



DIAGNOSTIC CHALLENGES IN RARE KIDNEY DISEASES

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Disclosure of Interests

No relevant disclosures

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Challenges rare kidney diseases (1)

- **Unknown genetic causes:**
 - 30-40% monogenic disease unsolved
 - Diagnostic odyssey
 - Limitations for genetic counseling, prenatal diagnosis, etc.
- **Data on prognosis limited:**
 - Loose/absent genotype-phenotype correlations
 - Absence of prognostic markers
- **Insufficient ontology:**
 - Heterogeneity
 - Imperfect prognostic value



Challenges rare kidney diseases (2)

- **Treatment:** for many only symptomatic
- **Carrier status:**
 - Females of X-linked diseases may be (severely) affected
 - Living-related kidney transplantation?
- **Health policy issues:**
 - Rare diseases
 - Access to expertise centres and genetic testing
 - Insurance coverage



Potential of genetic testing for diagnostics and research of rare kidney diseases

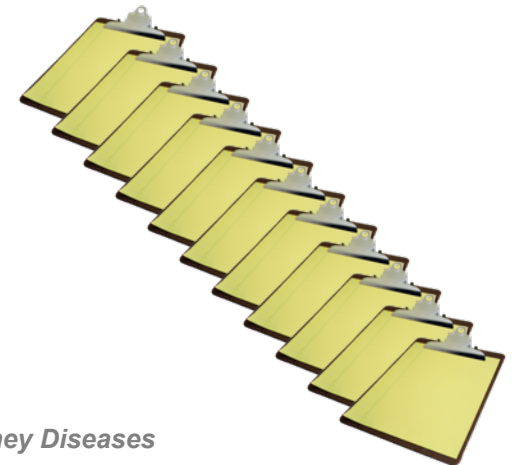
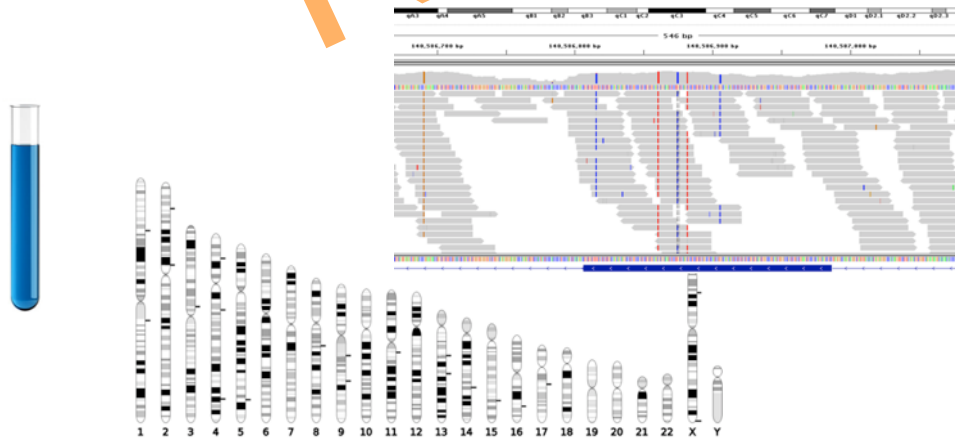
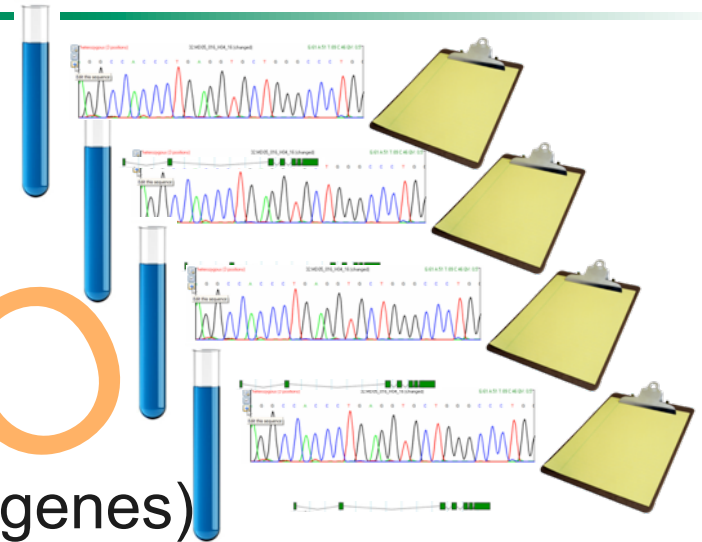


