonsense-mediated mRNA decay (NMD) of a transcript is expected (but not always occurring) if a there is a stop in translation more than 50 nucleotides before the last exon–exon junction (Lambert et al., 2020).
Nucleic acid	DNA/RNA
Nucleoside	Purine/pyrimidine base linked to a sugar (ribose/deoxyribose)

Nucleotide	The constituent of DNA/RNA (base, sugar, phosphate)
Open reading frame	DNA sequence without a stop codon in a particular reading frame
Paired-end sequencing	Sequencing both ends of a DNA fragment and comparing the nucleotide number between these sequences to the reference genome in order to detect rearrangements such as deletions or duplications
Penetrance	The proportion with which a genotype results in a phenotype
Phenocopy	Described in detail in Section 1.2.3
Phenotype	Observable characteristics (traits) of an individual
Pleiotropy	Variants in a gene lead to multiple phenotypic traits
Polygenic	A phenotype is the result of several genetic loci acting together
Polymorphism	A variant with an allele frequency ≥ 1.0% (strictly speaking, two or more variants in a population at a frequency too high to be due to repeated mutations)
Positive selection	A certain genotype is favored in evolution
Protein domain	A particular structure/functional unit within a protein
Pseudogene	A DNA sequence highly homologous to a protein-coding gene, but without function
Purine bases	Adenine and guanine
Pyrimidine bases	Cytosine, thymine, and uracil
Rare variant	A variant with an allele frequency < 1.0%

Read	In next-generation sequencing, a read is a DNA sequence relating to a specific DNA fragment (the number of reads is called “read depth”)
Recessive	A phenotype only manifesting if both copies of a gene are affected (homozygous/compound-heterozygous causative variants)
Segregation	In terms of pedigrees, the likelihood of inheriting a trait/phenotype from a parent.
Sequence similarity/homology	Quantity of overlap of nucleic acid/protein sequences
Single-nucleotide polymorphism	Polymorphism resulting from a single nucleotide change
Splicing	Removal of introns from a primary transcript and connecting of exons
Stop codon	UAA, UAG, UGA in mRNA; leads to the termination of protein translation
Synonymous variant	A nucleotide change not leading to an amino acid change
Trait	See Phenotype
Transcription factor	A protein which binds DNA modulating gene transcription
Trisomy	The condition of having three copies of a chromosome (e.g., trisomy 21)
Truncating variant	Variant which leads to a shortened gene product (also called protein-truncating variant; Rivas et al., 2015)
Untranslated regions	Parts of the mRNA at the 5’ and 3’ end not translated into a protein that have important regulatory functions

Variant of uncertain significance	A variant that cannot be deemed (likely) benign or (likely) pathogenic given current knowledge
X-inactivation	Inactivation of all but one X chromosome in cells with more than one X chromosome by epigenetic mechanisms (in females; also called lyonization, after geneticist Mary Lyon)

List of abbreviations and acronyms

aa	Amino acid
ACE	Angiotensin I-converting enzyme
ACGS	Association for Clinical Genomic Science (United Kingdom)
ACMG	American College of Medical Genetics and Genomics
AD	Autosomal dominant
ADAS	Autosomal dominant Alport syndrome
ADPKD	Autosomal dominant polycystic kidney disease
ADTKD	Autosomal dominant tubulointerstitial kidney disease
aHUS	Atypical hemolytic uremic syndrome
AR	Autosomal recessive
ARAS	Autosomal recessive Alport syndrome
ARPKD	Autosomal recessive polycystic kidney disease
AS	Alport syndrome
BMI	Body mass index
bp	Base pair(s)
CAKUT	Congenital anomalies of the kidney and urinary tract
cAMP	Cyclic adenosine monophosphate
Cas9	CRISPR-associated protein 9
cDNA	Complementary DNA
CI	Confidence interval
CKD	Chronic kidney disease
CM	Cap mesenchyme

CMA	Chromosomal microarray
CNV	Copy number variant
CoQ10	Coenzyme Q10
CRISPR	Clustered regularly interspaced short palindromic repeats
DCT	Distal convoluted tubule
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
DOI	Digital object identifier
dRTA	Distal renal tubular acidosis
EDTA	Ethylenediaminetetraacetic acid
EPO	Erythropoietin
ER	Endoplasmic reticulum
ES	Exome sequencing
ESRD	End-stage renal disease
EVAdb	Exome Variant and Annotation Database
EVS	Exome Variant Server
FGGS	Focal global glomerulosclerosis
FSGS	Focal segmental glomerulosclerosis
GBM	Glomerular basement membrane
GFR	Glomerular filtration rate
gnomAD	Genome Aggregation Database (see "Online Resources")
GS	Genome sequencing
GWAS	Genome-wide association studies
HGMD®	Human Gene Mutation Database (see "Online Resources")

HGNC	Human Genome Organisation Gene Nomenclature Committee
HPLC	High performance liquid chromatography
IgAN	IgA nephropathy
kb	Kilobase pairs
KDIGO	Kidney Disease: Improving Global Outcomes
KO	Knockout
LOEUF	Loss-of-function observed/expected upper bound fraction
LoF	Loss-of-function
LOVD	Leiden Open Variation Database (see "Online Resources")
Mb	Megabase
MCD	Minimal change disease
MELAS	Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
MGI	Mouse Genome Informatics (see "Online Resources")
MM	Metanephric mesenchyme
MODY	Maturity-onset diabetes of the young
MRI	Magnetic resonance imaging
mtDNA	Mitochondrial DNA
NC	Nephric cord
ND	Nephric duct
NGS	Next-generation sequencing
NMD	Nonsense-mediated mRNA decay
NPHP	Nephronophthisis

OMIM®	Online Mendelian Inheritance in Man® (see "Online Resources")
OR	Odds ratio
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RRT	Renal replacement therapy
SNP	Single-nucleotide polymorphism
SNV	Single-nucleotide variant
SRNS	Steroid-resistant nephrotic syndrome
suPAR	Soluble urokinase-type plasminogen activator
TAL	Thick ascending limb of the loop of Henle
TBMN	Thin basement membrane nephropathy
TMA	Thrombotic microangiopathy
TRIS	Tris(hydroxymethyl)aminomethane
tRNA	Transfer RNA
UB	Ureteric bud
URL	Uniform resource locator
UTI	Urinary tract infection
UTR	Untranslated region
VACTERL/VATER	V – Vertebral anomalies, A – Anorectal malformations, C – Cardiovascular anomalies, T – Tracheoesophageal fistula, E – Esophageal atresia, R – Renal and/or radial anomalies, L – Limb defects
VNTR	Variable-number tandem repeats
VUR	Vesicoureteral reflux
VUS	Variant of uncertain significance
XL	X-linked

XLAS	X-linked Alport syndrome

Online resources

ClinVar	https://www.ncbi.nlm.nih.gov/clinvar/ (database of genomic variation relating to human disease)
DECIPHER	https://decipher.sanger.ac.uk/ (database of genomic variation relating to human disease, especially CNVs)
HGMD®	http://www.hgmd.cf.ac.uk (database of genomic variation relating to human disease)
GeneMatcher	https://genematcher.org/ (tool to connect researchers interested in the same gene)
gnomAD	https://gnomad.broadinstitute.org/ (database aggregating sequencing data from exome and genome sequencing projects; v.2.1.1 was used for this thesis)
LOVD	https://www.lovd.nl/ (database of genomic variation relating to human disease)
MGI	http://www.informatics.jax.org/ (database of KO mouse models)
Mutalyzer	https://mutalyzer.nl/ (tool to check sequence variant nomenclature)
OMIM®	https://omim.org/ (online catalog of human genes and associated diseases)
Primer3	http://primer3.ut.ee/ (online tool for primer design)
PubMed	https://pubmed.ncbi.nlm.nih.gov/ (database of biomedical literature)
RefSeq	https://www.ncbi.nlm.nih.gov/refseq/ (database of genomic, transcript, and protein reference sequences)

UCSC Genome Browser	https://genome.ucsc.edu/ (online genome browser hosted by the University of California, Santa Cruz, CA, United States of America [UCSC])
UniProt	https://www.uniprot.org/ (database of protein sequences)

1 INTRODUCTION

Although each hereditary nephropathy (= hereditary kidney disease) on its own is usually rare (affecting less than 1 in 2,000 people), hereditary kidney diseases in total affect nearly 10% of adults with chronic kidney disease (CKD) and more than 70% of CKD cases with onset below the age of 25 years. Therefore, hereditary nephropathies pose a substantial burden of disease (Devuyst et al., 2014; Groopman et al., 2019; Vivante & Hildebrandt, 2016). This thesis examines the application of exome sequencing (ES), that is, the sequencing of the exonic (protein-coding) regions of the human genome, in 260 genetically unsolved index patients with a clinically presumed hereditary nephropathy across all major disease groups (recruitment criteria are defined in Section 3.1). The introduction describes the most important hereditary kidney diseases/disease entities and the application of next-generation sequencing, specifically exome sequencing, in hereditary nephropathies. The aim of this thesis is addressed after the introduction, in Chapter 2.

Of note, the terms “monogenic,” “familial,” and “hereditary,” used interchangeably in this thesis, denote a single-gene/copy number variant (CNV) cause for a disease inheritable by Mendelian laws. In this thesis, the neutral term “variant” instead of “mutation” for a change in human nucleotide sequence is used, as is preferred by the American College of Medical Genetics and Genomics (ACMG; Richards et al., 2015). Furthermore, wherever the expression “causative variant” is employed, this includes both pathogenic and likely pathogenic variants/CNVs according to the ACMG criteria for sequence variant/CNV interpretation (and amendments) with a fitting genotype (Section

3.3.2; Richards et al., 2015; Riggs et al., 2020). Additionally, only gene names approved by the Human Genome Organisation Gene Nomenclature Committee (HGNC) are used (https://www.genenames.org/). The term "exome sequencing" instead of "whole exome sequencing" (WES) is employed. Although common in the literature, WES is a) a tautology and b) misleading, as (short-read-based) exome sequencing is not able to capture the entirety of the protein-coding regions of the human genome, the "whole exome," as there are regions/genes with (partially) insufficient coverage or mapping (assignment of sequenced DNA to a reference genome), for example, due to pseudogenes or homologous regions (e.g., *PKD1*; Section 1.2.1; Ali et al., 2019; Prior et al., 2019).

Wherever phenotype numbers of the Online Mendelian Inheritance in Man® (OMIM®) catalog (see "Online resources") are used, these are provided in brackets; these are called "MIM phenotype number" and also abbreviated as "[MIM XXXXXX]" throughout the thesis. Finally, as only that individual of a family, in which genetic studies had been started, was included in the study cohort of this thesis (i.e., not other relatives), the individuals of the study cohort are referred to as "index patients" or "index cases," or simply "patients" or "cases."

1.1 Hereditary nephropathies

Hereditary kidney diseases comprise clinically and genetically heterogeneous conditions affecting renal compartments such as the glomerulus (e.g., Alport syndrome [AS], hereditary focal segmental glomerulosclerosis/steroid-resistant nephrotic syndrome [FSGS/SRNS]) and tubuli (e.g., Bartter syndrome), altering embryonic development of the kidney (e.g., congenital anomalies of the kidney and urinary tract [CAKUT]), disturbing renal structure (e.g., ciliopathies), or occurring as part of metabolic (e.g., Fabry disease) and mitochondrial disorders (e.g., mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes [MELAS]; Devuyst et al., 2014; Mehta & Jim, 2017; Seidowsky et al., 2013). Furthermore, renal anomalies can be a frequent feature of chromosomal aberrations, for example, monosomy X (Turner syndrome) or trisomy 21 (Down syndrome), which are not focused on in this thesis (Mehta & Jim, 2017).

In the following sections, an overview of hereditary nephropathies is presented: Autosomal dominant tubulointerstitial kidney disease (ADTKD), AS, CAKUT, ciliopathies, FSGS/SRNS, VACTERL/VATER (V – Vertebral anomalies, A – Anorectal

malformations, C – Cardiovascular anomalies, T – Tracheoesophageal fistula, E – Esophageal atresia, R – Renal and/or radial anomalies, L – Limb defects), and other hereditary kidney diseases not fitting the aforementioned entities (tubulopathies, inherited metabolic disorders, mitochondrial disorders, atypical hemolytic uremic syndrome). These disease groups are used analogously to describe the ES study cohort in the results section of the thesis.

1.1.1 Autosomal dominant tubulointerstitial kidney disease (ADTKD)

ADTKD is an umbrella term for several rare hereditary kidney diseases that feature a slow, progressive loss of renal function (end-stage renal disease [ESRD] in adulthood, mean age 45 years) with unremarkable urinary sediment (marginal hematuria or proteinuria). As ADTKD is a late-onset disease, reproduction is preserved, and pedigree analysis can reveal affected individuals of both sexes in each generation compatible with an autosomal dominant inheritance pattern. Renal biopsy can show unspecific fibrosis of the tubular interstitium (hence tubulointerstitial kidney disease) and is not diagnostic of disease. Renal cysts are prevalent in some patients but do not lead to enlarged kidneys, in contrast to autosomal dominant polycystic kidney disease (ADPKD; Section 1.1.4). The expressivity of disease can be highly variable within and across families. Treatment is symptomatic, and kidney transplantation is curative, as there is no recurrence in the transplant (Bleyer et al., 2017; Devuyst et al., 2019).

As clinical course and renal biopsy are unspecific, genetic testing is the mainstay of ADTKD work-up (Devuyst et al., 2019). Genes currently associated with ADTKD are *UMOD* (16p12.3), *MUC1* (1q22), *HNF1B* (17q12), *REN* (1q32.1), and *SEC61A1* (3q21.3), with ADTKD-*UMOD*, -*MUC1*, and -*REN* being the major forms (Table 1). Cases in which clinical suspicion of ADTKD is high but a genetic diagnosis cannot be achieved have been designated ADTKD-NOS (“not otherwise specified”; Bleyer et al., 2017; Devuyst et al., 2019). Furthermore, heterozygous causative variants in *DNAJB11* (3q27.3), a protein of the primary cilium (Section 1.1.4), have been identified in adult patients with renal insufficiency and normal-sized polycystic kidneys, and represent a phenotypic overlap of ADTKD and ADPKD (Bergmann et al., 2018; Cornec-Le Gall et al., 2018). No exact data on the prevalence of ADTKD exist (Devuyst et al., 2019). However, a study of over 3,000 adults with CKD was able to diagnose ADTKD-*UMOD*

in 3% of genetically solved cases, which was the third largest monogenic disease group after ADPKD and AS (Groopman et al., 2019). The prevalence of ADTKD (including ADTKD-NOS) has been estimated at 0.54% of ESRD patients in Ireland (Cormican et al., 2019).

Gene (chromosomal location)	**Encoded protein**	**Protein expression**	**Protein function**	**Genotypic characteristics**	**Pathogenesis of renal disease**	**Phenotypic characteristics**	**First report**
UMOD (16q12)	Uromodulin (Tamm-Horsfall protein)	• Kidney (TAL, DCT)	• Most abundant protein in urine • Decreases kidney stone formation • Protection against UTIs • Urine concentration	• Dominant negative/toxic effect • Usually missense variants substituting conserved cysteine residues (disruption of disulfide bonds)	• ER retention of misfolded protein • ER stress	• Hyperuricemia • Gout	Hart et al., 2002
MUC1 (1q22)	Mucin 1	• Secretory epithelia (kidney: TAL, DCT, collecting duct)	• Protection of luminal epithelial surface • Cell signaling	• Toxic effect • Duplication of cytosine in a cytosine tract of the VNTR region of *MUC1* (leading to a frameshift)	• Accumulation of truncated mucin 1 in renal tubular cells	• None	Kirby et al., 2013
HNF1B (17q12)	Hepatocyte nuclear factor 1β	• Kidney • Pancreas • Liver • Lung • Intestine • Urogenital tract	• Transcription factor	• Haploinsufficiency • Usually whole-gene deletions in ADTKD • About 50% of cases are *de novo* • Can be part of 17q12 deletion syndrome [MIM 614527][a]	• Increased renal fibrosis by activation of a TWIST2-dependent transcriptional network	• Can also manifest as CAKUT • Extrarenal disease possible (cognitive impairment, MODY, pancreatic hypoplasia, abnormal liver function)	Lindner et al., 1999
REN (1q32.1)	Preprorenin	• Kidney (juxtaglomerular apparatus)	• Precursor of prorenin and renin	• Dominant negative/toxic effect • Homozygous LoF variants cause AR renal tubular dysgenesis [MIM 267430][a]	• Impairment of preprorenin processing • ER stress	• Anemia in first year of life (EPO responsive) • Hyporeninemic hypoaldosteronism (hyperkalemia, hypotension)	Gribouval et al., 2005
SEC61A1 (3q21.3)	α1 subunit of SEC61	• Ubiquitous	• Subunit of heterotrimeric channel • Part of the ER translocon • Transport of secretory proteins to ER	• Missense variants leading to ER translocon malfunction	• Accumulation of SEC61α mutant in ER • Erroneous localization of mutant proteins to Golgi apparatus • Affects post-translational processing of proteins (including uromodulin, mucin 1 and renin)	• Congenital anemia (only two families described)	Bolar et al., 2016

Table 1: ADTKD-associated genes with respective protein function, genotypic and phenotypic characteristics. ADTKD, autosomal dominant tubulointerstitial kidney disease; AR, autosomal recessive; CAKUT, congenital anomalies of the kidney and urinary tract; DCT, distal convoluted tubule; EPO, erythropoietin; ER, endoplasmic reticulum; LoF, loss-of-function; MODY, maturity onset diabetes of the young; TAL, thick ascending limb of the loop of Henle; UTI, urinary tract infection; VNTR, variable number tandem repeats.

[a]MIM phenotype number (https://www.omim.org/).

Adapted from Devuyst et al., 2019, with data from Adam et al., 2012; Ayasreh et al., 2018; Bingham et al., 2001; Bleyer et al., 2014; Bolar et al., 2016; Bollee et al., 2011; Chan et al., 2018; Devuyst et al., 2017; Gribouval et al., 2005; Gudbjartsson et al., 2010; Hart et al., 2002; Horikawa et al., 1997; Kirby et al., 2013; Lang et al., 2017; Lindner et al., 1999; Mefford et al., 2007; Mo et al., 2004; Patton et al., 1995; Roehlen et al., 2018; Schaeffer et al., 2017; Scolari et al., 2004; Tokonami et al., 2018; Vylet'al et al., 2006; Williams et al., 2009; Zivna et al., 2009. Table (excerpt) published (Riedhammer, Schmaderer, et al., 2020).

1.1.2 Alport syndrome (AS)

AS was first described by Arthur Cecil Alport as "hereditary familial congenital haemorrhagic nephritis" in 1927 (Alport, 1927). Causative variants in the genes *COL4A3*, *COL4A4*, and *COL4A5* have been associated with AS and result in alterations of heterotrimeric α-chains 3 (*COL4A3*), 4 (*COL4A4*), and 5 (*COL4A5*) of type IV collagen (Barker et al., 1990; Mochizuki et al., 1994). Type IV collagen α-chains feature an N-terminal 7S domain, a triple-helical domain with recurring Gly-X-Y motifs (X and Y denoting variable amino acids), and a C-terminal non-collagenous domain, named NC1 (Figure 1; Chew & Lennon, 2018). The α3α4α5 heterotrimer constitutes the main component of the mature GBM, but also of basement membranes in the eye (cornea, lens, and retina) and ear (cochlea; Kashtan, 2017). The pathogenesis of AS on a molecular level involves the impaired conversion of immature heterotrimeric collagen α1α1α2 to mature α3α4α5 in the renal GBM. Collagen α1α1α2 is more prone to proteolytic degradation than collagen α3α4α5 (Chew & Lennon, 2018; Kalluri et al., 1997).

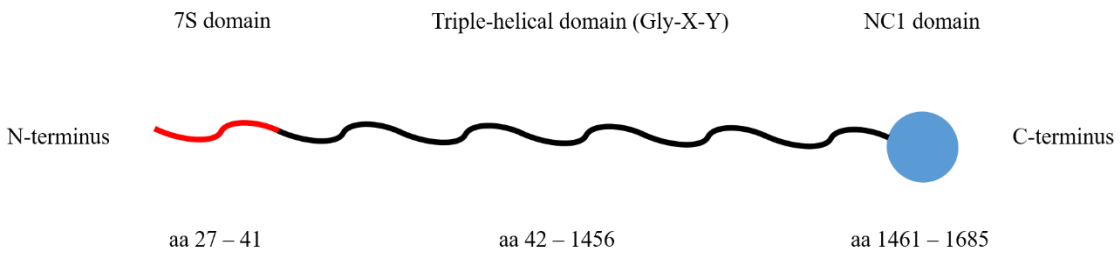

Figure 1: Protein structure of a single α-chain 5 of type IV collagen (COL4A5, RefSeq accession number NP_000486.1). Adapted from Chew & Lennon, 2018, and with data from https://www.uniprot.org/. aa, amino acid; NC1, non-collagenous 1. Figure published (Riedhammer, Schmaderer, et al., 2020).

The prevalence of AS is about 1 in 50,000 live births (Watson & Bush, 2020). *COL4A5* is an X-linked gene (Xq22.3), and X-linked AS (XLAS) represents two thirds of cases; 15% are autosomal recessive AS (ARAS; biallelic causative variants in the genes *COL4A3* [2q36.3] and *COL4A4* [2q36.3]), and about 20% are described as autosomal dominant AS (ADAS; heterozygous causative variants in *COL4A3* and *COL4A4*; Kashtan, 2019). In one publication, ADAS was even diagnosed in 31% of genetically solved AS families (15 of 48; Fallerini et al., 2014). However, there is disagreement as to

whether the term "ADAS" in patients with heterozygous causative variants in *COL4A3* and *COL4A4* should be employed: Consensus guidelines advocate not to use it, since it can be misleading, as true heterozygous carriers only have isolated hematuria, not the full AS phenotype. In contrast, patients with AS phenotypically but only a heterozygous causative variant in *COL4A3*/*COL4A4* may have a second causative variant in non-analyzed regions of the respective genes (e.g., an intronic variant). Instead of ADAS, the term "thin basement membrane nephropathy" (TBMN) for heterozygous carriers of causative variants in *COL4A3* and *COL4A4* and a mild phenotype (see above) is preferred by some (Savige, 2018; Savige et al., 2019). Others encourage the use of the term ADAS, as it is seen as a more specific and generally understood diagnosis, which expedites the surveillance of renal function and therapy initiation, if needed (Kashtan et al., 2018). In this thesis, the umbrella term "type-IV-collagen-related nephropathy" is used and comprises the two poles of disease severity, AS and TBMN (Imafuku et al., 2020). Index patients with type-IV-collagen-related nephropathy were allocated to either an AS or TBMN subgroup by clinical and histopathological criteria (Section 3.1.2).

To add to the genetic complexity of type-IV-collagen-related nephropathy, one study, compiling data from eleven AS pedigrees, proposed a digenic inheritance in AS (e.g., a heterozygous causative variant in *COL4A3* and a heterozygous causative variant in *COL4A4* segregating with disease; Mencarelli et al., 2015). Additionally, genetic modifiers, for example, the missense variant NM_014625.3:c.686G>A, p.(Arg229Gln) in *NPHS2* or the missense variant NM_199180.2:c.1057G>A, p.(Val353Met) in *KIRREL2* (also called *NEPH3*), have been introduced as aggravating genetic factors in TBMN (Stefanou et al., 2015; Tonna et al., 2008; Voskarides et al., 2017).

The clinical phenotype of AS is comprised of hematuria (first clinical sign); progressive decline of renal function, with 90% on dialysis by age 40; bilateral high-frequency sensorineural hearing loss with onset in childhood or adolescence (not at birth), developing in 80–90% of cases by age 40; and ocular changes such as anterior lenticonus, maculopathy, and bilateral cataracts, which develop in 30–40% of patients. Anterior lenticonus is viewed as pathognomonic for AS. This data comes from males with XLAS but should be applicable to patients with ARAS as well (Jais et al., 2000; Kashtan, 2019). Concerning kidney biopsy, there are only unspecific changes on light microscopy (e.g., FSGS, interstitial fibrosis), but there is distinctive ultrastructural pathology: In early stages, thinning of the glomerular basement membrane (GBM; < 250–330 nm in adults,

< 200–250 nm in children) can be observed. With further progression of the disease, diffuse lamellation and splitting of the lamina densa of the glomerular capillary wall and thickening of the glomerular capillary wall, all of which are pathognomonic, become evident (Kashtan, 2019; Nozu et al., 2019).

Characteristically for an X-linked disorder, XLAS typically affects males (hemizygous causative variant in *COL4A5*). However, females with a heterozygous causative variant in *COL4A5* can show a broad spectrum of disease severity, ranging from clinically asymptomatic to TBMN to AS (Kashtan, 2007). This is believed to correlate with skewed X-inactivation in females (preferential X-inactivation of one X chromosome). Still, even though there is mouse data supporting this (intuitively understandable) mechanism of variable expressivity of XLAS in females, proof in humans is missing (Rheault et al., 2010; Vetrie et al., 1992). Females with a heterozygous causative variant in *COL4A5* are at a higher risk of developing ESRD compared to the general population: 95% have hematuria, 12% reach ESRD before 40 years of age, and 30% reach it before 60 years of age (cross-sectional study of about 300 women with heterozygous causative variants in *COL4A5*; Jais et al., 2003). The lifetime risk of ESRD in women in the general population is 1.8% (Turin et al., 2012).

As seen in other monogenic diseases associated with changes in collagen structure (e.g., classic Ehlers-Danlos syndrome), typical causative variants in AS are glycine substitutions in the triple-helical domain of type IV collagen (Gly-X-Y motif; Figure 1). Genotype–phenotype correlations exist for XLAS: Broadly speaking, loss-of-function (LoF) variants such as nonsense and frameshift variants, and large rearrangements lead to a more severe disease than missense variants (Figure 2; Bekheirnia et al., 2010; Gross et al., 2002; Jais et al., 2000). Furthermore, large deletions at the 5’ ends of *COL4A5* and *COL4A6* (neighboring genes), not ranging beyond the second intron of *COL4A6*, can result in AS with diffuse leiomyomatosis of the esophagus (Antignac & Heidet, 1996; Mothes et al., 2002).

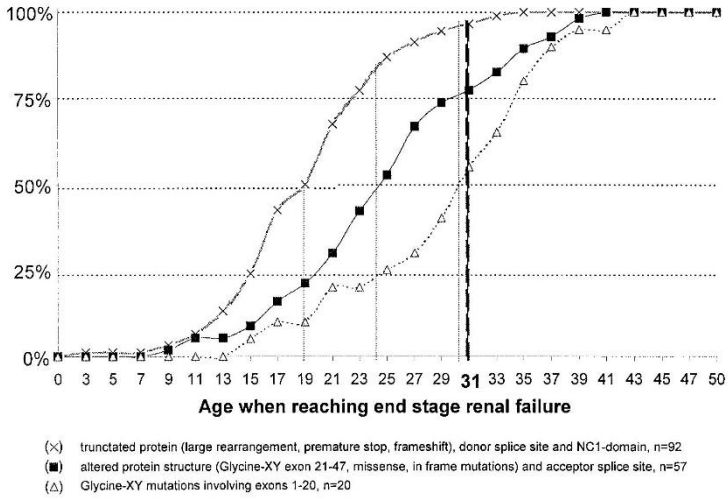

Fig. 3. Age when reaching ESRF in patients with identified mutations with altered protein structure and acceptor splice site mutations *vs* a truncated protein, donor splice site mutations and mutations involving the NC1-domain. Numbers of patients reaching ESRF differ significantly between 17 and 37 years of age ($P = 0.01$) as well as numbers of patients with adult type of AS (truncated protein, 7.5%, *vs* altered protein structure, 24.6%, *vs* glycine-XY mutations of exons 1–20, 45%; $P = 0.01$).

Figure 2: Genotype–phenotype correlation in X-linked Alport syndrome (XLAS). From Gross et al., 2002.

Management of AS consists of an interdisciplinary approach to evaluate organ involvement (kidney, eyes, and ears). In terms of renal disease, annual examination for proteinuria/microalbuminuria is advised in patients with genetic diagnosis of AS or in offspring of AS patients at risk of disease (e.g., the son of a female patient with a heterozygous causative variant in *COL4A5*). Angiotensin I-converting enzyme (ACE) inhibition has been shown to suspend kidney failure (retrospective registry data, 283 AS patients) and should be initiated when proteinuria develops (or microalbuminuria in patients with an LoF variant or a family history with onset of ESRD before 30 years of age; Gross et al., 2012; Kashtan et al., 2013). A prospective double-blind, randomized, placebo-controlled phase-three trial (EARLY PRO-TECT Alport, 66 children with AS) showed that ACE inhibition is safe in AS patients and also indicated a risk reduction for disease progression, although this finding did not reach significance (hazard ratio 0.51, 95% confidence interval [CI] 0.12–2.20; Gross et al., 2020). Therapies under ongoing investigation in double-blind, randomized, placebo-controlled trials include treatment with anti-microRNA-21 (HERA, clinicaltrials.gov identifier NCT02855268) and bardoxolone methyl (CARDINAL, NCT03019185).

1.1.3 Congenital anomalies of the kidney and urinary tract (CAKUT)

CAKUT entails various anomalies of renal development, including minor forms such as vesicoureteral reflux (VUR) and more serious manifestations such as (bilateral) kidney dysplasia or agenesis (van der Ven, Vivante, et al., 2018). CAKUT is the major reason for renal replacement therapy (RRT) in children (41.3% of children aged 0–14 years in need of RRT), and its prevalence is about 3 to 6 per 1,000 live births (Chesnaye et al., 2014; Pohl et al., 2002). CAKUT can be limited to the kidney and urinary tract (isolated) or can be part of a syndromic disease (Vivante et al., 2014). CAKUT has been listed as a distinct clinical feature in more than 200 syndromes (van der Ven, Vivante, et al., 2018). Moreover, several CAKUT phenotypes can exist in one person (e.g., kidney dysplasia and VUR), a situation which has been termed "complex CAKUT" (van der Ven, Vivante, et al., 2018; Verbitsky et al., 2019).

CAKUT is caused by perturbation of renal morphogenesis, which can be due to environmental, epigenetic, and genetic factors and the interplay of these (Nicolaou et al., 2015). The development of the mammalian kidney is an intricate process: The kidney originates from the intermediate mesoderm, and its development follows three major steps, from the *pronephros* to the *mesonephros* to the *metanephros*. The latter progresses to the mature kidney (Skorecki et al., 2016). The most important reason for CAKUT is believed to be disturbed communication between the ureteric bud (UB) and the metanephric mesenchyme (MM; Ichikawa et al., 2002). Figure 3 provides an overview of the major stages of kidney development.

Although alterations in renal development can be multifactorial in nature, there are also hereditary forms of CAKUT, that is, CAKUT resulting from single-gene defects ("monogenic CAKUT"). This is underscored by familial clustering of CAKUT, knockout (KO) mouse models recapitulating CAKUT phenotypes, and complex heritable syndromes involving CAKUT (Bulum et al., 2013; Vivante et al., 2014). Hence, it is not surprising that about 40 different monogenic causes for (isolated/syndromic) CAKUT have been described thus far, with autosomal dominant (AD), autosomal recessive (AR), and X-linked (XL) modes of inheritance (van der Ven, Vivante, et al., 2018). The associated genes typically encode transcription factors (Table 2).

Unfortunately, diagnostic yield of genetic studies in CAKUT cohorts is only about 12%, increasing to 20–27% in consanguineous families (van der Ven, Connaughton, et al., 2018; Vivante et al., 2017).

CNVs were found to play a major role in CAKUT: in 16.6% of 192 cases with renal hypodysplasia in one study (Sanna-Cherchi et al., 2012). Another study showed that there was a significant burden of rare CNVs (frequency ≤ 1%, size ≥ 100 kilobase pairs [kb]) in CAKUT (odds ratio [OR] 1.28 [95% CI 1.18–1.39, $p = 4.01 \times 10^{-9}$]; 1,044 cases vs. 6,767 controls; Verbitsky et al., 2019).

Due to the large number of KO mouse models with CAKUT phenotypes, further monogenic CAKUT forms are thought to exist. By means of comprehensive sequencing (e.g., exome or genome sequencing), more disease-associated genes will be described in the future, further unraveling the complex process of kidney development (Vivante et al., 2014).

