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The genetics of kidney diseases: where do we stand?

(A clinician's perspective)

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SPP SOCIEDADE PORTUGUESA DE PEDIATRIA Sociedade de Nefrologia

IPNA

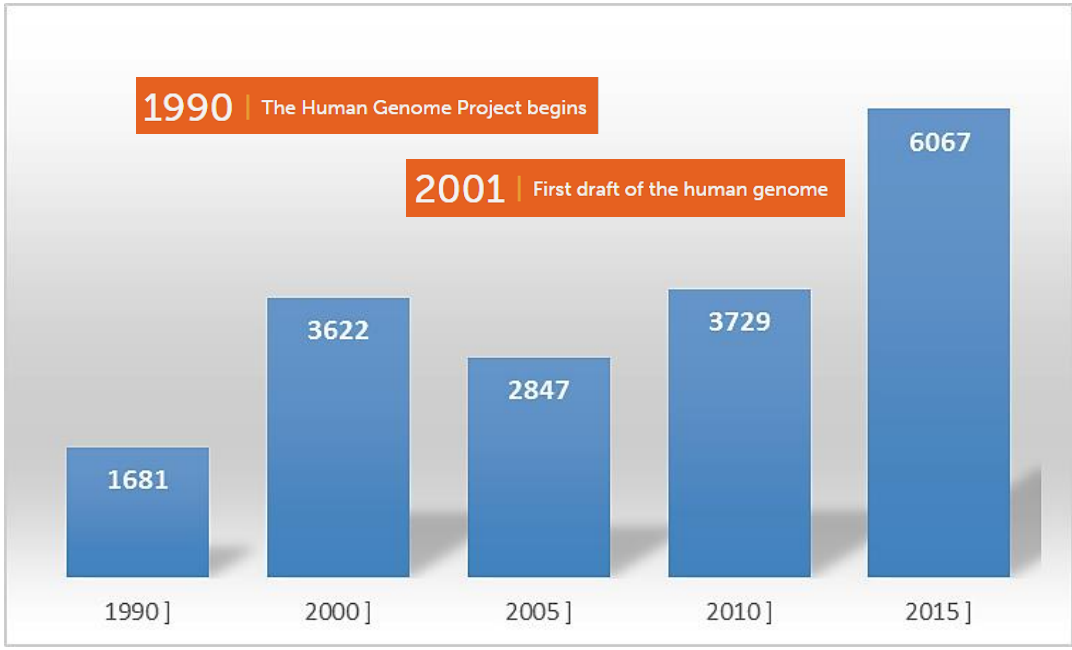
O autor declara ausência de potenciais conflitos de interesses

(de acordo com o ponto 24. do documento UEMS 2012/30 “Accreditation of Live Educational Events by the EACCME”)

PubMed-indexed published research on the genetics of kidney diseases

Number of publications on the genetics of kidney diseases, retrieved from the PubMed database

<1990	[1990-2000]	[2001-2005]	[2006-2010]	[2011-2015]	[Q1-4/2016]	Total
1681	**3622**	**2847**	**3729**	**6067**	*1449*	*18124*



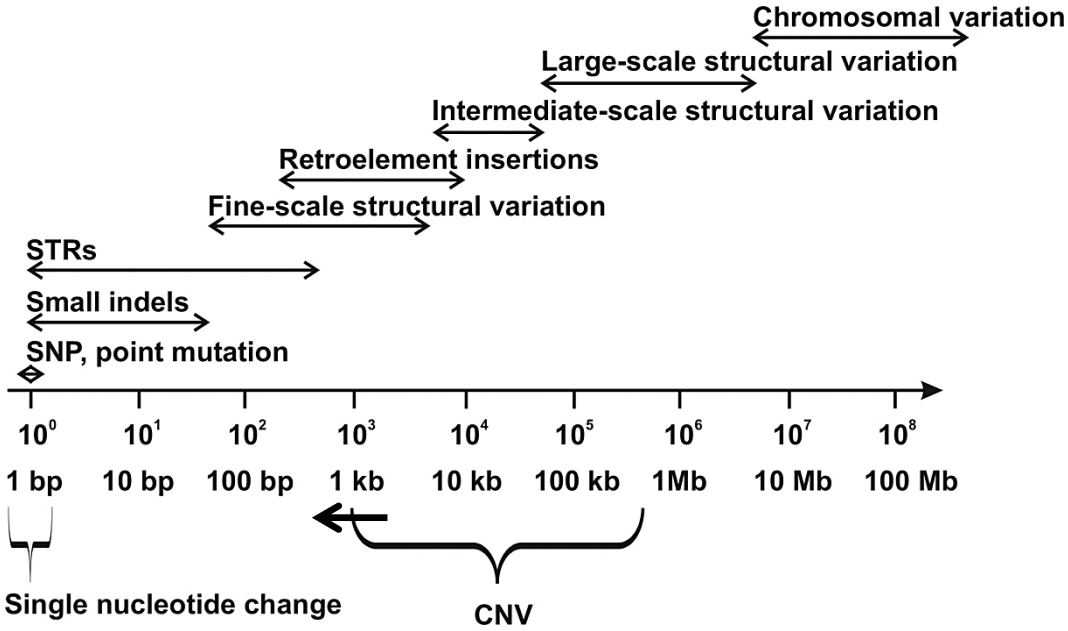
PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed Advanced Search Builder

Search (((((((kidney[Title/Abstract]) OR renal[Title/Abstract])) AND ((disease*[Title/Abstract]) OR disorder[Title/Abstract]))) OR ((nephropath*[Title/Abstract]) OR glomerulopath*[Title/Abstract]))) AND ((((genetic*[Title/Abstract]) OR hereditary[Title/Abstract]) OR inherited[Title/Abstract]) OR familial[Title/Abstract])

Genetic variation and genetic contribution to human disease

The spectrum of variation in the human genome



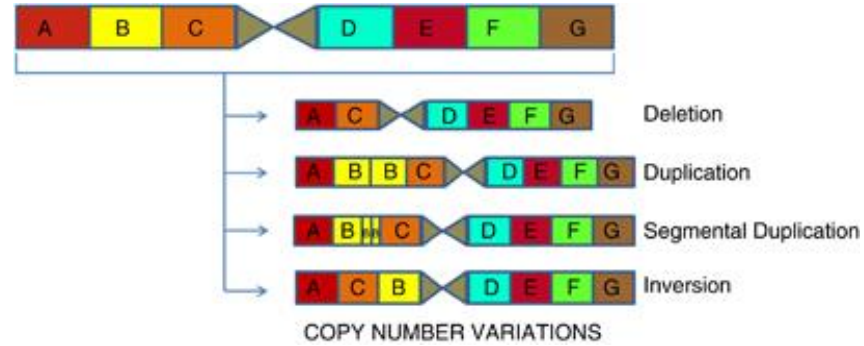
STRs: short tandem repeats; SNP: single nucleotide polymorphism; CNV: copy number variation.

Pollex RL & Hegele RA: Copy number variation in the human genome and its implications for cardiovascular disease. *Circulation* 2007;115:3130-3138.

Number of SNPs identified in the 1000 Genomes Project phase I overall: ~38 x 10^6 ; per individual sample: 3.6 x 10^6.

[www.1000genomes.org/category/phase-1/]

Types of genomic variants



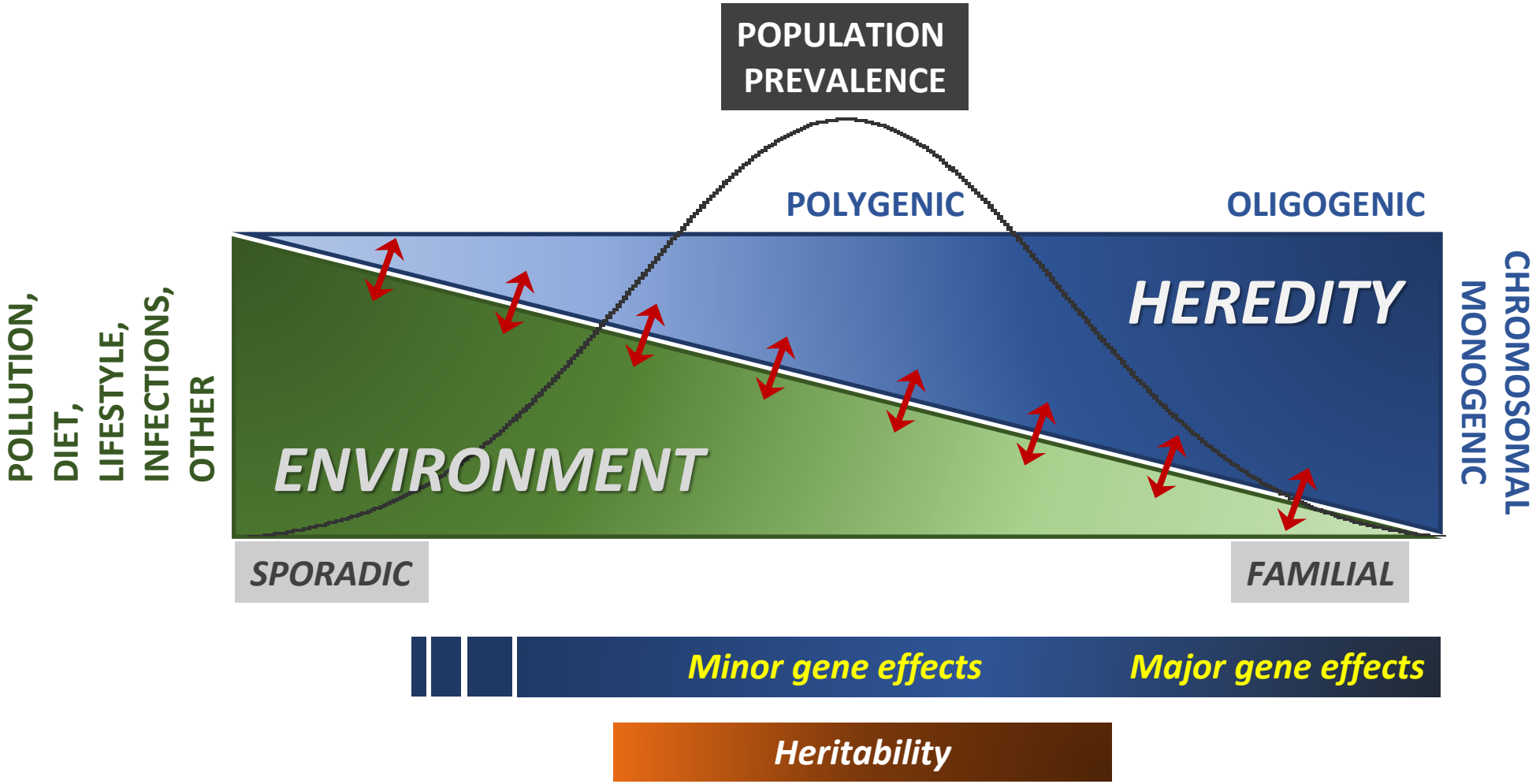
Genomic variants in form of CNVs can be classified primarily as deletion, duplication, segmental duplication and inversion. These variations can encompass the entire gene or a segment of a particular gene represented in the figure.

Almal SH & Padh H: Implications of gene copy-number variation in health and diseases. *J Hum Genet* 2012:57:6-13.

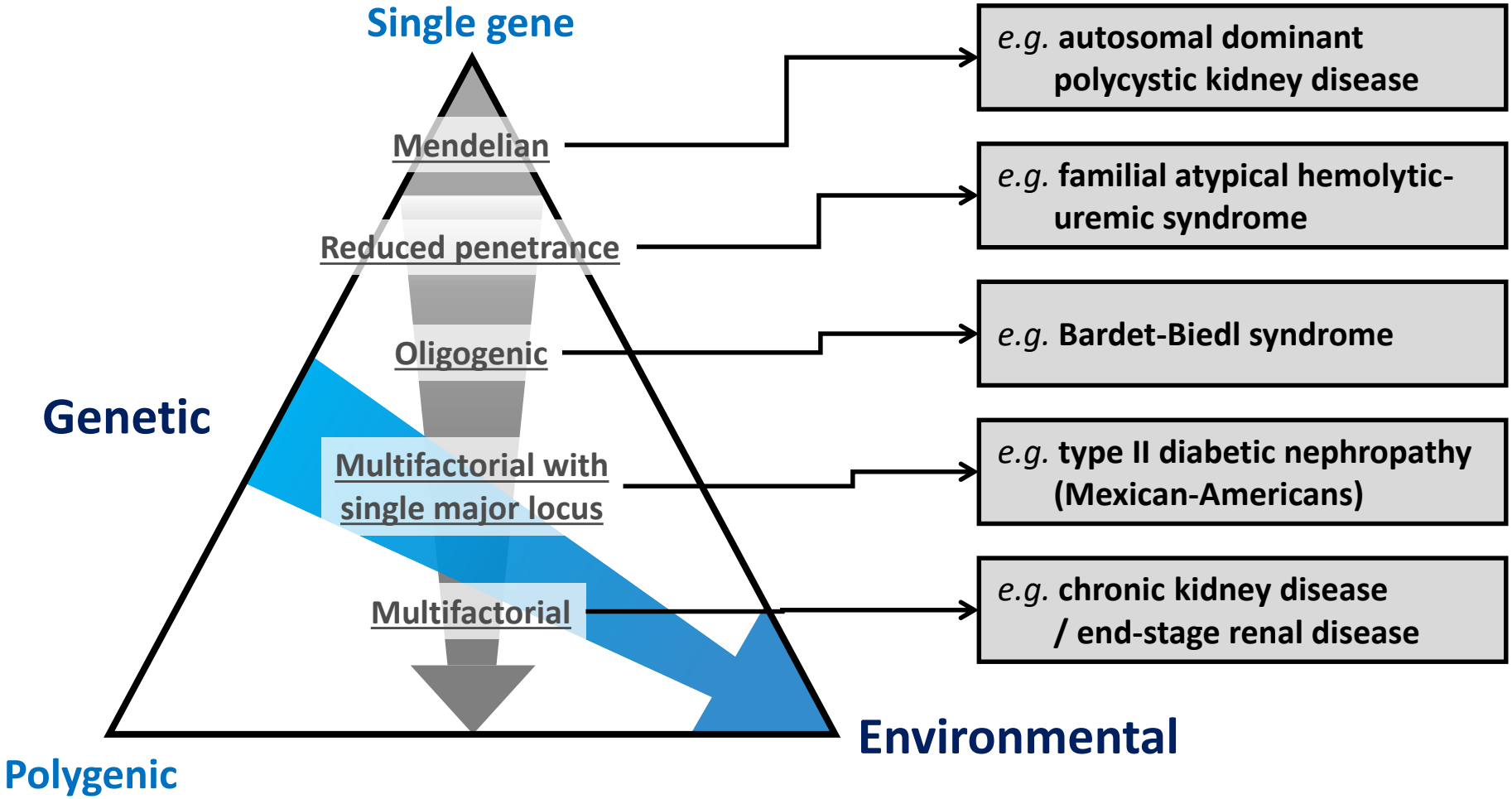
Number of reported common CNVs: 356,817.

Zhao M & Zhao Z: CNVannotator: a comprehensive annotation server for copy number variation in the human genome. *PLoS ONE* 2013;8:e80170.