- Accelerate diagnostic process
- Change classical diagnostic paradigm “from phenotype to genotype”
- Discover novel disease genes
- Discover new diseases
- Better understanding of biological basis of diseases
- Shift in phenotypic boundaries and reclassification of some kidney diseases /improve disease ontology
- Clues for prognosis and treatment

Next Generation Sequencing (NGS) changing paradigm of clinical genetic testing

From one test per gene to one test for all (involved) genes

- Disease-specific multi-gene panels
- Whole Exome sequencing (WES: all genes)
- Whole Genome sequencing (WGS: complete DNA)



Predicted clinical utility NGS for rare kidney diseases

■ Establish diagnosis:

- End diagnostic odyssey in puzzling cases
- Bringing peace of mind to family
- Avoiding further expensive and fruitless testing
- Reversed/deep phenotyping
- Improving diagnostics for genetically heterogeneous disorders (nephrotic syndrome, ciliopathies, etc)
- Mode of inheritance → cascade testing
- Enables genetic counseling, prenatal diagnosis, PGD etc.
- Benefits for carrier testing (living-related donors)

■ “Genetics first” approach

- No need for other diagnostic procedures such as renal biopsy?
- Exome first” approach may be economically feasible and may even become cost-saving (*Shashi et al., Gen Med 2014; Monroe et al., Genet Med 2016*)



Predicted clinical utility NGS for rare kidney diseases

- **Broadening phenotypic spectrum gene mutations**
 - Shift in phenotypic boundaries
 - Reclassifications of some kidney diseases
- **Therapeutic/Prognostic value:**
 - Response to eculizumab in aHUS
 - Response immunosuppressive therapy & risk post-transplant disease recurrence in steroid-resistant nephrotic syndrome
 - Identify new targets for therapy

Current diagnostic yield whole exome sequencing (WES)

THE NEW ENGLAND JOURNAL OF MEDICINE

ORIGINAL ARTICLE

Clinical Whole-Exome Sequencing for the Diagnosis of Mendelian Disorders

Yaping Yang, Ph.D., Donna M. Muzny, M.Sc., Jeffrey G. Reid, Ph.D.,
Matthew N. Bainbridge, Ph.D., Alecia Willis, Ph.D., Patricia A. Ward, M.S.,
Alicia Braxton, M.S., Joke Beuten, Ph.D., Fan Xia, Ph.D., Zhiyv Niu, Ph.D.,
Matthew Hardison, Ph.D., Richard Person, Ph.D., Mir Reza Bekheirnia, M.D.,
Magalie S. Leduc, Ph.D., Amelia Kirby, M.D., Peter Pham, M.Sc., Jennifer Scull, Ph.D.,
Min Wang, Ph.D., Yan Ding, M.D., Sharon E. Plon, M.D., Ph.D.,
James R. Lupski, M.D., Ph.D., Arthur L. Beaudet, M.D.,
Richard A. Gibbs, Ph.D., and Christine M. Eng, M.D.

**Causative genetic defect identified in 25-35% of
patients with a suspected genetic disorder**

Cost-effectiveness WES: HTA study UMC Utrecht

371 patients with ID

	No. (median)	No. (mean)	Costs (median)	Costs (mean)
Health-care visits	38	61	2,144	3,012
Imaging	8	16	771	1,439
Genetics	6	7	5,745	6,588
Metabolics	6	6	2,777	2,818
Biochemical investigations	5	28	355	2,034
Day admission	2	4	309	517
Total			14,153	16,409

Values are given in USD.