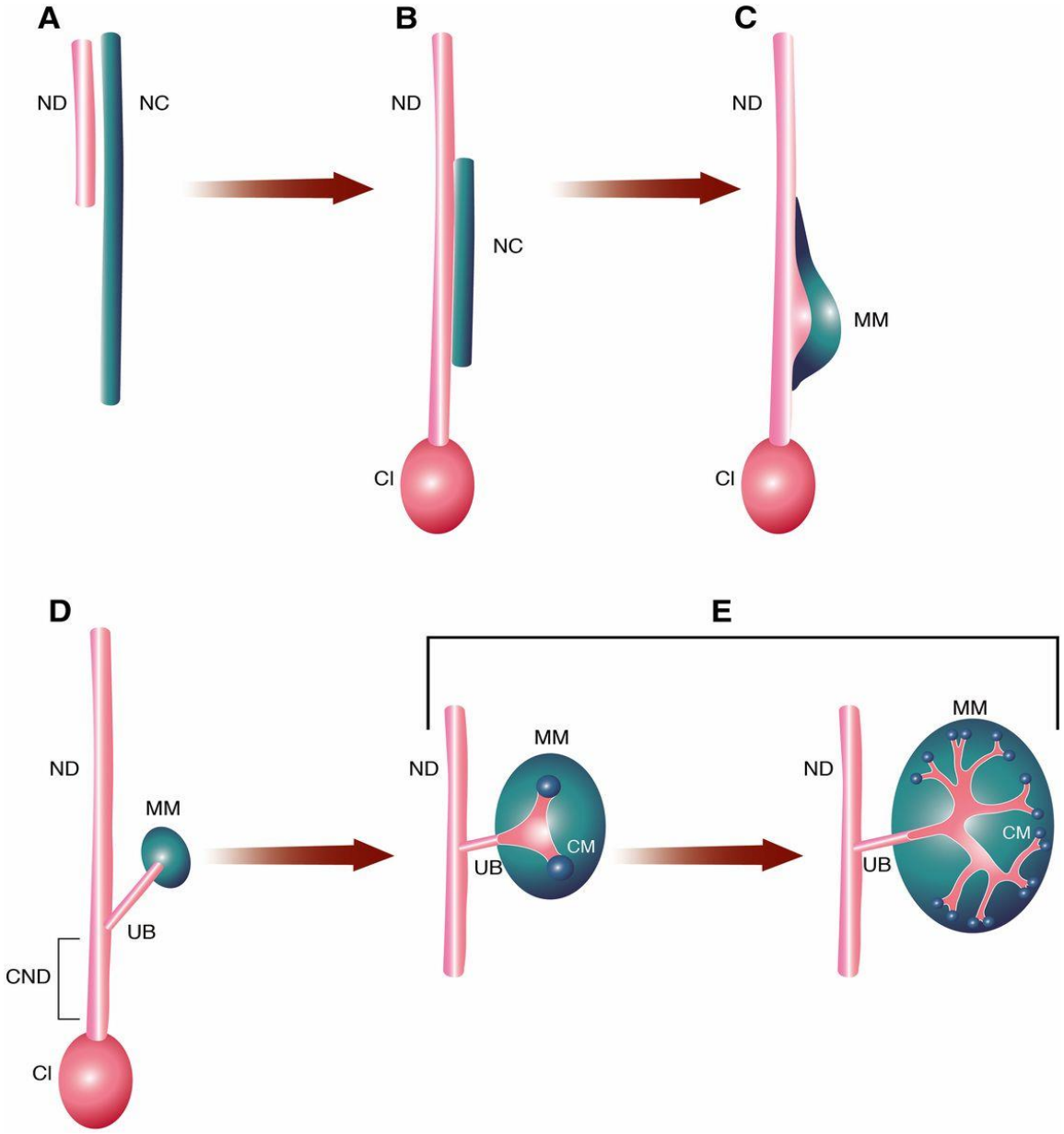

Figure 3: Embryonal development of the kidney. A) The nephric duct (ND) and nephric cord (NC) are the progenitors of the mature reno-urinary system. They derive from the intermediate mesoderm. B) With embryonic growth, the cloaca (Cl) forms at the caudal end of the ND, which later gives rise to the bladder. C) The NC is rearranged to the metanephric mesenchyme (MM). D) At the fifth week of human gestation, the ureteric bud (UB) protrudes to the MM. E) Mutual signaling from the UB and MM then promotes branching of the UB, which develops into the urinary collecting system (i.e., collecting ducts, renal pelvis, and ureter). The MM adjacent to UB progresses to become the cap mesenchyme (CM), eventually transitioning from mesenchyme into epithelium. These epithelial cells subsequently develop into the different parts of the nephron (glomerulus, proximal and distal tubules). CND, common nephric duct (caudal end of the ND, between UB and Cl). From van der Ven, Vivante, et al., 2018, and with data from Costantini, 2012; Little et al., 2016; Short & Smyth, 2016; Skorecki et al., 2016.

Gene (chromosomal location)	Mode of inheritance	Protein function	Associated monogenic disease(s) in human [MIM phenotype number][a]	Renal and urinary tract phenotype	Extrarenal manifestation(s)	First report
BMP4 (14q22.2)	AD	Regulatory molecule	• Microphthalmia, syndromic 6 [607932] • Orofacial cleft 11 [600625]	• Hypoplastic kidneys	• Microphthalmia • Dysmorphic facies • Cleft lip and palate	Bakrania et al., 2008
EYA1 (8q13.3)	AD	Transcriptional coactivator	• Branchiootorenal syndrome 1 [602588] • Otofaciocervical syndrome [166780] • Branchiootorenal syndrome 1, with or without cataracts [113650]	• Renal dysplasia/aplasia	• Hearing loss • Ear malformations • Branchial cysts	Abdelhak et al., 1997
GATA3 (10p14)	AD	Enhancer-binding protein	• Hypoparathyroidism, sensorineural deafness, and renal dysplasia [146255]	• Renal dysplasia • Unilateral renal agenesis	• Hearing loss • Hypoparathyroidism • Uterine/vaginal anomalies in females	Van Esch et al., 2000
HNF1B[b] (17q12)	AD	Transcription factor	• Diabetes mellitus, noninsulin-dependent [125853] • Renal cysts and diabetes syndrome [137920]	• Renal hypodysplasia • Renal cysts • Renal agenesis	• Diabetes mellitus • Uterine anomalies in females • Gout	Lindner et al., 1999
PAX2 (10q24.31)	AD	Transcription factor	• Papillorenal syndrome [120330] • Glomerulosclerosis, focal segmental, 7 [616002]	• Renal hypoplasia • VUR • Adult-onset FSGS (no genotype-phenotype correlation [papillorenal syndrome vs. FSGS])	• Optic nerve coloboma	Sanyanusin et al., 1995
ROBO2 (3p12.3)	AD	Transmembrane receptor	• Vesicoureteral reflux 2 [610878]	• Hypoplastic kidneys • VUR	• None	Lu et al., 2007
SALL1 (16q12.1)	AD	Transcriptional repressor	• Townes-Brocks syndrome 1 [107480]	• Renal hypodysplasia • VUR	• Dysmorphic facies • Imperforate anus • Triphalangeal thumb	Kohlhase et al., 1998
SOX17 (8q11.23)	AD	Transcription factor	• Vesicoureteral reflux 3 [613674]	• VUR • Megaureter	• None	Gimelli et al., 2010
TNXB[c] (6p21.33-p21.32)	AD	Glycoprotein of the extracellular matrix	• Vesicoureteral reflux 8 [615963]	• VUR	• Joint hypermobility	Gbadegesin et al., 2013
DSTYK[d] (1q32.1)	AD	Dual serine/threonine and tyrosine protein kinase	• Congenital anomalies of kidney and urinary tract 1 [610805]	• Renal hypodysplasia • VUR	• Epilepsy	Sanna-Cherchi et al., 2013
ACE (17q23.3)	AR	Angiotensin I-converting enzyme	• Renal tubular dysgenesis [267430]	• Renal tubular dysgenesis	• Microcephaly • Hypotension • Pulmonary hypoplasia	Gribouval et al., 2005
AGT (1q42.2)	AR	Angiotensinogen	• Renal tubular dysgenesis [267430]	• See *ACE*	• See *ACE*	Gribouval et al., 2005
AGTR1 (3q24)	AR	Angiotensin receptor 1	• Renal tubular dysgenesis [267430]	• See *ACE*	• See *ACE*	Gribouval et al., 2005
REN[e] (1q32.1)	AR	Renin (enzyme)	• Renal tubular dysgenesis [267430]	• See *ACE*	• See *ACE*	Gribouval et al., 2005
FRAS1 (4q21.21)	AR	Extracellular matrix protein	• Fraser syndrome 1 [219000]	• Renal agenesis	• Cryptophthalmos • Ear malformations • Mental retardation • Genital anomalies	McGregor et al., 2003
FREM2 (13q13.3)	AR	Integral membrane protein	• Fraser syndrome 2 [617666] • Cryptophthalmos, unilateral or bilateral, isolated [123570]	• Renal agenesis	• Cryptophthalmos • Genital anomalies	Jadeja et al., 2005
ANOS1 (Xp22.31)	XL	Adhesion molecule	• Hypogonadotropic hypogonadism 1 with or without anosmia (Kallmann syndrome 1) [308700]	• Unilateral renal agenesis	• Anosmia • Hypogonadotropic hypogonadism • Small testes • Azoospermia	Hardelin et al., 1993

Table 2: Overview of principal CAKUT-associated genes with respective protein function and phenotypes. AD, autosomal dominant; AR, autosomal recessive; FSGS, focal segmental glomerulosclerosis; VUR, vesicoureteral reflux; XL, X-linked.

[a]https://www.omim.org/.

[b]Monoallelic causative variants in *HNF1B* have also been associated with ADTKD (Table 1), and *HNF1B* can be deleted as part of 17q12 deletion syndrome [MIM 614527].

[c]Biallelic causative variants in *TNXB* have been associated with AR Ehlers-Danlos syndrome, classic-like, 1 [MIM 606408].

[d]Biallelic causative variants in *DSTYK* have been associated with AR Spastic paraplegia 23 [MIM 270750].

[e]Monoallelic causative variants in *REN* have been associated with ADTKD [MIM 613092] (Table 1).

Adapted from Vivante et al., 2014, with data from Abdelhak et al., 1997; Bakrania et al., 2008; Gbadegesin et al., 2013; Gimelli et al., 2010; Gribouval et al., 2005; Hardelin et al., 1993; Jadeja et al., 2005; Kohlhase et al., 1998; Lindner et al., 1999; Lu et al., 2007; McGregor et al., 2003; Sanna-Cherchi et al., 2013; Sanyanusin et al., 1995; Van Esch et al., 2000. Table published (Riedhammer, Schmaderer, et al., 2020).

1.1.4 Ciliopathies

The cilium (plural cilia) is an evolutionarily conserved organelle protruding from the cell body of eukaryotes. Cilia are differentiated into motile and immotile. Spermatozoa, for example, have motile cilia for locomotion. Motile cilia are also needed to clear mucus from the airways (Reiter & Leroux, 2017). Immotile cilia, also called "primary cilia," serve as signal transducing sensors on the apical cell surface. Primary cilia are found on nearly all human cells and are vital for cell differentiation and embryonic development (Braun & Hildebrandt, 2017; Hildebrandt et al., 2011). Figure 4 schematically depicts a primary cilium with its basic components.

As cilia are found on nearly all cell types in nearly all organs (e.g., brain, eyes, kidney, skeleton, respiratory epithelia), ciliary dysfunction causes a large variety of phenotypes (Reiter & Leroux, 2017). As this thesis is on hereditary nephropathies, ciliopathies causing a renal phenotype are addressed in the following. Malfunction of primary (immotile) cilia plays a major role in hereditary kidney disease, with nephronophthisis (NPHP) and polycystic kidney disease constituting the main groups (Hildebrandt et al., 2011).

NPHP and NPHP-related ciliopathies (in the following summarized as NPHP) constitute a vastly genetically heterogeneous disease entity (> 90 disease-causing genes) and are inherited in an AR manner (some XL; Braun & Hildebrandt, 2017). They represent (alongside CAKUT and FSGS/SRNS) one of the most frequent causes of ESRD in pediatric patients (5% of cases; Vivante & Hildebrandt, 2016). A recurrent cause of NPHP is a homozygous deletion of the gene *NPHP1* (2q13), which can be identified in 20–25% of cases (other genes each only explain a few percent of cases at most; Halbritter et al., 2013). NPHP presents with an insidious childhood-onset decline of renal function: A rise in creatinine in the serum is not noticed before 9 years of age on average (Gretz et al., 1989). NPHP is further characterized by polyuria, polydipsia, anemia, and renal ultrasound pathology comprising small- or normal-sized kidneys with augmented parenchymal echogenicity, loss of corticomedullary differentiation, and several cysts situated at the corticomedullary junction. There are no pathognomonic findings on kidney biopsy, but typical histology includes a loss of tubular basement membrane integrity, tubular atrophy, and tubulointerstitial fibrosis. ESRD occurs until the age of 30. Later

onset of ESRD is uncommon, and other potential tentative diagnoses should be discussed in this case (Braun & Hildebrandt, 2017).

NPHP can also feature extrarenal manifestations in 10–20% of cases; these can include retinitis pigmentosa, brain malformations (e.g., cerebellar vermis hypoplasia in Joubert syndrome), skeletal dysplasia (e.g., Jeune syndrome), and liver fibrosis (Braun & Hildebrandt, 2017; Stokman et al., 2016). This pleiotropy of NPHP is reflected by the fact that one gene can be associated with several allelic disorders: *NPHP1*, for example, the most frequently mutated gene in NPHP (see above), is linked to Senior–Løken syndrome 1 [MIM 266900], an NPHP with retinal degeneration; to isolated renal NPHP [MIM 256100]; and to Joubert syndrome 4 [MIM 609583], an NPHP with a neuronal phenotype (see above).

One more important group of syndromic ciliopathies encompassing the kidney is Bardet–Biedl syndrome (AR inheritance), which in itself is highly genetically heterogeneous (> 20 disease-associated genes in OMIM®, numbered *BBS1–BBS21*). The phenotype includes mental retardation with behavioral issues, obesity, retinitis pigmentosa, hypogonadism, polydactyly, and cystic kidney disease (Braun & Hildebrandt, 2017). The proteins encoded by Bardet–Biedl syndrome genes are part of a complex important for ciliary transport, the “BBsome” (Braun & Hildebrandt, 2017; Jin & Nachury, 2009; Loktev et al., 2008). Furthermore, there is data suggesting digenic inheritance – in addition to AR inheritance – in Bardet–Biedl syndrome (Fauser et al., 2003; Katsanis, 2004).

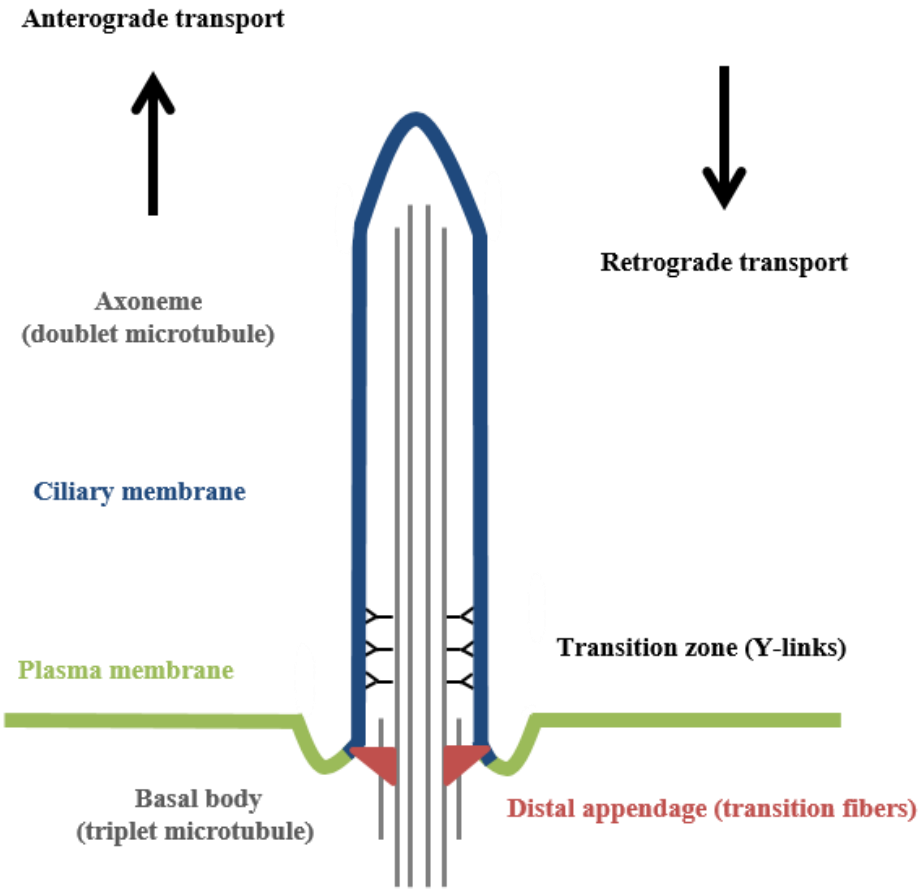

Figure 4: Primary cilium, basic components (schematic figure). Adapted from Hildebrandt et al., 2011, and Reiter & Leroux, 2017. Figure published (Riedhammer, Schmaderer, et al., 2020). The basal body (also called the "mother centriole") is comprised of nine triplet microtubules and is attached to the ciliary membrane by so-called distal appendages; the axoneme, the actual shaft of the cilium, has nine doublet microtubules and arises from the basal body. In motile cilia, the axoneme features two additional central microtubules (9 x 2 + 2) and dynein arms for movement. The transition zone is located at the proximal end of the axoneme and serves as a gatekeeper for the entry and release of proteins to and from the cilium and connects the axoneme to the ciliary membrane by links with the shape of a Y (Carvalho-Santos et al., 2011; Fisch & Dupuis-Williams, 2011; Reiter et al., 2012; Reiter & Leroux, 2017). The intraflagellar transport of proteins (along the axoneme) is important for maintenance of ciliary structure, which consists of an intricate network of components like kinesin-2 (anterograde transport) and dynein-2 (retrograde transport; Mourao et al., 2016).

The group of polycystic kidney diseases within ciliopathies consists of autosomal recessive polycystic kidney disease (ARPKD) and ADPKD. The phenotypic hallmark of ARPKD and ADPKD is enlarged kidneys containing copious (uncountable) cysts filled with fluid. This is an important difference compared to NPHP, in which kidneys are small to normal sized with only a few (countable) cysts (see above; Braun & Hildebrandt, 2017; Hildebrandt et al., 2011). ARPKD has a birth prevalence of 1 in 26,500, but this number can be higher in consanguineous populations (Alzarka et al., 2017; Bergmann et al., 2018). Biallelic causative variants in the gene *PKHD1* (6p12.3–p12.2) are responsible for ARPKD and can be identified in nearly 80% of cases (Melchionda et al., 2016; Ward et al., 2002). Furthermore, in several consanguineous families with a phenotype resembling ARPKD but negative *PKHD1* analysis, biallelic causative variants (missense, nonsense, and frameshift variants) in the gene *DZIP1L* (3q22.3) could be detected, making it another cause of ARPKD (< 1% of cases; Lu et al., 2017). *PKHD1* encodes fibrocystin, a protein with 4,074 amino acids (aa; longest transcript, RefSeq accession number NP_619639.3), which has a transmembrane domain, a long extracellular N-terminal, and a short intracellular C-terminal domain (Onuchic et al., 2002). Fibrocystin, located at the primary cilium, is expressed in the kidney (both fetal and adult), liver, and pancreas and is believed to be important for the differentiation of the collecting ducts of the kidney and the biliary ducts of the liver (Follit et al., 2010; Ward et al., 2002; Ward et al., 2003). As a large gene (about 470 kb in size), there is profound allelic heterogeneity in *PKHD1*-associated ARPKD, with both missense and LoF variants described, most of them only found in a single family (Bergmann et al., 2018). There is considerable variability in the expressivity of ARPKD, both inter- and intrafamilial. Thus, additional genetic and environmental factors may modify disease severity (Bergmann et al., 2005).

In its most severe form, ARPKD manifests *in utero*, right after birth or in early childhood with renal enlargement, parenchymal hyperechogenicity, loss of corticomedullary differentiation, and numerous small cysts deriving from the renal collecting ducts of both kidneys (difference compared to NPHP, for example, in which cysts are located at the corticomedullary junction). Patients can present with a so-called Potter sequence due to oligohydramnios leading to pulmonary hypoplasia, clubbed feet, facial dysmorphies, and spine and limb abnormalities. Death from respiratory failure occurs in about 20% of cases. In patients surviving the neonatal period/infancy, congenital hepatic fibrosis due to defects in the development of the biliary tree is a serious comorbidity in *PKHD1*-

associated ARPKD (Bergmann et al., 2018; Bergmann et al., 2005). Patients with causative variants in *DZIP1L* show a milder disease course, none of the reported individuals died perinatally (Lu et al., 2017). Management of ARPKD is symptomatic (treatment of hypertension, dialysis/kidney transplantation if ESRD develops, combined transplantation of liver and kidney in patients with ESRD and congenital hepatic fibrosis; Bergmann et al., 2018).

ADPKD, an AD cystic kidney disease, is the most frequent deadly monogenic disease, as it leads to ESRD, which is lethal without RRT (Bergmann et al., 2018). The prevalence of ADPKD in the European Union is estimated at 1 in 2,525, which would render ADPKD a rare disease (less than 1 in 2,000; Bergmann et al., 2018; Willey et al., 2017). However, autopsy studies have yielded a much higher rate of ADPKD (about 1 in 500), indicating that many (mild) cases may not be diagnosed during the lifetime (Chan, 1993). In contrast to ARPKD (and NPHP), cysts are scattered throughout both kidneys in ADPKD (growing from epithelial cells; Bergmann et al., 2018). There are accepted ultrasound-criteria for the diagnosis of ADPKD in adolescents/adults (Pei et al., 2009).

Although the development of cysts can begin *in utero*, with their continuous expansion leading to renal insufficiency, ESRD typically only occurs in adulthood (50% of patients have ESRD by 60 years of age). The decline of renal function is preceded by clinical symptoms such as deficient concentration of urine (up to 60% of children), hypertension (20–40% of children), abdominal pain (60% of adults), nephrolithiasis (20–25% of adults), gross hematuria (up to 60% of adults), and urinary tract infections (UTIs; 30–50% of adults; Ecder & Schrier, 2009; Grantham, 2008; Ho et al., 2012; Massella et al., 2018; Torres et al., 2007). Cysts can also develop in other organs: Polycystic liver disease can be diagnosed in more than 80% of patients by age 30, 10% of adults have cysts in the pancreas, and up to 40% of male patients have cysts in the seminal vesicle (Chebib et al., 2016; Grantham, 2008; Luciano & Dahl, 2014; Torra et al., 2008). In terms of mortality and morbidity, a particularly important extrarenal manifestation of ADPKD is intracranial aneurysms, which are more frequent in ADPKD patients compared to the general population (10% vs. 2%). They are even more abundant in ADPKD patients with a family history of intracranial/subarachnoid hemorrhage (22%; Pirson et al., 2002; Rinkel et al., 1998).

ADPKD is an autosomal dominant disease with locus heterogeneity: The most commonly mutated gene in ADPKD-patients is *PKD1* (16p13.3; 80% of cases). About 15% of ADPKD patients harbor heterozygous causative variants in *PKD2* (4q22.1; Bergmann et al., 2018; Mochizuki et al., 1996). The residual cases are due to causative variants in the genes *GANAB* (11q12.3), *DNAJB11* (3q27.3), and – as recently described – *ALG9* (11q23.1) or in yet unknown ADPKD-associated genes, or else are non-monogenic (Besse et al., 2019; Cornec-Le Gall et al., 2018; Harris & Torres, 2018; Porath et al., 2016). *PKD2*-associated ADPKD is a milder disease than *PKD1*-associated ADPKD, with a median age at ESRD of 78 years (vs. 58 years in *PKD1*-associated ADPKD; Cornec-Le Gall et al., 2013; Cornec-Le Gall et al., 2017). Interestingly, while biallelic LoF variants in *PKD1*/*PKD2* are believed to be embryonically lethal (analogously to KO mice), there are severe ADPKD cases in which compound-heterozygous causative variants in *PKD1*/*PKD2* have been identified. In these cases, there is (at least) one hypomorphic variant that leaves residual expression of the encoded protein (Audrezet et al., 2016; Bergmann et al., 2011; Hopp et al., 2012; Losekoot et al., 2012; Lu et al., 1997; Rossetti et al., 2009; Wu et al., 2000). While penetrance is complete – that is, occurrence of bilateral cysts if a certain age is reached – there is extensive variable expressivity in ADPKD concerning the course of disease and extrarenal features (both inter- and intrafamilial). Furthermore, *de novo* events occur in about 10–15% of cases (negative family history of ADPKD; Bergmann et al., 2018).

PKD1 encodes polycystin 1 (4,303 aa, RefSeq accession number NP_001009944.2), and *PKD2* encodes polycystin 2 (968 aa, RefSeq accession number NP_000288.1). These are both membrane-bound multispan proteins that form a complex intracellularly at their respective C-terminal ends. This complex is located at various components of the cell, as well as at the primary cilium. Polycystin 1 has receptor-like properties with a large extracellular domain (3,074 aa), while polycystin 2 is a cation channel, and their interaction is believed to be important for the regulation of intracellular Ca^{2+} and multiple cell-signaling pathways. In the pathogenesis of ADPKD, the cilium is viewed as a flow sensor enabling calcium entry when there is flow and reducing calcium entry in the absence of flow. Loss of the polycystin complex reduces calcium influx. Therefore, the development of cysts is dosage-dependent, and it is an accepted hypothesis that a somatic mutation of the second *PKD1/PKD2*-allele in the kidney (the first allele being altered in the germline) leads to disease (with additional influences like environmental factors).

Loss of *PKD1/PKD2* expression leads to dysregulated calcium homeostasis in renal tubular epithelial cells, with increased cyclic adenosine monophosphate (cAMP) levels. Vasopressin (via the vasopressin V2 receptor) is a main driver of renal tubular cAMP production and ADPKD patients have increased vasopressin levels (Bergmann et al., 2018; Harris & Torres, 2014; Terryn et al., 2011).

Management of ADPKD is largely symptomatic (control of hypertension, antibiotic treatment and drainage of infected cysts, management of pain due to enlarged cysts, monitoring of cyst growth [preferentially by MRI], dialysis, and kidney transplantation). However, therapy directly targeting cyst growth is also available by blocking the vasopressin V2 receptor (and reducing cAMP production subsequently; see above) with the receptor antagonist Tolvaptan. This drug has shown efficacy in reducing cystic growth (total kidney volume) and glomerular filtration rate (GFR) decline in ADPKD patients in a landmark double-blind, randomized, placebo-controlled, three-year phase-three trial (TEMPO 3:4 Trial; Torres et al., 2012). Decision on the administration of Tolvaptan is based on the progression of GFR decline and kidney enlargement over time, clinical symptoms, and type of causative genetic variant (truncating vs. non-truncating; Gansevoort et al., 2016).

It is debatable if asymptomatic children of genetically solved ADPKD-patients should undergo predictive genetic testing. A genetic diagnosis can have major influence on access to certain insurance and professions, and there is the "right not to know" for children that must be respected. However, there are treatable symptoms that occur in childhood, such as hypertension and proteinuria (see above), which usually are not recognized in early stages. A current international consensus states that surveillance of treatable childhood manifestations (i.e., hypertension, proteinuria) without genetic testing or renal ultrasound (which is also considered diagnostic; see above) is an acceptable management approach (Gimpel et al., 2019).

Of note, there are disease entities overlapping with ciliopathies phenotypically: Heterozygous causative variants in *DNAJB11* (which encodes a ciliary protein) can lead to a phenotype between ADTKD and ADPKD (see Section 1.1.1), and NPHP can mimic ("phenocopy") CAKUT (Bergmann et al., 2018; Cornec-Le Gall et al., 2018; van der Ven, Connaughton, et al., 2018). This is further elaborated upon in Section 1.2.3.

Moreover, the complex genetic architecture of *PKD1*, which impairs proper analysis by (short-read-based) ES, is discussed in Section 1.2.1 (Ali et al., 2019).

1.1.5 Hereditary focal segmental glomerulosclerosis and steroid-resistant nephrotic syndrome (FSGS/SRNS)

First and foremost, FSGS is not a single-disease entity, but describes a renal pathology characterized by a loss of podocytes (the visceral epithelial cells of the glomerulus) with subsequent deposition of extracellular matrix and, eventually, obliteration of the glomerular capillary tuft (glomerulosclerosis). On light microscopy, these changes can be "focal," that is, affecting not the entirety of a renal biopsy specimen, and "segmental," that is, with pathology limited to certain parts of the glomerulus. Electron microscopy typically shows podocyte foot-process effacement. FSGS should not be mixed with focal global glomerulosclerosis (FGGS), which comes with aging of kidneys and hypertensive nephropathy (De Vriese et al., 2018).

FSGS has a worldwide incidence of 0.2–1.8/100,000 population per year, but these numbers should be interpreted with caution, as there are differences in the availability of kidney biopsy and the assessment of indication for kidney biopsy (McGrogan et al., 2011; Rosenberg & Kopp, 2017). For example, in the United States of America, FSGS was the prime pathology among 2,501 kidney biopsies in adults between 2000 and 2011 (39%), and incidence rose from 1.6 to 5.3/100,000 population per year in this period. Furthermore, there was a profound inclination for FSGS in the African American population (49.8% of kidney biopsy diagnoses; Sim et al., 2016).

SRNS, on the other hand, is the clinical description of a nephrotic syndrome (for a definition, see below) not in remission after four weeks of daily prednisone (at 2 mg/kg/d; definition in children). It is in second place of ESRD etiologies up to the age of 20 (the first being CAKUT) and has a monogenic cause in about 30% of cases presenting before the age of 25 years (Nourbakhsh & Mak, 2017; Sadowski et al., 2015). The typical histologic correlate of SRNS in the kidney (if biopsied) is FSGS (Sadowski et al., 2015). Of note, minimal change disease (MCD), with no or minor glomerular pathology on light microscopy and the leading reason for nephrotic syndrome in children, characteristically presents as steroid-sensitive nephrotic syndrome but can evolve to FSGS if it is relapsing and steroid-resistance occurs (Vivarelli et al., 2017).

Reduction of podocyte number resulting in the histologic lesions of FSGS can be due to a plethora of etiologies including monogenic, environmental (e.g., viral infection, medication) and circulating factors (Rosenberg & Kopp, 2017). There are various classifications of FSGS, but the most important distinctions are primary (idiopathic) FSGS, genetic FSGS (also called hereditary or familial FSGS), and secondary FSGS (including infection- and medication-associated FSGS and maladaptive [due to reduced nephron quantity or increased strain on normal nephrons] FSGS; De Vriese et al., 2018; Rosenberg & Kopp, 2017).

The clinical hallmark of FSGS is proteinuria (> 150 mg/d), typically presenting as nephrotic syndrome (proteinuria > 50 mg/kg/d [> 3.5 g/d in a 70-kg adult], reduced serum albumin concentration associated with hyperlipidemia and edema; Topham, 2009). It is important to distinguish the different forms of FSGS (primary, genetic, secondary): Primary FSGS is supposed to be triggered by a circulating factor (immune-mediated). Soluble urokinase-type plasminogen activator (suPAR) is a prominent representative of these proposed circulating factors, but its role is disputed (Saleem, 2018; Wei et al., 2011). The mainstay of therapy of primary FSGS presenting with nephrotic syndrome is immunosuppressive therapy, with corticosteroids and, if necessary, other immunosuppressive drugs such as calcineurin inhibitors applied (Beaudreuil et al., 2017; Korbet, 2012). Furthermore, primary FSGS has a high risk of recurrence in kidney transplants (in about one-third of cases; Uffing et al., 2020). Genetic FSGS, in contrast, has a very low rate of recurrence (if any) in the kidney transplant but does not respond to immunosuppressive regiments usually (there are exceptions to this rule; De Vriese et al., 2018; Morello et al., 2019; Trautmann et al., 2018). In secondary FSGS, in turn, treatment of the underlying cause is crucial (e.g., cessation of FSGS-associated medication; Rosenberg & Kopp, 2017).