Classification of human diseases according to the nature of their underlying causality



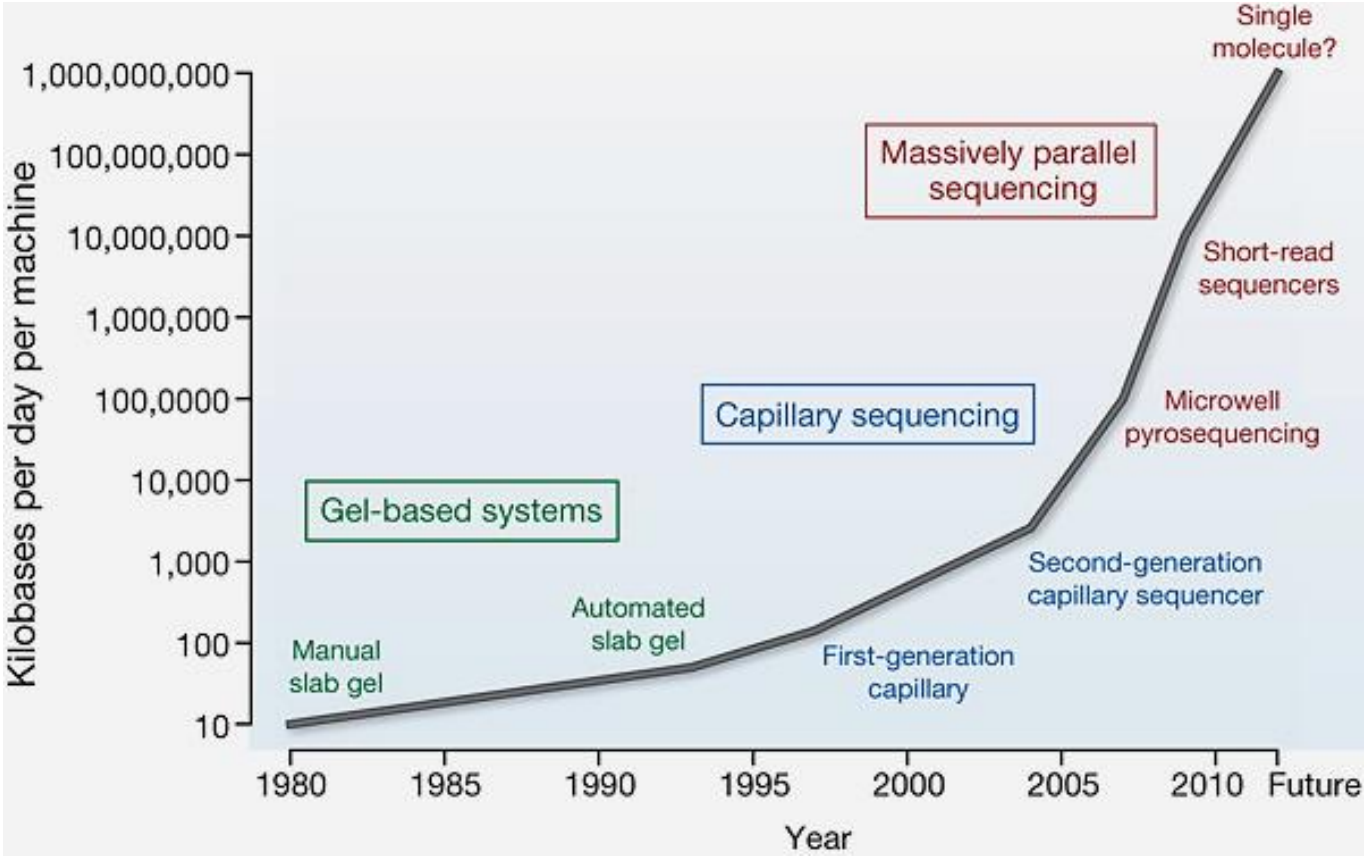
Genetic and environmental influences in the causation of human diseases, as exemplified by kidney disorders



Modified from Strachan T & Read AP, *Human Molecular Genetics* 2. Wiley-Liss, New York, 1999.

Methodological advances

Improvements in the rate of DNA sequencing over the past 30 years and into the future

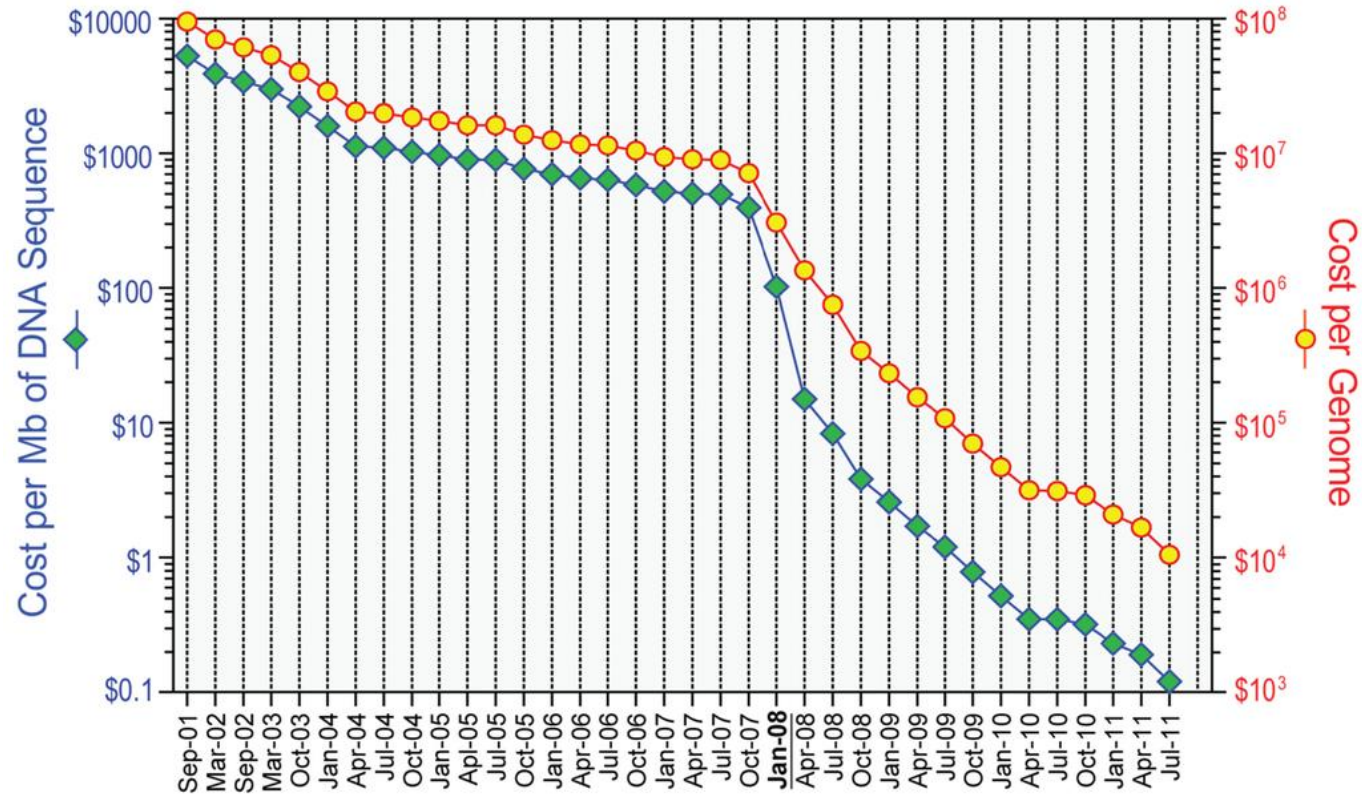


From slab gels to capillary sequencing and second-generation sequencing technologies, there has been a more than a million-fold improvement in the rate of sequence generation over this time scale.

Stratton MR *et al.*: The cancer genome. *Nature* 2009;458:719-724.

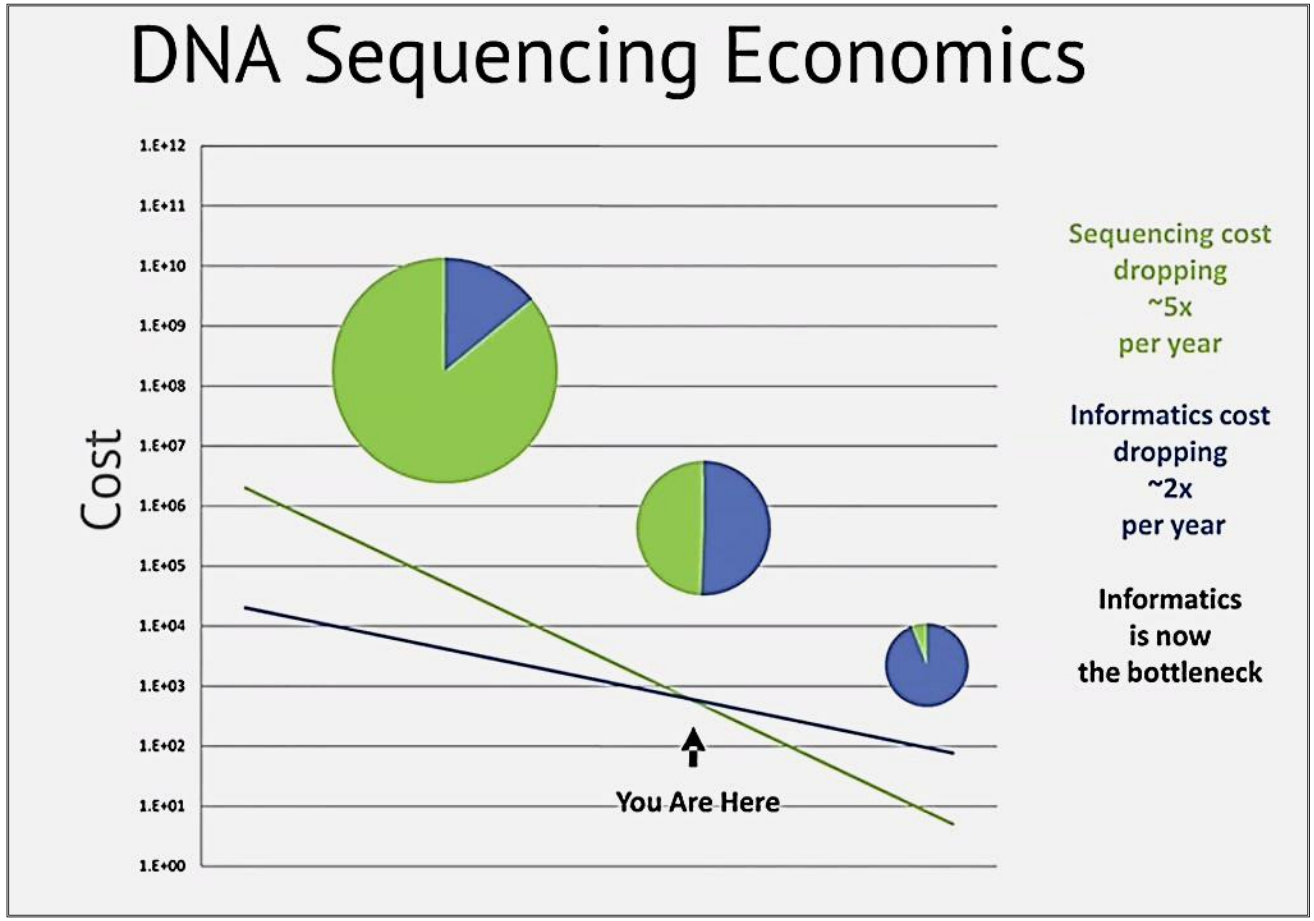
The plummeting cost of genome sequencing

The cost-accounting data, available at the website of National Human Genome Research Institute (NHGRI), are summarized relative to two metrics: (1) the cost of determining one megabase (10^6 bases) of DNA sequence of a specified quality, and (2) the cost of sequencing a human-sized genome (i.e., 3,000 Mb). Of note, the sudden and profound decrease beginning in January 2008 represents the time when the NHGRI sequencing centers transitioned from Sanger-based chemistry and capillary-based instruments to next-generation DNA sequencing technologies.



Li L *et al*.: Cancer genome sequencing and its implications for personalized cancer vaccines. *Cancers* 2011;3:4191-4211.

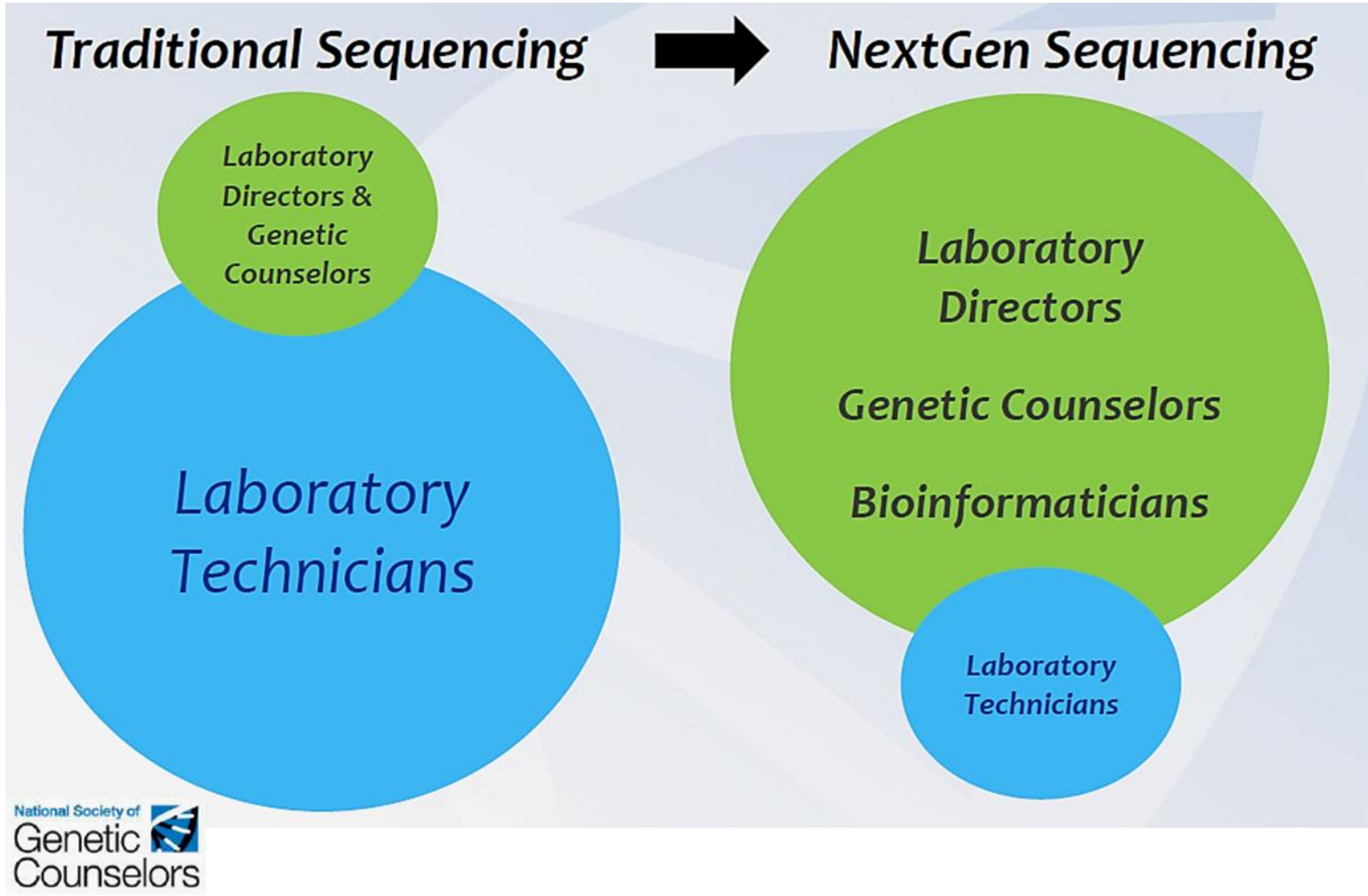
Informatics is now the bottleneck!