Average costs entire traditional diagnostic trajectory: **\$16,409** (range: \$6,343 to \$47,841)

Largest proportion of costs: previous genetic tests

Trio WES: diagnostic yield 35%, costs \$3,972

WES: average cost savings \$3,547 for genetic & metabolic investigations in diagnosed patients



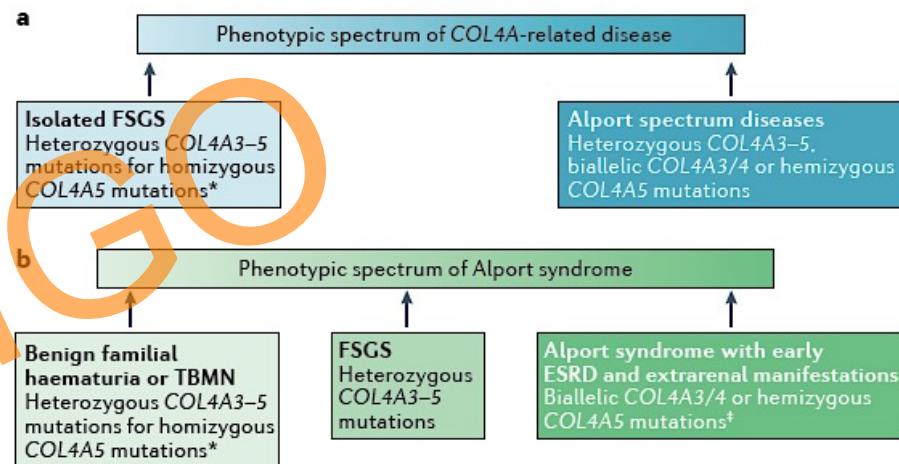
Conclusion HTA-WES

- Implementation “WES first” approach in diagnostics may be cost-efficient
- “WES first” approach may decrease total time diagnostic process.
- We also need to perform long term HTA (POST-WES).

NGS in patients with presumed genetic renal disorders revealed “unexpected” gene mutations

Broadening phenotypic spectrum across and within current kidney disease categories

- *COL4A3-5* genes (Alport syndrome) mutations in patients presenting with FSGS
- *PAX-2* mutations (renal-coloboma syndrome) in patients presenting with FSGS
- *DGKE* mutations in SRNS, MPGN and aHUS
- Phenotypic heterogeneity ciliopathies

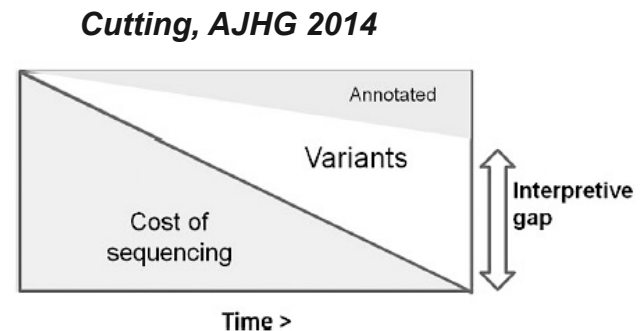


Stokman et al., Nat Rev Nephrol-2016, in press

**New aetiological insights
May have implications for management**

NGS limitations

- **Costs, workforce, training, throughput**
- **Differences in access** to diagnostic testing and **insurance coverage**
- **Technical challenges:** likely to be solved with improving technology
- **Interpretation difficulties:** Ability to interpret the disease implications of individual variants has not kept pace with the ease with which we find them
- **Legal/social/ethical difficulties**



Interpretation difficulties: how to define pathogenicity of identified variants?

- In silico tools for predicting pathogenicity (i.e. SIFT, Polyphen) < 80% accuracy
- Databases of common variants in “healthy” individuals (dbSNP, 1000 Genomes project, Exome Aggregation Consortium (ExAC), Exome Sequencing Project (ESP)/ underrepresentation certain ethnicities
- Human genomes contain ~100 highly penetrant disease-causing mutations, with 20 genes completely inactivated (*MacArthur et al., Science 2012*)
- Mutation and variation databases: lots of differences in annotation of variants (*McCarthy et al., Genome Med 2014*)
- Pathogenicity previously reported mutations called into question (*Piton et al., AJHG 2013, Nicolaou et al., KI 2015*) upon reanalysis because they have relatively high frequency in control Exomes/genomes