As SRNS is a clinical description which can lead to FSGS on a histologic level and the common pathological pathway is podocyte injury, these two entities are also summarized by the term “monogenic podocytopathies” (Boyer et al., 2017). There is a marked locus heterogeneity in monogenic podocytopathies, as more than 30 genes have been associated with these. Encoded proteins are important for podocyte dynamics (actin cytoskeleton, e.g., *ACTN4*), slit diaphragm integrity (e.g., *NPHS1*, *NPHS2*), or cell signaling (e.g., *PLCE1*; De Vriese et al., 2018).

Figure 5 shows the distribution of these proteins across the glomerular filtration barrier, and Table 3 is a list of typical monogenic podocytopathy-associated genes.

The diagnostic yield of exome sequencing is nearly 30% in pediatric and adolescent cases with SRNS (see above; Sadowski et al., 2015). Moreover, if SRNS manifests in the first year of life, about two thirds of patients can be genetically diagnosed by testing only four genes (*NPHS1*, *NPHS2*, *WT1*, and *LAMB2*; Hinkes et al., 2007). In adult patients with FSGS, a genetic cause can be established in 20% of cases by ES, as shown by one study of 193 patients (mean age of onset of kidney disease 34 years) with FSGS on kidney biopsy, or proteinuria and relatives with FSGS on kidney biopsy (Yao et al., 2019). Genes typically associated with adult-onset FSGS are *INF2*, *TRPC6*, and *ACTN4*, all inherited in an AD manner (whereas childhood-onset SRNS characteristically shows AR inheritance). Notably, the study of monogenic causes of FSGS has recently encountered criticism as the attribution of FSGS to a case is not sufficiently based on histologic criteria, but mostly on reported diagnoses (Howie, 2020).

Apart from monogenic causes, there are also risk alleles for FSGS. Most importantly, two alleles in the gene *APOL1* (22q12.3) NM_003661.4:c.1024A>G, p.(Ser342Gly) and c.1164_1169del, p.(Asn388_Tyr389del), named G1 and G2, confer a profound susceptibility to primary FSGS, and are exclusively found in people of African descent. The two variants are in negative linkage disequilibrium and are therefore never located on the identical chromosome (not monoallelic; Genovese et al., 2010). Allele frequency of the G1 variant is up to 40% in Ghana, and a homozygous G1/G1, homozygous G2/G2, or compound heterozygous G1/G2 genotype (called a high-risk genotype) is prevalent in 13% of African Americans in the United States of America (Freedman et al., 2018). These high-risk genotypes result in an OR of 17 for FSGS and even 29 for HIV-associated nephropathy; these are exceedingly high odds for common variants in a non-monogenic disease (Kopp et al., 2011). The lifetime risk for primary FSGS in individuals with a high-risk genotype is 4% (compared to 0.8% in non-carriers; Kopp et al., 2011). The reason for the high allele frequencies of the G1 and G2 variants in Western sub-Saharan Africa is supposed to be positive selection, as these variants convey trypanolytic activity against *Trypanosoma brucei rhodesiense*, a parasite causing African trypanosomiasis (also called "African sleeping sickness"). The 13%-prevalence of the high-risk genotype in African

Americans is seen as one contributor to the elevated incidence rates of primary FSGS in this population in the United States of America (see above; Freedman et al., 2018).

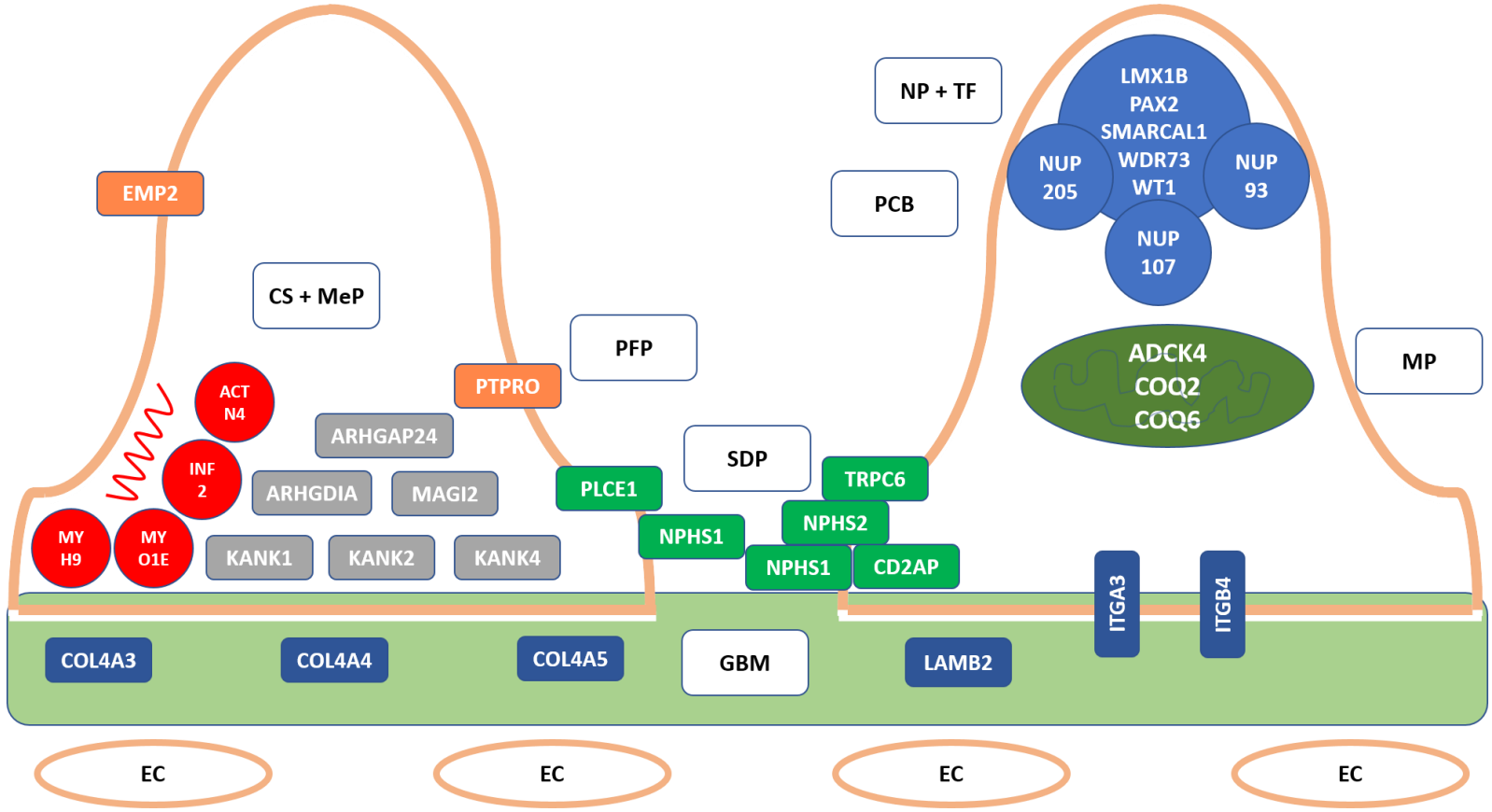

Figure 5: Selection of FSGS/SRNS-associated genes and their distribution across the glomerular filtration barrier. Figure by PD Dr. Julia Hoefele (Hoefele et al., 2018). CS (red and grey) + MeP (orange), cytoskeletal and membrane-associated proteins; EC, endothelial cell; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane (genes of GBM in dark blue); MP, mitochondrial proteins (dark green); NP + TF, nuclear proteins and transcription factors (light blue); PCB, podocyte cell body (orange line); PFP, podocyte foot process (orange line towards slit diaphragm); SDP, slit diaphragm proteins (light green); SRNS, steroid-resistant nephrotic syndrome.

Gene (chromosomal location)	Mode of inheritance	Encoded protein	Protein Function	Associated monogenic disease(s) in human [MIM phenotype number][a]	Phenotype	First report
NPHS1 (19q13.1)	AR	Nephrin	• Vital element of the slit diaphragm	• Nephrotic syndrome, type 1, also called Finnish congenital nephrosis [256300]	• Congenital SRNS	Kestila et al., 1998
NPHS2 (1q25.2)	AR	Podocin	• Transmembrane protein, important for recruiting nephrin to slit diaphragm	• Nephrotic syndrome, type 2 [600995]	• Childhood to adolescene to even early adulthood (SRNS and FSGS)	Boute et al., 2000
PLCE1 (10q23.33	AR	Phospholipase Cε1	• Cell signaling, interaction with nephrin	• Nephrotic syndrome, type 3 [610725]	• Early childhood SRNS (kidney biopsy shows diffuse mesangial sclerosis)	Hinkes et al., 2006
MYO1E (15q22.2)	AR	Nonmuscle myosin 1e	• Component of the podocyte foot process	• Glomerulosclerosis, focal segmental, 6 [614131]	• Childhood-onset FSGS	Mele et al., 2011
LAMB2 (3p21.31)	AR	Laminin-β2	• Extracellular matrix glycoprotein • Cell adhesion, differentiation, signaling	• Pierson syndrome [609049]	• Congenital or childhood-onset SRNS (kidney biopsy shows diffuse mesangial sclerosis) • Microcoria, blindness • Neurodevelopmental delay • Individuals with LoF alleles show more severe phenotype than those with missense alleles	Zenker et al., 2004
COQ2 (4q21.23)	AR	Coenzyme Q2 4-hydroxybenzoate polyprenyl transferase	• Enzyme, biosynthesis of CoQ10 in mitochondria	• Coenzyme Q10 deficiency, primary, 1 [607426]	• Childhood-onset SRNS amenable to treatment with CoQ10 • Sensorineural deafness • Encephalopathy	Quinzii et al., 2006
COQ6 (14q24.3)	AR	Coenzyme Q6 monooxygenase	• Enzyme, biosynthesis of CoQ10 in mitochondria	• Coenzyme Q10 deficiency, primary, 6 [614650]	• Childhood-onset SRNS amenable to treatment with CoQ10 • Sensorineural deafness	Heeringa et al., 2011
COQ8B (19q13.2)	AR	aarF domain containing kinase 4	• Enzyme, biosynthesis of CoQ10 in mitochondria	• Nephrotic syndrome, type 9 [615573]	• Childhood-onset SRNS amenable to treatment with CoQ10	Ashraf et al., 2013
WT1 (11p13)	AD	Wilms tumor 1	• Transcription factor	• Nephrotic syndrome, type 4 [256370] • Denys-Drash syndrome [194080] • Frasier syndrome [136680] • Wilms tumor, type 1 [194070]	• DDS and FS represent a spectrum of disease including: FSGS/diffuse mesangial sclerosis, gonadoblastoma, gonadal dysgenesis, male pseudohermaphroditism • Most causative variantes in DDS are in exon 8 or 9 of *WT1* (dominant negative effect) • 74% of children with DDS develop Wilms‘ tumor (nephroblastoma) • Recurrent nearsplice variant in *WT1* causes FS (donor splice site of exon 9) • Can present as isolated FSGS/diffuse mesangial sclerosis with nephrotic syndrome • Germline causative variants in *WT1* are identified at low frequency in isolated Wilms‘ tumors (2.1%)	Hastie, 1992; Barbaux et al., 1997; Jeanpierre et al., 1998
PAX2[b] (10q24.31)	AD	Paired box 2	• Transcription factor	• Glomerulosclerosis, focal segmental, 7 [616002] • Papillorenal syndrome [120330]	• CAKUT with optic nerve coloboma • Adult-onset FSGS • No genotype-phenotype correlation (papillorenal syndrome vs. FSGS)	Barua et al., 2014
ACTN4 (19q13.2)	AD	α–Actinin-4	• Protein of the cytoskeleton	• Glomerulosclerosis, focal segmental, 1 [603278]	• Adult-onset FSGS	Kaplan et al., 2000
TRPC6 (11q22.1)	AD	Transient receptor potential cation channel 6	• Calcium channel, receptor-activated • Interaction with nephrin and podocin	• Glomerulosclerosis, focal segmental, 2 [603965]	• Adult-onset FSGS	Reiser et al., 2005
INF2 (14q32.33)	AD	Inverted formin 2	• Involved in actin dynamics	• Glomerulosclerosis, focal segmental, 5 [613237] • Charcot-Marie-Tooth disease, dominant intermediate E [613237]	• Adolescence- or adult-onset FSGS • Charcot-Marie-Tooth disease (hereditary motor and sensory neuropathy)	Brown et al., 2010; Boyer et al., 2011

Table 3: Selection of different hereditary FSGS/SRNS-associated genes with respective protein function and phenotypes. Note that *COL4A3*, *COL4A4*, and *COL4A5* are not listed, although causative variants in these genes can lead to FSGS. These genes are specifically addressed in Section 1.1.2. AD, autosomal dominant; AR, autosomal recessive; CAKUT, congenital anomalies of the kidney and urinary tract; CoQ10, coenzyme Q10; DDS, Denys-Drash syndrome; FS, Frasier syndrome; FSGS, focal segmental glomerulosclerosis; SRNS, steroid-resistant nephrotic syndrome.

[a]https://www.omim.org/.

[b]See also Table 2.

Adapted from De Vriese et al., 2018, with data from Ashraf et al., 2013; Barbaux et al., 1997; Barbosa et al., 1999; Barua et al., 2014; Boute et al., 2000; Boyer et al., 2011; Brown et al., 2010; Dome & Huff, 2016; Hastie, 1992; Heeringa et al., 2011; Hinkes et al., 2006; Jeanpierre et al., 1998; Kaplan et al., 2000; Kestila et al., 1998; Little et al., 2004; Mele et al., 2011; Quinzii et al., 2006; Reiser et al., 2005; Zenker et al., 2004. Table published (Riedhammer, Schmaderer, et al., 2020).

1.1.6 VACTERL/VATER association

VACTERL is an acronym describing the non-random appearance of certain birth defects: V – Vertebral anomalies, A – Anorectal malformations, C – Cardiovascular anomalies, T – Tracheoesophageal fistula, E – Esophageal atresia, R – Renal and/or radial anomalies, L – Limb defects. If no cardiovascular anomalies or limb defects occur, the association is called VATER. The incidence of VACTERL/VATER is estimated at < 1 to 9 per 100,000 infants. However, this number is dependent on clinical diagnostic criteria, and ascertainment bias can be assumed (Solomon, 2011). VACTERL/VATER is etiologically highly heterogeneous and involves both monogenic and multifactorial causes (Solomon, 2018). There are no generally accepted criteria for clinical diagnosis of VACTERL/VATER, but a widely used standard is that at least three components of the association should be present (Solomon, 2018). With this requirement, several monogenic syndromes can be grouped in/overlap with the VACTERL/VATER spectrum: Townes-Brocks syndrome (renal, cardiac, limb and anorectal malformations) associated with causative variants in *SALL1*. Or CHARGE syndrome (*CHD7*), featuring cardiac, skeletal, renal, and anorectal anomalies. Or Alagille syndrome (*JAG1*, *NOTCH2*) characterized by vertebral, cardiac, and renal abnormalities (Solomon, 2011; Solomon et al., 2012). The contribution of genetics in VACTERL/VATER is emphasized by familial clustering and data from monozygotic twin studies (Reutter et al., 2016). However, because of the multiple organ systems involved and the etiological heterogeneity, it is a challenging task to unravel the genetic and environmental determinants of VACTERL/VATER, apart from the overlap with known monogenic syndromes (Saisawat et al., 2014; Solomon, 2018).

1.1.7 Other hereditary nephropathies

Distinct hereditary nephropathy disease groups investigated in this thesis are ADTKD, AS, CAKUT, ciliopathies, FSGS/SRNS, and VACTERL/VATER. Nonetheless, there are several more entities when discussing hereditary nephropathies which are briefly described in the following.

1.1.7.1 Tubulopathies

Tubulopathies show marked genetic heterogeneity. Bartter syndrome and Gitelman syndrome are prominent representatives and affect electrolyte transporters in the thick ascending limb of the loop of Henle (TAL) and the distal convoluted tubule (DCT) of the

renal tubular system (Devuyst et al., 2014). Associated genes in Bartter syndrome encode electrolyte transporters like the sodium–potassium-2-chloride co-transporter – targeted by loop diuretics – in the TAL (NKCC2, gene *SLC12A1* [15q21.1], Bartter syndrome, type 1), and causative variants lead to profound loss of sodium and potassium in the urine, resulting in hypokalemic alkalosis with hypercalciuria and nephrocalcinosis (Simon, Karet, et al., 1996). Causative variants in the gene encoding the thiazide-sensitive sodium-chloride cotransporter (NCCT, *SLC12A3* [16q13]) in the DCT are linked to Gitelman syndrome, which features renal potassium and magnesium wasting (Simon, Nelson-Williams, et al., 1996). Bartter syndrome (several types) and Gitelman syndrome are (mainly) inherited in an AR manner and therefore typically manifest in infancy/(early) childhood or – in case of Bartter syndrome – even *in utero* (Gitelman syndrome can become apparent only in adolescence/adulthood; Seyberth & Schlingmann, 2011).

Inherited tubular diseases of the collecting duct include AR distal renal tubular acidosis (dRTA) with defective H^+ secretion of the intercalated cells (causative variants in genes *ATP6V1B1* [2p13.3] and *ATP6V0A4* [7q34]; phenotype of hyperchloremic non-anion gap metabolic acidosis, nephrolithiasis, nephrocalcinosis, and sensorineural hearing loss; Both et al., 2014); and AD Liddle syndrome (causative variants in genes *SCNN1A* [12p13.31], *SCNN1B* [16p12.2], and *SCNN1G* [16p12.2]), with increased renal sodium reabsorption (increased expression of epithelial sodium channel in the collecting duct) leading to early-onset hypertension (Shimkets et al., 1994; Tetti et al., 2018). A hereditary disorder of the proximal tubule is Dent disease (associated genes *CLCN5* [Xp11.23] and *OCRL* [Xq26.1]), an XL tubulopathy (hence male predominance) presenting with tubular proteinuria, aminoaciduria, hypercalciuria, hyperphosphaturia, and glycosuria (also called renal Fanconi syndrome; Devuyst & Thakker, 2010).

1.1.7.2 Inherited metabolic disorders

Inherited metabolic disorders can also manifest with a profound renal phenotype. One example is Fabry disease, an XL multi-system lysosomal storage disease (gene *GLA* [Xq22.1]), characterized by lysosomal accumulation of glycosphingolipid due to deficiency of the enzyme α-galactosidase A. Glycosphingolipid deposition in the kidney causes proteinuria and progressive renal function loss (other organs involved are heart, nervous system, skin, gastrointestinal tract, and eyes). Fabry disease can be treated with enzyme replacement therapy (Germain, 2010).

1.1.7.3 Mitochondrial disorders

Due to their pleiotropy, hereditary mitochondrial disorders can have various effects on the kidney. These disorders can be the result of causative variants in both nuclear and mitochondrial DNA encoded proteins. Renal phenotypes include SRNS (*COQ2*, *COQ6*, and *COQ8B*; Table 3), FSGS in MELAS (typical mitochondrial DNA variant NC_012920.1:m.3243A>G in *MT-TL1*), and tubulopathy (O'Toole, 2014).

1.1.7.4 Atypical hemolytic uremic syndrome (complement-mediated hemolytic uremic syndrome)

aHUS is an exceedingly rare (incidence of 0.5/million/year) but devastating complement-mediated renal disease (hence more aptly called complement-mediated hemolytic uremic syndrome) affecting all age groups. aHUS presents with microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. Fifty percent of untreated patients progress to ESRD. The histologic correlate on kidney biopsy is thrombotic microangiopathy (TMA). The pathophysiologic hallmark of aHUS is the dysregulation of the alternative pathway of complement, leading to endothelial injury. The complement system is an element of the innate immune system which consists of different activation pathways (Feitz et al., 2018; Karpman et al., 2017; Kavanagh et al., 2006). Familial occurrence of aHUS underlines genetic influence and, in addition to autoantibodies against complement factor H, several genes encoding components of the alternative pathway of complement have been implicated in aHUS pathogenesis (e.g., *CFH* [1q31.3], *CFB* [6p21.33], *C3* [19p13.3], *CFI* [4q25], *CD46*[1q32.2], *THBD* [20p11.21], and rearrangements of *CFH* and *CFH*-related genes at the *CFH* locus; Feitz et al., 2018; Valoti et al., 2015). However, aHUS is not a clear-cut monogenic disorder (as it has a penetrance of about 50%): Individuals do not necessarily develop aHUS even if they carry rare deleterious alleles in one of the mentioned genes. Additional triggers such as infection, malignant hypertension, pregnancy, or autoimmune disease are needed to develop the disease (Feitz et al., 2018; Kavanagh et al., 2006; Schaefer et al., 2018).

Accordingly, the above-mentioned genes are all associated with susceptibility to aHUS in OMIM®. Therefore, results from genetic analyses of complement genes are difficult to interpret: One retrospective study of 57 aHUS patients from the French aHUS registry, who received renal transplantation, showed that patients with “mutations” (no minor allele frequency [MAF] or exact variant provided) in complement genes *CFH*, *MCP*, *CFI*, *C3*, *CFB*, and *THBD* had a significantly higher risk of aHUS recurrence in the transplant

within a five-year follow-up time (p = 0.009). However, this was not a gene-specific analysis (Le Quintrec et al., 2013). Another retrospective study of 851 patients from the Global aHUS registry showed that carrying variants (MAF < 1.0%; regardless of zygosity; n = 247) in seven aHUS genes (*C3*, *CFH*, *CFI*, *CFB*, *MCP*, *DGKE*, *THBD*) did not result in an overall significantly higher ESRD rate. However, a significantly larger proportion of patients positive for *CFH* variants (n = 95) progressed to ESRD in a period of 15 years from first aHUS presentation (Schaefer et al., 2018).

A major leap forward for establishing a connection between variation in complement-associated genes and aHUS was achieved by statistical analysis of rare variant burden (MAF < 0.01%) in 13 genes (*CFH*, *CFI*, *CD46*, *C3*, *CFB*, *CFHR1*, *CFHR3*, *CFHR4*, *CFHR5*, *CFP*, *PLG*, *THBD*, and *DGKE*) in 1,231 aHUS cases. Only protein-altering variants were included (nonsense, frameshift, canonical splice site, missense variants, and in-frame indels). This study showed a significant burden of rare protein-altering variants (compared to controls in the Exome Variant Server [EVS] and/or the database of the Exome Aggregation Consortium [ExAC]) in the genes *CFH*, *CFI*, *CD46*, *C3*, *DGKE*, and *CFB*. Rare variant cases in *CFH* had an OR of 12 compared to controls (Osborne et al., 2018). A next step to improve the interpretation of rare variants identified in aHUS-associated genes was the investigation of 93 complement and coagulation genes in 400 patients with aHUS. There was significant enrichment (compared to the Genome Aggregation Database [gnomAD] and 599 internal controls) for rare variants (MAF < 0.01%; nonsense, frameshift, canonical splice site, missense variants and in-frame indels) in *CFH*, *C3*, *CD46*, *CFI*, and *DGKE*, and a pathogenic influence of a variant was improbable when MAF was > 0.1%. Consequently, many variants reported in the literature as causative should be reclassified due to high MAF (Bu et al., 2018). However, in both aforementioned studies, only 13 and 93 genes, respectively, were analyzed for rare variants, hence, this data cannot be interpreted as significant to a genome-wide degree.

As already described, aHUS is prompted by dysregulation of the alternative pathway of complement. The current mainstay of therapy is plasma exchange and, more specifically recognizing the etiology of aHUS, terminal inhibition of the complement cascade by the humanized monoclonal IgG antibody Eculizumab (an annual cost of about €500,000; Legendre et al., 2013). As aHUS can recur, especially after kidney transplantation (about a 50% recurrence rate), there is a huge debate as to whether and when Eculizumab can be

discontinued (Ariceta, 2019; Kavanagh et al., 2006). In a nutshell, no high-quality prospective and controlled trials have led to algorithms to determine when to cease Eculizumab administration in aHUS (Ariceta, 2019).

1.2 Exome sequencing

ES denotes the NGS-based assessment of the protein-coding regions of the human genome and has been successfully employed for diagnosing monogenic disease in a variety of medical specialties, including nephrology (Groopman et al., 2018; Petersen et al., 2017). In the following, ES, its utility in the detection of causative variants in monogenic diseases, its role in the discovery of novel disease-associated genes, and its limitations are described in general. Then, the application of ES in several hereditary nephropathy disease groups is delineated. After this, the benefit of comprehensive genetic testing (such as ES) for the identification of phenocopies in hereditary nephropathies is described. Note that the following sections refer to short-read-based NGS (for definition, see below), which is routinely used for targeted gene panels/ES; long-read NGS, which can overcome the limitations of short-read NGS, is discussed briefly in Section 1.3.

1.2.1 Exome sequencing in general, its utility and limitations

The basis of ES is next-generation sequencing (NGS), which is characterized by the sequencing of DNA molecules in a parallel fashion (so-called massively parallel sequencing) in a separating chamber, that is, the flow cell (Majewski et al., 2011). This procedure is a fundamental leap of efficiency in comparison to Sanger sequencing, in which only a single DNA fragment is analyzed by terminating DNA polymerization in a nucleotide-specific manner (Sanger et al., 1977). While the sequencing of the human genome by Sanger sequencing in the framework of the Human Genome Project was associated with costs of about $2.7 billion in 2001, NGS enabled the genome to be sequenced for $10,000 in 2011 (Lander et al., 2001; Majewski et al., 2011; Petersen et al., 2017).

In order to sequence multiple samples in parallel by NGS, adaptor oligonucleotides acting as molecular bar codes are added to fragmented DNA, which is called “library preparation.” In ES, after library preparation, the exonic regions of the human genome are selectively captured and enriched, whereafter the actual sequencing is carried out,

typically as short-read sequencing (reads of 100 base pairs [bp]; Figure 6; Goodwin et al., 2016; Teer & Mullikin, 2010; Yohe & Thyagarajan, 2017).

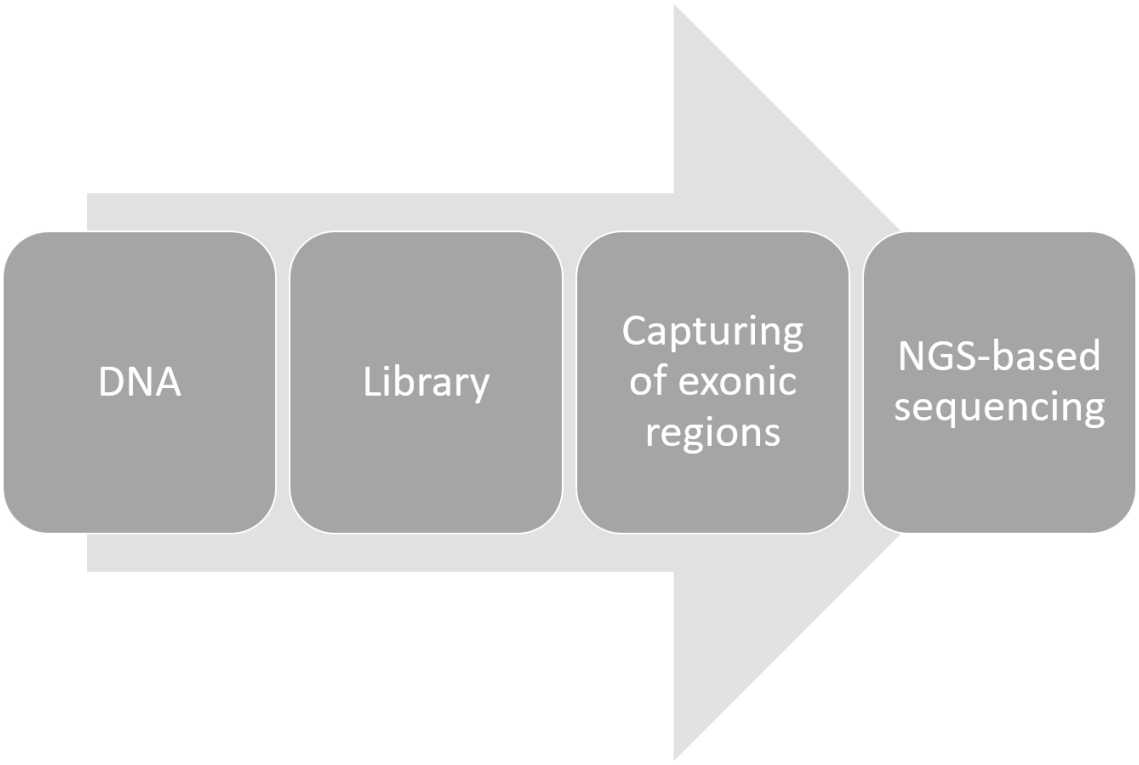

Figure 6: Essential steps of exome sequencing (based on short-read NGS). NGS, next-generation sequencing. Adapted from Yohe & Thyagarajan, 2017.

ES enables the unbiased and comprehensive analysis of the protein-coding regions of the genome (about 1–2% of the genome) and has proven to be a cost-effective (compared to genome sequencing [GS]) and efficient technique to diagnose monogenic disease: It is estimated that about 85% of causative variants are located in the exome (Majewski et al., 2011). Within about ten years from the first publication of ES in 2009, the number of disease-associated genes listed in OMIM® has nearly doubled (Ng et al., 2009; Petersen et al., 2017).

In ES, only the exonic parts of the genome are analyzed, as a result of which the intronic/intragenic/untranslated region and other non-coding variants can be missed (if not specifically targeted for; Groopman et al., 2018). General limitations of (short-read-based) NGS are also pertinent to ES: Regions of high sequence homology, for example, due to pseudogenes, cannot be sufficiently interpreted, as variants cannot be confidently

aligned to the gene of interest. This is the case for *PKD1*, the most important gene associated with ADPKD (Section 1.1.4), in which homologous pseudogenes prevent proper analysis (Ali et al., 2019). Furthermore, variants in repetitive regions (e.g., variable number tandem repeat [VNTR] regions) cannot be reliably detected by short-read-based NGS. This is true for *MUC1*, the gene associated with ADTKD-*MUC1* (Section 1.1.1), in which the causative variant is an insertion of a cytosine nucleotide in a cytosine tract of the VNTR region of *MUC1* (Groopman et al., 2018; Kirby et al., 2013). Moreover, in short-read-based NGS, repeat expansions (e.g., trinucleotide repeat expansions in neurologic disorders such as Huntington's disease) elude detection (Fernandez-Marmiesse et al., 2018). Gene segments abundant of the nucleotide bases guanine and cytosine ("GC-rich"), which pair with three hydrogen bonds, can also lead to insufficient coverage in short-read-based NGS (Groopman et al., 2018). Examples of GC-rich regions are exon 1 of *COL4A3* (ARAS) and the gene *PKD1* (ADPKD), which has a large GC content in general (Moriniere et al., 2014; Thongnoppakhun et al., 2004).

Not only can single-nucleotide variants (SNVs) and small insertions and deletions of nucleotide bases ("indels") be detected by ES, but CNVs down to the size of one exon can be as well (Plagnol et al., 2012). This is a better resolution than can be achieved by chromosomal microarray (CMA), which is still considered the gold-standard for CNV detection. Genomic imbalances only down to a size of 30–50 kb can be detected by CMA (Coe et al., 2007; Haraksingh et al., 2017). However, while the recall rate of CMA-detected CNVs by ES is about 90% for deletions, it is only 65% for duplications. In addition, naturally, only regions covered by ES can be evaluated for CNVs (Marchuk et al., 2018).

Not necessarily a limitation, but a notable "side effect" of ES (and other comprehensive genomic testing methods) are so-called secondary findings. These are causative variants identified in disease-associated genes that are not related to the reason for testing. There is a list of actionable disease-associated genes (for which therapies or surveillance programs exist) issued by the ACMG, for which the detection of a causative variant (e.g., a causative variant in the breast-cancer-predisposition gene *BRCA1*) should be reported as a secondary finding (if the patient has not declined reporting when consenting to ES; Kalia et al., 2017). Reportable secondary findings occur in about 2% of patients analyzed by ES (Groopman et al., 2019).

Table 4 summarizes the benefits and disadvantages of different molecular genetic methods utilized in the diagnosis of hereditary kidney diseases. As GS is not routinely used in hereditary kidney diseases, it is not mentioned in the table but is addressed in Section 1.3.