The cost of sequencing is falling more than twice as quickly as the cost of computing, so that the cost of sequencing has more to do with data analysis than data collection.

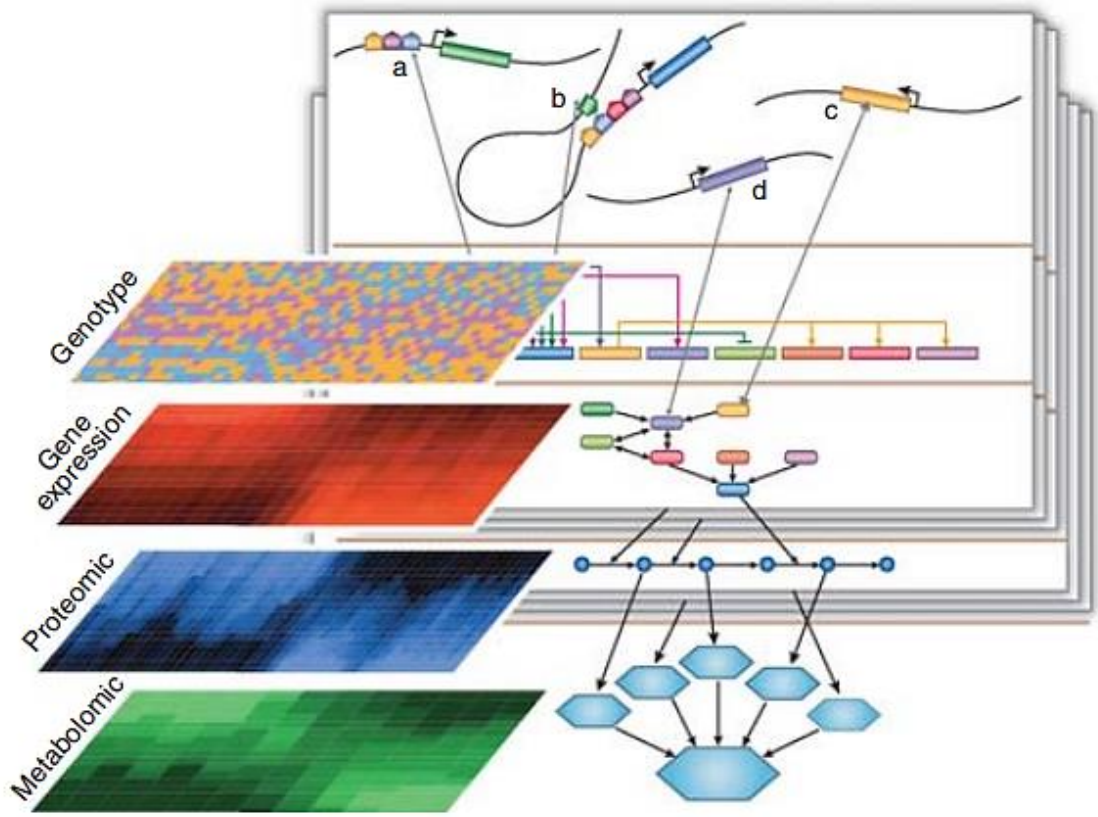
[McAuley R, accessed at: http://insider1673.rssing.com/chan-4569069/all_p4.html]

Paradigm shift in molecular genetics!



[Gonzalez K, Ambry Genetics; accessed at: http://www.ambrygen.com/sites/default/files/pdfs/2012_NSGC%20webinar_SK%20KG%284%29.pdf]

Systems genetics strategy for studying systems effects of candidate variants



Various high-throughput technologies allow observation of the state of the molecular mechanism of the cell as quantitative measures of macromolecules (shown are RNA, proteins, and metabolites) that can be used in quantitative trait locus (QTL) analysis.

Keller BJ *et al*.: A systems view of genetics in chronic kidney disease. *Kidney Int* 2012;81:14-21.

Clinical use and gene-finding applications

Types of disease-causing mutations and standard laboratory methods for their identification

Types of mutation
Missense/nonsense
Splicing
Regulatory
Small deletions*
Small insertions*
Small indels*
Repeat variations
Gross insertions/duplications
Complex rearrangements
Gross deletions

*Small defects are ≤20 bp in size.

[http://www.hgmd.cf.ac.uk/ac/index.php]

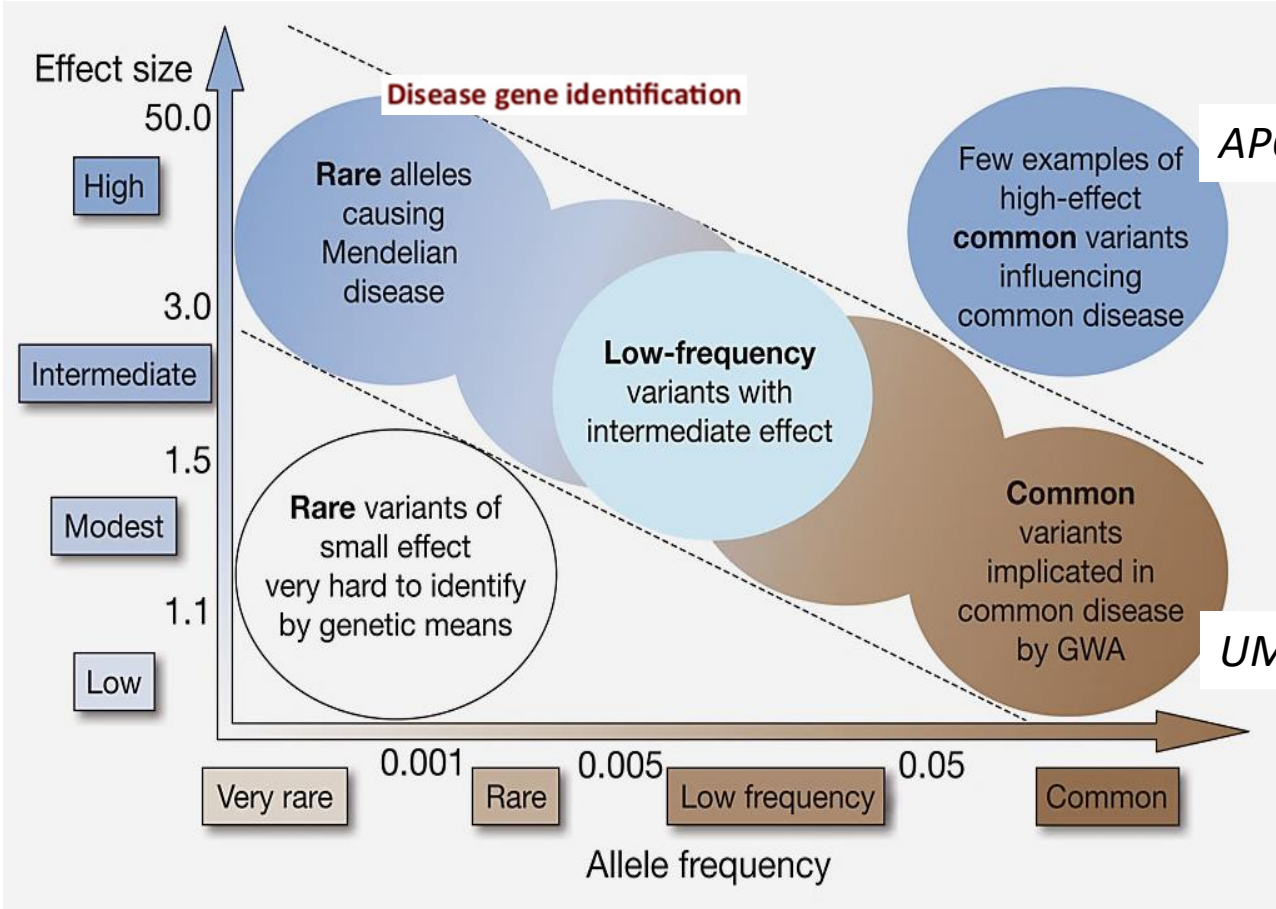
DNA sequencing

- Single-gene Sanger sequencing
- Next Generation Sequencing
  - Targeted sequencing
  - Whole exome sequencing (WES)
  - [Whole genome sequencing (WGS)]

Deletion/duplication analysis

- Multiplex ligation-dependent probe amplification (MLPA)
- Quantitative polymerase chain reaction
- Targeted chromosomal microarray

Feasibility of identifying genetic variants by risk allele frequency and strength of genetic effect (odds ratio)



APOL1

UMOD; CUBN

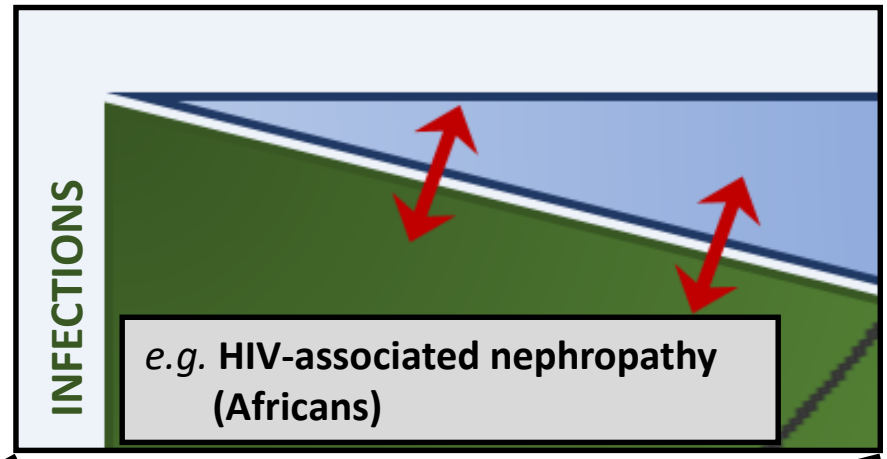
Disease gene identification

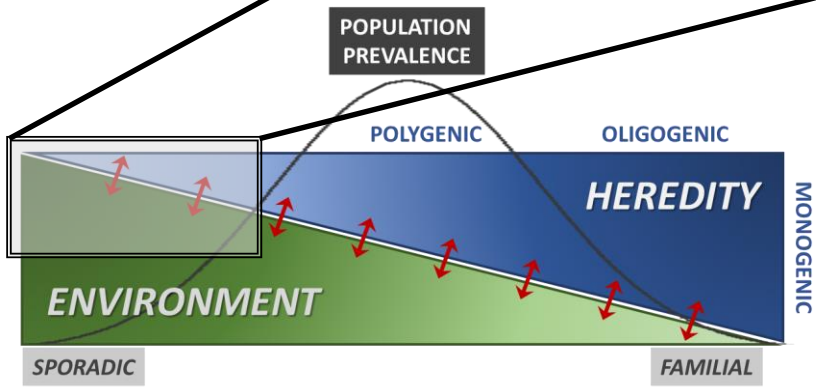
1. Linkage studies
2. Homozygosity mapping
3. Exome & whole genome sequencing
4. Genome-wide association studies
5. Detection of structural variation
6. Transcriptomics

Manolio TA *et al.*: Finding the missing heritability of complex diseases. *Nature* 2010;461:747-753.

Elucidating the role of genetic modulation of environmental kidney diseases

Genetic modulation of environmental kidney diseases: HIV-associated nephropathy in Africans





Nat Genet. 2008 Oct;40(10):1175-84. doi: 10.1038/ng.226. Epub 2008 Sep 14.

MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis.

Kopp JB[1], Smith MW, Nelson GW, Johnson RC, Freedman BI, Bowden DW, Oleksyk T, McKenzie LM, Kajiyama H, Ahuja TS, Berns JS, Briggs W, Cho ME, Dart RA, Kimmel PL, Korbet SM, Michel DM, Mokrzycki MH, Schelling JR, Simon E, Trachtman H, Vlahov D, Winkler CA.

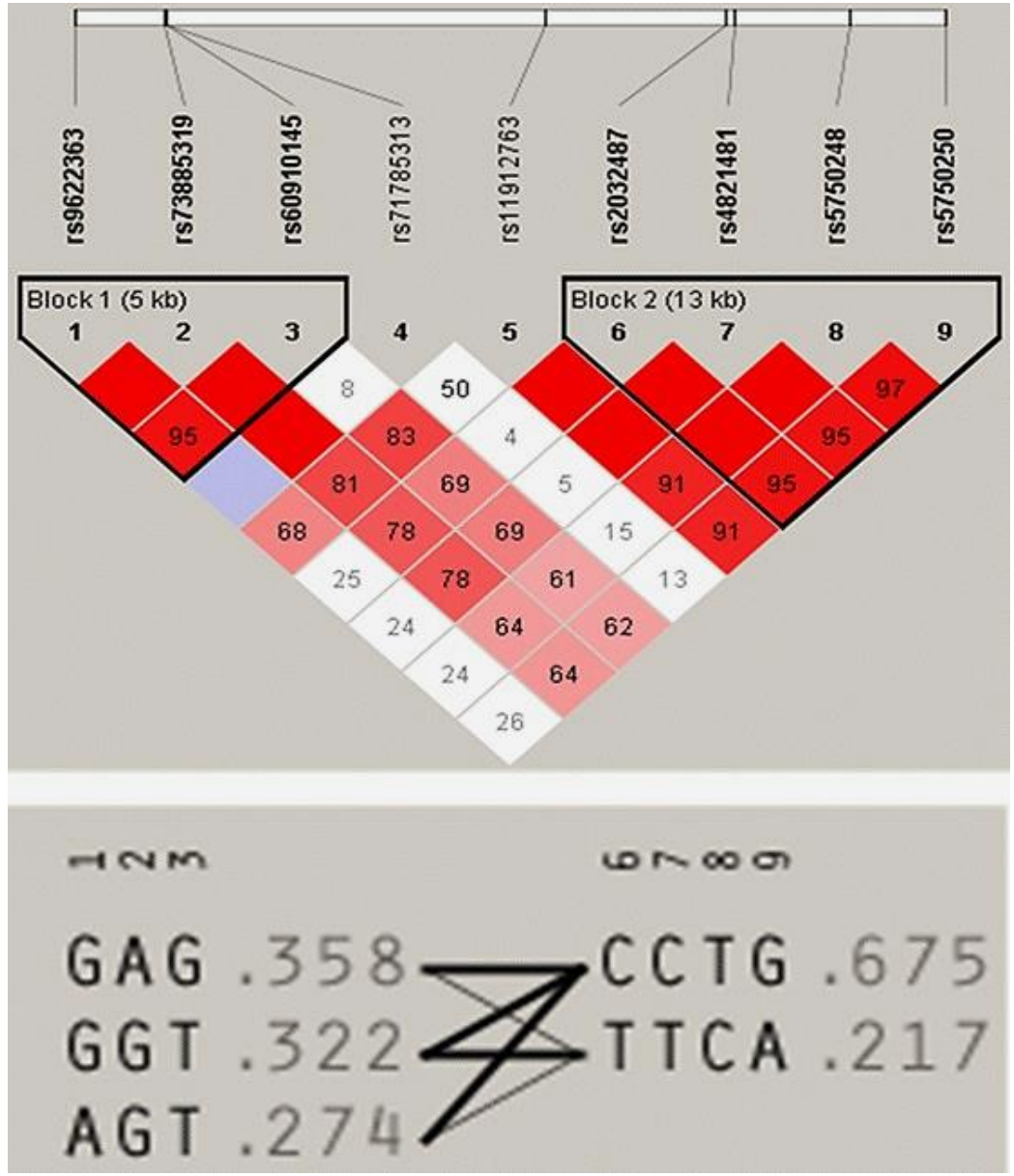
Author information

[1]Kidney Disease Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA.