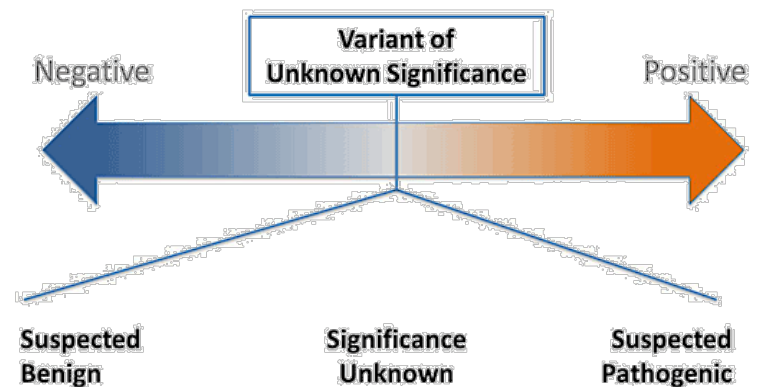
Interpretation difficulties

Complexity of variant interpretation

Many Variants of Unknown Significance (VUS)

- Segregation analysis
- Functional studies
- Data sharing !!!

Range of **VUS** results



NGS: legal/social/ethical/ issues

- Ownership, storage and access to data
- Data sharing/confidentiality and privacy
- Genome sequencing (including bioinformatic analysis) is still too expensive for routine use in research/diagnostics
- Differences in access to diagnostic testing and insurance coverage
- Informed consent: impossible to counsel patients about full range of findings that might result from WES/WGS sequencing



WES/WGS: unsolicited findings (UFs)

- Unanticipated findings not related to initial reason for genetic analysis
- Rate reportable UFs range from 1 to 8.8%
- UFs may also have important implications for unaffected family members
- Disagreement on release of UFs
 - What information should be returned ?



Amendola et al., *Genome Res* 2015

There is still room for single gene testing

Indication

Minimal locus heterogeneity

Distinctive features (i.e. family history, biochemistry, biopsy...) point to one gene

Epigenetic abnormalities

Examples

CTNS for cystinosis
FN1 for Glomerulopathy with fibronectin deposition

AVPR2 in X-linked NDI
KCNJ10 in EAST syndrome
LMX1B in Nail-Patella syndrome

Beckwith Wiedemann syndrome



When to consider NGS-based multi-gene disease panels?

Genetic heterogeneity

- **Alport syndrome:** *COL4A5, COL4A3, COL4A4*
Turnaround time from 6 months to 6 days (*Artuso et al., EJHG 2012*)
- **Nephrotic syndrome:** 27 known genes
Disease-associated variants in 30% of screened NS patients (*Sadowski et al, JASN 2014*)

Disorders with overlapping phenotypes

- **Bartter/Gitelman syndrome:**
SLC12A1, KCNJ1, BSND, CLCNKB, SLC12A3, HNF1B

Disorders associated with genes from common pathway or structure

- **Renal ciliopathies** (Nephronophthisis, BBS, Joubert, OFD,)

When to consider WES/WGS?

Phenotype indistinct, no clear hypothesis about underlying cause but suggestive of genetic condition

- i.e. unexplained CKD

Gene panel testing revealed no causative mutations (second tier)