Method	Indication	Advantages	Disadvantages
Sanger sequencing	• Targeted sequencing of regions not covered by NGS (e.g., GC-rich segments) • Phenotype is highly suggestive of a single-gene disorder (e.g., Fabry disease, gene *GLA*) • Sequencing of a known variant (e.g., familial variant) • Confirmation of a variant detected by NGS	• Detection of SNVs and insertions and deletions up to 10 bp • No secondary findings	• Limited detection of mosaics • No detection of CNVs • Time- and money-consuming and hence not reasonable in genetically heterogeneous disorders
Targeted gene panel (NGS-based analysis of a set of preselected genes)	• Suspicion of a disorder with a limited number of disease-associated genes (e.g., AS)	• Optimization for region of interest possible • Secondary findings unlikely • High sensitivity for SNVs (95–99%) and small insertions and deletions (90–95%) • CNV-analysis possible (resolution one exon)	• Panels need to be updated constantly as new disease-associated genes are described • Limited utility if genetic heterogeneity is large (sequential panel sequencing necessary) • No generally accepted standard protocol for CNV detection exists
Exome sequencing (NGS-based analysis of the protein-coding regions of the human genome)	• Suspicion of a genetically heterogeneous disorder (e.g., ciliopathy, syndromic CAKUT)	• Unbiased analysis of all protein-coding regions of the human genome • High sensitivity for SNVs (95–99%) and small insertions and deletions (90–95%) • CNV-analysis possible (resolution one exon)	• Secondary findings (side effect, not a clear disadvantage) • No generally accepted standard protocol for CNV detection exists
Chromosomal microarray (molecular karyotyping)	• Suspicion of a structural aberration of the human genome (e.g., 17q12 deletion syndrome)	• Higher resolution than karyotyping (CNVs to a size of 30–50 kb detectable vs. 5 Mb resolution limit in karyotyping)	• No detection of SNVs or duplications and deletions below resolution limit • Complex chromosomal rearrangements without a change in dosage are missed (e.g., balanced translocations; karyotyping needed [this disadvantage is also applicable to short-read-based NGS methods])

Table 4: Overview of different molecular genetic methods routinely used to diagnose hereditary kidney diseases. In the case of NGS (targeted gene panel and exome sequencing), short-read-based NGS is meant. GC-rich segments are gene segments abundant with the nucleotide bases guanine and cytosine. AS, Alport syndrome; bp, base pairs; CAKUT, congenital anomalies of the kidney and urinary tract; CNV, copy number variant; kb, kilobase pairs; Mb, megabase pairs; NGS, next-generation sequencing; SNV, single-nucleotide variant. Adapted from Groopman et al., 2018, and with data from de Ravel et al., 2007. Different versions of the table have been published (Riedhammer & Hoefele, 2019; Riedhammer, Schmaderer, et al., 2020).

1.2.2 Exome sequencing in hereditary nephropathies

ES has been widely used to diagnose hereditary nephropathies and to discover new disease-associated genes. Limitations of detection are discussed above, in Section 1.2.1. Table 5 lists exome sequencing/NGS studies in different hereditary nephropathy disease groups and their respective diagnostic yields (causative variant identified).

Disease group	Number of index cases/families	Diagnostic yield	Comment	Reference
AS	**27**	**81%**	Targeted NGS-based gene panel, not ES.	Mallett et al., 2017
CAKUT	**232**	**13%**	Diagnostic yield rises in severe CAKUT (renal agenesis, renal dysplasia; 17%), reportedly consanguineous cases (20%) and syndromic CAKUT (29%).	van der Ven, Connaughton, et al., 2018
Ciliopathies	**79**	**63%**	In 76% of cases parents were consanguineous.	Braun et al., 2016
SRNS (onset < 25 years of age)	**300**	**28%**	Diagnostic yield of 48% in cases with congenital SRNS	Warejko et al., 2018
FSGS (adult-onset)	**179**	**20%** (about 50% of likely pathogenic and pathogenic variants in *COL4*-genes, mainly heterozygous carriers)	*In silico* panel of 109 genes from ES data. Onset of kidney disease was 34 ± 16 years (± SD). Inclusion criteria were FSGS on kidney biopsy or proteinuria and relative with FSGS on kidney biopsy.	Yao et al., 2019
VACTERL/VATER	**271**	**14%**	Commercial lab data, VACTERL denoted as part of clinical characteristics.	Solomon, 2018

Table 5: Overview of diagnostic yields of ES/NGS-based panels (short-read) in major hereditary nephropathy disease groups. AS, Alport syndrome; CAKUT, congenital anomalies of the kidney and urinary tract; ES, exome sequencing; FSGS, focal segmental glomerulosclerosis; NGS, next-generation sequencing; SD, standard deviation; SRNS, steroid-resistant nephrotic syndrome; VACTERL/VATER, V – Vertebral anomalies, A – Anorectal malformations, C – Cardiovascular anomalies, T – Tracheoesophageal fistula, E – Esophageal atresia, R – Renal and/or radial anomalies, L – Limb defects.

Furthermore, an ES study of 3,037 patients with CKD (91.6% older than 21 years; either ESRD or creatinine > 1.5 mg/dl in men, > 1.3 mg/dl in women or proteinuria/hematuria, pointing towards active glomerular disease, present) diagnosed a hereditary nephropathy in 9.3% of cases, comparable to yields for monogenic forms in cancer cohorts (Figure 7). This study showed that if the clinical diagnosis was diabetic or hypertensive nephropathy, a genetic diagnosis could only be established in 1.6 and 2.5% of cases, respectively, indicating that there is only a small fraction of monogenic causes to be found when these clinical diagnoses are made (Groopman et al., 2019). Interestingly, in patients with a "nephropathy of unknown origin," a genetic diagnosis could be made in about every sixth case (17.1%, 48 of 281 cases) and this category was an independent predictor of a positive genetic result (other independent predictors were familial occurrence and cystic/congenital kidney disease; Groopman et al., 2019). This finding is backed by two studies. One study was of 57 patients with ESRD of unknown origin on the kidney transplant waiting list, in which NGS-based targeted analysis of 209 genes linked to ESRD led to a genetic diagnosis in 12% of cases (Ottlewski et al., 2019). The other was an ES-study of 114 families with CKD, in which a genetic diagnosis could be made in 16 families with etiologically undetermined CKD (38% of solved cases; Connaughton et al., 2019). This underscores that NGS-based targeted sequencing and ES can help to classify renal disease of unknown origin, which in turn can have major clinical implications, such as the assessment of previously unrecognized extrarenal manifestations (e.g., ocular and otologic evaluation in AS), causal therapy (enzyme replacement in Fabry disease), and tailored therapy (e.g., discontinuation of immunosuppression in monogenic SRNS with rapid need of kidney transplantation), and prognosis (e.g., risk of disease recurrence in the kidney transplant in hereditary FSGS [negligible] vs. primary FSGS [one-third of cases]; Germain, 2010; Groopman et al., 2019; Trautmann et al., 2018; Uffing et al., 2020). Clinical implications were present in 89% of 167 patients (a sub-cohort with detailed clinical information) with a genetic diagnosis in the publication by Groopman et al., 2019.

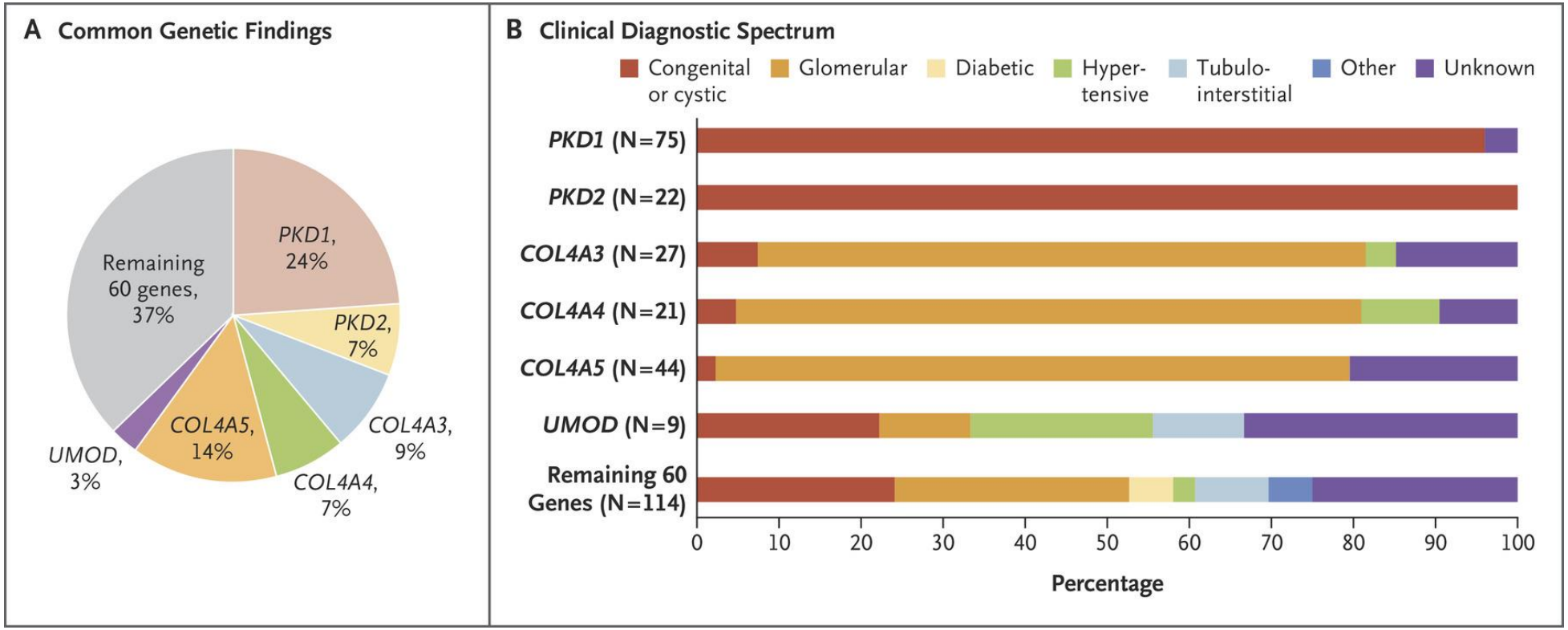

Figure 7: A) Distribution of disease-associated genes, in which causative variants were identified by ES in a cohort of 3,037 patients with CKD (91.6% > 21 years old). About two-thirds of hereditary nephropathies are due to causative variants in six genes. B) Spectrum of clinical pre-categorization of patients with an identified monogenic disease. Note the clinical heterogeneity of ADTKD-*UMOD*. 312 hereditary nephropathies were diagnosed in 307 patients (five patients had two diagnoses). From Groopman et al., 2019.

1.2.3 Comprehensive genetic testing for the detection of phenocopies in hereditary nephropathies

A “phenocopy” is generally defined as a phenotype not explained by an established genotype (e.g., a causative variant running in a family) but by a different genotype or environmental factors (Strachan & Read, 2018). The term is widely used in linkage studies, in which it denotes a proband who is affected by the same disease phenotypically as other family members but does not carry the known causative variant like the other affected relatives (Lescai & Franceschi, 2010). In a wider sense, “phenocopy” has been used in several medical specialties to illustrate a condition in which the phenotype of a patient resembles a specific hereditary disease/disease group with a known genetic cause/known set of disease-associated genes (e.g., Huntington's disease) with failure to identify the typical genotype (a heterozygous CAG repeat expansion in *HTT*) but detection of a different causative variant (a hexanucleotide repeat expansion in *C9orf72*) and recategorization of the disease by genetic diagnosis (Huntington's disease to frontotemporal dementia; Biancalana et al., 2018; Hensman Moss et al., 2014; Mariani et al., 2016; Turner et al., 2010; Ulirsch et al., 2018).

According to this broad definition, which is employed in this thesis to describe the recategorization of disease by genetic diagnosis, phenocopies have been detected in hereditary nephropathy cohorts with selected disease groups: In a cohort of 70 families with histopathological diagnosis of FSGS and familial occurrence (analyzed by ES or targeted sequencing of 2,400 podocyte-enriched genes), 10% had heterozygous or compound-heterozygous (likely) pathogenic variants not in genes associated with hereditary FSGS but the ARAS genes *COL4A3* and *COL4A4* (Malone et al., 2014). Furthermore, an ES study of 300 families (335 individuals) with SRNS revealed phenocopies in nearly 4% of families. Causative variants were found in genes *COL4A5* and *COL4A3* mimicking SRNS but also *GLA*, the gene linked to Fabry disease, which can be treated by enzyme replacement therapy (Warejko et al., 2018). In 8 of 36 patients (22%) with a phenotype resembling ARPKD, causative variants could be found in other genes than *PKHD1*, the gene associated with ARPKD (Szabo et al., 2018). Finally, causative variants in NPHP-genes (e.g., *NPHP1*, *NPHP4*, and *TMEM213*) can lead to a CAKUT-like phenotype with small cystic kidneys mimicking renal hypo-/dysplasia (van der Ven, Connaughton, et al., 2018).

1.3 Genomics in hereditary nephropathies – outlook

GS has not been implemented in routine diagnostics in hereditary nephropathies due in part to the question of cost. However, it has several advantages: First, it can detect non-coding variants, which have been described in several monogenic kidney diseases (King et al., 2002; Lo et al., 2011; Mele et al., 2015). Second, it is not subject to any capturing bias, which allows more even coverage of coding regions compared to ES (e.g., of homologous areas in the ADPKD-gene *PKD1*; Section 1.2.1; Groopman et al., 2018; Mallawaarachchi et al., 2016).

The addition of RNA sequencing ("transcriptome") to ES (and GS) can help identify and functionally characterize variants elusive to genomic diagnostics (e.g., in AR diseases: deep intronic variants leading to aberrant splicing resulting in the loss of one allele and hence monoallelic expression of a second [likely] pathogenic variant on the other allele at the RNA level; Kremer et al., 2017).

Furthermore, long-read sequencing, that is, NGS-based sequencing of reads up to several hundred kilobase or even megabase pairs (as opposed to short-read sequencing with reads of a length of only 100 bp) is becoming increasingly prominent in genomics (Pollard et al., 2018). For hereditary nephropathies, long-read sequencing might be a promising tool – if adequately accurate nucleotide-sequencing can be achieved – to diagnose ADTKD-*MUC1*, for example (Section 1.2.1; Groopman et al., 2018).

1.4 Genetics of non-monogenic kidney diseases

To illustrate the contrast to hereditary nephropathies, the genetics of non-monogenic (multifactorial) kidney diseases are briefly described in the following.

Monogenic disease is caused by rare variants (allele frequency < 1.0%) with a high effect size (OR > 10) for developing a certain phenotype. ES is an excellent tool to discover rare variants in monogenic disease (Section 1.2.1; Petersen et al., 2017). By contrast, genetic predisposition (susceptibility) for multifactorial disease is determined by common variants (allele frequency > 5.0%) with a low effect size (OR 1.05–1.30; Tam et al., 2019). Remarkable exemptions are *APOL1* risk alleles G1 and G2: These are common genetic variants conferring a high-risk for non-monogenic kidney diseases (OR of 17 for primary FSGS and 29 for HIV-associated nephropathy), probably due to positive selection (Section 1.1.5; Kopp et al., 2011; Wuttke & Kottgen, 2016).

Genome-wide association studies (GWAS) have proven very effective in detecting common variants (loci) contributing to non-monogenic diseases/traits (Figure 8; McCarthy et al., 2008; Tam et al., 2019). Genome-wide data from large case-control cohorts (hundreds to hundreds of thousands to over a million individuals, depending on the disease/trait investigated and the expected effect size) is typically generated by SNP arrays (like the Illumina® Infinium™ Global Screening Array featuring more than 600,000 markers; about $40 per sample) with imputed genotypes (Tam et al., 2019). GWAS have identified disease-associated loci in various non-monogenic diseases, such as major depressive disorder or type-2 diabetes (Hyde et al., 2016; Zhao et al., 2017).

As for multifactorial kidney diseases, GWAS have led to the identification of risk loci for non-monogenic nephrolithiasis, IgA nephropathy (IgAN), idiopathic membranous nephropathy and traits such as hyperuricemia and CKD in general (Dehghan et al., 2008; Kiryluk et al., 2014; Kottgen et al., 2009; Palsson et al., 2019; Stanescu et al., 2011; Wuttke et al., 2019). Interestingly, some detected risk loci are also associated with monogenic disease (e.g., *UMOD* locus) or suggest a link between genetic variation and autoantibody formation (PLA_2R1 autoantibodies and *HLA–DQA1* risk allele in idiopathic membranous nephropathy; Stanescu et al., 2011; Wuttke & Kottgen, 2016; Wuttke et al., 2019). Hence, GWAS might aid in unraveling the pathophysiology of non-monogenic kidney diseases to expedite targeted therapy development (Wuttke & Kottgen, 2016).

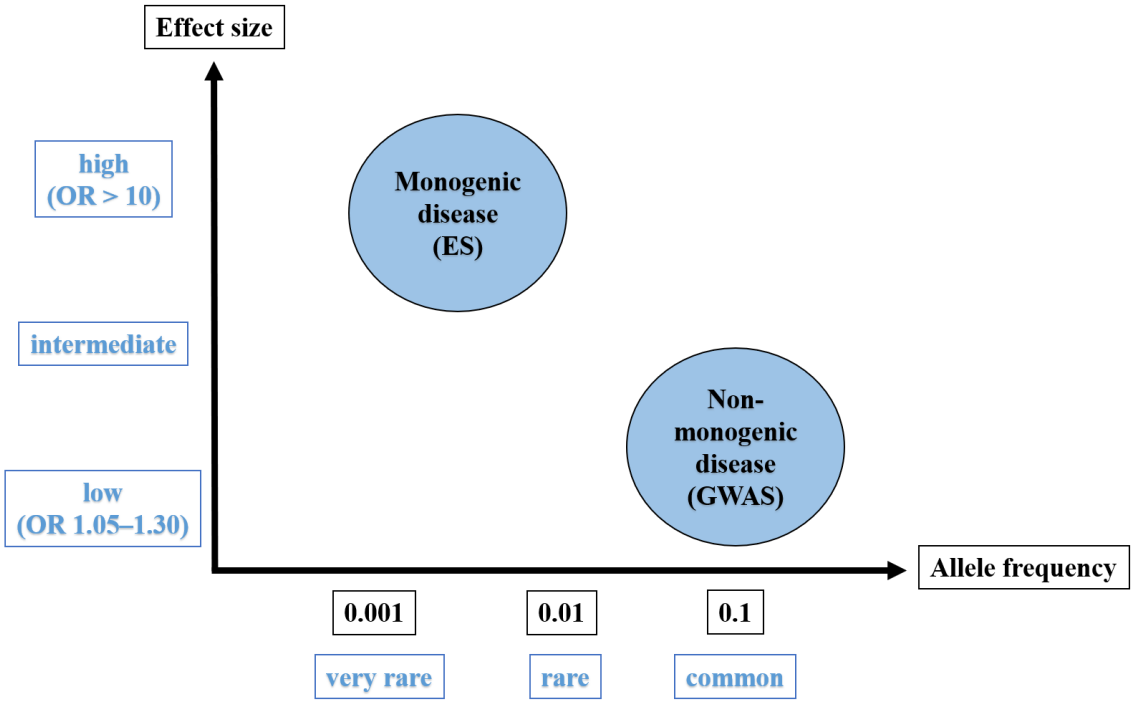

Figure 8: Illustration of the relationship of effect size and allele frequency in monogenic vs. non-monogenic (multifactorial) disease. ES, exome sequencing; GWAS, genome-wide association study; OR, odds ratio. Adapted from McCarthy et al., 2008, with data from Tam et al., 2019. Figure published (Riedhammer, Schmaderer, et al., 2020).

2 AIM OF THIS THESIS

The aim of this thesis is to evaluate the application of ES in hereditary kidney disease in a cohort of 260 phenotypically well-ascertained (index) patients (= [index] cases) featuring children, adolescents, and adults. Each index case had either a phenotype in accordance with/the clinical tentative diagnosis of a specific hereditary nephropathy or had renal disease and fulfilled specific criteria such as positive family history or syndromic disease pointing towards a hereditary nephropathy (Section 3.1.1).

Furthermore, this thesis intends to delineate the utility of ES in uncovering phenocopies in hereditary nephropathies and prioritizing novel disease-associated genes and to improve clinical decision-making in patients with a presumed hereditary nephropathy.

3 PATIENT COHORT, MATERIAL, AND METHODS

3.1 Patient cohort

3.1.1 Patient recruitment and consent

This is a cross-sectional study of 260 (index) patients (= [index] cases) analyzed by ES. All index cases were from unrelated families and were recruited between October 2015 and February 2019. All index cases (and their respective families) were genetically unsolved and had either a) a phenotype in accordance with/the clinical tentative diagnosis of a distinct hereditary kidney disease/disease entity; or b) renal involvement without phenotypic overlap with a specific hereditary kidney disease/disease entity, but met at least one of the following inclusion criteria:

- First manifestation before the age of 18 years
- Familial occurrence (at least one similarly affected individual in a family in addition to index case)
- Reported parental consanguinity
- Syndromic disease, that is, involvement of an additional organ system apart from the kidney

Exclusion criteria:

- Cases with renal involvement without phenotypic overlap with a specific hereditary kidney disease/disease entity NOT meeting any of the inclusion criteria mentioned above
- Renal involvement which could clearly be attributed to a secondary cause (e.g., ESRD due to trauma or medication-associated or infection-associated FSGS)
- Ongoing or cancelled analysis (e.g., at the referring clinician's request)
- Cases previously solved genetically (ES was done additionally for other purposes, e.g., to identify further variants modifying the phenotype)
- Missing consent

Patients were recruited either directly at the Institute of Human Genetics of the Klinikum rechts der Isar, Technical University of Munich, Munich, Germany, which is a tertiary care center, or they were recruited by external human geneticists and (pediatric) nephrologists and referred to the institute. The index cases are part of a larger renal disease ES cohort, called "NephroGen" (*n* = 330 index cases recruited between October 2015 and February 2019), at the Institute of Human Genetics of the Klinikum rechts der Isar, Technical University of Munich, Munich, Germany. Some cases of the NephroGen cohort had to be excluded from the present thesis when applying the criteria mentioned above. See Figure 13 in Section 4.1.1 for a flowchart of included and excluded patients.

The recruitment, genetic analysis by ES, storage of data, and publication of results was approved by the local Ethics Committee of the Technical University of Munich and performed according to the standard of the Helsinki Declaration of 2013 (World Medical, 2013). Written informed consent was acquired by all participants or their legal guardians. Biological samples were only collected after written informed consent was obtained.

Importantly, all unsolved cases in the ADTKD disease group received external *MUC1* testing (insertion of a cytosine nucleotide in a cytosine tract of the VNTR region of *MUC1*), as ADTKD-*MUC1* cannot be diagnosed by short-read-based ES. Furthermore, all cases with the clinical tentative diagnosis of ADPKD received external *PKD1* testing prior to ES, as *PKD1* cannot be confidently analyzed by short-read-based ES (Section 1.2.1).

The Online Supplementary Table (URL/DOI in the appendices) lists all cases with inclusion criteria applied, as well as all excluded cases along with the reason for exclusion.

3.1.2 Phenotype ascertainment

Phenotypes were ascertained by reviewing medical reports and filling out a standardized questionnaire (Figure 9). Index cases were assigned to specific disease groups corresponding to their phenotype/clinical tentative diagnosis (NOT their genetic diagnosis).

These disease groups were:

- ADTKD
- CAKUT
- Ciliopathy
- FSGS/SRNS
- Type-IV-collagen-related nephropathy
- VACTERL/VATER
- Other (all phenotypes/clinical tentative diagnoses not fitting any of the aforementioned disease groups)

The disease group type-IV-collagen-related nephropathy comprises both AS and TBMN. To assign a case to the TBMN subgroup, one of the subsequent criteria had to be met:

- A thin basement membrane but not AS on kidney biopsy (Section 1.1.2) in the index patient (or a biopsied similarly affected relative with a fitting inheritance pattern) and a clinical presentation/family history consistent with TBMN (i.e., only microscopic hematuria without CKD/with only late-onset ESRD [> 50 years of age] in the index patient/family).
- Clinical tentative diagnosis of TBMN due to the phenotype of the index patient and family history in cases in which no kidney biopsy was performed (i.e., only microscopic hematuria without CKD/with only late-onset ESRD [> 50 years of age] in the index patient/family).

A thinned GBM on kidney biopsy alone cannot diagnose TBMN reliably. This is due to the fact that, in early stages of AS, only thinning of the GBM can be present, and TBMN

can be falsely assumed if only biopsy findings are used for diagnosis (Kashtan, 2019). Therefore, information on clinical presentation and family history were mandatory to assign a case to the "TBMN subgroup" (see above). Cases with biopsy-proven AS, a clinical tentative diagnosis of AS as per the referring clinician without phenotypic and/or family history information to change the clinical tentative diagnosis to TBMN were assigned to the "AS subgroup." Patients in the AS subgroup with only a heterozygous (likely) pathogenic variant in *COL4A3* or *COL4A4* were designated as "unsolved case" (due to a non-fitting genotype; see Online Supplementary Table).

A

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Klinikum rechts der Isar Technische Universität München

Laboratory number:_____

Questionnaire for Study
Identification of Genes and Modifiers in Hereditary Disorders
of the Kidney and Urinary Tract

Patient's data (if applicable patient's label)	
Last name	First name
Date of birth (day/month/year)	Telephone number
Street	Zip code
Place of residency	Country

Are the parents consanguineous?
☐ yes ☐ no

Kidney disease occurring in relatives?
☐ Mother: _____ ☐ Sister: _____
☐ Father: _____ ☐ Brother:_____
☐ Others: _____

Diagnosis

Date of first diagnosis ____(day)/____(month)/____(year)
Diagnosis(es) _____
Molecular genetic and cytogenetic results _____

Extrarenal manifestations

☐ Microcephaly ☐ Deafness/hearing impairment ☐ Polydactyly
☐ Vermis aplasia/hypoplasia ☐ Ocular changes ☐ Skeletal malformations
☐ Mental retardation ☐ Heart defects ☐ Growth retardation
☐ Others:_____

Laboratory results at time of first diagnosis

Blood:
☐ Serum creatinine ____ mg/dl
☐ Serum urea ____ mg/dl
☐ GFR ____ ml/min/1,73m²
☐ Immun serologic abnormalities
→ if so, which ones:_____
☐ Others: _____

Urine:
☐ Creatinine clearance ____ ml/min
☐ Microhematuria ☐+ ☐++ ☐+++
☐ Macrohematuria
☐ Proteinuria (Age) ____ g/g creatinine

Figure 9: Standardized questionnaire for phenotype ascertainment. A) First page.

B

Last name_____First name_____ born___(day)/___(month)/____(year)

Ultrasound

☐ Normal ☐ Abnormalities:_____

Kidney biopsy

Has a kidney biopsy been performed? ☐ yes ☐ no

First biopsy ____(day)/____(month)/____(year)

Second biopsy ____(day)/____(month)/____(year)

☐ Minimal change nephropathy ☐ Thin Basement Membrane Nephropathy (TBMN)

☐ Focal segmental glomerulosclerosis (FSGS) ☐ Alport syndrome

☐ Diffuse mesangial sclerosis ☐ Nephronophthisis

☐ Mesangioproliferative glomerulonephritis ☐ C3 glomerulopathy

☐ No result ☐ Other results:_____

Dialysis and kidney transplantation

Current need for dialysis? ☐ yes ☐ no

☐ Hemodialysis since: ____(month)/____(year)

☐ Peritoneal dialysis since: ____(month)/____(year)

Has a kidney transplantation been performed? ☐ yes ☐ no

☐ First transplantation ____(day)/____(month)/____(year)

☐ Second transplantation ____(day)/____(month)/____(year)

Transplant failure due to: ☐ Relapse ☐ Rejection

Did arterial hypertension exist before dialysis/transplantation? ☐ yes ☐ no

Remarks

We thank you for your effort.

Please fill out the following spaces so that we can reach you for further inquiries.

Name:

Date: Signature: Business address:

Figure 9: Standardized questionnaire for phenotype ascertainment. B) Second page.

3.2 Sample processing, polymerase chain reaction, and Sanger sequencing

The methods described in this section were primarily performed by medical technical assistants of the Institute of Human Genetics at the Klinikum rechts der Isar of the Technical University of Munich, Munich, Germany, and the Institute of Human Genetics at the Helmholtz Zentrum München – German Research Center for Environmental Health, Neuherberg, Germany.

3.2.1 DNA isolation

For DNA isolation from whole blood specimen, 2–3 ml blood was drawn from a peripheral vein into a tube containing EDTA as an anticoagulant.

3.2.1.1 Manual DNA isolation

For manual DNA isolation from whole blood (mainly used for infectious material; otherwise, automated DNA isolation; see below), the Gentra Puregene DNA Purification Kit (QIAGEN, Hilden, Germany) was used. First, erythrocytes were lysed by adding 1,350 µl of Red Blood Cell Lysis Solution (RBC Lysis Solution) to 450 µl of patient blood with subsequent mixing. After a 5-min incubation step at room temperature, the solution was centrifuged at 16,000 x g for 30 s. Nucleated cells thus became visible as a white pellet. The supernatant was discarded, and the pellet was resuspended in the residual liquid (about 20 µl) by vortexing. The next step was lysis of the nucleated cells and denaturing the proteins by adding 450 µl Cell Lysis Solution und 2 µl Proteinase K and subsequently mixing. This solution was incubated at 55 °C for 1 h. After this, 150 µl Protein Precipitation Solution was added and vortexed for 20 s. This solution was centrifuged for 1 min at 16,000 x g, and a dark-brown pellet became visible. The supernatant contained DNA and was transferred into a new tube, with 450 µl 100% isopropanol in order to precipitate DNA. The tube was mixed by inverting, and DNA became visible as threads or a clump. The solution was then centrifuged at 16,000 x g for 1 min, and the supernatant was discarded. The DNA was washed with 450 µl 70% ethanol and centrifuged again at 16,000 x g for 1 min. The supernatant was discarded, and the pellet was air dried for 5 min at room temperature. The pellet was resuspended and mixed in 50 µl of DNA Hydration Solution. In order to completely dissolve DNA, the solution was incubated at 65 °C for 1 h.

3.2.1.2 Automated DNA isolation

DNA was extracted from whole blood with the automated nucleic acid purification instrument Chemagic™ 360 (PerkinElmer, Waltham, MA, United States of America) using the manufacturer's protocol. In short, transiently magnetized rods attract beads after binding to nucleic acid (DNA) from homogenized and lysed cells. Bead-bound DNA can then be washed and eluted from the beads by buffers. Empty beads are then removed by the magnetized rods, and DNA remains in a collecting tube.

3.2.2 DNA quantification and quality check

DNA quality and quantity were measured with a NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, United States of America).

Specifically, the light absorption (absorbance) of DNA at wavelengths of 260 nm and 280 nm was measured. An absorbance ratio A260/280 of about 1.8 is considered to indicate "pure DNA." Absorbance at and above 2.0 indicates contamination with RNA. A second measure of DNA purity was A260/230. Pure DNA has values of 2.0–2.2. If A260/230 is below 2.0, there can be contamination with substances absorbing light at 230 nm.

DNA quantity was calculated automatically by applying a modified Beer–Lambert law (see NanoDrop™ 1000 Spectrophotometer User's Manual):

$$c = (A * \varepsilon)/b$$

c: nucleic acid concentration (ng/ml)

A: absorbance units (AU)

ε: wavelength-dependent extinction coefficient in ng*cm/µl (double-stranded DNA: 50 ng*cm/µl)

b: pathlength in cm (For the NanoDrop™ 1000 pathlengths of 1 mm and 0.2 mm are used)

For nucleic acid, data is normalized to a 1.0-cm (10.0-mm) path (see NanoDrop™ Technical Bulletin T042).

3.2.3 Polymerase chain reaction

3.2.3.1 Basic method

Polymerase chain reaction (PCR) is used for the amplification of specific DNA fragments (template). Primers (oligonucleotides complementary to the target DNA sequence), a thermostable DNA polymerase and free nucleotides (deoxyribonucleotide triphosphates, dNTPs) are the essential components of any PCR. Primers mark the starting point of replication. For this thesis, primers were designed with Primer3 (http://primer3.ut.ee/).