Abstract

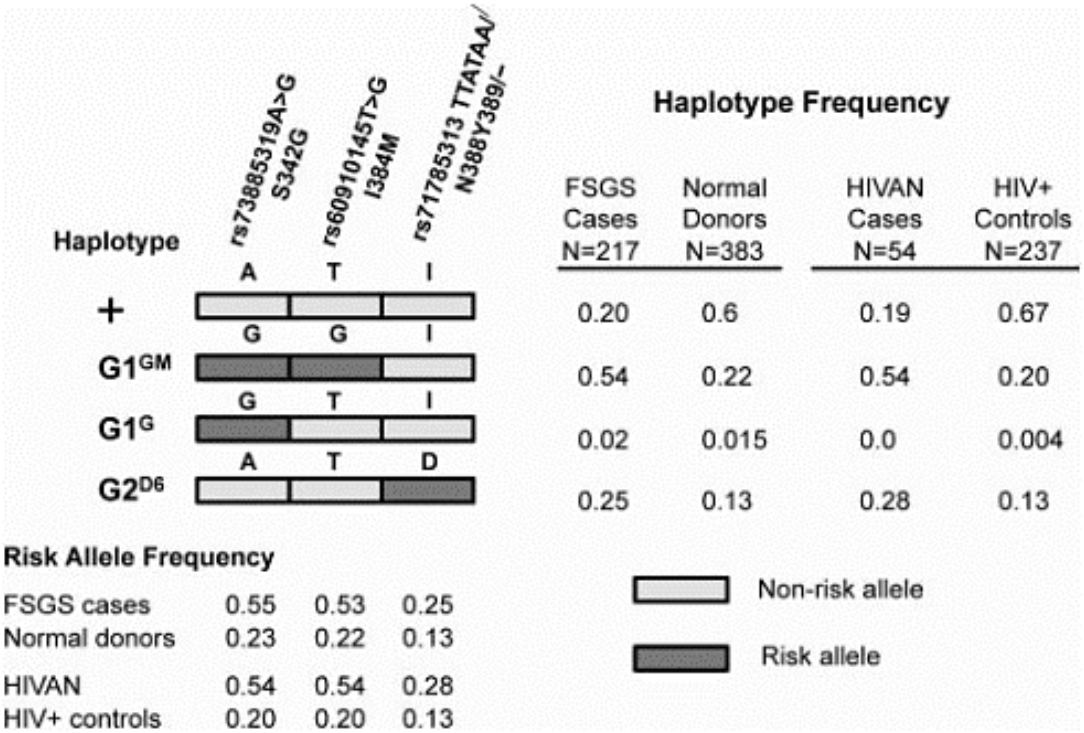
The increased burden of chronic kidney and end-stage kidney diseases (ESKD) in populations of African ancestry has been largely unexplained. To identify genetic variants predisposing to idiopathic and HIV-1-associated focal segmental glomerulosclerosis (FSGS), we carried out an admixture-mapping linkage-disequilibrium genome scan on 190 African American individuals with FSGS and 222 controls. We identified a chromosome 22 region with a genome-wide logarithm of the odds (lod) score of 9.2 and a peak lod of 12.4 centered on MYH9, a functional candidate gene expressed in kidney podocytes. Multiple MYH9 SNPs and haplotypes were recessively associated with FSGS, most strongly a haplotype spanning exons 14 through 23 (OR = 5.0, 95% CI = 3.5-7.1; P = 4 x 10(-23), n = 852). This association extended to hypertensive ESKD (OR = 2.2, 95% CI = 1.5-3.4; n = 433), but not type 2 diabetic ESKD (n = 476). Genetic variation at the MYH9 locus substantially explains the increased burden of FSGS and hypertensive ESKD among African Americans.

Plot of linkage disequilibrium between single nucleotide polymorphisms in the *APOL1/MYH9* gene and their haplotypes



Tayo BO *et al*.: Genetic variation in *APOL1* and *MYH9* genes is associated with chronic kidney disease among Nigerians. *Int Urol Nephrol* 2013;45:485-494.

Three *APOL1* allelic variants are strongly associated with HIV-related collapsing glomerulopathy



In a recessive model, APOL1 variants conferred 17-fold higher odds for FSGS and 29-fold higher odds for HIVAN.

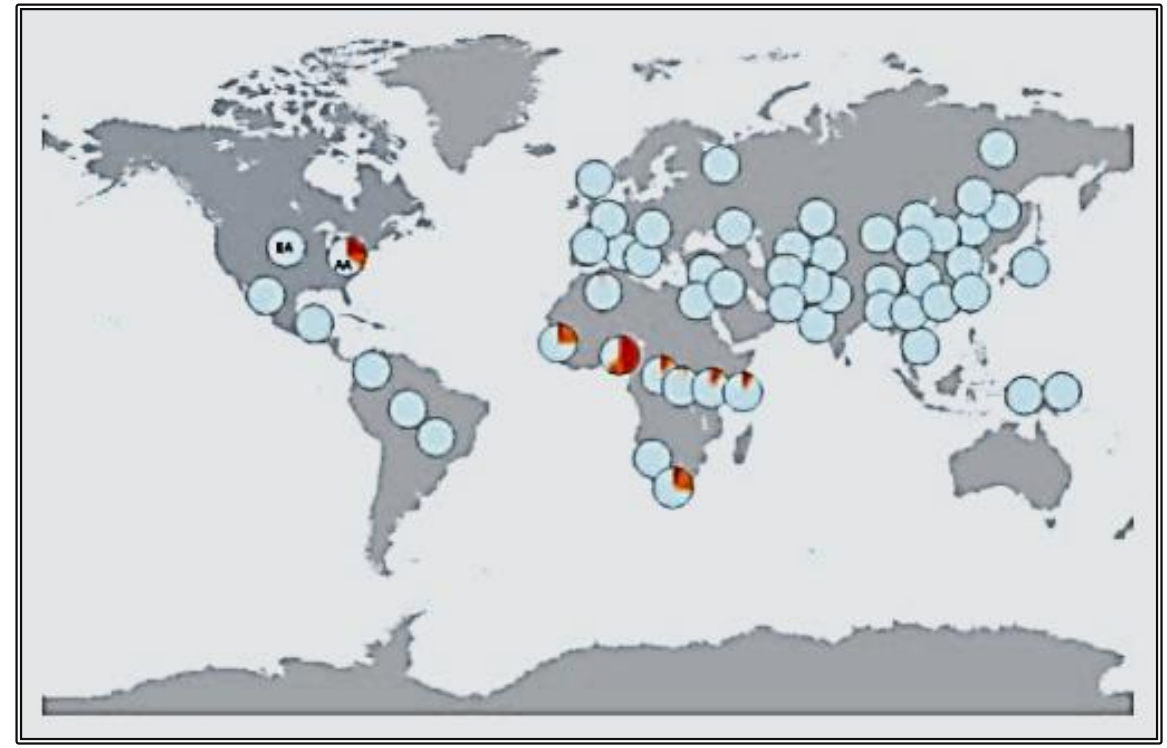
Distribution of haplotypes and risk alleles.

Four APOL1 haplotypes were observed in the African American study group: the major haplotype, denoted as (+) and comprised of three nonrisk alleles; the $G1^{GM}$ haplotype with two missense risk alleles; the rare $G1^{G}$ haplotype with one missense risk allele; and the $G2^{D6}$ haplotype with the 6 bp deletion risk allele. The risk allele and haplotype frequencies are shown for FSGS and HIVAN case and control groups in African Americans, including healthy blood donors and HIV individuals with no kidney disease.

FSGS, focal segmental glomerulosclerosis; HIV, human immunodeficiency virus; HIVAN, HIV-associated nephropathy.

Kopp JB *et al.*: *APOL1* genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol* 2011;22:2129-2137.

Worldwide frequency distribution of the *APOL1* variants associated with increased risk of idiopathic focal segmental glomerulosclerosis, HIV-associated nephropathy, and nondiabetic end-stage renal disease in African Americans

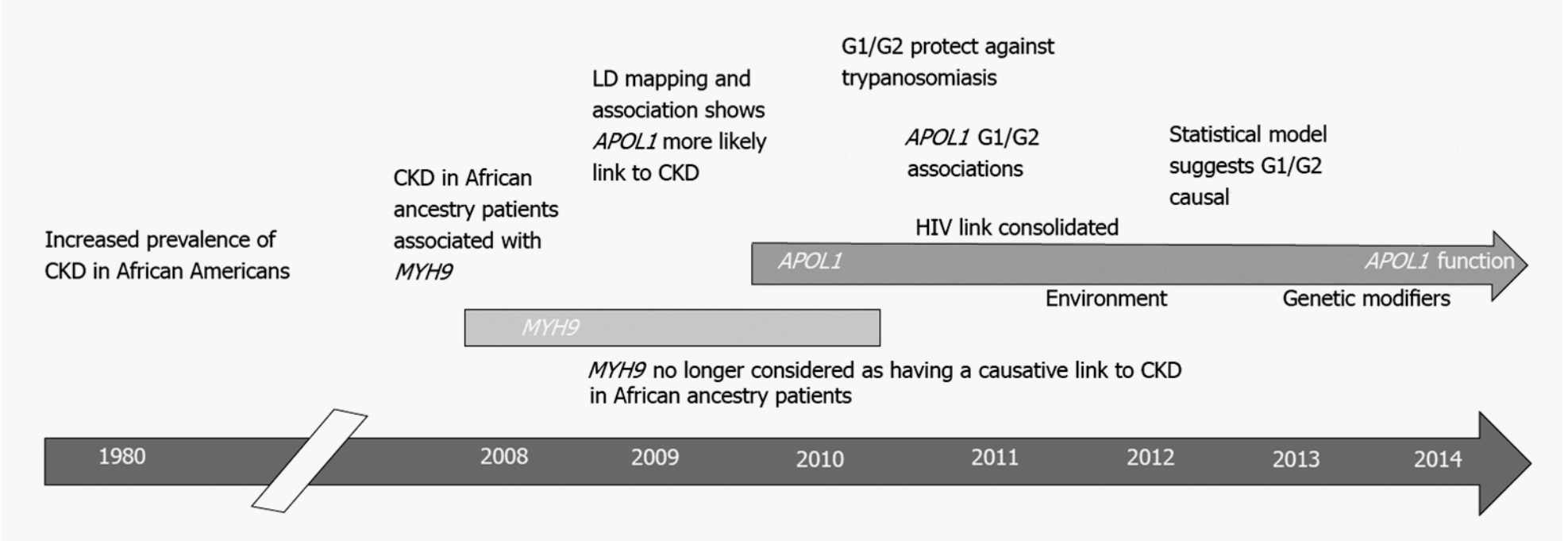


Genotypes of G1 and G2 were determined for 54 diverse human populations including African Americans (AA) and European Americans (EA). The allele frequencies of G1 (red), G2 (orange), and wild-type alleles (light blue) in each population are depicted in pie charts overlaid upon a world map.

HIV, human immunodeficiency virus.

Kopp JB *et al.*: *APOL1* genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol* 2011;22:2129-2137.

Historical timeline reflecting the discovery of genetic association to chronic kidney disease in populations with African ancestry

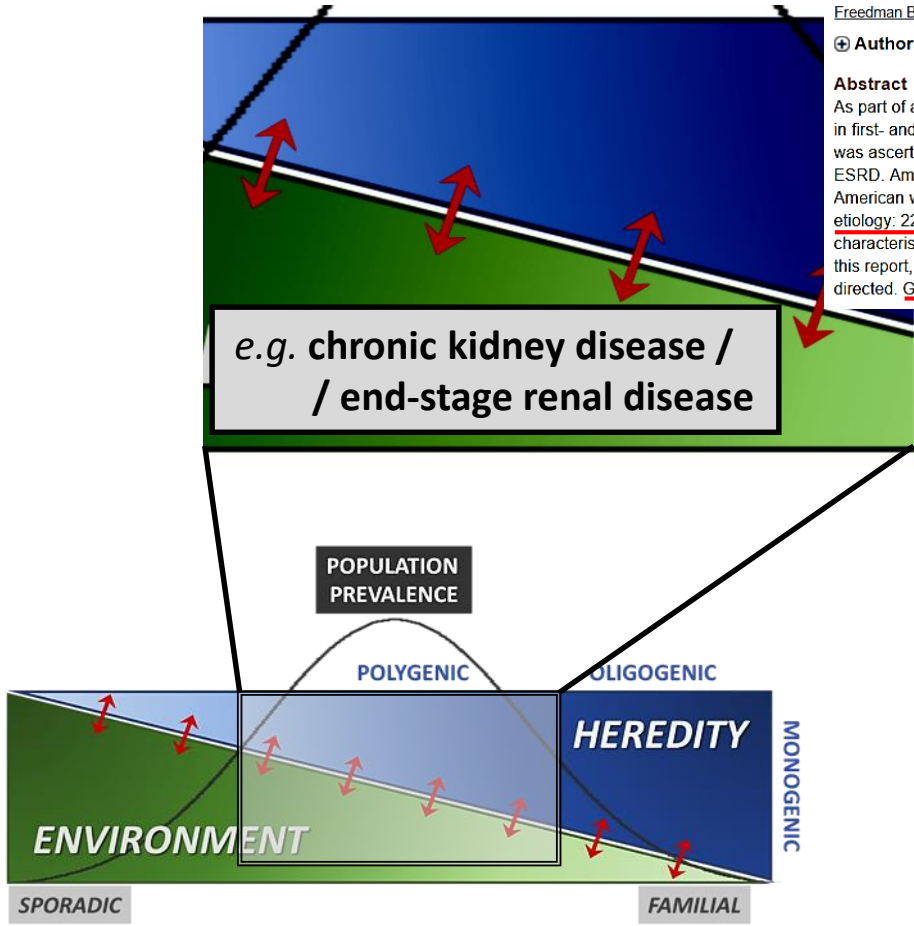


CKD: chronic kidney disease; HIV: human immunodeficiency virus; LD: linkage disequilibrium; APOL1: apolipoprotein L1 gene; G1/G2: APOL1 allelic variants; MYH9: non-muscle myosin heavy chain 9 gene.

Modified from Kasembeli NA *et al*.: African origins and chronic kidney disease susceptibility in the human immunodeficiency virus era. *World J Nephrol* 2015;4:295-306.

Elucidating the polygenic contribution to kidney function and multifactorial kidney diseases

Family history of end-stage renal disease among incident dialysis patients



J Am Soc Nephrol. 1997 Dec;8(12):1942-5.

Family history of end-stage renal disease among incident dialysis patients.

Freedman BI[1], Soucie JM, McClellan WM.