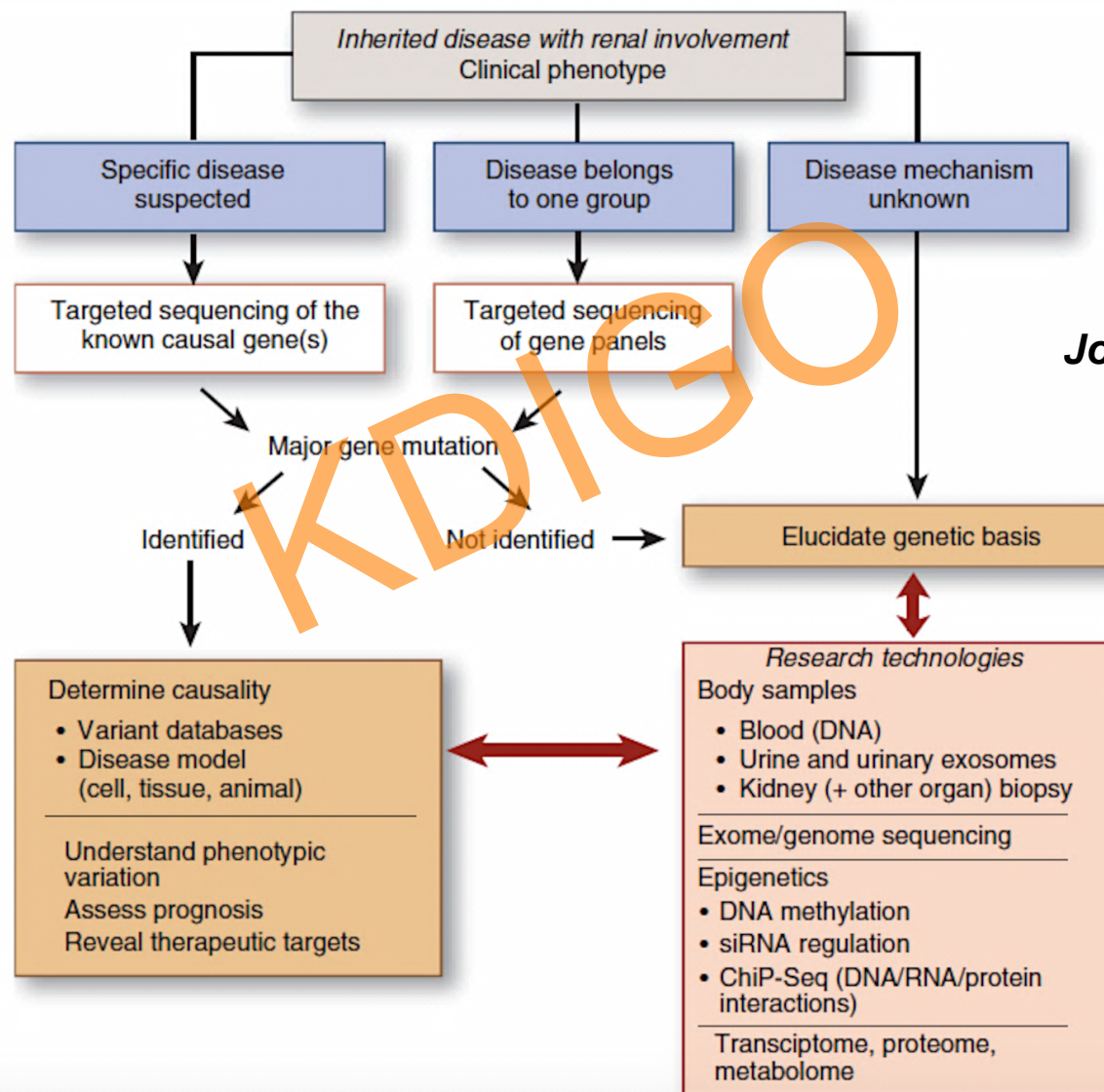
- discover new genes/unexpected genetic variants



WES in clinical practice is time-consuming

Clinical Evaluation	<ul style="list-style-type: none">• Define phenotype through history and exam• Differential diagnosis
Test Selection	<ul style="list-style-type: none">• Match phenotype to test• Consider diagnostic yield, cost, etc.
Test Approval	<ul style="list-style-type: none">• Institutional review• Insurance prior authorization
Consent	<ul style="list-style-type: none">• Discuss limitations, possible results• Discuss option to receive secondary findings
Evaluate Results	<ul style="list-style-type: none">• Check databases, review literature, etc.• Re-evaluate phenotype
Return of Results: Diagnosis Found	<ul style="list-style-type: none">• Delivery of genetic diagnosis to patient/family• Return of secondary findings• Provide support for coping• Consider management: assess need for additional testing, change in therapies• Genetic counseling for family planning
Return of Results: No Diagnosis Found	<ul style="list-style-type: none">• Inform about negative results, including explanation of any candidate variants• Provide support for coping• Consider further options for evaluation• Offer reassessment of exome data: research basis or future clinical reanalysis (if available)