By using a thermal cycler, double-stranded DNA is first denatured into single strands by heating the probe to 95 °C (breaking of DNA hydrogen bonds). The next step is the annealing of primers to complementary DNA sequences at 50–60 °C. The last step is synthesis of a new DNA strand (extension) at the free 3'-OH-end of the primer by the thermostable DNA polymerase adding free nucleotides (complexed with Mg^{2+}) in a 5'-to-3' direction (typically, *Taq* polymerase, a DNA polymerase I from *Thermus aquaticus*, a thermophilic microorganism, is used). Newly synthesized double-stranded DNA molecules are again templates for subsequent replications. These three steps (denaturing, annealing and extension) are carried out 25–30 times (cycles).

3.2.3.2 Materials and procedure

The following mastermix was used for a standard PCR:

- 1.2 µl Primer Mix
- 18.8 µl HPLC-grade water
- 3.0 µl 10x PCR buffer
- 3.0 µl dNTP-Mix (2 mM)
- 1.8 µl $MgCl_2$ (25 mM)
- 0.2 µl Taq-Polymerase (5 U/µl)

The mastermix was prepared on ice and mixed thoroughly. Then, 2.0 µl of DNA template (10 ng/µl) was added, for a total reaction volume of 30 µl. The following PCR program was used:

Cycles	Temperature	Duration (min:s)	Effect
1	95 °C	4:00	Initial denaturation
15	94 °C	0:30	Denaturation
	Touchdown 65–55 °C	0:30	Annealing
	72 °C	0:15	Extension
20	94 °C	0:30	Denaturation
	58 °C	0:30	Annealing
	72 °C	0:15	Extension
1	72 °C	3:00	Final extension

In the "touchdown" protocol, the annealing temperature is decreased by 1 °C each cycle, which increases primer specificity (Don et al., 1991).

3.2.3.3 Agarose gel electrophoresis

The PCR product was detected with agarose gel electrophoresis. A 2.7% gel (better separation of smaller DNA fragments compared to lower-concentrated gels) was prepared by adding 2.7 g of agarose powder to 100 ml 1x TBE buffer (Tris/Borate/EDTA) and microwave heating for 1–3 min until the agarose powder was completely dissolved. The solution was cooled down to about 50 °C, then 2.5 µl Midori Green Advance DNA stain (NIPPON Genetics Europe GmbH, Düren, Germany) was added for the later visualization of DNA under ultraviolet light. Next, the solution was poured into a casting tray with well combs. These combs were removed once the gel had solidified. Five µl BlueJuice™ (Thermo Fisher Scientific, Inc., Waltham, MA, United States of America) was mixed with a 1 µl PCR reaction and carefully pipetted into the wells. Electrophoresis was done at 230 V for 30 min. The negatively charged phosphate backbone of DNA leads to the migration of DNA molecules towards the anode (positively charged). Migration is

dependent on size: Smaller-sized DNA fragments migrate faster than larger DNA fragments (Aaij & Borst, 1972).

3.2.3.4 PCR purification

For the removal of primers, free nucleotides, and DNA polymerase, MultiScreen 96-well filter plates (Merck Millipore, Burlington, MA, United States of America) were used. PCR products were briefly centrifuged, 190 µl HPLC-grade water was added, and the solution was pipetted into the filter plate. The closed plate was placed on a vacuum manifold, and a vacuum of 10 mmHg was applied, which leads to the binding of DNA to the filter membrane. Pure PCR products were eluted with HPLC-grade water.

3.2.4 Sanger sequencing

3.2.4.1 Basic method

Sanger sequencing was used for targeted sequencing of a variant previously identified by ES (e.g., segregation of a variant in parents to determine inheritance or to confirm a *de novo* status). The DNA template was generated by PCR as described in Section 3.2.3. Sanger sequencing is named after biochemist Frederick Sanger, who introduced this method in 1977 (and received the Nobel Prize in Chemistry in 1980 for this achievement). It employs the principle of DNA chain termination (Sanger et al., 1977): Similar to PCR, primers and DNA polymerase are needed. Importantly, in Sanger sequencing, modified dideoxynucleotide triphosphates (ddNTPs) are added as free nucleotides. Specifically, fluorescence-labelled ddNTPs were used ("dye-terminator sequencing"). ddNTPs lack a free 3'-OH-end, which is pivotal for the elongation of DNA by the DNA polymerase (the formation of a phosphodiester bond between two nucleotides). Whenever a fluorescence-labelled ddNTP (ddATP, ddTTP, ddGTP, ddCTP) is incorporated into the DNA strand by the DNA polymerase, elongation is terminated, and a fluorescence signal is emitted. The synthesized strand (complementary to template) is then separated by capillary electrophoresis and sequenced in an automated DNA-sequencer to yield a so-called "peak trace chromatogram" (example in Figure 10).

3.2.4.2 Materials and procedure

For Sanger sequencing, the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Inc., Waltham, MA, United States of America) was used. The reaction was carried out in a 96-well microtiter plate. The following reactants were employed:

- 1 µl primer (specific for region of interest)
- 2 µl 5x Sequencing-Buffer
- 0.75 µl BigDye™ Terminator Mix v3.1
- Purified PCR product (Section 3.2.3.4; volume dependent on PCR product quantity; see below)
- HPLC-grade water (volume dependent on template volume; see below)

PCR product quantity	Template (volume)	HPLC-grade water (volume)
< 5 ng	2 µl	4 µl
5–20 ng	1 µl	5 µl
> 20 ng	0.5 µl	5.5 µl

Total reaction volume was 9.75 µl. The sealed plate was then put in a thermo cycler and the following program was run:

Temperature	Duration (min:s)	Cycles	Effect
96 °C	1:00	1	Denaturation
96 °C	0:10	25	Denaturation
50 °C	0:05		Annealing
60 °C	1:30		Extension
15 °C	Permanently	1	Hold

After the cycle sequencing reaction, sequencing products were precipitated by adding 100 µl 100% ethanol to the respective wells and then mixing. The plate was sealed, incubated

at room temperature for 15 min, and then centrifuged at 1,500 x g at 4 °C for 30 min. The seal was then removed, and the plate was inverted onto absorbent paper without dislodging the pellet. The inverted plate was then centrifuged at 185 × g for 1 min. Then, 30 µl of 70% ethanol was added to each well and the plate (right-side up) was centrifuged at 2500 x g at 4 °C for 15 min. The plate was inverted on absorbent paper and again centrifuged at 185 × g for 1 min. The plate was then air dried for no longer than 5 min at room temperature. Finally, a purified sequence reaction was dissolved in 25 µl HPLC-grade water.

3.2.4.3 Sequencing

Sequencing (capillary electrophoresis and fluorescence detection) was done with an Applied Biosystems™ 3730xl DNA Analyzer (Thermo Fisher Scientific, Inc., Waltham, MA, United States of America). Chromatograms were analyzed with Chromas v2.6.4 (Technelysium Pty Ltd., South Brisbane, Australia) or SeqPilot v.4.4.0 (JSI medical systems, Ettenheim, Germany).

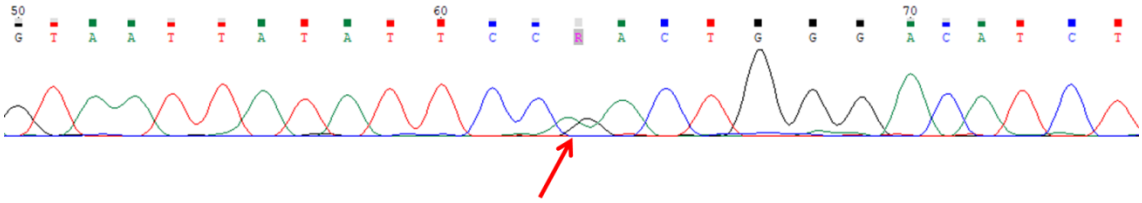

Figure 10: Example of a chromatogram showing a heterozygous nucleotide change G>A (arrow). Missense variant in *NPHS2* NM_014625.2:c.413G>A, p.(Arg138Gln). Chromas software v2.6.4 (Technelysium Pty Ltd, South Brisbane, Australia).

3.3 Exome sequencing

The basic principle of ES, its utility, and its limitations are described in the introduction, in Section 1.2.1. Sample processing for subsequent ES is described in Section 3.2. Three µg of DNA was needed for ES, which was performed at the Next-Generation Sequencing Core Facility at the Helmholtz Zentrum München – German Research Center for Environmental Health, Neuherberg, Germany.

3.3.1 Method

Exonic regions were either targeted and enriched using a Sure Select Human All Exon V5 (50 Mb) Kit (Agilent Technologies, Inc., Santa Clara, CA, United States of America) and sequenced as 100 bp paired-ends runs on a HiSeq2500 (Illumina, Inc., San Diego, CA, United States of America; 11% of cases) or targeted and enriched using a Sure Select Human All Exon V6 (60 Mb) Kit (Agilent Technologies, Inc., Santa Clara, CA, United States of America) and sequenced as 100 bp paired-ends runs on a HiSeq4000 (Illumina, Inc., San Diego, CA, United States of America; 89% of cases). Reads were aligned to the Genome Reference Consortium Human Build 37 (GRCh37), also called "hg19" synonymously, with the Burrows-Wheeler Aligner (v.0.7.5a; Li & Durbin, 2009).

SNVs and indels (smaller than one exon) were called with SAMtools (version 0.1.19; Li et al., 2009). For the detection of CNVs (starting from the size of one exon), the algorithm “ExomeDepth” was used. ExomeDepth compares normalized read depth data of a single ES sample with a reference ES data set to identify CNVs. The reference data set was regularly updated and came from the Munich Exome Server (over 20,000 exomes in 03/2020). CNV noise of up to 2.5 was accepted for analysis (Plagnol et al., 2012). Comparison of ExomeDepth with CMA, the accepted gold standard for the detection of larger CNVs (beginning at a size of 30 kb; Table 4), shows that ExomeDepth has a recall rate (sensitivity) of about 90% for the detection of deletions, but only 65% for the detection of duplications. ExomeDepth can only detect CNVs in regions covered by ES (not in intragenic regions, for example; Marchuk et al., 2018). Mitochondrial DNA was obtained from off-target reads (Griffin et al., 2014).

3.3.2 Variant interpretation

Exome analysis was performed with the custom-made tool "Exome Variant and Annotation Database" (EVAdb; Institute of Human Genetics, Helmholtz Zentrum München – German Research Center for Environmental Health, Neuherberg, Germany). For every analysis, in a first step, a search for nonsynonymous variants (i.e., nonsense, frameshift, canonical splice site, missense, initiation codon, stoploss variants, and indels) and CNVs was conducted. If no causative variant(s) could be detected, a second EVAdb-based analysis, using the GATK haplotype caller (**G**enome **A**nalysis **T**ool**k**it) for SNV detection, was performed (https://gatk.broadinstitute.org/hc/en-us). If the case was still unsolved, the search was extended to near-splice, synonymous, intronic, and untranslated-region (UTR) variants (provided that there was coverage). Variants were visualized with IGV v.2.3.68 (**I**ntegrative **G**enomics **V**iewer; http://software.broadinstitute.org/software/igv/home). For the detection of AD, *de novo* and mitochondrial variants, only variants with a MAF < 0.1% (compared to in-house controls of the Munich Exome Sever with > 20,000 exomes and 125,748 exome and 15,708 genome sequences of gnomAD v.2.1.1) were considered. For AR and XL variants (homozygous, hemizygous, or [putatively] compound heterozygous), a MAF threshold of < 1.0% was used. There are pathogenic variants in hereditary kidney disease with a MAF of more than 1.0%, for example, the missense variant NM_014625.3:c.686G>A, p.(Arg229Gln) in *NPHS2* (also called p.R229Q). Therefore, in unsolved cases, a further analysis for AR and XL nonsynonymous variants up to a MAF of 3.0% was performed. The p.(Arg229Gln) variant can cause SRNS when *in trans* with specific 3' *NPHS2* variants (Miko et al., 2018; Tory et al., 2014).

A Phred accuracy score was used for the assessment of SNV/mapping quality:

$$Q = -10log_{10}P$$

Q: phred score

P: probability of an erroneous base call

A score of 30 indicates that the probability of a false base call (or false mapping) is 1 in 1,000. If the SNV quality score was below 30 and/or the mapping quality score was below 30 and/or the coverage of a variant was below 10 reads, the variant was discarded. If the coverage of a variant of interest was between 10 and 19 reads, the variant was mandatorily confirmed by Sanger sequencing (Section 3.2.4).

The following *in silico* tools to predict functional consequences of variants were used: SIFT (**S**orting **I**ntolerant **F**rom **T**olerant), PolyPhen-2 (**Poly**morphism **Phen**otyping v2) and CADD (**C**ombined **A**nnotation-**D**ependent **D**epletion; Adzhubei et al., 2010; Kircher et al., 2014; Ng & Henikoff, 2003). Thresholds for *in silico* prediction of a damaging/deleterious effect of a variant were SIFT ≤ 0.05, PolyPhen-2 between 0.85 and 1.0, and CADD ≥ 15. Furthermore, conservation of an amino acid/nucleotide across 100 vertebrate species was checked with the UCSC Genome Browser (100 vertebrates Basewise Conservation by PhyloP). In trio ES, that is, the analysis of an index patient with his/her parents, inheritance of variants could be viewed directly (*de novo*, compound-heterozygosity, XL inheritance, etc.). Otherwise, inheritance was determined by the Sanger sequencing of parents (if the DNA of the parents was available; see Online Supplementary Table).

All identified sequence variants are listed in the Online Supplementary Table (URL/DOI in the appendices) and are reported with their genomic position (GRCh37/hg19), transcript (RefSeq accession number), cDNA/mtDNA and protein position (if applicable), using the website Mutalyzer (https://mutalyzer.nl/; den Dunnen et al., 2016). CNVs are reported with their (approximate) genomic position and cytogenetic band.

Detected variants/CNVs were compared with public databases for causative variants such as ClinVar, DECIPHER (the **D**atabas**e** of genomi**c** var**i**ation and **P**henotype in **H**umans using **E**nsembl **R**esources), the Human Gene Mutation Database (HGMD® Professional 2019.4), and the Leiden Open Variation Database (LOVD; see "Online resources"). Only variants/CNVs rated as "likely pathogenic" or "pathogenic" according to the ACMG criteria for sequence variant/CNV interpretation and with a genotype in agreement with the mode of inheritance are called "causative variants" and led to a "solved case" (Figure 11; Richards et al., 2015; Riggs et al., 2020). Current amendments of the ACMG classification ("Association for Clinical Genomic Science [ACGS] Best Practice Guidelines for Variant Classification 2019") and recommendations for the interpretation

of LoF variants were also applied (Abou Tayoun et al., 2018). "Genotype in agreement with the mode of inheritance" means, for example, that a heterozygous (likely) pathogenic variant in a gene associated with an AR disease is not considered causative until a second (putatively) compound-heterozygous (likely) pathogenic variant can be detected. Cases in which no causative variant(s) could be identified (i.e., benign, likely benign variants, variants of uncertain significance [VUS], non-fitting genotype), were designated as "unsolved case."

For ACMG criterion PP3 ("multiple lines of computational evidence support a deleterious effect on the gene or gene product"), two out of three *in silico* prediction tools had to be above/below the set thresholds. In one case (HN-F684-II-1), CADD showed a clearly deleterious effect (CADD = 34) of the missense variant NM_001080463.1:c.11333C>T, p.(Ala3778Val) in *DYNC2H1*, but SIFT and PolyPhen-2 did not. As the altered amino acid was completely conserved across 100 species (UCSC browser), PP3 was still applied.

ACMG criterion PM3 ("for recessive disorders, detected *in trans* with a pathogenic variant") was applied if the variant on the opposite chromosome (*in trans*) was a (likely) pathogenic variant (of any kind) previously reported in another patient with an overlapping phenotype. If the variant *in trans* was a (likely) pathogenic LoF variant (i.e., nonsense, frameshift, canonical splice site variant with predicted nonsense-mediated mRNA decay [NMD], large rearrangements) and a loss of function was a known mechanism of disease, PM3 was used regardless of any previous report of the variant.

ACMG criterion PM1 ("located in a mutational hot spot and/or critical and well-established functional domain") was used not only for missense variants, but also for in-frame indels located in a specific protein domain (Richards et al., 2015).

Secondary findings were only reported if a causative variant was identified in one of the 59 medically actionable genes determined by the ACMG (Kalia et al., 2017).

ACMG STANDARDS AND GUIDELINES

RICHARDS *et al* | Interpretation of sequence variants

Table 3 Criteria for classifying pathogenic variants

Evidence of pathogenicity	Category
Very strong	PVS1 null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease Caveats: • Beware of genes where LOF is not a known disease mechanism (e.g., *GFAP*, *MYH7*) • Use caution interpreting LOF variants at the extreme 3′ end of a gene • Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact • Use caution in the presence of multiple transcripts
Strong	PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change Example: Val→Leu caused by either G>C or G>T in the same codon Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level PS2 De novo (both maternity and paternity confirmed) in a patient with the disease and no family history Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, and so on, can contribute to nonmaternity. PS3 Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well established. PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls Note 1: Relative risk or OR, as obtained from case–control studies, is >5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0. See the article for detailed guidance. Note 2: In instances of very rare variants where case–control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.
Moderate	PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation PM2 Absent from controls (or at extremely low frequency if recessive) (**Table 6**) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium Caveat: Population data for insertions/deletions may be poorly called by next-generation sequencing. PM3 For recessive disorders, detected in *trans* with a pathogenic variant Note: This requires testing of parents (or offspring) to determine phase. PM4 Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before Example: Arg156His is pathogenic; now you observe Arg156Cys Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level. PM6 Assumed de novo, but without confirmation of paternity and maternity
Supporting	PP1 Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease Note: May be used as stronger evidence with increasing segregation data PP2 Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) Caveat: Because many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant. PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology PP5 Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

LOF, loss of function; OR, odds ratio.

8 GENETICS in MEDICINE

Figure 11: American College of Medical Genetics and Genomics (ACMG) guideline for sequence variant interpretation (excerpt). From Richards et al., 2015.

3.3.3 Algorithm to identify variants in novel potentially disease-associated genes

As the AMCG criteria should not be applied to variants in genes not yet associated with a disease ("candidate genes"), an algorithm (Figure 12) was used to prioritize variants in candidate genes in unsolved cases (Richards et al., 2015). Quality parameters and *in silico* prediction tools were used, as described in Section 3.3.2. gnomAD constraint metrics were employed for gene assessment: A loss-of-function variant observed/expected ratio (depending on gene and sample size) with a loss-of-function observed/expected upper-bound fraction (LOEUF) < 0.35 indicates that a gene is haploinsufficient; that is, heterozygous LoF variants (with expected NMD) will cause disease (Karczewski et al., 2020). For missense variants, a Z-score > 3.09 indicates selection against missense variants in the respective gene (Lek et al., 2016). For functional and KO mouse data, a PubMed and Mouse Genome Informatics (MGI) search was conducted (see "Online resources"). Note that a lack of functional/mouse data was not a reason to exclude variants.

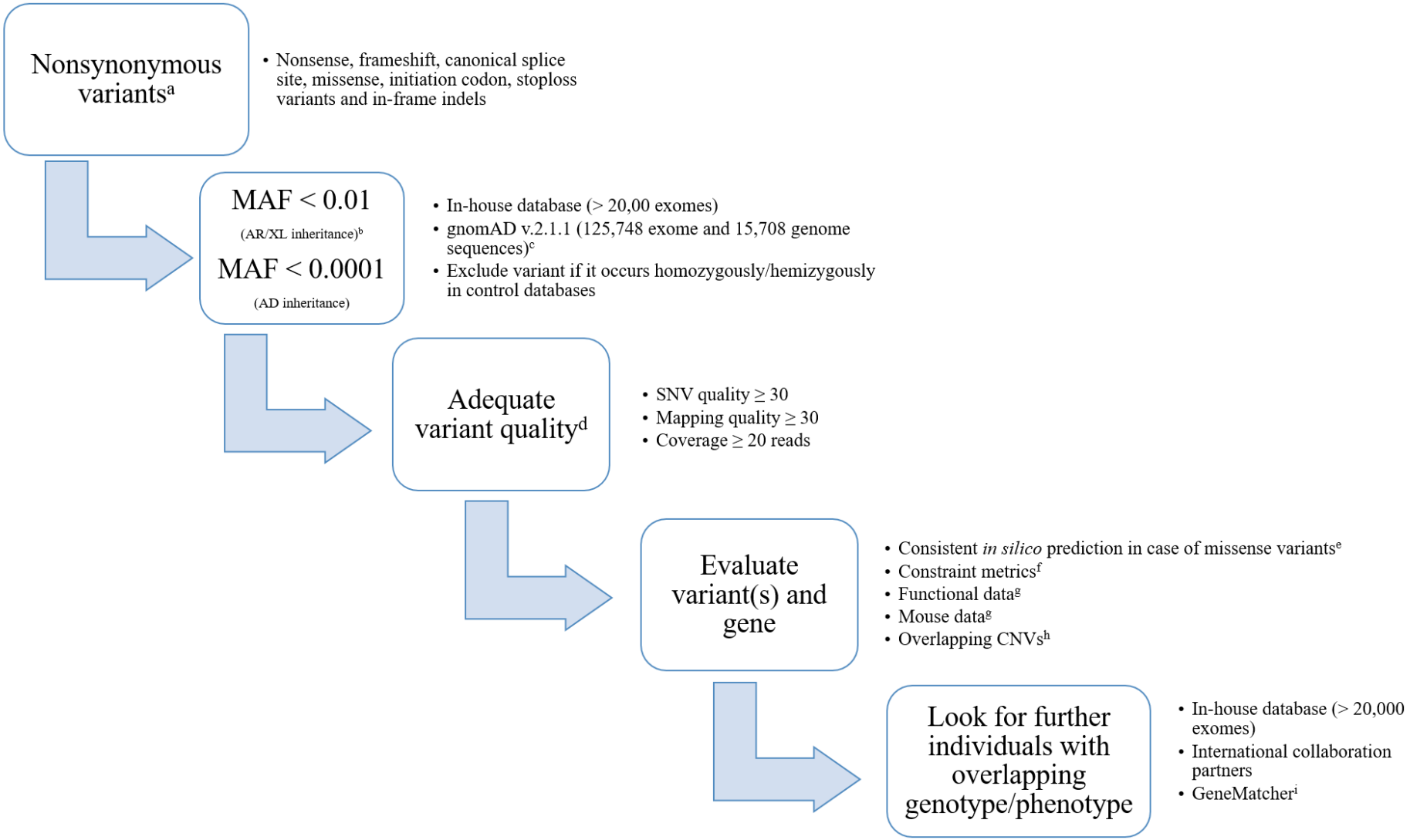
Nonsynonymous variants[a]
• Nonsense, frameshift, canonical splice site, missense, initiation codon, stoploss variants and in-frame indels
MAF < 0.01
(AR/XL inheritance)[b]
MAF < 0.0001
(AD inheritance)
• In-house database (> 20,00 exomes)
• gnomAD v.2.1.1 (125,748 exome and 15,708 genome sequences)[c]
• Exclude variant if it occurs homozygously/hemizygously in control databases
Adequate variant quality[d]
• SNV quality ≥ 30
• Mapping quality ≥ 30
• Coverage ≥ 20 reads
Evaluate variant(s) and gene
• Consistent *in silico* prediction in case of missense variants[e]
• Constraint metrics[f]
• Functional data[g]
• Mouse data[g]
• Overlapping CNVs[h]
Look for further individuals with overlapping genotype/phenotype
• In-house database (> 20,000 exomes)
• International collaboration partners
• GeneMatcher[i]

Figure 12: Algorithm for prioritizing variants in candidate genes (used in unsolved cases). AD, autosomal dominant; AR, autosomal recessive; CNV, copy number variant; gnomAD, Genome Aggregation Database; MAF, minor allele frequency; SNV, single-nucleotide variant; XL, X-linked.

[a]CNVs are investigated analogously. Extend search to near-splice, synonymous, intronic and untranslated-region (UTR) variants if no relevant nonsynonymous variant is identified.

[b]Homozygous/(putatively) compound heterozygous/hemizygous variants.

[c]https://gnomad.broadinstitute.org/.

[d]See Section 3.3.2 for further explanation of quality parameters.

[e]SIFT ≤ 0.05, PolyPhen-2 0.85–1.0, CADD ≥ 15 (Section 3.3.2). Consistency means that two out of three *in silico* prediction tools had to be above/below the set thresholds. This was not the case for variant NM_022648.4:c.4076A>G, p.(Tyr1359Cys) in *TNS1* (only CADD = 15) in case ATS-F536-II-1. As the variant *in trans* NM_022648.4:c.1097A>G, p.(Tyr366Cys) clearly had a damaging effect on *in silico* prediction (SIFT = 0.00, PolyPhen-2 = 0.98, CADD = 26) and the *Tns1* knock-out mouse had a fitting renal phenotype, the gene was still prioritized (see Table 7 for further information).

[f]Gene-specific constraint metrics pointing towards haploinsufficiency/missense variant constraint (see above).

[g]Search in PubMed (https://pubmed.ncbi.nlm.nih.gov/) and MGI (http://www.informatics.jax.org/).

[h]Search in PubMed (https://pubmed.ncbi.nlm.nih.gov/), DECIPHER (https://decipher.sanger.ac.uk/), and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).

[i]https://genematcher.org/.

3.4 Statistical analysis and graphical visualization

Categorical data are displayed as absolute and relative frequencies. Continuous variables are presented as median and interquartile range (IQR, 25^{th}–75^{th} percentile). The IQR is provided in brackets. There were no normally distributed variables in the dataset. Fisher's-Exact Test was used for a comparison of categorical data. A Mann–Whitney *U* test and a Kruskal–Wallis test were employed to compare continuous variables as appropriate. All tests were two-tailed. Binomial logistic regression was done to evaluate the influence of independent variables on the dichotomous dependent variable "solved case yes/no." In detail, independent variables were "age at first manifestation," "age at ES," "ES with parents," "sex," "non-Finnish European descent," "reported family history," "reported parental consanguinity," and "extrarenal manifestation." All independent variables were input in the model in one step. There was no multicollinearity of independent variables (bivariate Pearson correlation and linear regression). For all statistical tests, a *p*-value of less than 0.05 was considered significant.

Statistical analyses were made with SPSS® Statistics 23 (IBM®, Ehningen, Germany). Plots were generated with R v3.5.2 software (R Foundation for Statistical Computing, Vienna, Austria). Further graphical visualization was done with PowerPoint® 2013 (Microsoft®, Redmond, WA, United States of America). Figure 16 was created with the free web-based tool SankeyMATIC (http://sankeymatic.com/build/).

4 RESULTS

Parts of the study cohort and the results have been published in the American Journal of Kidney Diseases (Riedhammer, Braunisch, et al., 2020).

4.1 Exome sequencing in a cohort of 260 genetically unsolved index cases with a clinically presumed hereditary nephropathy

4.1.1 Cohort description

The cohort of this cross-sectional study comprised 260 index cases, in which no genetic diagnosis had been established prior to ES. Patients were recruited for ES between October 2015 and February 2019, and either 1) they had the tentative clinical diagnosis of a specific hereditary kidney disease/a phenotype overlapping with a specific hereditary kidney disease (236/260, 91%) or 2) their phenotype did not overlap with a specific hereditary kidney disease but there was renal disease and the inclusion criteria outlined in Section 3.1.1 were met (24/260, 9%). These patients are part of the larger NephroGen exome sequencing cohort, with overall 330 cases. Figure 13 provides a detailed flowchart of included and excluded index patients of the NephroGen cohort. The Online Supplementary Table (URL/DOI in the appendices) lists all included and excluded cases.

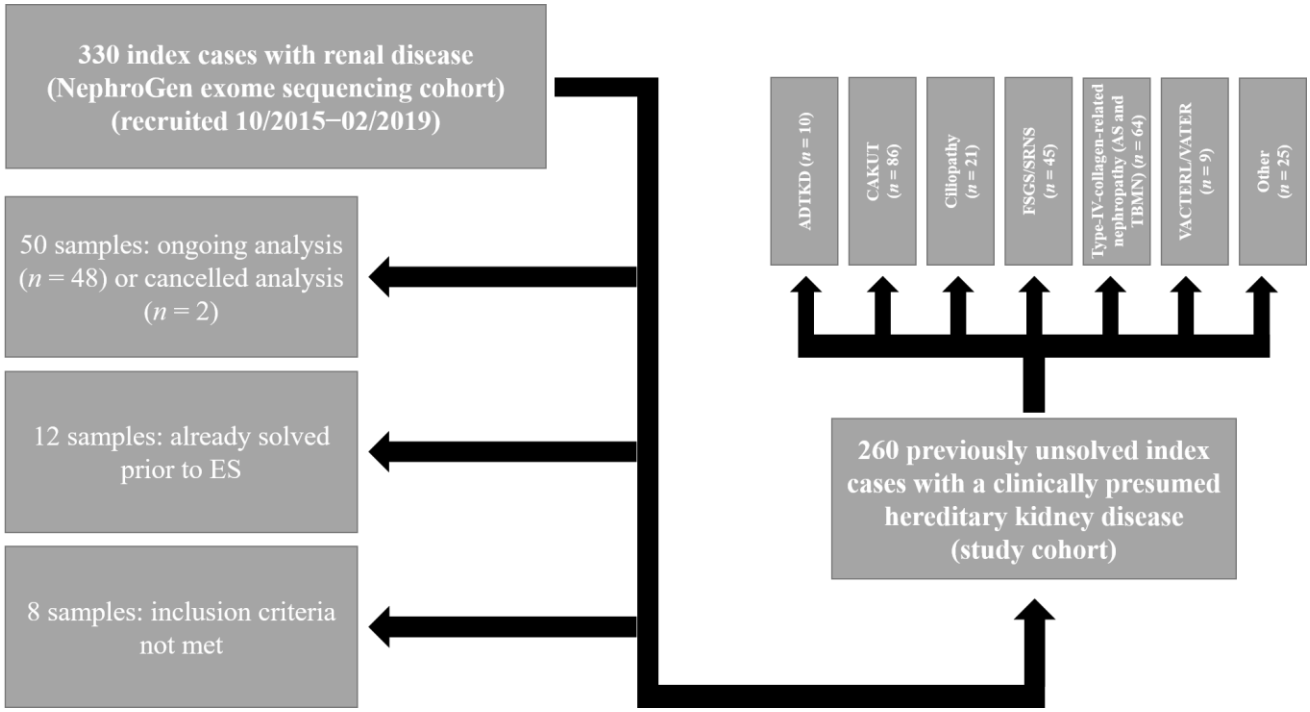

Figure 13: Flowchart to illustrate the inclusion of index cases of the larger NephroGen exome sequencing cohort. "Already solved prior to ES" means that a causative variant/causative variants establishing a genetic diagnosis were already identified prior to ES (see Online Supplementary Table for all included and excluded cases). ADTKD, autosomal dominant tubulointerstitial kidney disease; AS, Alport syndrome; CAKUT, congenital anomalies of the kidney and urinary tract; FSGS, focal segmental glomerulosclerosis; SRNS, steroid-resistant nephrotic syndrome; TBMN, thin basement membrane nephropathy; VACTERL/VATER, V – Vertebral anomalies, A – Anorectal malformations, C – Cardiovascular anomalies, T – Tracheoesophageal fistula, E – Esophageal atresia, R – Renal and/or radial anomalies, L – Limb defects.

Ten of 260 index cases (4%) were assigned to the ADTKD disease group according to their clinical tentative diagnosis/phenotype, 86 of 260 (33%) to the CAKUT disease group, 21 of 260 (8%) to Ciliopathy, 45 of 260 (17%) to FSGS/SRNS, 64 of 260 (25%) to type-IV-collagen-related nephropathy (including AS and TBMN), 9 of 260 (4%) to VACTERL/VATER, and 25 of 260 (10%) clinical tentative diagnoses/phenotypes did not fit any of these disease groups and were assigned to the "Other" group (e.g., aHUS, tubulopathies, unclear syndromes with renal involvement; Figure 14).

Of the index cases, 218 of 260 (84%) were of non-Finnish European descent, and 96 of 260 (37%) had a reported family history. Those with reportedly consanguineous parents (22/260, 9%) had a positive family history in 12 of 22 (55%) cases, and those with non-consanguineous parents (238/260, 92%) had a positive family history in 84 of 238 (35%, $p = 0.104$). Baseline characteristics of the cohort are summarized in Table 6.