⊕ Author information

Abstract

As part of a larger study of genetic risk factors for the occurrence of renal failure, the prevalence of a family history of end-stage renal disease (ESRD) in first- and second-degree relatives of all incident dialysis patients treated in Georgia, North Carolina, and South Carolina (ESRD Network 6) in 1994 was ascertained. Family histories were obtained from 4365 dialysis patients (83% of those eligible), and 856 (20%) reported having a family history of ESRD. Among race-sex groups, 14.1% of Caucasian men, 14.6% of Caucasian women, 22.9% of African-American men, and 23.9% of African-American women reported a first- or second-degree relative with ESRD (P = 0.001). The prevalence of relatives with ESRD varied by the reported etiology: 22.2% in diabetes mellitus; 18.9% in hypertension, 22.7% in glomerulonephritis; and 13.0% of other etiologies (P = 0.001). Patient characteristics independently associated with family history of ESRD included race, younger age, higher levels of education, and etiology of ESRD. In this report, it is concluded that a large proportion of incident ESRD cases have close relatives with ESRD in whom preventive actions might be directed. Genetic analyses in multiply affected families may identify the inherited factors contributing to progressive renal failure.

Prevalence of Stages of Chronic Kidney Disease and Levels of Kidney Function in the US

	Stages of CKD		Levels of Kidney Function		
	N (1000's)*	(%)	GFR (mL/min/1.73 m²)	N (1000's)*	(%)
1	10,500[a] 5,900	5.9[a] 3.3	≥90	114,000	64.3
2	7,100[a] 5,300	4.0[a] 3.0	60–89	55,300	31.2
3	7,600	4.3	30–59	7,600	4.3
4	400	0.2	15–29	400	0.2
5	300	0.2	<15 (or dialysis)	300	0.2

* Data for Stages 1–4 from NHANES III (1988–1994). Population of 177 million with age ≥20 years. Data for Stage 5 from USRDS (1998),[2] includes approximately 230,000 patients treated by dialysis, and assumes 70,000 additional patients not on dialysis. Percentages total >100% because NHANES III may not have included patients on dialysis. GFR estimated from serum creatinine using MDRD Study equation based on age, gender, race and calibration for serum creatinine.

[a] For Stages 1 and 2, kidney damage was assessed by spot albumin-to-creatinine ratio >17 mg/g (men) or >25 mg/g (women) on one occasion (larger prevalence estimate) or on two measurements (smaller prevalence estimate). Albuminuria was persistent in 54% of individuals with GFR ≥90 mL/min/1.73 m² (n = 102) and 73% of individuals with GFR 60–89 mL/min/1.73 m² (n = 44).

US: United States.

[http://www2.kidney.org/professionals/kdoqi/guidelines_ckd/p4_class_g1.htm]

Familial aggregation is evidence for a genetic component to end-stage renal disease

Year / US State	Total number of patients*	Patients providing family history information	Patients reporting having family history of ESRD**
1995	4,328	3,205 (74.05%)	671 (20.94%)
1996	5,468	3,618 (66.17%)	790 (21.84%)
1997	5,842	3,475 (59.48%)	772 (22.22%)
1998	6,307	3,249 (51.51%)	729 (22.44%)
1999	6,650	2,887 (43.41%)	645 (22.34%)
2000	7,201	3,070 (42.63%)	699 (22.77%)
2001	7,506	2,846 (38.92%)	682 (23.96%)
2002	7,418	2,225 (29.99%)	593 (26.65%)
Total:	50,720	24,575 (45.45%)	5,581 (22.71%)

US: United States. ESRD: end-stage renal disease.
(*) Incident dialysis patients without a primary ESRD diagnosis attributed to Mendelian diseases or urological causes.
(**) Family history of ESRD in first- and second-degree relatives.

Freedman BI *et al.*: Population-based screening for family history of end-stage renal disease among incident dialysis patients. *Am J Nephrol* 2005;25:529-535.

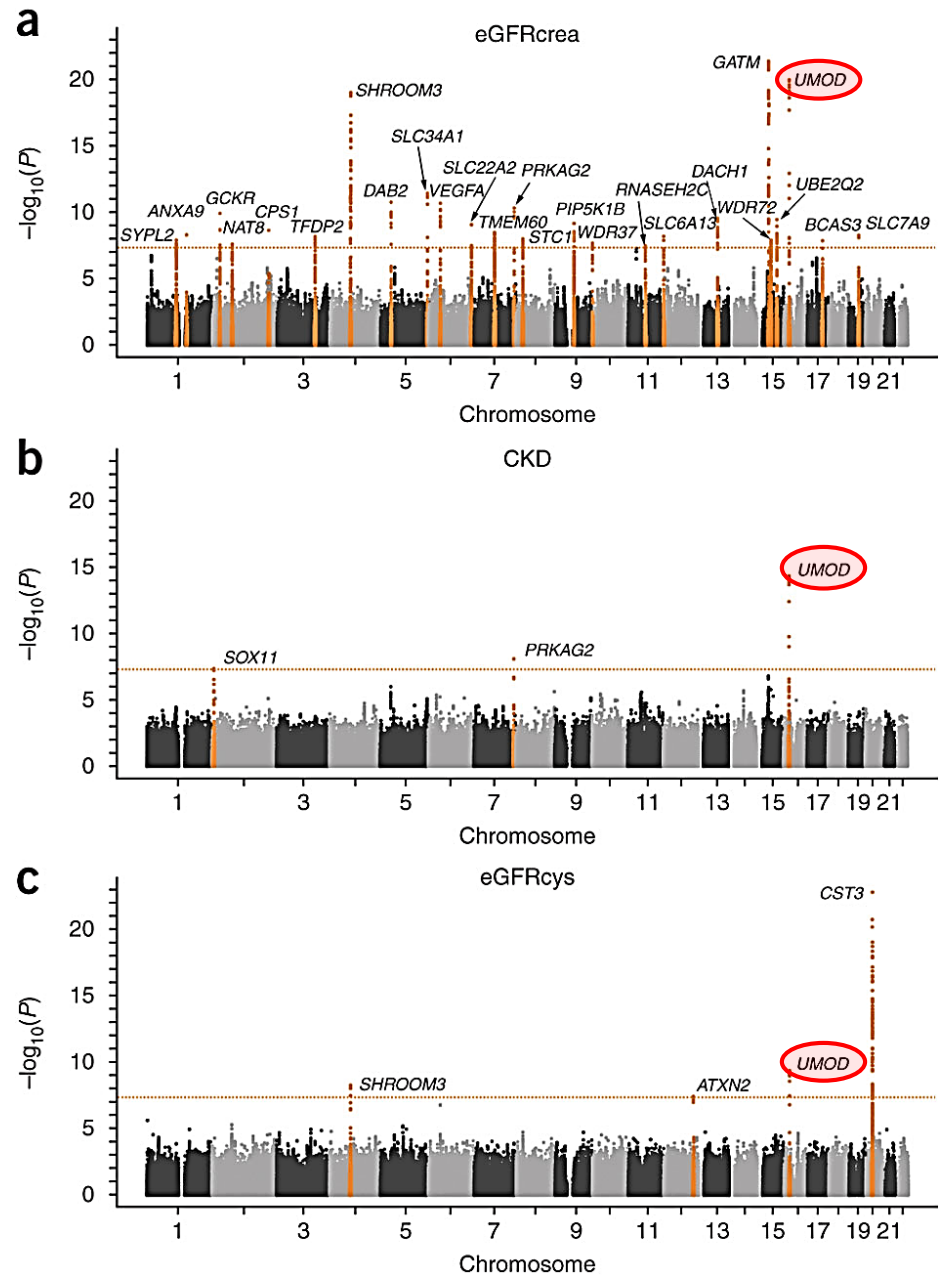
New susceptibility loci for reduced renal function identified by meta-analysis of genome-wide association studies in individuals of European ancestry

Manhattan plots showing the significance levels for each of the single nucleotide polymorphisms (SNPs) tested. The SNP locations on the plot reflect their position across the 23 human chromosomes. The dotted line indicates the genome-wide significance threshold at $P = 5 \times 10^{-8}$.

a. eGFRcrea – Glomerular Filtration Rate estimated from serum creatinine

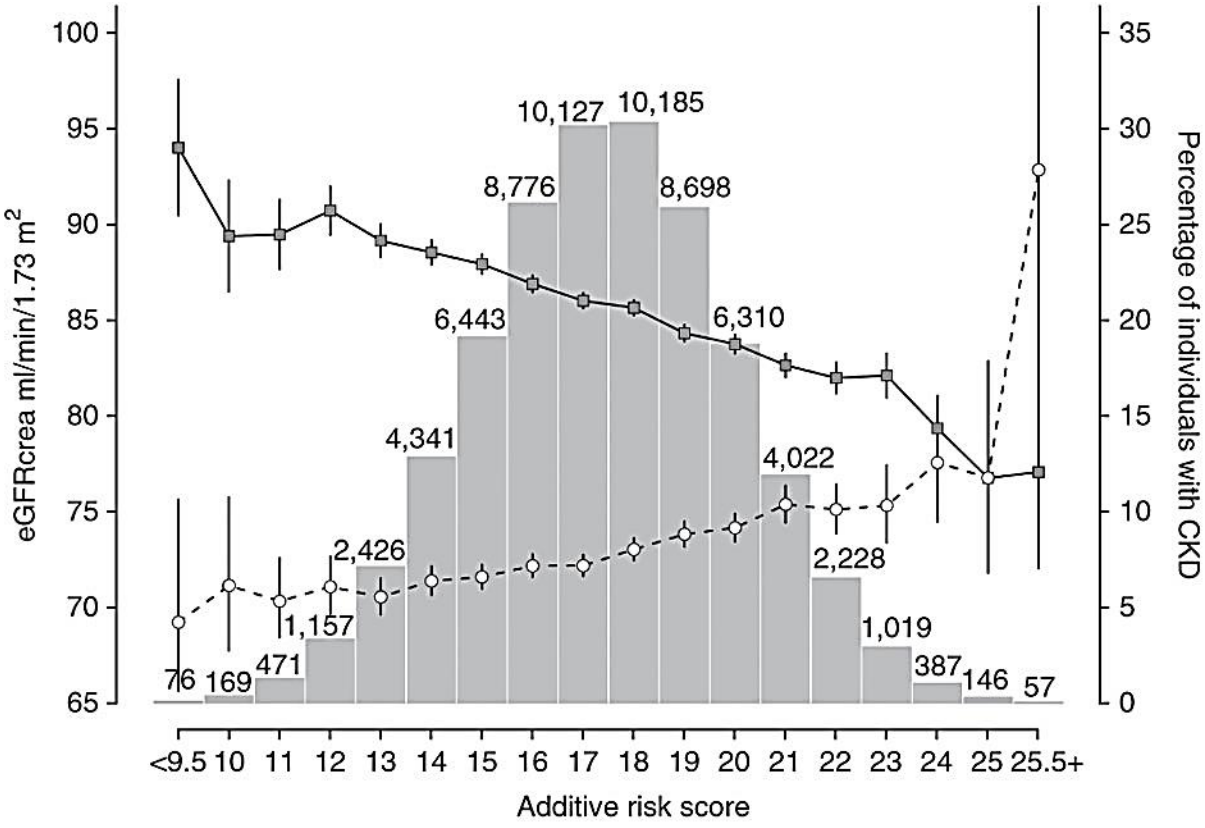
b. CKD – Chronic Kidney Disease

c. eGFRcys – Glomerular Filtration Rate estimated from serum cystatin.



Köttgen A *et al.*: New loci associated with kidney function and chronic kidney disease. *Nat Genet* 2010;42:376-384.

Distribution of the additive genetic risk score and mean estimated glomerular filtration rate and chronic kidney disease prevalence per risk score category



Köttgen A *et al.*: New loci associated with kidney function and chronic kidney disease. *Nat Genet* 2010;42:376-384.

The additive risk score was calculated by summing the dosages of estimated glomerular filtration rate (eGFR)-lowering alleles of the 16 single nucleotide polymorphisms identified in a meta-analysis of genome-wide association data from 20 predominantly population-based studies, which enrolled 67,093 individuals of European ancestry.

Grey squares indicate mean eGFR calculated from serum creatinine (eGFRcrea); white circles indicate chronic kidney disease (CKD) prevalence. Error bars represent 95% confidence intervals. The number of individuals in each risk score category is indicated.

Exemplary types of monogenic contributions to causation of kidney diseases

Rough numbers for three kidney phenotypes and three specific genetic loci found to influence these phenotypes

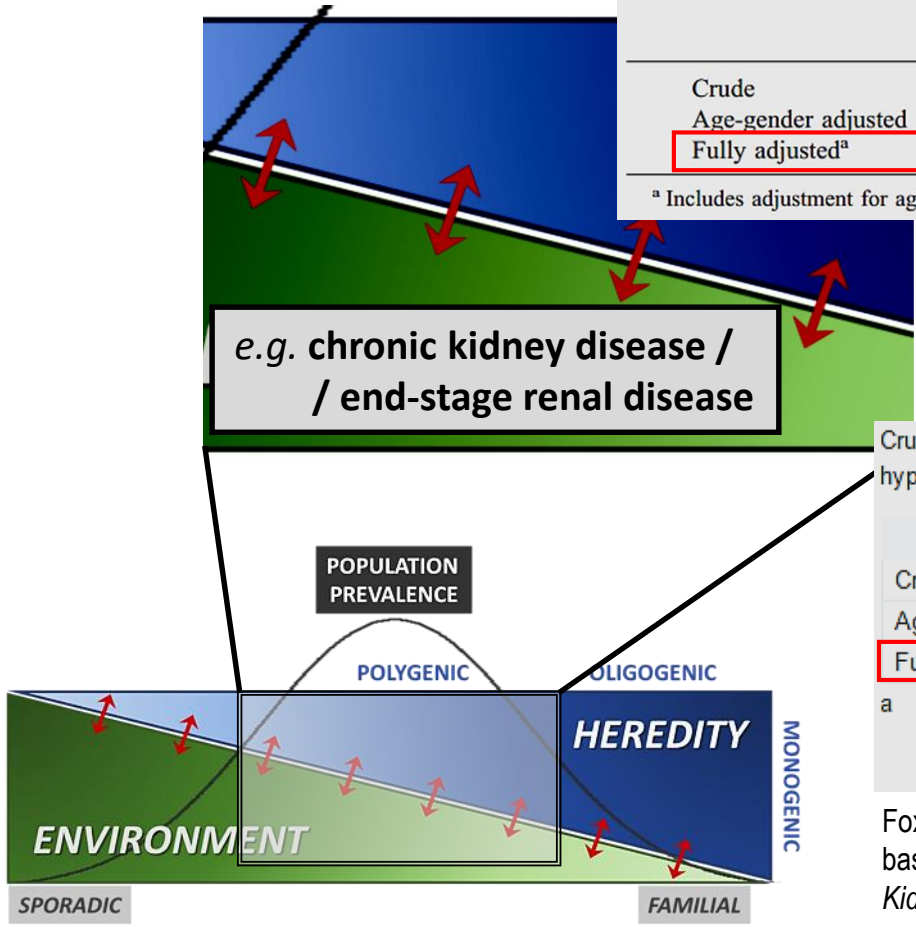
Phenotype	Gene variant example	Risk allele frequency	High-risk genotype frequency	Relative risk
CKD	*UMOD*	0.80	0.96[A]	1.25[B]
H-ESRD and FSGS	*APOL1*	0.33	0.11	7–10[C]
PKD	*PKD1*	0.001[D]; negligible[E]	0.001[D]; negligible[E]	~1,000

[A]High-risk genotypes combined. [B]Additive model. [C]Recessive model. [D]For any disease-causing mutation. [E]For any one specific mutation

CKD: chronic kidney disease; H-ESRD: hypertension-associated end-stage renal disease; FSGS: focal segmental glomerulosclerosis; PKD: polycystic kidney disease; UMOD: uromodulin gene; APOL1 apolipoprotein L1 gene; PKD1: polycystin-1 gene.