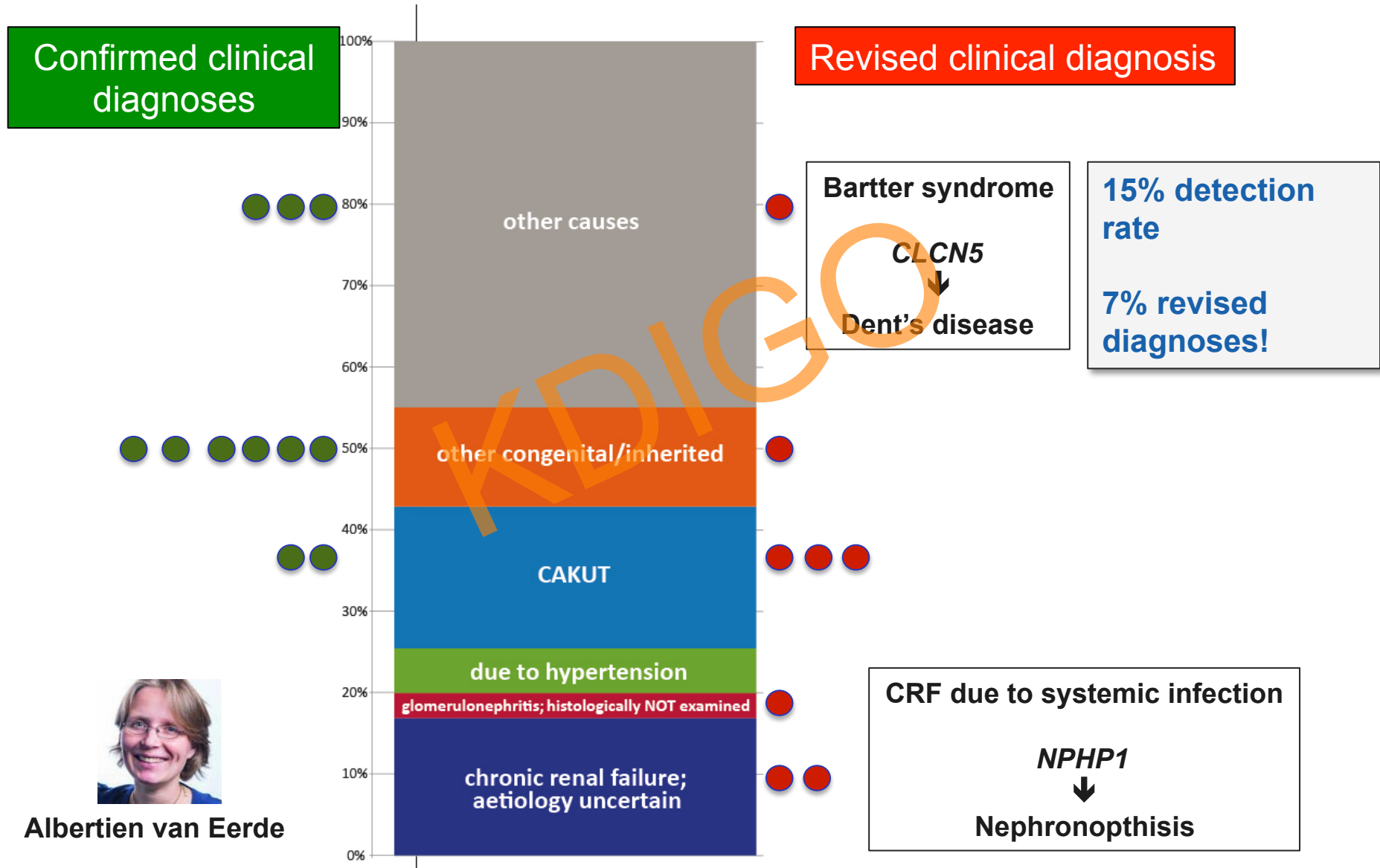
Diagnostic paradigm for rare inherited renal diseases & potential use of research technologies.



Joly et al., KI 2015

Diagnosis on clinical grounds/genetic evidence or both?