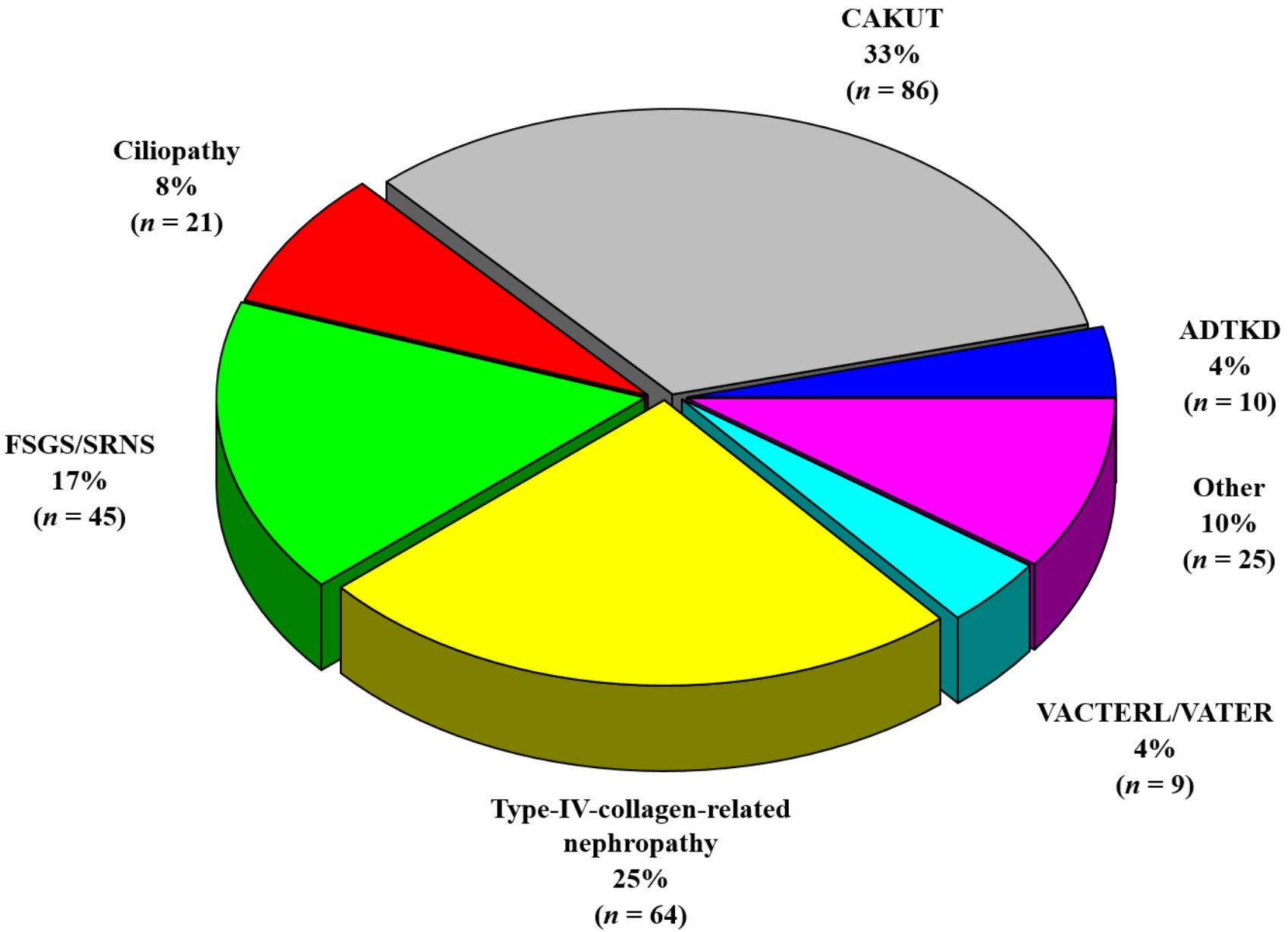
CAKUT
33%
(*n* = 86)
ADTKD
4%
(*n* = 10)
Other
10%
(*n* = 25)
VACTERL/VATER
4%
(*n* = 9)
Type-IV-collagen-related
nephropathy
25%
(*n* = 64)
FSGS/SRNS
17%
(*n* = 45)
Ciliopathy
8%
(*n* = 21)

Figure 14: Distribution of clinical disease groups of the study cohort consisting of 260 index cases with a clinically presumed hereditary nephropathy analyzed by exome sequencing (ES). Please note that “type-IV-collagen-related nephropathy” includes both Alport syndrome (AS) and thin basement membrane nephropathy (TBMN). ADTKD, autosomal dominant tubulointerstitial kidney disease; CAKUT, congenital anomalies of the kidney and urinary tract; FSGS, focal segmental glomerulosclerosis; SRNS, steroid-resistant nephrotic syndrome. VACTERL/VATER, V – Vertebral anomalies, A – Anorectal malformations, C – Cardiovascular anomalies, T – Tracheoesophageal fistula, E – Esophageal atresia, R – Renal and/or radial anomalies, L – Limb defects.

Parameter	Total cohort (n = 260)	Clinical disease groups						p
		ADTKD (n = 10)	CAKUT (n = 86)	Ciliopathy (n = 21)	FSGS/SRNS (n = 45)	Type-IV-collagen-related nephropathy (n = 64)	VACTERL/ VATER (n = 9)	Other (n = 25)	
Age at first manifestation[a] (years)	2.0 [0.0–11.0]	17.0 [10.8–40.3]	0.0 [-]	0.0 [0.0–10.5]	10.0 [3.5–18.0]	6.0 [4.0–15.0]	0.0 [-]	13.0 [3.0–32.5]	< 0.001
Age at ES (years)	15.0 [5.0–28.5]	49.0 [41.8–65.3]	10.0 [1.0–24.0]	10.0 [0.8–26.0]	24.0 [13.0–37.5]	16.0 [8.0–26.0]	10.0 [1.0–12.5]	23.0 [4.5–37.0]	< 0.001
ES with parents[b] (%)	86 (33)	0 (0)	35 (41)	12 (57)	17 (38)	9 (14)	6 (67)	7 (28)	< 0.001

Male sex (%)	149 (57)	5 (50)	57 (66)	9 (43)	21 (47)	39 (61)	5 (56)	13 (52)	ns
Non-Finnish European descent (%)	218 (84)	10 (100)	74 (86)	19 (91)	32 (71)	59 (92)	6 (67)	18 (72)	0.01
Reported family history (%)	96 (37)	8 (80)	15 (17)	8 (38)	13 (29)	43 (67)	0 (0)	9 (36)	< 0.001
Reported parental consanguinity (%)	22 (9)	0 (0)	7 (8)	2 (10)	7 (16)	1 (2)	1 (11)	4 (16)	ns
Extrarenal manifestation (%)	79 (30)	0 (0)	34 (40)	12 (57)	3 (7)	13 (20)	9 (100)	8 (32)	< 0.001
Number of solved cases[c] (%)	77 (30)	4 (40)	8 (9)	12 (57)	10 (22)	35 (55)	0 (0)	8 (32)	< 0.001
Number of phenocopies[d] (%)	12 (5)	1 (10)	0 (0)	1 (5)	3 (7)	4 (6)	0 (0)	3 (12)	0.04

Table 6: Baseline characteristics of the study cohort. Interquartile range (IQR) provided in brackets. “Type-IV-collagen-related nephropathy” includes Alport syndrome (AS) and thin basement membrane nephropathy (TBMN). ADTKD, autosomal dominant tubulointerstitial kidney disease; CAKUT, congenital anomalies of the kidney and urinary tract; FSGS, focal segmental glomerulosclerosis; ns, not significant ($p \geq 0.05$); SRNS, steroid-resistant nephrotic syndrome; VACTERL/VATER, V – Vertebral anomalies, A – Anorectal malformations, C – Cardiovascular anomalies, T – Tracheoesophageal fistula, E – Esophageal atresia, R – Renal and/or radial anomalies, L – Limb defects.

[a]All cases of clinical disease groups “CAKUT” and “VACTERL/VATER” had congenital onset of manifestation (age of manifestation set to “0,” no IQR).

[b]This means the sequencing of index case and both of his/her biological parents (“trio”).

[c]= Diagnostic yield (i.e., likely pathogenic/pathogenic variant[s]/CNV as per ACMG and amendments with a fitting genotype; see Section 3.3.2).

[d]See Section 4.3 for detailed information on identified phenocopies. In phenocopy cases, disease category according to genetic diagnosis is different from the disease category according to the clinical tentative diagnosis/phenotype.

4.1.2 Diagnosis of a hereditary kidney disease by exome sequencing

Diagnosis of a hereditary kidney disease (likely pathogenic or pathogenic variant/CNV as per ACMG criteria and current amendments and fitting genotype; see Section 3.3.2) could be made by ES in 77 of 260 cases (diagnostic yield of 30%). Diagnostic yield differed significantly among disease groups ($p < 0.001$): 4 of 10 (40%) in ADTKD, 8 of 86 (9%) in CAKUT, 12 of 21 (57%) in Ciliopathy, 10 of 45 (22%) in FSGS/SRNS, 35 of 64 (55%) in type-IV-collagen-related nephropathy, and 8 of 25 (32%) in "Other." None of the cases assigned to the VACTERL/VATER disease group could be genetically solved. Figure 15 presents the diagnostic yield in the different disease groups.

In 3 of 260 (1% of total cases, 4% of solved cases; HN-F56-II-1, ATS-F247-II-1, HN-F695-II-1), a second monogenic disease apart the hereditary kidney disease could be diagnosed by ES (dual diagnosis; see Online Supplementary Table). An actionable secondary finding as per ACMG was detected in 4 of 260 (2%) cases (Kalia et al., 2017). Furthermore, 52 of 260 (20%) were known to have genetic testing (of any kind; not establishing a genetic diagnosis for the renal phenotype) prior to ES.

The Online Supplementary Table (URL/DOI in the appendices) provides detailed phenotypic and genotypic information (including applied ACMG criteria for variants) on all cases included in this thesis. It also includes information on previous genetic testing and which cases have been published.

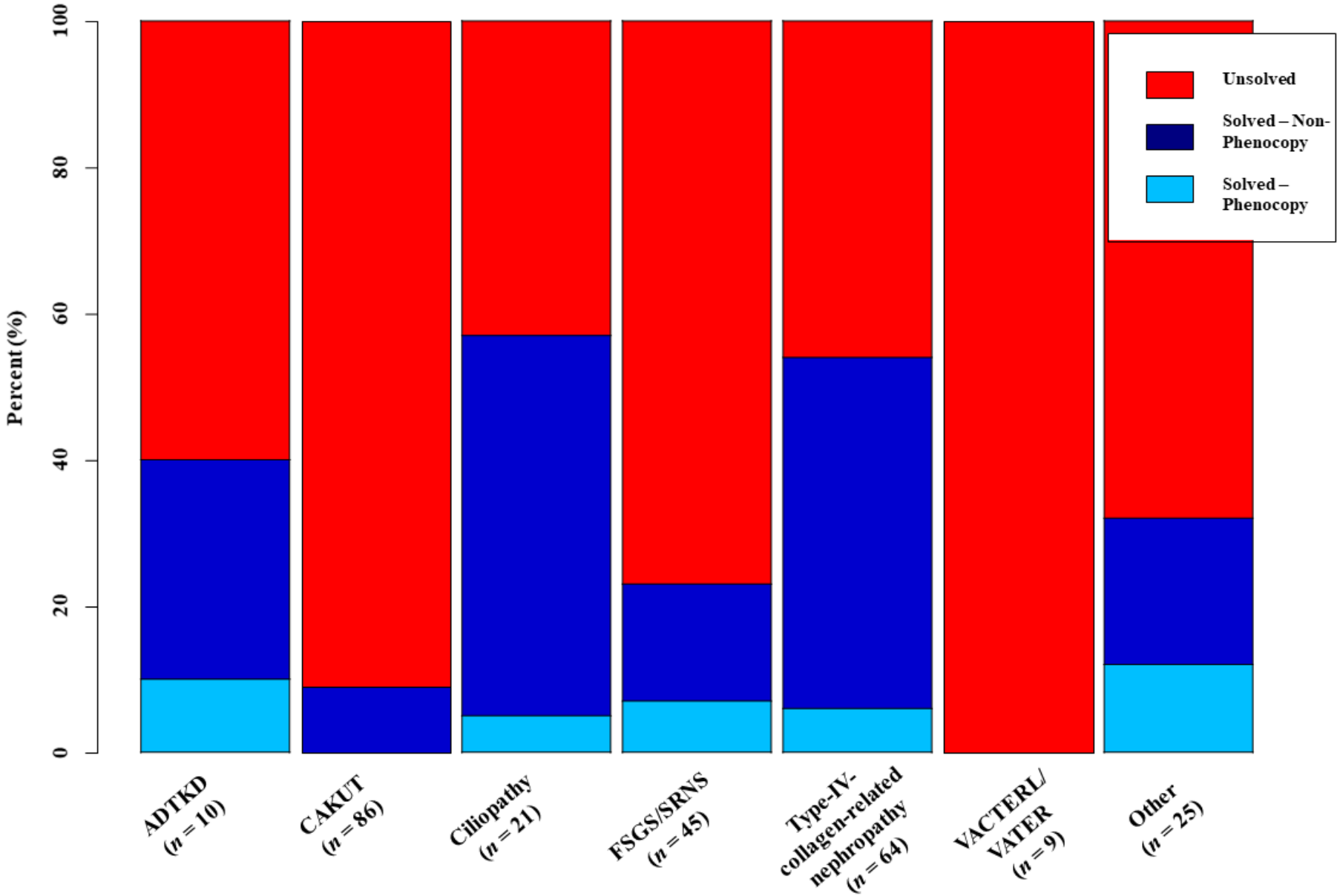
Percent (%)
0
20
40
60
80
100
Unsolved
Solved – Non-Phenocopy
Solved – Phenocopy
ADTKD (n = 10)
CAKUT (n = 86)
Ciliopathy (n = 21)
FSGS/SRNS (n = 45)
Type-IV-collagen-related nephropathy (n = 64)
VACTERL/ VATER (n = 9)
Other (n = 25)

Figure 15: Diagnostic yield of respective clinical disease groups. Cases were categorized by clinical tentative diagnosis/phenotype into the mentioned disease groups. Solved cases are those in which a genetic diagnosis could be made by ES. In phenocopy cases, the disease category according to genetic diagnosis is different from the disease category according to the clinical tentative diagnosis/phenotype. Solved cases are separated into "Solved – Non-Phenocopy" (dark blue) and "Solved – Phenocopy" (light blue). Fractions are given in percentages. No cases in the VACTERL/VATER clinical disease group could be solved. The diagnostic yield of ES in the total cohort (*n* = 260 index cases) was 30%. ADTKD, autosomal dominant tubulointerstitial kidney disease; AS, Alport syndrome; CAKUT, congenital anomalies of the kidney and urinary tract; FSGS, focal segmental glomerulosclerosis; SRNS, steroid-resistant nephrotic syndrome; TBMN, thin basement membrane nephropathy; VACTERL/VATER, V – Vertebral anomalies, A – Anorectal malformations, C – Cardiovascular anomalies, T – Tracheoesophageal fistula, E – Esophageal atresia, R – Renal and/or radial anomalies, L – Limb defects.

4.2 Differences in type-IV-collagen-related nephropathy classified as Alport syndrome versus thin basement membrane nephropathy

There was a significant difference in the number of solved cases (diagnostic yield) of type-IV-collagen-related nephropathy clinically classified as AS (30/46) compared to classified as TBMN (5/18; 65% vs. 28%; p = 0.01; see Section 3.1.2 for detailed information on classification of cases as AS or TBMN). One case, clinically classified as TBMN, was a phenocopy (HN-F56-II-1, hemizygous causative variant in *CLCN5*; see Table 7). The remaining four solved TBMN cases had heterozygous causative variants in *COL4A3* (ATS-F688-II-1), *COL4A4* (cases ATS-F503-II-1 and ATS-F673-II-1), and *COL4A5* (female patient, ATS-F274-III-14). Median age at first manifestation was significantly lower in AS compared to TBMN cases, that is, 5.5 years [3.0–9.0] versus 16.0 years [5.0–32.3] (p = 0.001). TBMN cases had no extrarenal manifestations, in contrast to 13 of 46 in AS cases (0% vs. 28%; p = 0.01). TBMN cases had a reported family history in 7 of 18, AS cases in 36 of 46 (39% vs. 78%; p = 0.006).

4.3 Detection of phenocopies and recategorization of disease by exome sequencing

4.3.1 Description of detected phenocopies

In the total cohort, there was a phenocopy in 12 out of 260 (5%) index cases. This means that the categorization of disease according to the clinical tentative diagnosis/phenotype differed from the categorization by genetic diagnosis (Section 1.2.3). In nearly every sixth solved case (12/77, 16%) a phenocopy could be identified.

Detection of phenocopies differed significantly by clinical disease group (p = 0.04): Four cases (HN-F20-II-1, HN-F56-II-1, HN-F203-II-2, ATS-F719-II-2) presented as type-IV-collagen-related nephropathy and were recategorized as FSGS/SRNS (three cases) and "Other" (one case, tubulopathy) by genetic diagnosis. Three (ATS-F9-II-1, ATS-F29-III-1, ATS-F261-II-1) were assigned to the FSGS/SRNS disease group and recategorized as type-IV-collagen-related nephropathy (AS). Three cases were allocated to the "Other" disease group by clinical presentation: In one, there was the clinical suspicion of hereditary dRTA, a tubulopathy, turning out to be a neuroendocrine defect (prohormone processing disorder) by the identification of compound heterozygous causative missense

variants in the gene *PCSK1* (HN-F46-II-1). The second case (ATS-F486-II-1) presented clinically as IgAN (microscopic hematuria with intermittent infection-associated macroscopic hematuria), a multifactorial disease, and had AS genetically (causative hemizygous frameshift variant in *COL4A5*). In the third case (HN-F542-II-1), the patient was clinically diagnosed with a tubulopathy (with epilepsy) but had a mitochondriopathy genetically.

Another case (HN-F683-I-2) was categorized into the ADTKD disease group by clinical presentation and family history, but the genetic diagnosis was AS (causative heterozygous canonical splice site variant in *COL4A5*). Finally, in one case (HN-F13-II-1), there was the clinical suspicion of ARPKD, a ciliopathy, which turned out to be chromosome 17q12 deletion syndrome involving the CAKUT gene *HNF1B*.

Table 7 provides a detailed description of all twelve phenocopy cases. Figure 16 illustrates the recategorization of disease by ES.

ID	Sex	Clinical (tentative) diagnosis	Disease group (based on clinical presentation)	Age and phenotype at first manifestation	Previous genetic tests[a]	Genetic diagnosis [MIM phenotype number][b]	Age at genetic diagnosis	Gene/CNV (transcript/cytogenetic band)	Nucleotide and amino acid change	gnomAD v.2.1.1 MAF[c]	ClinVar rating[d]	Applied ACMG criteria/ACMG CNV score[e]	Zygosity/ heteroplasmy	Inheritance
ATS-F9-II-1	m	FSGS (FSGS on kidney biopsy at 29 years of age)	FSGS/SRNS	26 years (nephrotic proteinuria)	-	Alport syndrome, autosomal recessive [203780]	32 years	*COL4A3* (NM_000091.4)	c.2126-1G>C, p.(?) c.4421T>C, p.(Leu1474Pro)	Not listed 0.003 (0.005 in non-Finnish Europeans)	Not listed 1x likely pathogenic, 5x VUS, 1x likely benign	PVS1/PM2 PS4_moderate (*in trans* with pathogenic variants)/PM1/PM3/PP3	Compound heterozygous	Mother and father
HN-F13-II-1	f	ARPKD	Ciliopathy	Prenatal (hyperechogenic kidneys on ultrasound)	-	Chromosome 17q12 deletion syndrome [614527]	4 months	1.2 Mb deletion (comprising *HNF1B*) (chr17q12)[f]	-	-	overlapping and similarly sized CNVs rated as pathogenic	1.00 (pathogenic)	Heterozygous	Mother
HN-F20-II-1	m	AS and sensorimotor neuropathy	Type-IV-collagen-related nephropathy	4 years (neuropathy) 15 years (proteinuria and sensorineural deafness)	*PMP22, MPZ, COL4A3, COL4A4, COL4A5, WT1, NPHS2*	Glomerulosclerosis, focal segmental, 5 [613237] Charcot-Marie-Tooth disease, dominant intermediate E [614455]	26 years	*INF2* (NM_022489.3)	c.224_256del, p.(Asp75_Ser85del)	Not listed	Not listed	PM1/PM2/PM4	Heterozygous	Undetermined
ATS-F29-III-1	f	MCD (MCD on kidney biopsy at 12 years of age)[g]	FSGS/SRNS	3 years (proteinuria, hematuria)	*COL4A3, COL4A4, COL4A5*	Alport syndrome, autosomal recessive [203780]	21 years	*COL4A3* (NM_000091.4)	c.1831G>A, p.(Gly611Arg) c.4421T>C, p.(Leu1474Pro)	Not listed 0.003 (0.005 in non-Finnish Europeans)	Likely pathogenic 1x likely pathogenic, 5x VUS, 1x likely benign	PS4_supporting/ PM1_strong/PM2/ PP3 PS4_moderate (*in trans* with pathogenic variants)/PM1/PM3/PP3	Compound heterozygous	Mother and father
HN-F46-II-1	f	dRTA	Other	3 weeks (hyperchloremic acidosis, hypercalciuria, nephrocalcinosis)	*ATP5V0A4, ATP6V1B1, CA2, SLC4A1, SLC4A4, VIPAS39, VPS33B, OCRL*	Obesity with impaired prohormone processing [600955]	2 years	*PCSK1* (NM_000439.4)	c.1346T>C, p.(Leu449Pro) c.1688C>G, p.(Pro563Arg)	Not listed Not listed	Not listed Not listed	PM1_supporting/PM2/ PP3/PP4_strong[h] PM1_supporting/PM2/PP3 /PP4_strong[h]	Compound heterozygous	Mother and father
HN-F56-II-1	m	TBMN (TBMN on kidney biopsy at 4 years of age; no family history of AS)	Type-IV-collagen-related nephropathy	4 years (microscopic hematuria, proteinuria, hypercalciuria, nephrocalcinosis)	-	Dent disease[i] [300009]	5 years	*CLCN5* (NM_000084.4)	c.292C>T, p.(Arg28*)	Not listed	Pathogenic	PVS1/ PS4_moderate/PM2	Hemizygous	Mother

HN-F203-II-2	f	AS (AS on kidney biopsy at 3 years of age)	Type-IV-collagen-related nephropathy	2 years (microscopic hematuria)	*COL4A3, COL4A4, COL4A5, MYH9*	Glomerulosclerosis, focal segmental, 6 [614131]	34 years	*MYO1E* (NM_004998.3)	c.2060T>C, p.(Leu687Ser)	Not listed	Not listed	PM1_supporting/PM2/PP1_moderate/PP3	Homozygous	Mother and father
ATS-F261-II-1	f	FSGS (FSGS on kidney biopsy at 32 years of age, CKD IV at 42 years of age)	FSGS/SRNS	5 years (hematuria and proteinuria)	-	Alport syndrome, X-linked [301050]	42 years	*COL4A5* (NM_000495.4)	c.645+1G>T, p.(?)	Not listed	Not listed	PVS1_strong/PM2	Heterozygous	Undetermined
ATS-F486-II-1	m	IgAN (clinical suspicion, no kidney biopsy performed because of young age)	Other	3 years (microscopic hematuria with intermittent infection-associated macroscopic hematuria)	-	Alport syndrome, X-linked [301050]	9 years	*COL4A5* (NM_000495.4)	c.225delA, p.(Gln76Lysfs*79)	Not listed	Not listed	PVS1/PM2	Hemizygous	Mother
HN-F542-II-1	f	Rasmussen encephalopathy with tubulopathy and CKD	Other	3 years (first seizure and renal salt wasting)	Mitochondrial disease panel	Maternally inherited epilepsy with tubulointerstitial kidney disease [-]	4 years	*MT-TF* (NC_012920.1)	m.616T>C	-	Pathogenic[i]	-	Homoplasmic in blood and urine	Mother (92% heteroplasmy level in blood)
HN-F683-I-2	f	ADTKD (MCKD; also-affected daughter; kidney transplantation at 66 years of age)	ADTKD	8 years (medullary cystic kidney disease)	*MUC1*	Alport syndrome, X-linked [301050]	72 years	*COL4A5* (NM_000495.4)	c.609+1G>C, p.(?)	Not listed	Not listed	PVS1_strong/PM2	Heterozygous	Undetermined
ATS-F719-II-2	m	AS (similarly affected siblings, kidney transplantation at 27 years of age)	Type-IV-collagen-related nephropathy	3 years (hearing impairment, declining renal function)	*COL4A5* (18 out of 51 exons sequenced)	Coenzyme Q10 deficiency, primary, 6 [614650]	38 years	*COQ6* (NM_182476.2)	c.1079G>T, p.(Arg360Leu) c.1237G>T, p.(Glu413*)	Not listed Not listed	Not listed Not listed	PM2/PM3/ PP1_moderate/PP3 PVS1/PM2/PP1_moderate	Compound heterozygous	Mother and father

Table 7: Summary of phenocopies identified by exome sequencing (ES). All cases were genetically solved by ES. Cases were categorized by clinical tentative diagnosis/phenotype into the mentioned disease groups. Phenocopy means that the disease category according to genetic diagnosis is different from the disease category according to the clinical tentative diagnosis/phenotype. ADTKD, autosomal dominant tubulointerstitial kidney disease; ARPKD, autosomal recessive polycystic kidney disease; AS, Alport syndrome; CAKUT, congenital anomalies of the kidney and urinary tract; CKD, chronic kidney disease; CNV, copy number variant; dRTA, distal renal tubular acidosis; FSGS, focal segmental glomerulosclerosis; IgAN, IgA nephropathy; MCD, minimal change disease; MCKD, medullary cystic kidney disease; SRNS, steroid-resistant nephrotic syndrome; TBMN, thin basement membrane nephropathy; VUS, variant of uncertain significance.

[a]All inconspicuous.

[b]https://www.omim.org/.

[c]https://gnomad.broadinstitute.org/.

[d]https://www.ncbi.nlm.nih.gov/clinvar/.

[e]Richards et al., 2015; Riggs et al., 2020. See Figure 11 for an excerpt of the American College of Medical Genetics and Genomics (ACMG) guideline for sequence variant interpretation.

[f]Approx. chr17:g.34842544-36104874.

[g]Included in the FSGS/SRNS disease group and not in disease group "type-IV-collagen-related nephropathy," as the genetic analysis of type-IV-collagen-related nephropathy–associated genes *COL4A3*, *COL4A4*, and *COL4A5* at 12 years of age was inconspicuous, and kidney biopsy at 19 years of age also showed podocytopathic changes alongside glomerular basement membrane changes (and MCD can evolve to FSGS; Section 1.1.5).

[h]Rated as strong because of a highly specific phenotype consisting of hyperphagia, diabetes insipidus, and an elevated proinsulin/insulin ratio in blood.

[i]A second monogenic disease was diagnosed in the patient: compound heterozygous likely pathogenic missense variants in *ADA2* NM_017424.2:c.140G>C, p.(Gly47Ala), and c.752C>T, p.(Pro251Leu). Genetic diagnosis of “Polyarteritis nodosa, childhood-onset” [MIM 615688].

[j]Also rated "confirmed pathogenic" on MITOMAP (https://mitomap.org//cgi-bin/search_allele?starting=616).

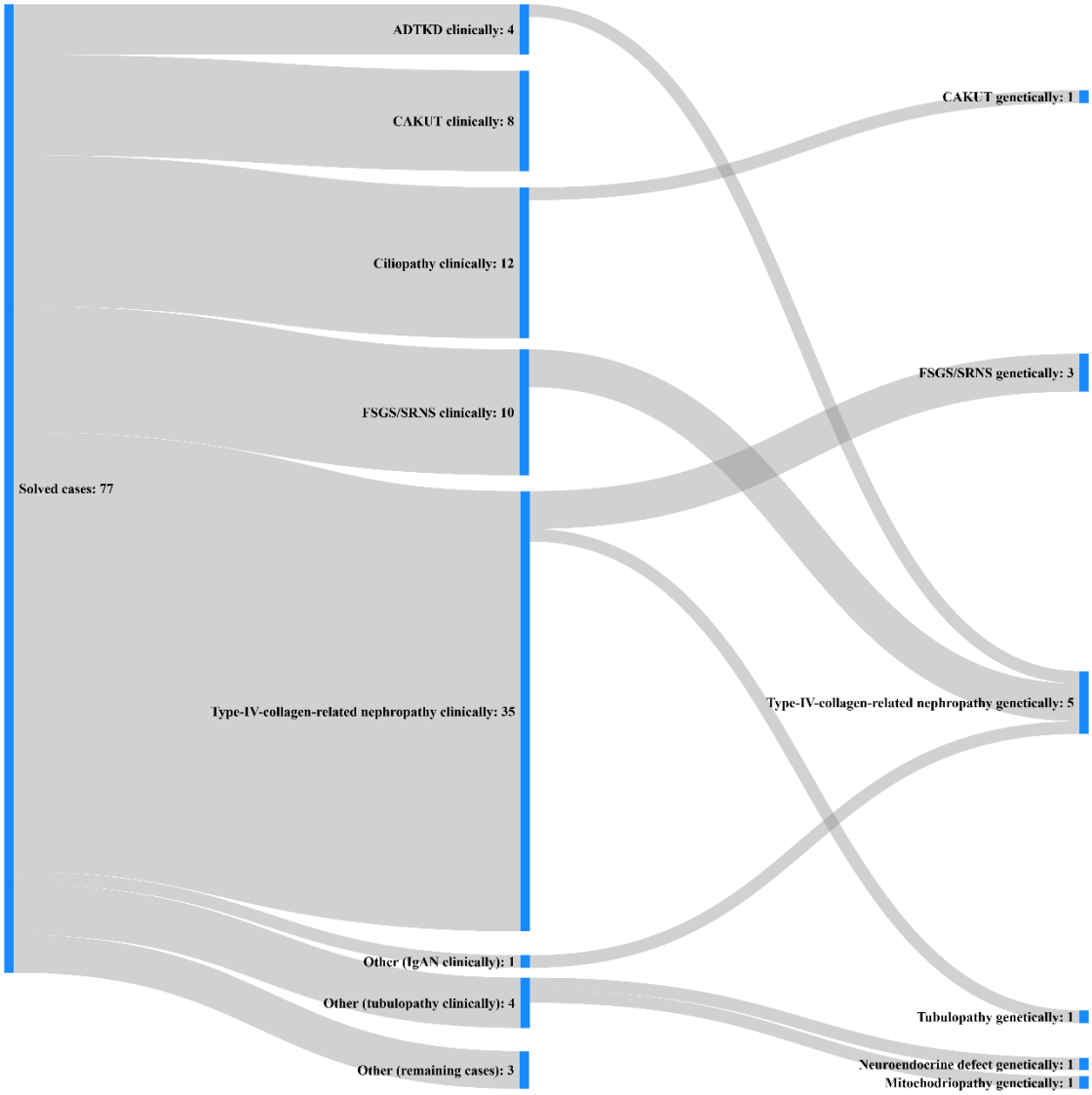

Figure 16: Recategorization of disease by the identification of phenocopies (*n* = 12). Phenocopy means that the disease category according to genetic diagnosis is different from the disease category according to the clinical tentative diagnosis/phenotype. Please note that tubulopathy and IgAN phenotypes were separated from the "Other" clinical disease group in this figure to visualize phenocopies. "Type-IV-collagen-related nephropathy" includes Alport syndrome (AS) and thin basement membrane nephropathy (TBMN). ADTKD, autosomal dominant tubulointerstitial kidney disease; CAKUT, congenital anomalies of the kidney and urinary tract; FSGS, focal segmental glomerulosclerosis; IgAN, IgA nephropathy; SRNS, steroid-resistant nephrotic syndrome.

4.3.2 Phenocopy detection rate of targeted NGS panels

Narrowly targeted NGS panels (≤ 25 kb; billable panel size in the German statutory health insurance system) would not have genetically diagnosed any of the aforementioned phenocopy cases. A comprehensive panel for hereditary nephropathies (299 genes) would have diagnosed 10 of 12 (83%) phenocopy cases. However, cases HN-F46-II-1 (*PCSK1*) and HN-F542-II-1 (*MT-TF*) and the second monogenic disease (*ADA2*) in HN-F56-II-1 would not have been diagnosed. See Online Supplementary Table (URL/DOI in the appendices) for the different genes included in the panels and the commercial provider.