Friedman DJ & Pollak MR: Genetics of kidney failure and the evolving story of *APOL1*. *J Clin Invest 2011;121:3367-3374.*

Heritability of renal function measures and of the urinary albumin excretion



Heritability results with 95% confidence intervals for serum creatinine, GFR, and creatinine clearance

	Serum Creatinine	GFR	Creatinine Clearance
Crude	0.21 (0.08 to 0.34)	0.36 (0.23 to 0.50)	0.72 (0.57 to 0.85)
Age-gender adjusted	0.29 (0.15 to 0.43)	0.31 (0.18 to 0.45)	0.56 (0.42 to 0.69)
Fully adjusted[a]	0.29 (0.15 to 0.44)	0.33 (0.19 to 0.47)	0.46 (0.32 to 0.60)

[a] Includes adjustment for age, gender, body mass index, HDL cholesterol, diabetes, systolic BP, HTN treatment, and current smoking.

Fox CS *et al*.: Genomewide linkage analysis to serum creatinine, GFR, and creatinine clearance in a community-based population: the Framingham Heart Study. *J Am Soc Nephrol* 2004;15:2457-2461.

Crude, age-adjusted, and multivariable-adjusted heritability estimates in the overall sample and hypertension-enriched sample

	Overall sample	Hypertension-enriched sample
Crude	0.20	0.25
Age-adjusted	0.17	0.20
Fully adjusted[a]	0.16	0.20

a Heritability for gender-specific residuals for urinary albumin/creatinine ratio, adjusted for age, body mass index, tobacco use, diabetes, systolic blood pressure, hypertension treatment, and serum creatinine.

Fox CS *et al*.: Genome-wide linkage analysis to urinary microalbuminuria in a community-based sample: the Framingham Heart Study. *Kidney Int* 2005;67:70-74.

The mysterious case of the "missing heritability" in the genome-wide association studies

- Heritability estimates for complex diseases may be inflated due to methodological problems.
- The single nucleotide polymorphism (SNP) sets used in current genome-wide association studies (GWAS) offer poor tagging, especially for rare variants and structural variations; this would both reduce the number of associations detected, and underestimate the true effect sizes of the detected loci.
- Many susceptibility loci simply have very small effect sizes, so that many have not been detected due to the inadequate statistical power of current studies.
- Gene-gene and gene-environment interactions account for a substantial portion of the heritability estimates, but these interactions have been largely neglected in GWAS do date.

Sham PC & Cherry SS: Genetic architecture of complex diseases.
In Zeggini E & Morris A (Eds.), *Analysis of Complex Disease Association Studies: A Practical Guide*, (1st ed., pp 1-13); Academic Press, London, 2011.

Identifying monogenic causes of kidney disease; understanding the underlying pathobiology, reviewing nosology and improving phenotypic characterization; developing new therapies

Degree of genetic causality in mono- and poly-genic kidney diseases

Feature	Monogenic recessive diseases	Monogenic dominant diseases	Polygenic and/or complex diseases
Penetrance	Full	Full or incomplete	Low
Predictive power of a mutation	Almost 100%	High	Low
Onset	Predominantly during childhood	Childhood or adulthood	Predominantly during adulthood
Disease frequency	Low	Low	High
Number of affected individuals needed for gene discovery	Few	Few	Hundreds to thousands
Gene mapping approaches	Homozygosity mapping* or linkage analysis	Linkage analysis	GWAS
WES or WGS	In consanguinity, single affected families are sufficient	WES in distant relatives to minimize shared variants	NA
Functional analysis in animal models (mice, zebrafish)	Easily feasible (gene knockdown, knockout)	Feasible	Difficult

GWAS, genome-wide association studies;
NA, not applicable;
WES, whole exome sequencing;
WGS, whole genome sequencing.
**Applicable to consanguineous families.*

Vivante A & Hildebrandt F. Exploring the genetic basis of early-onset chronic kidney disease. *Nat Rev Nephrol* 2016;12:133-146.

Monogenic and oligogenic kidney disorders

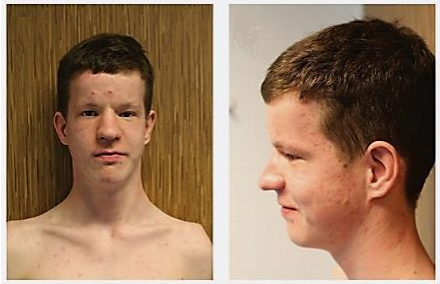
European Journal of Human Genetics (2015), 1–8

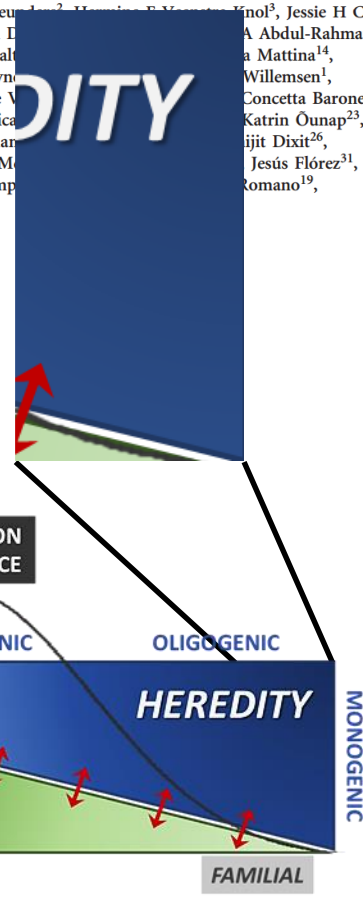
www.nature.com/ejhg

ARTICLE

The Koolen-de Vries syndrome: a phenotypic comparison of patients with a 17q21.31 microdeletion *versus* a *KANSL1* sequence variant

David A Koolen*,[1], Rolph Pfundt[1], Katrin Linda[1], Gea Beu… …[3], Jessie H Conta[4], Ana Maria Fortuna[5], Gabriele Gillessen-Kaesbach[6], Sarah D… …A Abdul-Rahman[9], Heather M Winesett[10], Wendy K Chung[11], Marguerite Dalt… …a Mattina[14], …Willemsen[1], …Concetta Barone[19], …Katrin Õunap[23], …ijit Dixit[26], … Jesús Flórez[31], …Romano[19],





e.g. **Ciliopathies**

http://www.kidney-international.org meeting report

Autosomal dominant tubulointerstitial kidney disease: diagnosis, classification, and management—A KDIGO consensus report

Kai-Uwe Eckardt[1], Seth L. Alper[2], Corinne Antignac[3,4], Anthony J. Bleyer[5], Dominique Chauveau[6], Karin Dahan[7], Constantinos Deltas[8], Andrew Hosking[9], Stanislav Kmoch[10], Luca Rampoldi[11], Michael Wiesener[1], Matthias T. Wolf[12] and Olivier Devuyst[13]

e.g. **Autosomal Dominant Tubulointerstitial Kidney Disease [genes: *UMOD* / *MUC1* / *REN* / *HNF1B* / other(s)]**

ARTICLE

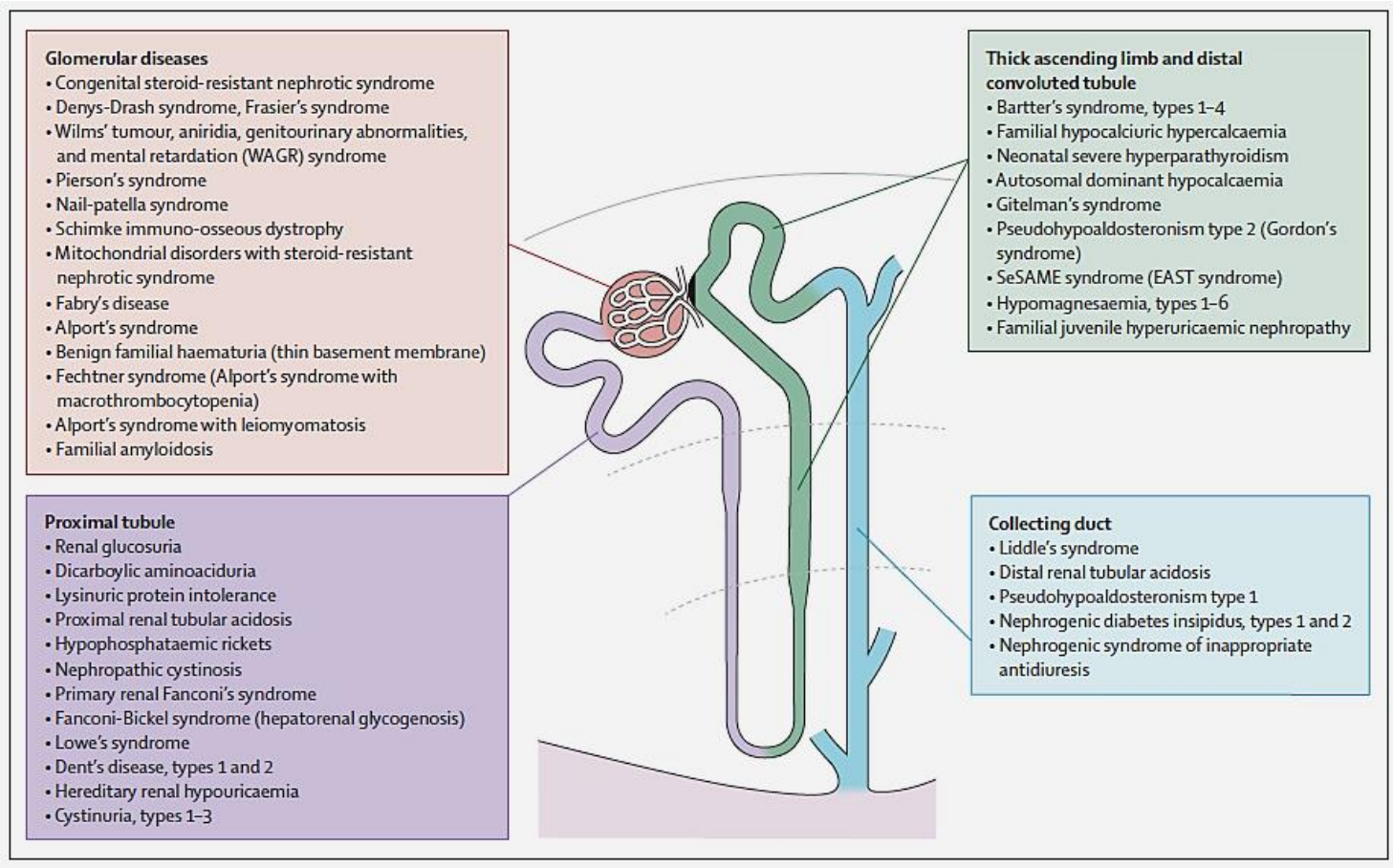
Mutations in *GANAB*, Encoding the Glucosidase IIα Subunit, Cause Autosomal-Dominant Polycystic Kidney and Liver Disease

Binu Porath,[1,16] Vladimir G. Gainullin,[1,16] Emilie Cornec-Le Gall,[1,2,3] Elizabeth K. Dillinger,[4] Christina M. Heyer,[1] Katharina Hopp,[1,5] Marie E. Edwards,[1] Charles D. Madsen,[1] Sarah R. Mauritz,[1] Carly J. Banks,[1] Saurabh Baheti,[6] Bharathi Reddy,[7] José Ignacio Herrero,[8,9,10] Jesús M. Bañales,[11] Marie C. Hogan,[1] Velibor Tasic,[12] Terry J. Watnick,[13] Arlene B. Chapman,[7] Cécile Vigneau,[14] Frédéric Lavainne,[15] Marie-Pierre Audrézet,[2] Claude Ferec,[2] Yannick Le Meur,[3] Vicente E. Torres,[1] Genkyst Study Group, HALT Progression of Polycystic Kidney Disease Group, Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease, and Peter C. Harris[1,4,*]

CrossMark The American Journal of Human Genetics *98*, 1193–1207, June 2, 2016 1193

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key	Gene/Locus	Gene/Locus MIM number
11q12.3	Polycyctic kidney disease 3	600666	AD	3	GANAB	104160

Inherited kidney disorders linked to nephron segments



Segmental distribution of rare inherited diseases of the kidney (does not include cystic and developmental disorders).

Urinalysis might point to the segmental origin of some kidney disorders. For example, glomerular diseases are usually characterised by albuminuria and dysmorphic red blood cells in urine; disorders of the proximal tubule by inappropriate urinary loss of low-molecular-weight proteins (eg, Clara Cell protein, β2-microglobulin, and vitamin D-binding protein), aminoacids, glucose, phosphate, uric acid, and calcium; disorders of the thick ascending limb by hypercalciuria and urinary concentrating defects; disorders of the distal convoluted tubule by inappropriate urinary loss of magnesium; and disorders of the collecting duct by inappropriate urinary concentration or dilution and defective potassium handling.

Devuyst O *et al*.: Rare inherited kidney diseases: challenges, opportunities, and perspectives. *Lancet* 2014;383:1844-1859.

Causes and genetic diagnosis of early-onset CKD

Diagnostic group	Indication to run a gene panel	Proportion of cases of early-onset CKD	Number of known causative genes	Percentage of cases caused by known genes (multiplied by fraction of all CKD)
CAKUT	CAKUT evident by renal imaging	49.1% (obstructive uropathy 20.7%; renal aplasia, hypoplastic or dysplastic kidneys 17.3%; reflux nephropathy 8.4%; prune belly syndrome 2.7%)	36	~17% (8.5%)*
SRNS	SRNS	10.4% (FSGS 8.7%; congenital nephrotic syndrome 1.1%; membranous nephropathy 0.5%; Denys–Drash syndrome 0.1%)	39	~30% (3%)
Chronic GN‡	Evidence of proteinuria and haematuria	8.1% (SLE nephritis 1.6%; familial nephritis (Alport syndrome) 1.6%; chronic GN 1.2%; MPGN type I 1.1%; MPGN type II 0.4%; IgAN 0.9%; idiopathic cresentic GN 0.7%; Henoch–Schönlein nephritis 0.6%)	10	~20% (4%)
Renal cystic ciliopathies	Increased echogenicity on renal ultrasound or presence of ≥2 renal cysts	5.3% (polycystic kidney disease 4.0%; medullary cystic kidney disease 1.3%)	95	~70% (3.7%)
aHUS	Microangiopathic haemolytic anaemia, thrombocytopaenia, and AKI	2.0%	9	~60% (1.2%)
Nephrolithiasis or nephrocalcinosis	Known stone disease or nephrocalcinosis	1.6% (cystinosis 1.5%; oxalosis 0.1%)	30	21% (0.4%)
Other	Other indications of genetic disease	23.5% (renal infarct 2.2%; pyelonephritis or interstitial nephritis 1.4%; Wilms tumour 0.5%; other systemic immunologic diseases 0.4%; granulomatosis with polyangiitis 0.4%; sickle cell nephropathy 0.2%; diabetic glomerulopathy 0.2%; other nonimmunologic causes 18.2%)	Not known	Not known
Total	—	100%	~219	(~20%)

Data are from the 2006 Annual Report of the North American Pediatric Renal Trials and Collaborative Studies.

aHUS; atypical haemolytic uraemic syndrome;
AKI, acute kidney injury; CAKUT, congenital anomalies of the kidneys and urinary tract; CKD, chronic kidney disease; FSGS, focal segmental glomerulosclerosis;
GN, glomerulonephritis; IgAN, IgA nephropathy; MPGN, membranoproliferative glomerulonephritis; SLE, systemic lupus erythematosus; SRNS, steroid-resistant nephrotic syndrome.
**10% of CAKUT can be caused by deleterious copy number variants.*
‡The estimates for chronic nephritis monogenic aetiologies are based only on the relative prevalence of Alport syndrome and MPGN, which together account for 20% of the aetiologies of chronic GN and for which a monogenic cause has been established in almost 100% of cases (in one of the following genes: Alport: COL4A3, COL4A4, COL4A5 and COL4A6; MPGN: Factor H, Factor I, MCP/CD46, CFHR5 and C3).