NGS panel sequencing reclassifies primary disease diagnoses in young CKD/ESRD



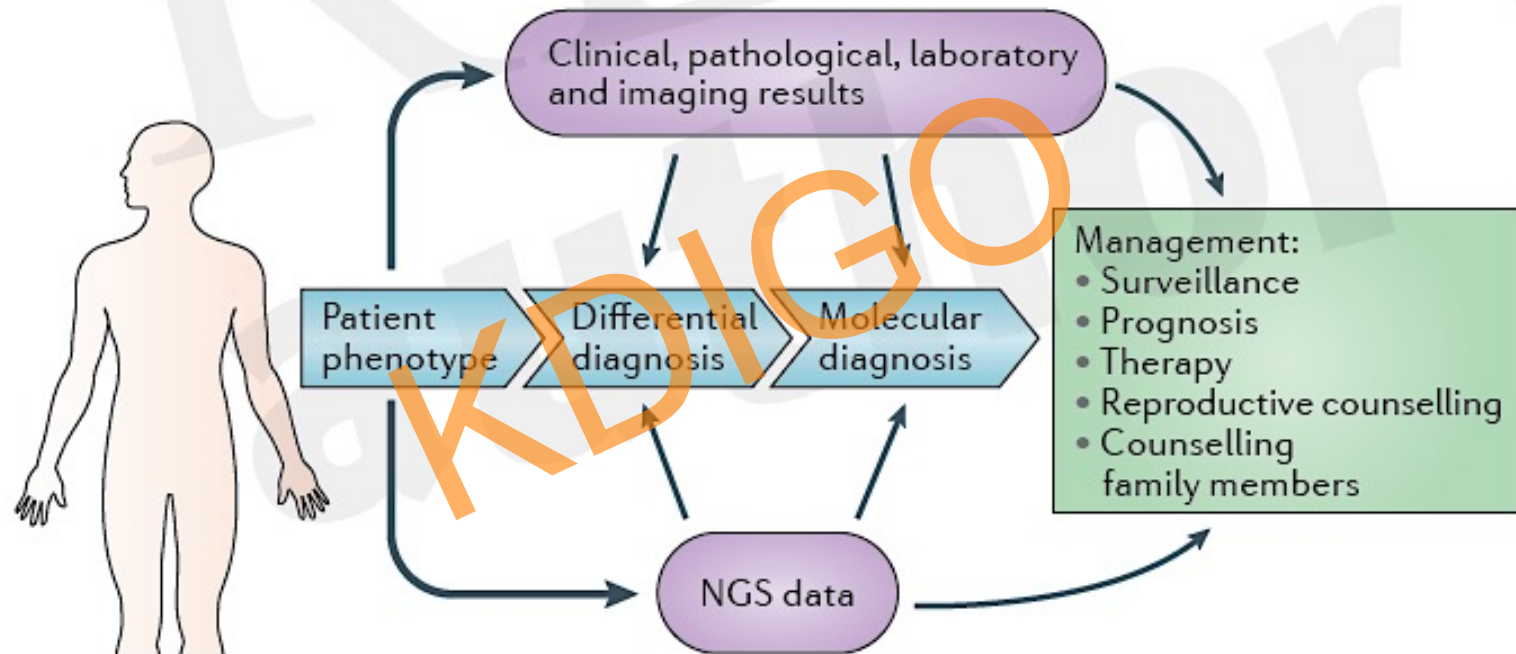
Albertien van Eerde

Conclusions

- NGS techniques have found their place in clinical practice of renal disorders, with implications for diagnosis, therapeutic decisions, genetic counseling, prenatal diagnosis, PGD
- NGS techniques have unraveled surprising, novel insights into phenotypic spectrum of gene mutations/ may lead to reclassification of some rare kidney diseases
- Important challenges in establishing pathogenicity of identified mutations by NGS techniques remain
- Data sharing initiatives are imperative to establish clinically useful genotype– phenotype correlations and to maximize the benefit of genetic testing
- Exome/Genome sequencing raises important ethical issues; especially how to deal with unsolicited findings

Conclusions

NGS diagnostics as part of routine diagnostic work-up



Stokman et al., Nat Rev Nephrol 2016, in press