4.4 Exome sequencing to prioritize novel hereditary-kidney-disease-associated genes

Unsolved cases were further investigated for potentially disease-causing variants in genes not associated with a monogenic hereditary kidney disease ("candidate gene"). For this, the variant search algorithm described in Section 3.3.3 was used. By this, in 8 of 260 (3%) index cases, a convincing candidate gene could be prioritized. Of note, 6 of 8 (75%) candidate genes were prioritized in the CAKUT clinical disease group. One case (HN-F75-II-1) has been published, and the gene (*PBX1*) is listed in OMIM® [MIM 617641] (Riedhammer et al., 2017). In five of the remaining seven cases, a manuscript is in preparation and the gene has been presented as a novel disease-associated gene at international conferences. See Table 8 for a complete account of prioritized genes with identified variants, the reasons for prioritization, and information on publication.

ID	Sex	Core phenotype	Disease group (based on clinical presentation)	Age at first manifestation	Prioritized gene (transcript)	Nucleotide and amino acid change	gnomAD v.2.1.1 MAF[a]	Renal phenotype in KO mouse[b]	Zygosity	Inheritance	Reason for prioritization of gene	Publication
HN-F25-II-1	m	SRNS at 7 years of age, primary FSGS on kidney biopsy at 8 years of age	FSGS/SRNS	7	*DAAM2* (NM_015345.3)	c.3080C>T, p.(Ser1027Leu)	0.0003	No	Homozygous	Mother and father	Encodes a member of the formin family important for actin dynamics (Hetheridge et al., 2012). Established hereditary FSGS/SRNS-associated gene *INF2* is also a member of the formin family. *In silico* prediction consistent for damaging effect of missense variant (SIFT = 0.00, PolyPhen-2 = 1.00, CADD = 27).	In preparation (co-author). Further FSGS/SRNS patients with biallelic variants in *DAAM2* found via international collaboration), poster (co-author) at ASN meeting 2019 (FR-PO787).
HN-F71-III-2	m	Bilateral renal hypoplasia, developmental delay, facial dysmorphies	CAKUT	Congenital	*FOXD2* (NM_004474.3)	c.789dup, p.(Gly264Argfs*228)	Not listed	Kidney hypoplasia and hydroureter; reduced penetrance	Homozygous	Mother and father	Encodes a transcription factor of the evolutionary conserved forkhead family (Kume et al., 2000). Homozygous frameshift variant, potentially causing NMD; KO in mouse leads to CAKUT phenotype; variant segregates in affected family members.	In preparation (first author). Oral presentation at virtual ESHG meeting 2020 (2020-A-1598-ESHG).
HN-F75-II-1	m	Bilateral renal dysplasia, developmental delay, growth retardation, skeletal anomalies	CAKUT	Congenital	*PBX1* (NM_002585.3)	c.413_419del, p.(Gly138Valfs*40)	Not listed	Renal hypoplasia/agenesis (also skeletal and multiple other congenital anomalies)	Heterozygous	*de novo*	Encodes a transcription factor important for organogenesis; *de novo* frameshift variant in a constrained gene (LOEUF = 0.26); KO mouse recapitulates phenotype of patient. Microdeletions encompassing *PBX1* were described in syndromic CAKUT patients just at the time of ES (Le Tanno et al., 2017).	Published (first author; Riedhammer et al., 2017).

HN-F312-II-3	m	Right renal hypoplasia, ESRD at 20 years of age	CAKUT	Congenital	*EIF3B* (NM_001037283.1)	c.1158-2A>G, p.(Tyr387_Lys430del)	Not listed	No (embryonic lethality before implantation)	Heterozygous	Not in mother (father deceased)	Constrained gene (LOEUF = 0.07). Encodes a conserved subunit of the eukaryotic translation initiation factor 3 complex (eIF3) important for protein synthesis and embryogenesis (Wagner et al., 2016). Variant leads to skipping of exon 7 (in-frame), disrupting EIF3E interaction domain (see UniProt[c]).	No (RNA sequencing/proteomics pending to check for consequences of in-frame skipping of exon 7).
HN-F527-II-1	m	Unilateral hydrouretero-nephrosis, cardiomyopathy, short stature	CAKUT	Congenital	*PLXNB2* (NM_012401.3)	c.4830_4831del, p.(Lys1611Glyfs*62)	Not listed	Renal hypoplasia and double ureters	Heterozygous	*de novo*	Constrained gene (LOEUF = 0.28). Encodes a transmembrane receptor important for kidney development. KO mouse recapitulates phenotype of patient (Perala et al., 2011).	In preparation (co-author). Further CAKUT patients with heterozygous variants in *PLXNB2* found via international collaboration, poster (co-author) at ASN meeting 2019 (FR-PO782).
ATS-F536-II-1	m	TBMN on kidney biopsy, microscopic hematuria, proteinuria (0.3 g/g creatinine), no CKD at 36 years of age	Type-IV-collagen-related nephropathy	33	*TNS1* (NM_022648.4)	c.4076A>G, p.(Tyr1359Cys) c.1097A>G, p.(Tyr366Cys)	0.00002 0.000004	Progressive cystic kidney degeneration	Compound heterozygous	Mother and father	Encodes a focal adhesion protein not essential for mouse embryogenesis. KO leads to postnatally healthy mice which develop renal failure with cystic degeneration of kidneys over time (Lo et al., 1997).	In preparation (co-author). Further patients with proteinuria/nephrotic syndrome and biallelic variants in *TNS1* found via international collaboration, poster (co-author) at ASN meeting 2019 (FR-PO789).
HN-F537-II-1	m	Left renal agenesis, right hydronephrosis III, facial dysmorphies	CAKUT	Congenital	*ARID5B* (NM_032199.2)	c.1489dup, p.(Ile497Asnfs*31)	Not listed	Multiple congenital anomalies including abnormal kidney morphology	Heterozygous	*de novo*	Constrained gene (LOEUF = 0.11). Encodes a member of the ARID protein family important for mammalian development (Patsialou et al., 2005). Several members of the ARID protein family have been associated with monogenic disease (Coffin-Siris syndrome).	No (skin biopsy pending for subsequent RNA sequencing and proteomics).

HN-F539-II-1	m	Bilateral renal dysplasia, imperforate anus	CAKUT	Congenital	*MAP4K4* (NM_001242559.1)	c.3023_3024del, p.(Val1008Glyfs*19)	Not listed	No (embryonic lethality during organogenesis)	Heterozygous	*de novo*	Constrained gene (LOEUF= 0.17). Encoded protein activates JNK pathway vital for embryonic development (Yao et al., 1999).	In preparation (co-author). Included in a cohort of patients with neurologic dysfunction and congenital malformations (mainly heart, also kidney) and heterozygous LoF variants in *MAP4K4*. Part of the cohort (this patient had not yet been included) was presented at ASHG meeting 2018 (PgmNr 353).

Table 8: Overview of novel hereditary-kidney-disease-associated genes prioritized by exome sequencing (ES). "Type-IV-collagen-related nephropathy" includes Alport syndrome (AS) and thin basement membrane nephropathy (TBMN). ASHG, American Society of Human Genetics; ASN, American Society of Nephrology; CAKUT, congenital anomalies of the kidney and urinary tract; ES, exome sequencing; ESHG, European Society of Human Genetics; FSGS, focal segmental glomerulosclerosis; IgAN, IgA nephropathy; KO, knockout; LOEUF, loss-of-function observed/expected upper bound fraction (see Section 3.3.3 for an explanation); SRNS, steroid-resistant nephrotic syndrome.

[a]https://gnomad.broadinstitute.org/.

[b]As per Mouse Genome Informatics (http://www.informatics.jax.org/).

[c]https://www.uniprot.org/.

4.5 Predictors of a solved case

To ascertain which parameters were predictive of a solved case in the study cohort (n = 260), binomial logistic regression with "solved case yes/no" as the dichotomous dependent variable and the parameters reported in Table 9 (see below) as the independent variables was performed (see Section 3.4 for further information). There was no multicollinearity of independent metric variables (no correlation coefficient of > 0.7, variance inflation factor not larger 5). The model was statistically significant (χ^2 = 56.0, p < 0.001), acceptably explained the variance in diagnostic yield (Nagelkerke R^2 = 0.27), and accurately classified 75% of cases. See Table 9, below, for significant independent predictors of a solved case.

Predictors of a solved case

Parameter	OR (95% CI)	*p*
Age at first manifestation (years)	0.97 (0.93-1.00)[a]	0.048
Age at exome sequencing (years)	1.01 (0.99-1.04)	ns
Exome sequencing with parents	0.71 (0.34-1.49)	ns
Sex (male)	0.54 (0.29-1.01)	ns[b]
Non-Finnish European descent	6.45 (0.80-52.15)	ns
Reported family history	6.61 (3.28-13.35)	<0.001
Reported parental consanguinity	2.94 (0.29-30.20)	ns
Extrarenal manifestation	3.21 (1.58-6.54)	0.001

Table 9: Binomial logistic regression to identify predictors of a positive genetic test (dependent variable "solved case yes/no"). χ^2 = 56.0 (p < 0.001); Nagelkerke R^2 = 0.27. OR, odds ratio; CI, confidence interval; ns, not significant ($p \geq 0.05$).

[a]95% CI: 0.933568-0.999620.

[b]p = 0.053.

5 Discussion

This thesis is intended to demonstrate the utility of exome sequencing in hereditary nephropathies. To this end, a cohort of 260 index cases was analyzed by ES. The cross-sectional study features children, adolescents, and adults. The median age at ES was 15 years [5.0–28.5]. Every patient in this cohort had either a phenotype in accordance with or the clinical tentative diagnosis of a hereditary kidney disease or had renal disease with specific criteria pointing towards a hereditary cause (e.g., familial occurrence of renal disease; see Section 3.1.1). In the following, the different findings (Chapter 4) of the study cohort are discussed, expounding upon the application and utility of ES in hereditary nephropathies. Furthermore, this chapter elaborates on how the results of this thesis can guide the genetic work-up of patients with a clinically presumed hereditary kidney disease.

5.1 Diagnostic yield in index patients with a clinically presumed hereditary nephropathy examined by exome sequencing

In the study cohort, a monogenic hereditary kidney disease could be diagnosed by ES in 77 of 260 (30%) cases. This is about a three-times-higher yield than in one study of more than 3,000 patients with CKD analyzed by ES (9.3%; Groopman et al., 2019). However, that was an unselected CKD cohort featuring mainly adult patients (91.6% over 21 years of age), whereas the study cohort of this thesis features children, adolescents and adults (median age

at ES 15 years [5.0–28.5]) with a clinically presumed hereditary nephropathy. Hence, a selection bias can be assumed.

The diagnostic yield differed significantly between disease groups ($p < 0.001$). Four of 10 (40%) cases categorized to the clinical disease group "ADTKD" could be solved. ES cannot unravel all ADTKD cases, as ADTKD-*MUC1* cannot be diagnosed by ES (Section 1.2.1; Groopman et al., 2018; Kirby et al., 2013). However, all unsolved ADTKD had negative *MUC1* testing. It is not surprising that 3 of 4 (75%) solved ADTKD cases had causative variants in *UMOD* (the remaining solved case was a phenocopy, HN-F683-I-2; see Table 7 and Section 5.3). The most common forms of ADTKD are ADTKD-*UMOD*, -*MUC1*, and -*REN* (Bleyer et al., 2017; Devuyst et al., 2019). As noted above, ADTKD-*MUC1* cannot be diagnosed by ES, and ADTKD-*REN* is a childhood-onset disease (Devuyst et al., 2019), whereas the small ADTKD cohort ($n = 10$) analyzed in this thesis has a median age at first manifestation of 17 years [10.8–40.3].

As for CAKUT, diagnostic yield is only 9% (8/86), which is comparable to yields reported in the literature. For example, one ES study of 232 CAKUT families could make a genetic diagnosis in 13% of cases (van der Ven, Connaughton, et al., 2018). It is noteworthy that all eight solved cases in this thesis had involvement of the kidney (8/63, diagnostic yield of 13% in this subgroup). None of the cases with solely urinary tract affection (e.g., VUR, hydronephrosis) could be genetically solved ($n = 23$). In the study by van der Ven, Connaughton, et al., 2018, cases with kidney involvement (defined as "severe" CAKUT phenotype, i.e., renal agenesis or renal dysplasia) also had a higher diagnostic yield of 17% (compared to 13% in the total cohort). However, the reason for the discrepancy of diagnostic yield between cases with kidney anomalies and cases with only urinary tract anomalies could also stem from the uneven distribution of kidney-anomaly cases (63/86, 73%) and urinary-tract-anomaly cases (23/86, 27%). Unfortunately, due to the small number of solved cases, no statistical evaluation of the CAKUT cohort could be done. It would be interesting to check predictors of a positive genetic result such as phenotype severity in a larger cohort.

As for ciliopathies, a diagnostic yield of 57% (12/21) is somewhat lower than that reported in an ES cohort of 79 families with nephronophthisis-associated ciliopathies (63%). Nonetheless, there, 76% of index cases had consanguineous parents, compared to 10% (2/21)

in the ciliopathy cohort of this thesis (Braun et al., 2016). This may be one explanation for the lower diagnostic yield in the ciliopathy cohort of this thesis, as NPHP and related disorders almost exclusively follow an AR inheritance. Additionally, the ciliopathy cohort of this thesis does not only feature NPHP and NPHP-related cases. Eleven of 21 (52%) cases of the ciliopathy disease group had a polycystic kidney disease phenotype (see Online Supplementary Table). The major ADPKD gene *PKD1* cannot be reliably analyzed by ES (Section 1.2.1), which is a limitation in the diagnostic capabilities of ES in ADPKD (Ali et al., 2019). Therefore, all index cases with the clinical tentative diagnosis of ADPKD received *PKD1* testing prior to ES. Nonetheless, there are cases such as HN-F62-II-1 in which bilateral multicystic kidneys and liver cysts were diagnosed in the first year of life. ES was inconspicuous, and ADPKD was not a clinical tentative diagnosis because of the young age at first manifestation. However, it is well known that there is pediatric-onset ADPKD, for example, if there is a hypomorphic allele *in trans* with a causative variant (Bergmann et al., 2011; Vujic et al., 2010). Therefore, in this case, early-onset ADPKD should be a differential diagnosis, and specific *PKD1* testing should follow an inconspicuous ES result.

The FSGS/SRNS cohort featured patients with a median age at disease onset of 10 years [3.5–18.0] and a median age at ES of 24 years [13.0–37.5]. The diagnostic yield was 22% (10/45). This is about the same diagnostic yield as reported in an ES study of 193 adults with FSGS (20%; Yao et al., 2019). However, it is lower than that reported in an SRNS cohort of 1,783 families analyzed by targeted NGS (30%; Sadowski et al., 2015). Yet, in the latter study, the median age of onset of proteinuria was 3.4 years, and a negative correlation of a positive genetic result and the age of onset of proteinuria could be shown, hence explaining the higher detection rate of monogenic FSGS/SRNS in that cohort. It is important to mention that in 22 of 45 (49%) cases of the FSGS/SRNS cohort of this thesis, there was no clear specification of FSGS (primary, secondary, or genetic). These cases were still included because of the clinical tentative diagnosis of hereditary FSGS. Additionally, in 18 of these 22 cases (82%), further criteria pointing towards a hereditary nephropathy apart the clinical tentative diagnosis “hereditary FSGS” were met (e.g., reported familial occurrence of disease, reported consanguinity, etc.; see Online Supplementary Table). Four cases with clearly documented primary FSGS on kidney biopsy were also included in the study cohort. In these cases, this was not counted as overlap with a hereditary kidney disease, as primary

FSGS is believed to be caused by a circulating factor and not monogenic gene defects (Section 1.1.5; De Vriese et al., 2018). These cases were included because other criteria such as early onset of disease (< 18 years of age) or reported family history were present (Section 3.1.1). Of note, one case with primary FSGS on kidney biopsy (HN-F26-II-5) could be genetically solved (homozygous causative variant in *COQ8B*), and in another (HN-F25-II-1), a new candidate gene could be prioritized (homozygous missense variant in *DAAM2*; see Table 8 in Section 4.4). This highlights that certain kidney biopsy results should not render a hereditary cause improbable and that other aspects alluding to a monogenic disease (such as family history, consanguinity, age at first manifestation, and extrarenal features) should also be included in the assessment of indication for genetic testing.

Diagnostic yield of the disease group type-IV-collagen-related nephropathy is discussed in depth in Section 5.2.

Concerning VACTERL/VATER association, none of the nine cases could be solved. This is not surprising, as VACTERL/VATER is not a clear-cut monogenic disease or entity. Rather, it designates the co-occurrence of multiple congenital anomalies in a non-random fashion in an individual. These can have a multifactorial and, sometimes, monogenic origin (Section 1.1.6). Hence, a low yield of 14% is reported in the literature when genetic tests are performed in patients with a phenotype of the VACTERL/VATER spectrum (Solomon, 2018). Furthermore, there is a problem with labeling patients as VACTERL/VATER or, for example, syndromic CAKUT patients. This is discussed in Section 5.6.

Diagnostic yield in the disease group “Other” was 32% (8/25). As this group features several different disease entities such as tubulopathies and aHUS, there is no specific discussion of it.

5.2 Type-IV-collagen-related nephropathy – Alport syndrome vs. thin basement membrane nephropathy

The term “type-IV-collagen-related nephropathy” represents a spectrum of hereditary hematuric disease and comprises two poles of disease severity: AS and TBMN (Imafuku et al., 2020). Patients with AS have progressive decline of renal function (ESRD by age 40 in 90% of males with XLAS), often in conjunction with extrarenal features such as ocular

involvement and hearing loss (Nozu et al., 2019). By contrast, TBMN, a pathology-derived term, follows a mild clinical course with isolated hematuria and, if at all, late-onset ESRD (> 50 years of age), rarely with proteinuria and extrarenal features. Heterozygous (likely) pathogenic variants in *COL4A3* and *COL4A4* have been associated with TBMN and females with heterozygous (likely) pathogenic variants in *COL4A5* can also present clinically with TBMN (Pierides et al., 2009). There is a major debate as to whether the term "TBMN" or "ADAS" should be used for heterozygous carriers of (likely) pathogenic variants in *COL4A3* and *COL4A4* (Section 1.1.2). In this thesis, the term "TBMN" is used, because the term ADAS

a) Elicits the connotation of a more severe phenotype than commonly associated with heterozygous (likely) pathogenic variants in *COL4A3* and *COL4A4*.
b) Is misleading, as it implicates a 50% recurrence risk of AS in offspring, which is not described in the literature so far (reduced penetrance, intrafamilial variability).
c) Could be ARAS instead if there is AS phenotypically (and on kidney biopsy) and a second (likely) pathogenic variant on the other allele was missed by conventional analysis of coding regions of *COL4A3* and *COL4A4* (e.g., intronic variant; Savige, 2018).

As stated in Section 3.1.2, cases of the type-IV-collagen-related nephropathy disease group were designated as either AS or TBMN based on family history and disease course in combination with histopathologic information (if available). Diagnostic yield in the disease group type-IV-collagen-related nephropathy was 55% (35/64). However, diagnostic yield increased if only AS cases were considered (30/46, 65%), which was a significantly higher detection rate than in the TBMN cases (5/18, 28%, $p = 0.01$). This underscores a more multifactorial etiology in TBMN. In three of the five solved cases classified as TBMN, heterozygous (likely) pathogenic variants in *COL4A3* (ATS-F688-II-1) and *COL4A4* (ATS-F503-II-1, ATS-F673-II-1) could be identified. Another solved case was a female patient with a heterozygous pathogenic glycine variant in *COL4A5* (ATS-F274-III-14) that arose *de novo* and led to a thinned GBM and FSGS/FGGS on kidney biopsy at 37 years of age, illustrating the broad phenotypic spectrum of heterozygous causative *COL4A5* variants in females. One solved case in the TBMN subgroup was a phenocopy (HN-F56-II-1). There was suspicion of TBMN due to a thinned GBM on kidney biopsy and a microscopic

hematuria (without a family history of AS), which turned out to be a tubulopathy (Dent disease) due to a hemizygous causative variant in *CLCN5* (Table 7). As written above for the FSGS/SRNS disease group, this underscores that kidney biopsy results can be misleading in terms of genetic work-up. Whereas patient HN-F56-II-1 had clear phenotypic aspects of Dent disease (microscopic hematuria, proteinuria, hypercalciuria, nephrocalcinosis), he was still referred to genetic testing with the clinical tentative diagnosis of TBMN. Of note, a thinned GBM was mentioned as a rare (in 5% of cases) renal pathology in Dent disease (Wang et al., 2016).

A diagnostic yield of 65% in AS cases is still lower than the yield reported in an NGS panel study of AS patients (80%; Mallett et al., 2017). However, in this thesis, some cases were allocated to AS merely by clinical tentative diagnosis of the referring clinician and lacked detailed phenotypic and family-history information (see Online Supplementary Table). Hence, diagnostic yield can change, for example, with more information on patients and their families being gathered and redistribution of cases from AS to TBMN.

The discrimination of AS and TBMN within the clinical disease group type-IV-collagen-related nephropathy in this thesis delineates the two poles of disease severity that give guidance in clinical assessment of patients in whom a type-IV-collagen-related nephropathy is presumed: A TBMN patient typically has microscopic hematuria (13/18 [72%] in the TBMN cohort of this thesis) but is significantly older at first manifestation than AS patients (16.0 years [5.0–32.3] vs. 5.5 years [3.0–9.0]; $p = 0.001$). A TBMN patient typically does not have extrarenal manifestations (0% of TBMN cases in the study cohort vs. 28% in AS patients; $p = 0.01$). A positive family history is significantly less reported in a TBMN patient than in an AS patient (39% vs. 78%; $p = 0.006$), which may be due to a subclinical phenotype in TBMN (microscopic hematuria without decline in kidney function). Of note, only two index cases of the TBMN subgroup reached ESRD, at 62 (HN-F325-II-1) and 65 years of age (ATS-F714-II-1). The median age at ES in the TBMN cohort was 16 years [7.0–43.5]; therefore, not only pediatric patients in the early stages of disease were tested. These findings underline the known milder phenotype in TBMN versus AS in a large cohort of unrelated patients with type-IV-collagen-related nephropathy. Furthermore, the results support the criteria-based discrimination of TBMN and AS proposed in Section 3.1.2. However, these

criteria must be verified in a larger prospective study of type-IV-collagen-related-nephropathy patients. Of note, none of the patients in the AS subgroup had reached ESRD yet. This is not surprising: The median age at ES in this subgroup was 15 years [8.0–22.0], whereas the median age at ESRD in XLAS is 25 years (similar in ARAS; Nozu et al., 2019).

5.3 Identifying phenocopies by exome sequencing and the clinical implications of the recategorization of disease

In this thesis, an important pitfall in the genetic work-up of patients with a presumed hereditary nephropathy is addressed: phenocopies. This pitfall involves the clinical assumption – due to phenotypical presentation and family history – of a certain hereditary kidney disease (e.g., biallelic causative variants in *PKHD1* in a patient with clinically presumed ARPKD) when this genotype is not present, and instead, another, unexpected one is (a causative variant in the CAKUT gene *HNF1B*, for example; case HN-F13-II-1). As explained in Section 1.2.3, this scenario is a commonly applied broad definition of a phenocopy.

In about one-sixth of solved cases (12/77, 16%) a phenocopy could be detected (Section 4.3.1). It is important to note that this an account of phenocopies in a cohort representing all principal categories of hereditary kidney diseases and featuring pediatric, adolescent, and adult patients (Devuyst et al., 2014). The median age at ES in the study cohort was 15.0 years [5.0–28.5]. Prior publications on phenocopies mainly focused on pediatric cohorts with selected clinically presumed hereditary nephropathies such as AS, FSGS/SRNS, CAKUT, or ciliopathies (Deltas et al., 2015; Malone et al., 2014; Sadowski et al., 2015; Schonauer et al., 2020; Szabo et al., 2018; van der Ven, Connaughton, et al., 2018; Warejko et al., 2018). In a study of 92 adult individuals (> 18 years of age) with CKD, ES was able to reclassify the clinical diagnosis in 27% of cases (Lata et al., 2018). Another ES study of 114 families representing 138 adult individuals (median age at testing 48 years) with CKD, revealed that the clinical tentative diagnosis could be corrected by ES in 21% of solved cases (Connaughton et al., 2019). However, in the publication by Connaughton et al., 2019, vague clinical tentative diagnoses (e.g., “chronic glomerulonephritis”) were seen as “corrected” if a genetic diagnosis could be made by ES. By contrast, all phenocopies reported in this thesis were phenotypically well ascertained and could be assigned to a specific hereditary kidney

disease category by clinical presentation or had the clinical tentative diagnosis of a hereditary nephropathy in the first place.

As expected, well-known phenocopies, for example, CAKUT presenting phenotypically as a ciliopathy (case HN-F13-II-1) could be detected in the study cohort of this thesis. Furthermore, FSGS is a histopathologic phenotype found in hereditary and non-hereditary kidney diseases (Braunisch et al., 2018; Rosenberg & Kopp 2017). Hence, unsurprisingly, genetically confirmed AS can present histologically as FSGS (Malone et al., 2014). This is true for cases ATS-F9-II-1 and ATS-F261-II-1. ATS-F9-II-1 had nephrotic-range proteinuria at 26 years and FSGS on kidney biopsy at 29 years of age, which is not the typical AS course. This could be due to the hypomorphic variant NM_000091.4:c.4421T>C, p.(Leu1474Pro) in *COL4A3 in trans* with the likely pathogenic LoF variant NM_000091.4:c.2126-1G>C, p.(?), with the result that a milder ARAS phenotype mimics hereditary FSGS clinically. It can be assumed that the variant p.(Leu1474Pro) is hypomorphic, as it cannot result in ARAS in a homozygous state: Its overall allele frequency is 0.003 in gnomAD v.2.1.1 (0.005 in non-Finnish Europeans), which would lead to a prevalence of ARAS caused by the homozygosity of this variant of about 1 in 111,000 (Hardy-Weinberg principle). ARAS has a prevalence of about 1 in 333,000 (prevalence of AS 1 in 50,000, about 15% are ARAS; Watson & Bush, 2020). This is also true for the phenocopy case ATS-F29-III-1, in which the variant p.(Leu1474Pro) was *in trans* with a likely pathogenic glycine variant NM_000091.4:c.1831G>A, p.(Gly611Arg). This patient had proteinuria and MCD on kidney biopsy at 12 years of age which can progress to FSGS (notice the later kidney biopsy age in ATS-F9-II-1; Vivarelli et al., 2017). Because of her clinical presentation, and because the AS-associated genes *COL4A3*, *COL4A4*, and *COL4A5* had already been analyzed and no causative variant(s) had been found, the case was allocated to the clinical disease group "FSGS/SRNS." The detection of causative variants in *COL4A3* in this case, ATS-F29-III-1, although *COL4A3* had already been analyzed with an inconspicuous result, highlights that genetic results can be falsely negative. Case ATS-F261-II-1, in turn, was a female with proteinuria and hematuria beginning in childhood that were not followed up on at that time. When kidney biopsy was done at 32 years of age, it revealed FSGS. CKD IV was reached at 42 years of age. Hence, the patient was believed to have slowly-progressive hereditary FSGS. The identification of the heterozygous likely pathogenic canonical splice site variant

NM_000495.4:c.645+1G>T, p.(?) in *COL4A5* led to the genetic diagnosis of XLAS, exemplifying the treacherous and potentially misleading phenotype of female patients with heterozygous causative variants in *COL4A5* (Kashtan, 2007). Case HN-F683-I-2 further illustrates this: Here, the clinical tentative diagnosis was medullary cystic kidney disease (called ADTKD today) for decades because of a dominant inheritance pattern (also-affected daughter) and slowly progressive decline in renal function (kidney transplantation at 66 years of age). ES could identify a heterozygous likely pathogenic canonical splice site variant NM_000495.4:c.609+1G>C, p.(?) in *COL4A5* establishing the genetic diagnosis of XLAS in this female patient.

XLAS can show extensive variable expressivity in females with heterozygous causative variants in *COL4A5*, but also in males with hemizygous causative variants, as demonstrated by case ATS-F486-II-1: Because of the clinical presentation of infection-associated macroscopic hematuria at 3 years of age, IgAN was suspected (although unusual at this early age; Bulut et al., 2012). Due to the very young age, kidney biopsy was not prioritized, and ES was done to check for hereditary causes. As IgAN is not a monogenic disease, an inconspicuous result was expected (Kiryluk et al., 2014). However, XLAS could be diagnosed by the detection of the hemizygous likely pathogenic frameshift variant NM_000495.4:c.225delA, p.(Gln76Lysfs*79) in *COL4A5*. It is well-known from genotype-phenotype correlation studies in males with XLAS that LoF variants such as frameshift variants in *COL4A5* lead to a more severe phenotype than, for example, missense variants involving a glycine residue of the triple-helical domain of COL4A5 (Bekheirnia et al., 2010; Gross et al., 2002). Hence, the type of variant could explain the clinical presentation of recurring macroscopic hematuria in case ATS-F486-II-1.

AS can mimic FSGS clinically, as shown above. Nonetheless, the opposite can also be true: Hereditary FSGS/SRNS can present as AS phenotypically. This is underlined by cases HN-F20-II-1, HN-F203-II-2, and ATS-F719-II-2. Case HN-F203-II-2 should be highlighted: This was a female with the clinical tentative diagnosis of AS because of microscopic hematuria manifesting at 2 years of age and AS on kidney biopsy at 3 years of age. Two brothers were similarly affected. Only in 2017, at the age of 34 years, ES could identify a homozygous causative variant in the index patient (and also her brothers) in *MYO1E*, a

hereditary FSGS-associated gene only published in 2011 (Mele et al., 2011). Hence, the diagnosis of AS from kidney biopsy was misleading. Unfortunately, the original biopsy report from 1986 could not be retrieved. This case, HN-F203-II-2, impressively demonstrates that the number of disease-associated genes is ever-increasing and repeated genetic testing is reasonable, especially if suspicion of a hereditary nephropathy is high. The same is true for case ATS-F719-II-2, in which clinical presentation and family history pointed towards AS but a mitochondrial disease due to compound-heterozygous causative variants in *COQ6* could be identified. *COQ6* became a disease-associated gene in 2011: The clinical tentative diagnosis of AS in index patient ATS-F719-II-2 was made in the early 1980s and never contested for over 30 years (Heeringa et al., 2011).

Another mitochondrial disease was case HN-F542-II-1, which was initially categorized as a hereditary salt-wasting tubulopathy with seizures by clinical presentation. As the ES performed in this study cohort also included mitochondrial DNA analysis, the causative homoplasmic mtDNA variant NC_012920.1:m.616T>C in *MT-TF* ($tRNA^{Phe}$) could be identified. Cases ATS-F719-II-2 and HN-F542-II-1 highlight the extensively variable phenotype of mitochondrial diseases.

It must be mentioned that clinical tentative diagnoses are made at a certain time point in the clinical course of a disease and can be altered if new phenotypic features become evident. This is true, for example, for case HN-F46-II-1. The initial presentation in the first year of life was a dRTA phenotype, hence, a hereditary tubulopathy was assumed, and the patient was recruited for ES. At 2 years of age, additional phenotypic features of hyperphagia and diabetes insipidus became evident, and compound-heterozygous causative missense variants in the gene *PCSK1* could be detected by ES. Biallelic causative variants in *PCSK1* are associated with the hereditary disease “obesity with impaired prohormone processing” [MIM 600955], a neuroendocrine defect. An elevated proinsulin/insulin ratio measured because of the genetic result (reverse phenotyping) supported the pathogenicity of the variants. dRTA is not a typical clinical feature of *PCSK1*-associated disease (Stijnen et al., 2016).

For clinical practice, a very important result of this thesis is that restricted NGS panels of ≤ 25 kb (billable panel size in the German statutory health insurance system) would not have solved any of the phenocopy cases (Section 4.3.2). Broad genetic testing by ES or

comprehensive NGS panels is warranted if a hereditary nephropathy, in which many disease entities have overlapping phenotypes (see the above paragraphs), is assumed in a patient – at least if initial narrow genetic testing was inconspicuous.