Vivante A & Hildebrandt F. Exploring the genetic basis of early-onset chronic kidney disease. *Nat Rev Nephrol* 2016;12:133-146.

Expanding clinical phenotype characterization by "reverse phenotyping"

Pediatr Nephrol. 2009 Dec;24(12):2369-73. doi: 10.1007/s00467-009-1299-2.

Dent's disease manifesting as focal glomerulosclerosis: Is it the tip of the iceberg?

Frishberg Y[1], Dinour D, Belostotsky R, Becker-Cohen R, Rinat C, Feinstein S, Navon-Elkan P, Ben-Shalom E.

Author information

Abstract

Dent's disease is an X-linked proximal tubulopathy. It often manifests in childhood with symptoms of Fanconi syndrome and low-molecular-weight proteinuria. We describe four boys from three unrelated families whose only presenting symptoms of Dent's disease were nephrotic-range proteinuria and histological findings of focal segmental and/or global glomerulosclerosis. In all families, a causal mutation in the CLCN5 gene, encoding a voltage-gated chloride transporter and chloride-proton exchanger, was identified. All three mutations are pathogenic: two are novel (p.Asp727fs and p.Trp122X), and one is a recurrent mutation, p.R648X. Given the atypical phenotype of these patients with Dent's disease, it is possible that this clinical entity is markedly underdiagnosed and that our report represents only the tip of the iceberg. The diagnosis of Dent's disease should be considered in all patients with nephrotic-range proteinuria without hypoalbuminemia or edema. Establishing the diagnosis of Dent's disease will prevent the administration of unnecessary immunosuppressive medications with their undesirable side effects.

J Am Soc Nephrol. 2013 Jul;24(8):1216-22. doi: 10.1681/ASN.2013020171. Epub 2013 May 16.

LMX1B mutations cause hereditary FSGS without extrarenal involvement.

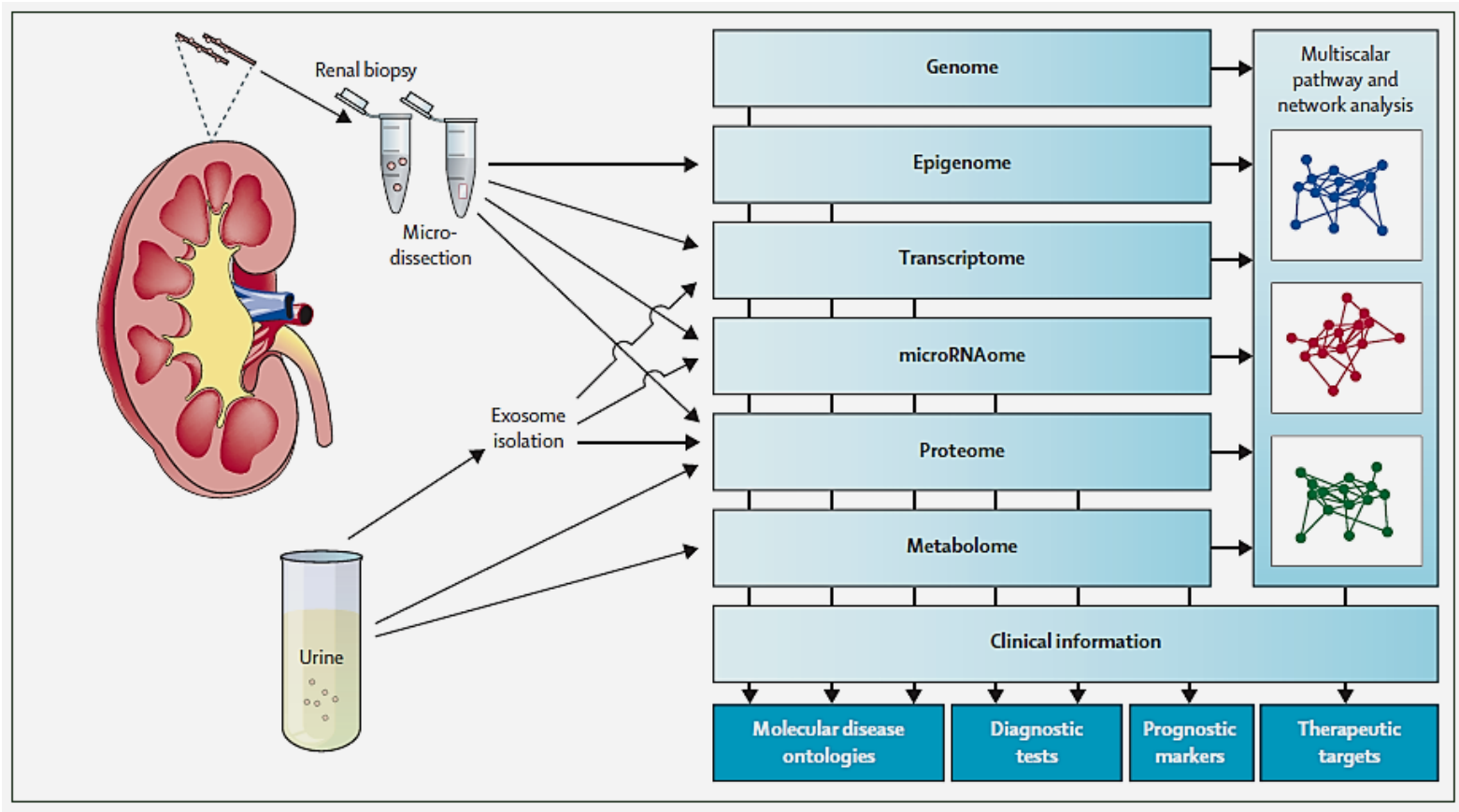
Boyer O[1], Woerner S, Yang F, Oakeley EJ, Linghu B, Gribouval O, Tête MJ, Duca JS, Klickstein L, Damask AJ, Szustakowski JD, Heibel F, Matignon M, Baudouin V, Chantrel F, Champigneulle J, Martin L, Nitschké P, Gubler MC, Johnson KJ, Chibout SD, Antignac C.

Author information

Abstract

LMX1B encodes a homeodomain-containing transcription factor that is essential during development. Mutations in LMX1B cause nail-patella syndrome, characterized by dysplasia of the patellae, nails, and elbows and FSGS with specific ultrastructural lesions of the glomerular basement membrane (GBM). By linkage analysis and exome sequencing, we unexpectedly identified an LMX1B mutation segregating with disease in a pedigree of five patients with autosomal dominant FSGS but without either extrarenal features or ultrastructural abnormalities of the GBM suggestive of nail-patella-like renal disease. Subsequently, we screened 73 additional unrelated families with FSGS and found mutations involving the same amino acid (R246) in 2 families. An LMX1B in silico homology model suggested that the mutated residue plays an important role in strengthening the interaction between the LMX1B homeodomain and DNA; both identified mutations would be expected to diminish such interactions. In summary, these results suggest that isolated FSGS could result from mutations in genes that are also involved in syndromic forms of FSGS. This highlights the need to include these genes in all diagnostic approaches to FSGS that involve next-generation sequencing.

Application of *omics* technologies in rare kidney diseases



Next-generation sequencing techniques and omics technologies, which can directly probe the kidney, will improve diagnostic efficiency for genetic renal diseases. Genomic studies and molecular profiling of kidney tissues, plain and exosome-enriched urine, and multiscalar bioinformatic analysis of crucial disease pathways, will allow the development of mechanistic renal disease ontologies, diagnostic tests, biomarkers, and novel therapeutic targets.

Devuyst O *et al.*: Rare inherited kidney diseases: challenges, opportunities, and perspectives. *Lancet* 2014;383:1844-1859.

Milestones in research of inherited kidney diseases

Milestones in nephrogenetics

1985 Mapping the first gene location for an inherited kidney disorder (autosomal dominant polycystic kidney disease, on chromosome 16)
1990 First detection of a point mutation at a specific locus single-gene disorder, *COL4A5*
1992 Molecular basis of nephrogenic diabetes insipidus described
1993 Identification of the tuberous sclerosis gene (*TSC2*)
1994 Cloning of the *PKD1* gene, responsible for about 85% of autosomal dominant polycystic kidney disease cases; challenging due to the size (46 exons) and complex organisation (presence of six highly homologous sequences of exons 1–33) of the gene on chromosome 16p13·3
1994 Liddle's syndrome reported to be due to activating mutation of the sodium channel ENaC
1996 Molecular basis for inherited kidney stone diseases identified
1996 Molecular basis of Bartter's and Gitelman's syndromes described
1996 Cloning of *PKD2*, the second gene involved in autosomal dominant polycystic kidney disease
1997 First nephronophthisis gene reported on
1998 Mutations in factor H reported to cause atypical haemolytic uraemic syndrome
1998 Molecular basis of cystinosis described
1999 Mutations in a paracellular protein (claudin-16) causes familial hypomagnesaemia with hypercalciuria
2000 Podocin (*NPHS2*) described as the major gene for steroid-resistant nephrotic syndrome
2001 Mutations in different genes shown to cause Bardet–Biedl syndrome (digenic inheritance)
2001 Mutations in WNK kinases shown to change regulation of sodium, potassium, and blood pressure
2002 Mutations in *UMOD* (Tamm-Horsfall protein) shown to cause familial juvenile hyperuricaemic nephropathy, an autosomal dominantly inherited form of interstitial nephritis
2005 Mutations in a cation channel (TRPC6) described to cause glomerular disease
2010 First success of exome sequencing in rare renal diseases (*SDCCA8* in Senior-Løken syndrome; retinal-renal ciliopathy)
2011 Broad spectrum and clinical heterogeneity of *HNF1B* gene mutations shown
2013 Description of *MUC1* as the cause of medullary cystic kidney disease type 1; the gene was missed by massive parallel sequencing, showing the need for refinement of analysis methods and assessment of clinical use of whole-exome sequencing for autosomal dominant heterogeneous disorders
2014 First description of mutation-dependent recessive inheritance in the case of *NPHS2*-associated steroid-resistant nephrotic syndrome

Milestones in treatment

1981 Oral cysteamine given for cystinosis
2000 Enzyme replacement therapy for Fabry's disease
2000 First in-vitro evidence that pharmacological chaperones can rescue cell-surface expression and function of misfolded vasopressin 2 receptors in nephrogenic diabetes insipidus
2005 First open-label, randomised, crossover, placebo-controlled trial for the effect of somatostatin analogue octreotide longacting release in autosomal dominant polycystic kidney disease
2008 Development of mTOR inhibitors for tuberous sclerosis
2009 Eculizumab for atypical haemolytic uraemic syndrome
2009 Proof-of-principle for use of bone marrow transplantation for treatment of mouse model with cystinosis
2009 Randomised, double-blind, placebo-controlled trial of the effect of somatostatin analogue lanreotide in polycystic liver disease associated with autosomal dominant polycystic kidney disease
2012 Global, randomised, double-blinded, placebo-controlled trial of the vasopressin 2 receptor antagonist tolvaptan in autosomal dominant polycystic kidney disease
2013 First randomised, single-blind, placebo-controlled, multicentre trial of octreotide longacting release for autosomal dominant polycystic kidney disease

Devuyst O *et al*.: Rare inherited kidney diseases: challenges, opportunities, and perspectives. *Lancet* 2014;383:1844-1859.

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European Commission > CORDIS > Projects & Results Service > Towards prevention of renal failure caused by inherited polycystic kidney disease

Towards prevention of renal failure caused by inherited polycystic kidney disease

Project reference: MR4*0193
Funded under: FP2-MHR 4C

Towards prevention of renal failure caused by inherited polycystic kidney disease

From 1989-07-01 **to** 1990-06-30

Objective

The aim of this Concerted Action was to relieve the burden on patients, their families, and society of one of the most frequent and expensive genetic diseases of man.

The Countries of the EEC spend roughly 4 billion ECU each year on renal replacement therapy, dialysis and transplantation. Throughout Europe around 5%, but in the Ferrara region of Italy, up to 20% of the patients with end stage renal failure have polycystic kidneys. Since the disease is caused by an autosomal dominant mutation, each child of a patient has a 50% risk to develop the disease. Cysts are present in the kidneys from the 12th week of gestation. Very slowly these cysts grow in size, thereby destroying the functional tissue. End stage renal failure usually occurs between 40 and 60, but in fact varies considerably between patients, even between members of the same family.

Genetic studies:
With recombinants obtained during family studies the PKD1 gene was localized to pGGG1 (D16S259) distal, and 26-6 (D16S125) proximal, a region of 750 kb on chromosome 16. Almost the entire region of 750 kb has been cloned in overlapping cosmids and yeast artificial chromosomes (YAC). The genetic heterogeneity of PKD was studied in a total of 328 families from all over Europe and the proportion of unlinked families was found to be 15%. The progression of PKD in p atients with unlinked PKD was slower indicating an intrinsic factor that is of influence on the age at which end stage renal failure is reached.

http://cordis.europa.eu/project/rcn/3861_en.html

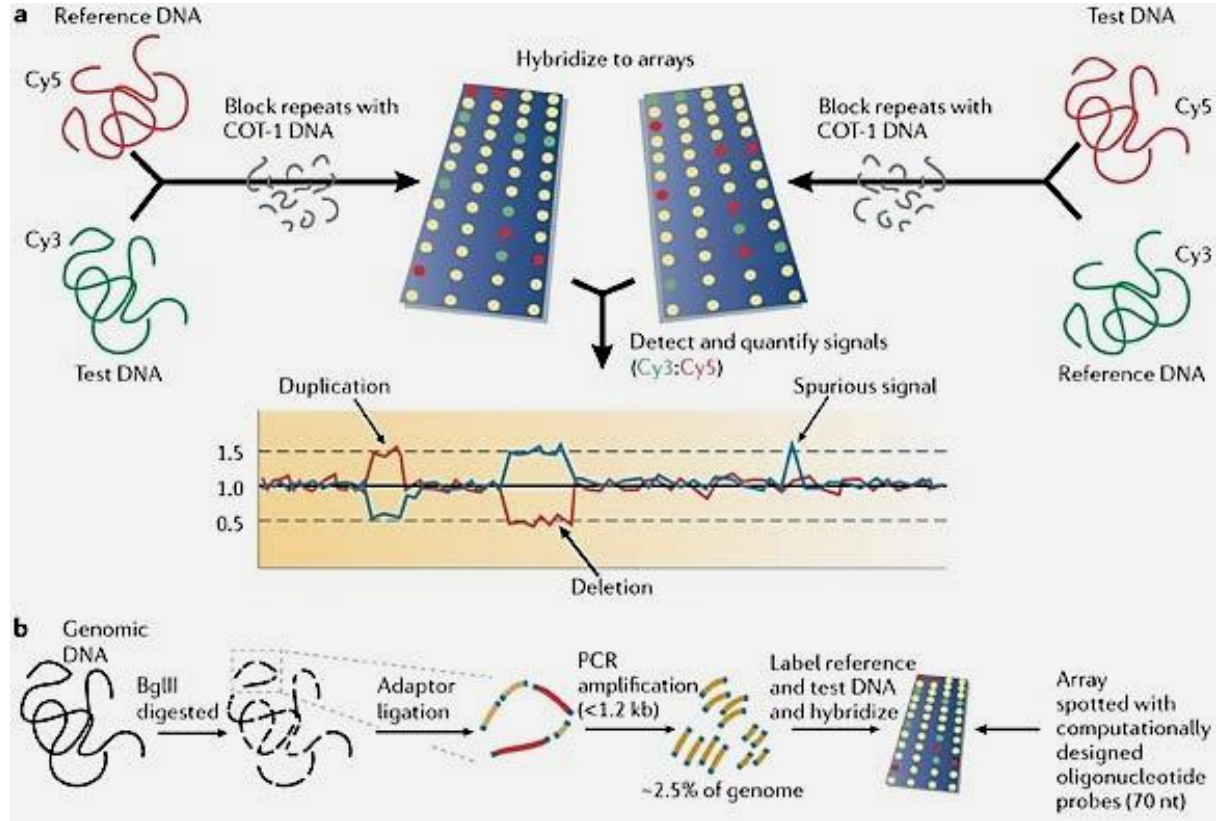
Thank you for your attention!

Porto, Ribeira do Douro

Array-based, genome-wide methods for the identification of copy-number variants

*a | In **array-based comparative genome hybridization** (array-CGH), reference and test DNA samples are differentially labelled with fluorescent tags (Cy5 and Cy3, respectively), and are then hybridized to genomic arrays after repetitive-element binding is blocked using COT-1 DNA. The array can be spotted with one of several DNA sources, including BAC clones, PCR fragments or oligonucleotides. After hybridization, the fluorescence ratio (Cy3:Cy5) is determined, which reveals copy-number differences between the two DNA samples. Typically, array-CGH is carried out using a 'dye-swap' method, in which the initial labelling of the reference and test DNA samples is reversed for a second hybridization (indicated by the left and right sides of the panel). This detects spurious signals for which the reciprocal ratio is not observed. An example output for a dye-swap experiment is shown: the red line represents the original hybridization, whereas the blue line represents the reciprocal, or dye-swapped, hybridization.*

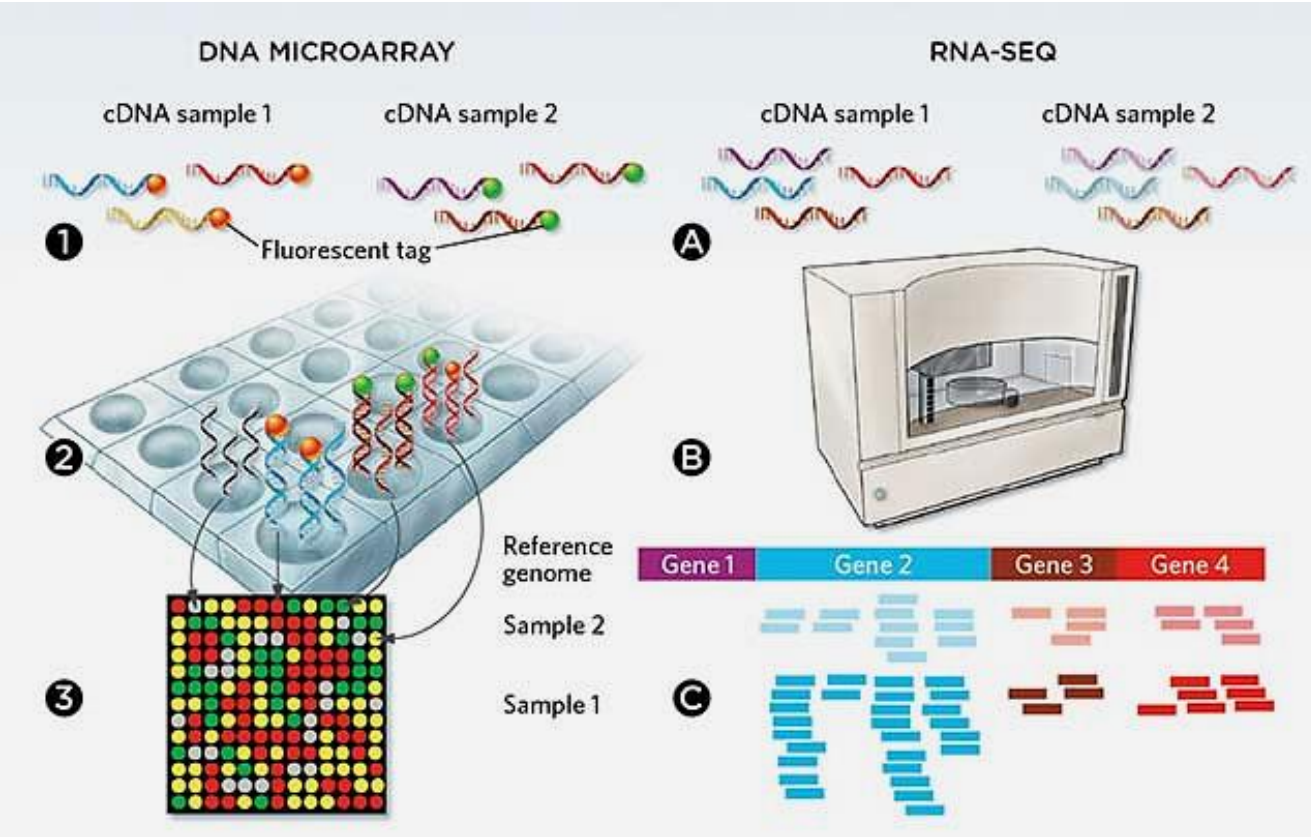
*b | **Representational oligonucleotide microarray analysis** (ROMA) is a variant of array-CGH in which the reference and test DNA samples are made into 'representations' to reduce the sample complexity before hybridization. DNA is digested with a restriction enzyme that has uniformly distributed cleavage sites (BglII is shown here). Adaptors (with PCR primer sites) are then ligated to each fragment, which are amplified by PCR. However, owing to the PCR conditions that are used, only DNA of less than 1.2 kb (yellow) is amplified. Fragments that are greater than this size (red) are lost, therefore reducing the complexity of the DNA that will be hybridized to the array. It is estimated that around 200,000 fragments of DNA are amplified, comprising approximately 2.5% of the human genome. In ROMA, an oligonucleotide array is used, which is spotted with computationally designed 70-nt probes. Each probe is designed to hybridize to one of the fragments in the representation.*



COT-1 DNA is a human placental DNA that is predominantly 50 to 300 bp in size and enriched for repetitive DNA sequences such as the *Alu* and *Kpn* family members. Cy3 and Cy5 are cyanine dyes.

Feuk L *et al.:* Structural variation in the human genome. *Nature Rev Genet 2006;7:*85-97.

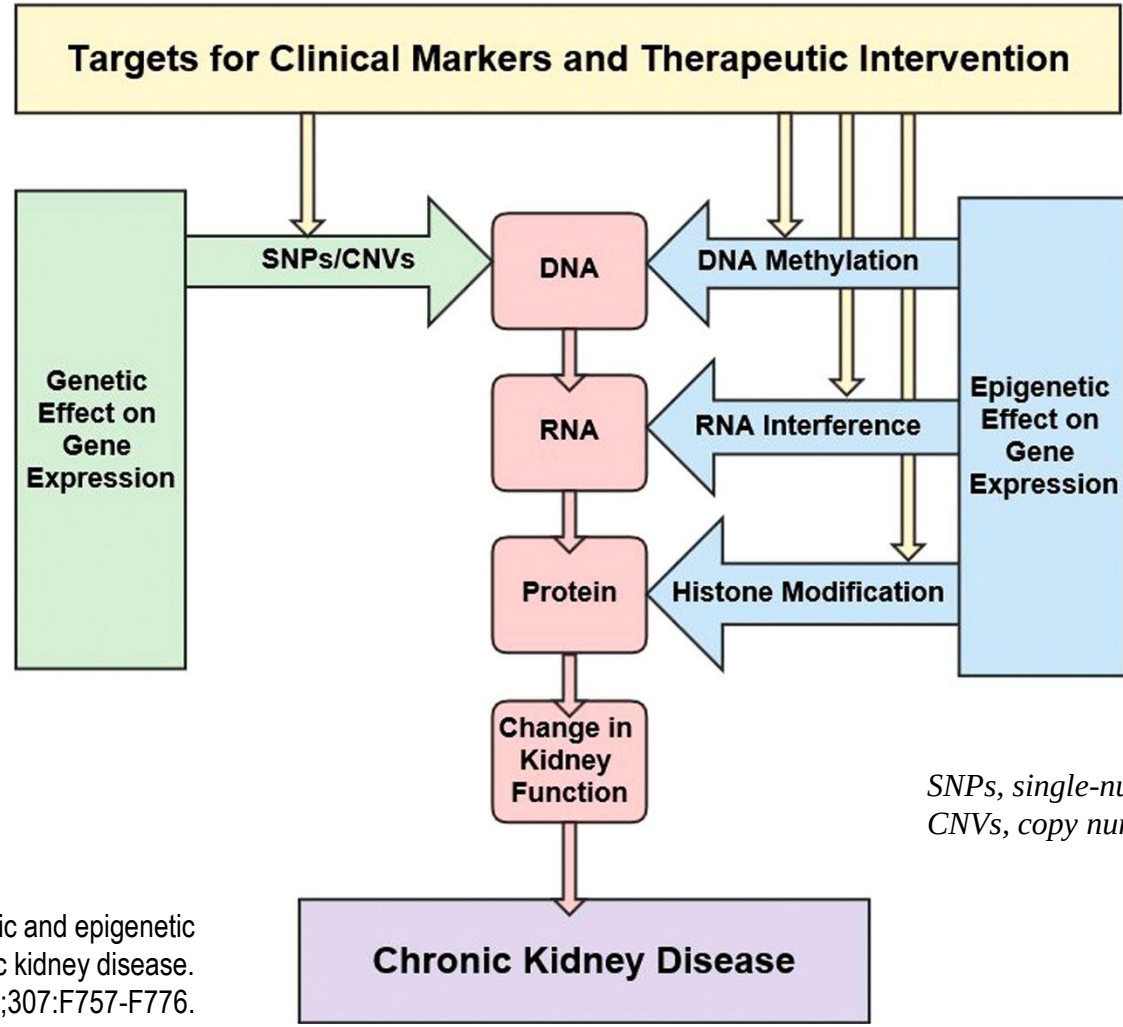
Measuring gene expression



DNA microarrays *consist of nucleic acid probes affixed to a surface. First, RNA is extracted from samples and converted into complementary DNA (cDNA), which is labeled with fluorescent tags (1). Next, labeled cDNA fragments hybridize with the nucleic acids on the array (2). Scanning the microarray measures the fluorescence level at each spot, revealing levels of gene expression (3). In* ***RNA-seq****, RNA is also extracted from samples, fragmented, and converted into cDNA in preparation for sequencing (A). Next, the cDNA library is sequenced (B). The resulting reads are mapped to the genome and gene expression is quantified (C).*

Yandell K: An array of options - A guide for how and when to transition from the microarray to RNA-seq. *The Scientist* 2015, June 1.

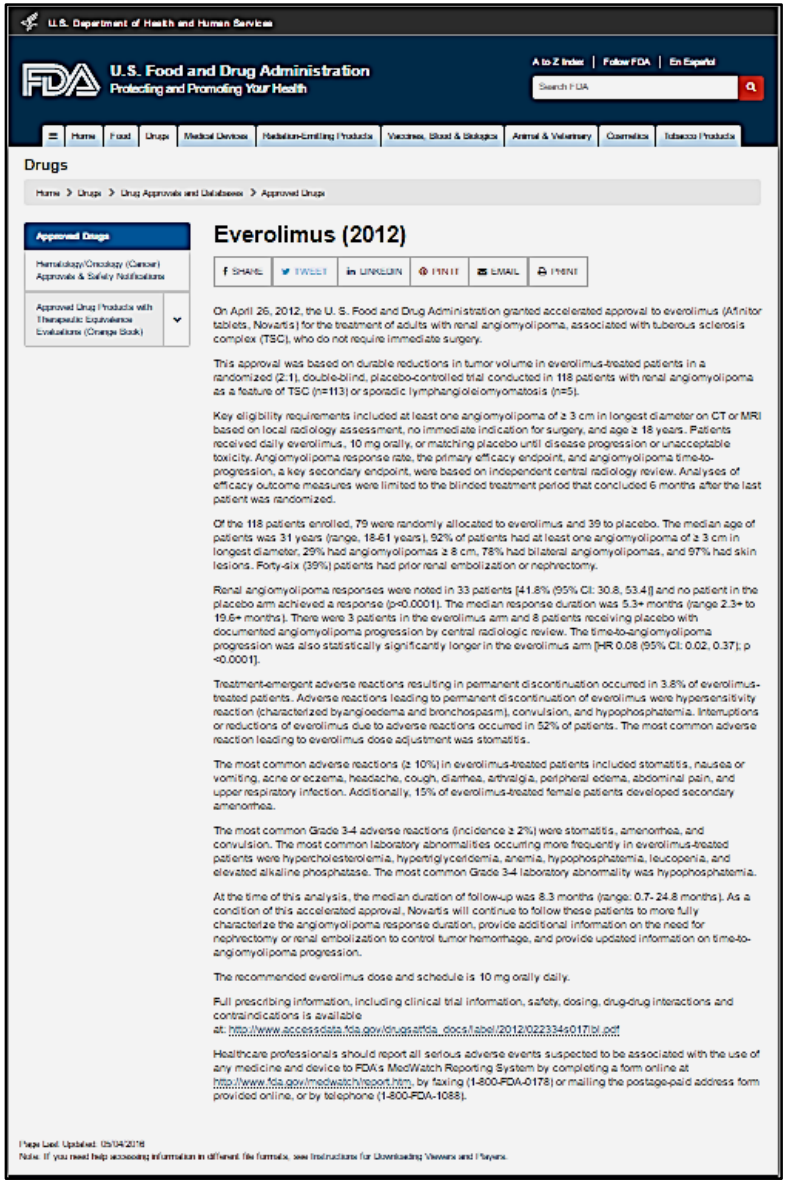
Potential genetic biomarkers for chronic kidney disease



SNPs, single-nucleotide polymorphisms; CNVs, copy number variations.

Smyth LJ *et al.*: Genetic and epigenetic factors influencing chronic kidney disease. *Am J Physiol Renal Physiol* 2014;307:F757-F776.

Recent therapeutic advancements



Everolimus

Approved for the treatment of adults with renal angiomyolipoma, associated with Tuberous Sclerosis Complex, who do not require immediate surgery.

[http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm302081.htm]

Tolvaptan

Approved to slow down cyst formation in Autosomal Dominant Polycystic Kidney Disease.

[http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2015/02/news_detail_002280.jsp&mid=WC0b01ac058004d5c1]