The recategorization from clinical tentative diagnosis to definite genetic diagnosis can have several important clinical implications, as exemplified by the identified phenocopies:

a) **Different clinical management** → focus on hormone supplementation in *PCSK1*-associated disease (case HN-F46-II-1); early ACE inhibition to reduce proteinuria in AS vs. avoidance of immunosuppression and swift kidney transplantation in FSGS/SRNS (e.g., case ATS-F29-III-1; Gross et al., 2012; Stijnen et al., 2016; Trautmann et al., 2017).
b) **Change in genetic counseling** → mitochondrial disease vs. autosomal disease (case HN-F542-II-1); XL vs. an AR disease (e.g., cases HN-F203-II-2 and ATS-F719-II-2)
c) **Different extrarenal manifestation** → liver fibrosis in ARPKD vs. developmental delay and maturity-onset diabetes of the young (MODY) in 17q12 deletion syndrome involving the CAKUT gene *HNF1B* (case HN-F13-II-1; Bergmann et al., 2018; Bockenhauer & Jaureguiberry, 2016).
d) **Causal treatment options** → CoQ10 supplementation in primary Coenzyme Q10 deficiency (case ATS-F719-II-2; Heeringa et al., 2011).

From a molecular genetic standpoint, grouping hereditary diseases by clinical presentation and not by their molecular genetic determinants if these are established can be seen as cumbersome and useless. However, clinicians must integrate phenotypic features into differential diagnoses and categorize the clinical picture in order to proceed with diagnostic and therapeutic decisions. Hence, it is an essential result of this thesis, that these categories are correct in most cases in which a genetic diagnosis can be made (65/77, 84% of solved cases), but in some cases (12/77, 16% of solved cases), a clinician must think outside the box. This is especially important if narrow and non-comprehensive genetic testing is standard procedure (Section 4.3.2).

5.4 Novel hereditary-kidney-disease-associated genes

Since its first report in 2009, ES has proven to advance the identification of disease-associated genes, as represented by a doubling of disease-associated genes in OMIM® within

a decade (Ng et al., 2009; Petersen et al., 2017). Using a stringent and reproducible search algorithm (Section 3.3.3), in this study cohort of 260 index patients with clinically presumed hereditary kidney disease, variants in novel potentially disease-associated genes ("candidate genes") could be prioritized in unsolved cases. In total, variants in eight candidate genes could be identified. Table 8 summarizes all candidate genes with the respective identified variant(s) and an explanation on prioritization. It is not surprising that 6 of 8 (75%) candidate genes were prioritized in CAKUT cases. This disease group has the largest number of index cases (n = 86) and the second lowest diagnostic yield (9%, only eight genetically solved cases). Furthermore, although there are already about 40 monogenic CAKUT-associated genes described in the literature, data from KO mouse models indicate that many more are to be present (Vivante et al., 2014).

As for the candidate genes *DAAM2*, *PLXNB2*, *TNS1*, and *MAP4K4*, further patients with an overlapping phenotype and genotype could be found either via direct communication with collaboration partners or via GeneMatcher (https://genematcher.org/). All of these projects have been presented at international conferences, and manuscripts are in preparation (co-authorship; Table 8). For the candidate genes *EIF3B* and *ARID5B*, follow-up is pending (RNA sequencing/proteomics in *EIF3B*, skin biopsy for RNA sequencing/proteomics in *ARID5B*). In the following, discussion will focus on the two candidate genes *PBX1* and *FOXD2*.

CAKUT-associated genes typically encode transcription factors important in renal organogenesis (van der Ven, Vivante, et al., 2018). *PBX1* and *FOXD2* are both transcription factors, and *Pbx1* as well as *Foxd2* KO mice show a CAKUT phenotype (Kume et al., 2000; Schnabel et al., 2003). In the case of *Pbx1*, KO mice also feature a profound skeletal phenotype recapitulating the extrarenal features of index patient HN-F75-II-1 (Selleri et al., 2001). In this patient, proband-only ES at 12 years of age could identify a heterozygous LoF variant NM_002585.3:c.413_419del, p.(Gly138Valfs*40) in *PBX1*, presumably leading to NMD (or a truncated protein without the DNA-binding domain; RefSeq accession number NP_002576.1). The variant is listed neither in gnomAD nor in the in-house database (Munich Exome Server), which contains over 20,000 exomes, and arose *de novo* (Sanger sequencing of parents). gnomAD reports a strong depletion for LoF variants for *PBX1* (LOEUF = 0.26).

These data supported a disease-causing effect of the frameshift variant in *PBX1* identified in patient HN-F75-II-1. Furthermore, at the time of ES, a publication reported heterozygous deletions only encompassing *PBX1* in patients with an overlapping syndromic CAKUT phenotype (Le Tanno et al., 2017). Taken together, there was convincing evidence that haploinsufficiency of *PBX1* causes syndromic CAKUT and *PBX1* could be reported as a novel disease-associated gene (Riedhammer et al., 2017). During the writing of the publication, five further syndromic CAKUT patients with deletions involving *PBX1* and heterozygous LoF variants (all *de novo*) were identified by targeted ES and published (Heidet et al., 2017). *PBX1* is now listed as a syndromic CAKUT gene in OMIM® [MIM 617641], and there is a second patient in the study cohort in which *PBX1*-associated disease due to a heterozygous causative nonsense variant could be diagnosed (HN-F305-II-1).

Index case HN-F71-III-2 came from a multiplex consanguineous Arabic family. CAKUT cohort studies showed that there is a higher diagnostic yield in patients with consanguineous parents compared to non-consanguineous cohorts (Vivante et al., 2017). The male index patient had congenital bilateral renal hypoplasia along with facial dysmorphies (retrognathia, micrognathia, dental abnormalities, dysplastic ears) and mental retardation. He had ESRD at 10 years of age and received allogenic kidney transplantation at 11 years of age. Three first cousins once removed had an overlapping syndromic CAKUT phenotype. Proband-only ES revealed a homozygous frameshift variant NM_004474.3:c.789dup, p.(Gly264Argfs*228), in the gene *FOXD2*. The variant is listed neither in gnomAD nor in the in-house database (Munich Exome Server), which contains over 20,000 exomes. It is very interesting that the variant leads to a large open reading frame, leaving the DNA-binding domain of FOXD2 intact (RefSeq accession number NP_004465.3). As *FOXD2* is a one-exon gene, NMD need not necessarily occur because of the premature stop codon, leaving the possibility of residual/altered function of the encoded protein due to the frameshift variant. The parents were heterozygous carriers, and an also-affected first cousin once removed carried the variant in a homozygous state (Sanger sequencing) as well. *Foxd2* encodes a transcription factor of the evolutionarily conserved forkhead family strongly expressed in murine developing kidney (Benayoun et al., 2011; Carlsson & Mahlapuu, 2002; Kume et al., 2000). Fox genes, including *Foxd2*, are also important for tongue and mandibular development (Jeong et al., 2004; Millington et al., 2017). *Foxd2* KO mice have kidney hypoplasia and hydroureter at a

reduced penetrance of 42%, which could be due to the redundancy of Fox genes in the developing kidney (Kume et al., 2000). The literature data in combination with the genotypic and segregation data favored the frameshift variant identified in *FOXD2* causing the syndromic CAKUT phenotype in index patient HN-F71-III-2 and his also-affected and genetically tested first cousin once removed. Unfortunately, a worldwide search for further patients with overlapping phenotype and fitting genotype did not yield any matches. Nonetheless, via a collaboration (Dr. Miriam Schmidts, University of Freiburg), *in vitro* functional studies were initiated (CRISPR/Cas9-mediated KO of *Foxd2* in murine ureteric bud-induced metanephric mesenchyme cells with subsequent RNA sequencing). A manuscript is currently in writing (first authorship), and the results of the functional studies have been presented as a talk at the 2020 virtual meeting of the European Society of Human Genetics (2020-A-1598-ESHG).

5.5 Whom to test? Predictors of a positive genetic test

For the clinician, which patient should be referred for genetic testing is an important question in the diagnostic work-up. Thanks to the size of the study cohort, with 260 index cases, predictors of a positive genetic test could be ascertained statistically (Section 4.5). The strongest significant predictor of a positive test was a reported family history (i.e., similarly affected relatives) with an OR of 6.61 (95% CI 3.28-13.35; $p < 0.001$). This is in line with findings from other cohorts. For example, one study of over 3,000 mainly adult patients with CKD analyzed by ES showed that a family history of kidney disease conferred an OR of 3.40 (2.40-4.70; $p < 0.001$) for a positive genetic test (Groopman et al., 2019). This result should prompt clinicians to take a thorough family history of their patients. A syndromic disease (i.e., extrarenal manifestation) was associated with an OR of 3.21 (1.58–6.54; $p = 0.001$) for a positive genetic result. It is known, for instance, from CAKUT cohorts, that syndromic disease leads to higher diagnostic yields (van der Ven, Connaughton, et al., 2018). Furthermore, an ES cohort with 3,040 cases showed a higher yield in “multiple congenital anomalies” cases (36%) compared to the diagnostic yield of the total cohort (29%; Retterer et al., 2016). Finally, lower age at first manifestation was linked to a higher detection rate of causative variants (OR 0.97 [0.93–1.00]), although this was borderline significant ($p = 0.048$). This is still convincing, as, for example, adult cohorts with FSGS analyzed by ES

lead to lower diagnostic yields than pediatric/adolescent cohorts with SRNS analyzed by targeted NGS (20% vs. 30%; Sadowski et al., 2015; Yao et al., 2019). This should remind clinicians to look specifically for early-onset symptoms. For example, an adult patient with FSGS should be asked at what age proteinuria was first noticed and whether there was SRNS and/or microscopic hematuria in childhood.

Exome sequencing with parents ("trio") was not a significant predictor of a solved case. This is in contrast to the ES study of 3,040 cases featuring various clinical disease groups mentioned a few sentences above. In this study, the diagnostic yield of proband-only ES was 24%, compared to 31% in trios (Retterer et al., 2016). However, in the study cohort of this thesis, there was a significantly uneven distribution of trios across the different clinical disease groups ($p < 0.001$; Table 7). About two thirds of trios (58/86, 67%) were performed in cases categorized into disease groups with diagnostic yields lower than the total cohort (30% of cases solved), that is, CAKUT (9%), FSGS/SRNS (22%), and VACTERL/VATER (no cases solved).

Age at ES was also not a significant predictor of a solved case. This is not surprising, as age of ES can be assumed to be dependent not only on disease severity, but also on various other factors such as healthcare system (access to ES/genetic testing in general), previous genetic testing, and inclusion of a hereditary kidney disease in the differential diagnosis (clinician-dependent).

Male sex had lower odds of a positive genetic result (OR 0.54 [0.29–1.01]), which was a borderline insignificant finding ($p = 0.053$). This result may be due to the fact that about two thirds (66%) of the index patients in the CAKUT disease group, which is the largest disease group of the study cohort (86/260, 33%) but which has the second-lowest diagnostic yield (9%), are male.

CAKUT cohorts, for example, have shown that consanguinity of parents results in higher diagnostic yields (Vivante et al., 2017). In the study cohort of this thesis, however, reported parental consanguinity was not indicative of a positive genetic test. This could be due to the overall limited number of cases with reported parental consanguinity (22/260, 9%).

As 84% of the study cohort was of non-Finnish European descent, it was expected that a positive OR for diagnostic yield could be calculated. This finding was not significant, however. It would not be convincing that Non-Finnish European descent is predictive of a positive genetic result, as there are no hereditary kidney diseases predominantly manifesting in non-Finnish Europeans (Devuyst et al., 2014).

In summary, significant independent predictors of a positive genetic result were reported family history, extrarenal manifestation, and lower age at first manifestation. This confirms most of the selection criteria applied if a case did not have a phenotype overlapping with a specific hereditary kidney disease or the clinical tentative diagnosis of a hereditary kidney disease (Section 3.1.1). Nonetheless, 91% of cases (236/260) were included because the clinical tentative diagnosis of a hereditary nephropathy was made. Indeed, if only these 236 cases were investigated for independent predictors of a positive genetic result, reported family history, extrarenal manifestation, and lower age at first manifestation stayed significant independent predictors (data not shown).

5.6 Limitations

Several limitations of this thesis need to be mentioned. First, the study cohort is very heterogeneous, which makes reproducibility difficult.

Furthermore, the FSGS/SRNS cohort of this thesis lacks sufficient histopathologic information in a substantial number of cases, which can be criticized (Section 5.1; Howie, 2020). This is underscored by the fact that the repeated phenotyping of several cases of the NephroGen cohort previously published as hereditary FSGS cases had to be excluded from the study cohort of this thesis, as they no longer met the inclusion criteria (cases HN-F175-, HN-F223-II-1, HN-F235-III-7, HN-F254-II-1, and HN-F326-II-2; see Online Supplementary Table; Riedhammer, Braunisch, et al., 2020).

The number of phenocopies is small, which limits generalizability. The study cohort is mainly of non-Finnish European descent (84%); hence, the results cannot simply be applied to other ethnic groups (Petrovski & Goldstein, 2016).

Moreover, it must be mentioned that CNVs can play a major role in hereditary nephropathies, especially in CAKUT (Section 1.1.3; Sanna-Cherchi et al., 2012; Verbitsky et al., 2019). ES

is limited in terms of CNV analysis, particularly for duplications: Whereas recall rate of CMA-detected CNVs by ES is high for deletions (about 90%), it is only 65% for duplications. And, of course, only coding regions can be evaluated for CNVs by ES (Marchuk et al., 2018). Hence, a considerable part of CAKUT diagnoses could be missed by ES. Only three unsolved CAKUT cases (HN-F646-II-1, HN-F647-II-1, HN-F514-II-2) had CMA prior to ES.

The allocation of cases to specific disease groups is highly dependent on the referring clinician (91% of cases were included because of the phenotype in accordance with or the clinical tentative diagnosis of a specific hereditary nephropathy). Therefore, there is a subjective nature to the assignment of disease category. This is highlighted by the VACTERL/VATER category. As noted in Section 1.1.6, there is no final agreement as to what defines the clinical diagnosis of VACTERL/VATER. If the rule is applied that at least three components of the association should be present, solved case HN-F502-II-1 (bilateral renal hypoplasia [R], imperforate anus [A], dysplastic ears, right triphalangeal thumb [L]), for example, could also have been grouped into the VACTERL/VATER category and not into CAKUT (Solomon, 2018). Hence, it is not surprising that the term "CAKUT in VACTERL" is present in the literature, which only adds to the confusion (Saisawat et al., 2014).

Limitations concerning diagnostic yield of ES in hereditary nephropathies have been discussed in Section 5.1.

5.7 Conclusion and outlook

The aim of this thesis (Chapter 2) was to illustrate the application of ES in diagnosing hereditary nephropathies and its usefulness in revealing phenocopies and to prioritize novel hereditary kidney disease-associated genes. These aims were achieved: When keeping its limitations in mind (especially *PKD1* and *MUC1*, Sections 1.2.1 and 5.1), ES can diagnose hereditary nephropathy across all major hereditary nephropathies (Devuyst et al., 2014). In contrast to narrowly designed targeted panels, ES has the capability to identify phenocopies, which can have crucial implications on genetic counseling, prognosis, and management of disease (Section 5.3). Furthermore, ES can make dual genetic diagnoses (Section 4.1.2). The unbiased approach of ES can help further unravel genetic diseases such as CAKUT by

prioritizing candidate genes, which can facilitate understanding of nephropathies in general and foster the development of targeted therapeutics in the future (Sections 4.4 and 5.4; van der Wijst et al., 2019).

Another aim of this thesis was to improve clinical decision-making in hereditary nephropathies: This thesis characterizes the differences of the two poles of disease severity of type-IV-collagen-related nephropathy, namely, AS and TBMN (Section 4.2). This can help better categorize patients and assess their prognosis. An ES-based burden test for rare variants (MAF < 1.0%) in TBMN/isolated microscopic hematuria cases versus controls could further elucidate genetic determinants of TBMN. In terms of whom to refer to genetic testing, this thesis provides vital statistical evidence of predictors of a positive genetic test (reported family history, extrarenal manifestation, and younger age at first manifestation), which supports clinical decision-making. These predictors should be specifically tested on the different clinical disease groups used in this thesis. Larger cohorts are needed to do so. Furthermore, the clinical criteria for the selection of cases (Section 3.1.1) and the assignment of type-IV-collagen-related-nephropathy cases to AS or TBMN (Section 3.1.2) should be evaluated by a prospective study.

One more finding of this thesis should be discussed in terms of future research: the recurrent missense variant p.(Leu1474Pro) in *COL4A3*. Because of its allele frequency in the general population (gnomAD), this variant cannot result in AS in a homozygous state (Section 5.3). However, *in trans* with another causative variant, it leads to a phenotype that is easily mistaken for hereditary FSGS, as exemplified by phenocopy cases ATS-F9-II-1 and ATS-F29-III-1 (and also non-phenocopy case ATS-F687-II-1, with no CKD at 13 years of age but nephrotic proteinuria; see Online Supplementary Table, and Sections 4.3.1 and 5.3). Therefore, especially in adult nephrology, there should be more cases with the *COL4A3* variant p.(Leu1474Pro) *in trans* with a causative variant and FSGS phenotypically: Heterozygous carrier frequency in non-Finnish Europeans of the variant (allele frequency of 0.005; gnomAD v.2.1.1) is 1 in 100 (0.01) and general carrier frequency of causative variants in *COL4A3* is about 1 in 333 (0.003; AS has a prevalence of 1 in 50,000, about 10% of AS cases are *COL4A3*-associated; Hardy-Weinberg principle). Hence, the probability of inheriting the p.(Leu1474Pro) and another causative *COL4A3* variant from one's parents is

0.5 x 0.01 x 0.5 x 0.003 = 0.0000075 (about 1 in 133,000 non-Finnish Europeans; Kashtan, 2019; Watson & Bush, 2020). Scrutinizing adult CKD cohorts (with FSGS and nephropathy of unknown origin) for this variant (and a second causative variant *in trans*) could help make genetic diagnoses which, for example, have implications on disease recurrence in kidney transplants (no recurrence to be expected in AS). However, the phenotypic spectrum of p.(Leu1474Pro) should be ascertained first by compiling more carriers of this variant (with a second causative variant *in trans*).

In conclusion, the results of this thesis enhance management and therapy in patients with hereditary nephropathies and help further unravel the molecular mechanisms of rare but also common kidney diseases, which will support therapeutic drug discovery in the future.

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7 APPENDICES

7.1 List of tables

Table 1 (page 29)	ADTKD-associated genes with respective protein function, genotypic and phenotypic characteristics.
Table 2 (page 38)	Overview of principal CAKUT-associated genes with respective protein function and phenotypes.
Table 3 (page 52)	Selection of different hereditary FSGS/SRNS-associated genes with respective protein function and phenotypes.
Table 4 (page 62)	Overview of different molecular genetic methods routinely used to diagnose hereditary kidney diseases.
Table 5 (page 63)	Overview of diagnostic yields of ES/NGS-based panels (short-read) in major hereditary nephropathy disease groups.
Table 6 (pages 98–99)	Baseline characteristics of the study cohort.
Table 7 (pages 106–107)	Summary of phenocopies identified by exome sequencing (ES).

Table 8 (pages 112–114) Overview of novel hereditary-kidney-disease-associated genes prioritized by exome sequencing (ES).

Table 9 (page 115) Binomial logistic regression to identify predictors of a positive genetic test (dependent variable “solved case yes/no”).

7.2 List of figures

Figure 1 (page 31) Protein structure of a single α-chain 5 of type IV collagen (COL4A5, RefSeq accession number NP_000486.1).

Figure 2 (page 34) Genotype–phenotype correlation in X-linked Alport syndrome (XLAS).

Figure 3 (page 37) Embryonal development of the kidney.

Figure 4 (page 42) Primary cilium, basic components (schematic figure).

Figure 5 (page 51) Selection of FSGS/SRNS-associated genes and their distribution across the glomerular filtration barrier.

Figure 6 (page 59) Essential steps of exome sequencing (based on short-read NGS).

Figure 7 (page 65) A) Distribution of disease-associated genes, in which causative variants were identified by ES in a cohort of 3,037 patients with CKD (91.6% > 21 years old). B) Spectrum of clinical pre-categorization of patients with an identified monogenic disease.

Figure 8 (page 69) Illustration of the relationship of effect size and allele frequency in monogenic vs. non-monogenic (multifactorial) disease.

Figure 9 (pages 75–76) Standardized questionnaire for phenotype ascertainment. A) First page. B) Second page.

Figure 10 (page 83) Example of a chromatogram showing a heterozygous nucleotide change G>A (arrow).

Figure 11 (page 88) American College of Medical Genetics and Genomics (ACMG) guideline for sequence variant interpretation (excerpt).

Figure 12 (page 90) Algorithm for prioritizing variants in candidate genes (used in unsolved cases).

Figure 13 (page 94) Flowchart to illustrate the inclusion of index cases of the larger NephroGen exome sequencing cohort.

Figure 14 (page 96) Distribution of clinical disease groups of the study cohort consisting of 260 index cases with a clinically presumed hereditary nephropathy analyzed by exome sequencing (ES).

Figure 15 (page 102) Diagnostic yield of respective clinical disease groups.

Figure 16 (page 110) Recategorization of disease by the identification of phenocopies (n = 12).

7.3 Publications pertinent to the thesis

2020

Riedhammer, K. M., Braunisch, M. C., Gunthner, R., Wagner, M., Hemmer, C., Strom, T. M., Schmaderer, C., Renders, L., Tasic, V., Gucev, Z., Nushi-Stavileci, V., Putnik, J., Stajic, N., Weidenbusch, M., Uetz, B., Montoya, C., Strotmann, P., Ponsel, S., Lange-Sperandio, B., & Hoefele, J. (2020). Exome Sequencing and Identification of Phenocopies in Patients With Clinically Presumed Hereditary Nephropathies. *Am J Kidney Dis*, *76*(4), 460-470. https://doi.org/10.1053/j.ajkd.2019.12.008

2017

Riedhammer, K. M., Siegel, C., Alhaddad, B., Montoya, C., Kovacs-Nagy, R., Wagner, M., Meitinger, T., & Hoefele, J. (2017). Identification of a Novel Heterozygous De Novo 7-bp Frameshift Deletion in PBX1 by Whole-Exome Sequencing Causing a Multi-Organ Syndrome Including Bilateral Dysplastic Kidneys and Hypoplastic Clavicles. *Front Pediatr*, *5*, 251. https://doi.org/10.3389/fped.2017.00251

7.4 Further publications

2020

Finsterer, J., Stollberger, C., Hasun, M., **Riedhammer, K.**, & Wagner, M. (2020). Multisystem Myotilinopathy, including Myopathy and Left Ventricular Noncompaction, due to the MYOT Variant c.179C>T. *Case Rep Cardiol*, *2020*, 5128069. https://doi.org/10.1155/2020/5128069

Hughes, J. J., Alkhunaizi, E., Kruszka, P., Pyle, L. C., Grange, D. K., Berger, S. I., Payne, K. K., Masser-Frye, D., Hu, T., Christie, M. R., Clegg, N. J., Everson, J. L., Martinez, A. F., Walsh, L. E., Bedoukian, E., Jones, M. C., Harris, C. J., **Riedhammer, K. M.**, Choukair, D., Fechner, P. Y., Rutter, M. M., Hufnagel, S. B., Roifman, M., Kletter, G. B., Delot, E., Vilain, E., Lipinski, R. J., Vezina, C. M., Muenke, M., & Chitayat, D. (2020). Loss-of-Function Variants in PPP1R12A: From Isolated Sex Reversal to Holoprosencephaly Spectrum and Urogenital Malformations. *Am J Hum Genet*, *106*(1), 121-128. https://doi.org/10.1016/j.ajhg.2019.12.004

Klinner, J., Kruger, M., Brunet, T., Makowski, C., **Riedhammer, K. M.**, Mollweide, A., Wagner, M., & Hoefele, J. (2020). Congenital lymphedema as a rare and first symptom of tuberous sclerosis complex. *Gene*, *753*, 144815. https://doi.org/10.1016/j.gene.2020.144815

Kummeling, J., Stremmelaar, D. E., Raun, N., Reijnders, M. R. F., Willemsen, M. H., Ruiterkamp-Versteeg, M., Schepens, M., Man, C. C. O., Gilissen, C., Cho, M. T., McWalter, K., Sinnema, M., Wheless, J. W., Simon, M. E. H., Genetti, C. A., Casey, A. M., Terhal, P. A., van der Smagt, J. J., van Gassen, K. L. I., Joset, P., Bahr, A., Steindl, K., Rauch, A., Keller, E., Raas-Rothschild, A., Koolen, D. A., Agrawal, P. B., Hoffman, T. L., Powell-Hamilton, N. N., Thiffault, I., Engleman, K., Zhou, D., Bodamer, O., Hoefele, J., **Riedhammer, K. M.**, Schwaibold, E. M. C., Tasic, V.,

Schubert, D., Top, D., Pfundt, R., Higgs, M. R., Kramer, J. M., & Kleefstra, T. (2020). Characterization of SETD1A haploinsufficiency in humans and Drosophila defines a novel neurodevelopmental syndrome. *Mol Psychiatry*. https://doi.org/10.1038/s41380-020-0725-5

Lorenz, G., Schul, L., Schraml, F., **Riedhammer, K. M.**, Einwachter, H., Verbeek, M., Slotta-Huspenina, J., Schmaderer, C., Kuchle, C., Heemann, U., & Moog, P. (2020). Adult macrophage activation syndrome-haemophagocytic lymphohistiocytosis: 'of plasma exchange and immunosuppressive escalation strategies' - a single centre reflection. *Lupus*, *29*(3), 324-333. https://doi.org/10.1177/0961203320901594

Staufner, C., Peters, B., Wagner, M., Alameer, S., Baric, I., Broue, P., Bulut, D., Church, J. A., Crushell, E., Dalgic, B., Das, A. M., Dick, A., Dikow, N., Dionisi-Vici, C., Distelmaier, F., Bozbulut, N. E., Feillet, F., Gonzales, E., Hadzic, N., Hauck, F., Hegarty, R., Hempel, M., Herget, T., Klein, C., Konstantopoulou, V., Kopajtich, R., Kuster, A., Laass, M. W., Lainka, E., Larson-Nath, C., Leibner, A., Lurz, E., Mayr, J. A., McKiernan, P., Mention, K., Moog, U., Mungan, N. O., **Riedhammer, K. M.**, Santer, R., Palafoll, I. V., Vockley, J., Westphal, D. S., Wiedemann, A., Wortmann, S. B., Diwan, G. D., Russell, R. B., Prokisch, H., Garbade, S. F., Kolker, S., Hoffmann, G. F., & Lenz, D. (2020). Defining clinical subgroups and genotype-phenotype correlations in NBAS-associated disease across 110 patients. *Genet Med*, *22*(3), 610-621. https://doi.org/10.1038/s41436-019-0698-4

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developmental delay, happy demeanor, distinctive facial features, and congenital anomalies. *Hum Mutat*, *40*(11), 1985-1992. https://doi.org/10.1002/humu.23844

Zweier, M., Begemann, A., McWalter, K., Cho, M. T., Abela, L., Banka, S., Behring, B., Berger, A., Brown, C. W., Carneiro, M., Chen, J., Cooper, G. M., Deciphering Developmental Disorders, S., Finnila, C. R., Guillen Sacoto, M. J., Henderson, A., Huffmeier, U., Joset, P., Kerr, B., Lesca, G., Leszinski, G. S., McDermott, J. H., Meltzer, M. R., Monaghan, K. G., Mostafavi, R., Ounap, K., Plecko, B., Powis, Z., Purcarin, G., Reimand, T., **Riedhammer, K. M.**, Schreiber, J. M., Sirsi, D., Wierenga, K. J., Wojcik, M. H., Papuc, S. M., Steindl, K., Sticht, H., & Rauch, A. (2019). Spatially clustering de novo variants in CYFIP2, encoding the cytoplasmic FMRP interacting protein 2, cause intellectual disability and seizures. *Eur J Hum Genet*, *27*(5), 747-759. https://doi.org/10.1038/s41431-018-0331-z

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Thiel, C., Wortmann, S., **Riedhammer, K.**, Alhaddad, B., Mayatepek, E., Prokisch, H., & Distelmaier, F. (2018). Severe ichthyosis in MPDU1-CDG. *J Inherit Metab Dis*, *41*(6), 1293-1294. https://doi.org/10.1007/s10545-018-0189-9

Westphal, D. S., **Riedhammer, K. M.**, Kovacs-Nagy, R., Meitinger, T., Hoefele, J., & Wagner, M. (2018). A De Novo Missense Variant in POU3F2 Identified in a Child with Global Developmental Delay. *Neuropediatrics*, *49*(6), 401-404. https://doi.org/10.1055/s-0038-1669926

7.5 Presentations at conferences

2020

Riedhammer, K. M., M. Nguyen, B. Alhaddad, S. J. Arnold, G. J. Kim, U. Heemann, M. Schmidts and J. Hoefele. ”Implication of *FOXD2* in autosomal recessive syndromic CAKUT.” Oral presentation at the annual meeting (virtual) of the European Society of Human Genetics (ESHG; 2020-A-1598-ESHG).

2019

Riedhammer, K. M., U. Schoenermarck, M. Fischereder, M. C. Braunisch, R. Günthner, L. Renders, U. Heemann and J. Hoefele. ”Phänotypische Variabilität bei Patienten mit molekulargenetisch gesicherter autosomal-dominanter tubulointerstitieller Nierenerkrankung (ADTKD).” Poster presentation at the annual meeting of the Deutsche Gesellschaft für Nephrologie (DGfN) in Dusseldorf, Germany (A1901/1125).

Riedhammer, K. M., M. C. Braunisch, R. Günthner, M. Wagner, B. Uetz, B. Lange-Sperandio and J. Hoefele. “Frequency and spectrum of phenocopies identified by exome sequencing in 239 cases with hereditary kidney disease.” Oral presentation at the annual meeting of the European Society of Human Genetics (ESHG) in Gothenburg, Sweden (2019-A-282-ESHG).

Riedhammer, K. M., M. C. Braunisch, R. Günthner, M. Wagner, B. Uetz, B. Lange-Sperandio and J. Hoefele. “Identifikation von Phänokopien in 15% der gelösten Fälle in einer Kohorte von 247 Familien mit hereditärer Nierenerkrankung.“ Poster presentation at the annual meeting of the Gesellschaft für Pädiatrische Nephrologie (GPN) in Cologne, Germany (Nieren- und Hochdruckkrankheiten 48(3): 117, 2019).

2018

Riedhammer, K. M., M. Stippel, R. Günthner, M. C. Braunisch, P. M. Herr, E. P. Macheroux, B. B. Beck, R. Satanovskij, V. Tasic and J. Hoefele. "Frequency of Pathogenic Variants in a Munich CAKUT cohort." Oral presentation at the annual meeting of the European Society for Paediatric Nephrology (ESPN) in Antalya, Turkey (OP-7).

Riedhammer K. M., G. Montini, J. Hoefele and S. Weber; European Society for Paediatric Nephrology Working Group on Congenital Anomalies of the Kidney and Urinary Tract. "EURECA - a European Registry of Familial CAKUT Cases." Oral presentation at the annual meeting of the European Society for Paediatric Nephrology (ESPN) in Antalya, Turkey (OP-24).

Riedhammer, K. M., M. Stippel, R. Günthner, M. C. Braunisch, P. M. Herr, E. P. Macheroux, B. B. Beck, R. Satanovskij, V. Tasic and J. Hoefele. "Next-Generation Sequencing in einer Kohorte mit CAKUT und hereditären Nierenerkrankungen." Poster presentation at the annual meeting of the Deutsche Gesellschaft für Nephrologie (DGfN) in Berlin, Germany (P003).

Riedhammer K. M., G. Montini, J. Hoefele and S. Weber. " EURECA - ein europäisches Register für familiäre CAKUT-Fälle." Poster presentation at the annual meeting of the Deutsche Gesellschaft für Nephrologie (DGfN) in Berlin, Germany (P004).

7.6 URL/DOI for Online Supplementary Table (featuring all index cases with detailed phenotypic and genotypic information)

URL

https://figshare.com/articles/dataset/Thesis_Dr_med_-_Exome_sequencing_in_hereditary_nephropathies_-_Supplementary_Table/14134886

DOI

https://doi.org/10.6084/m9.figshare.14134886.v1