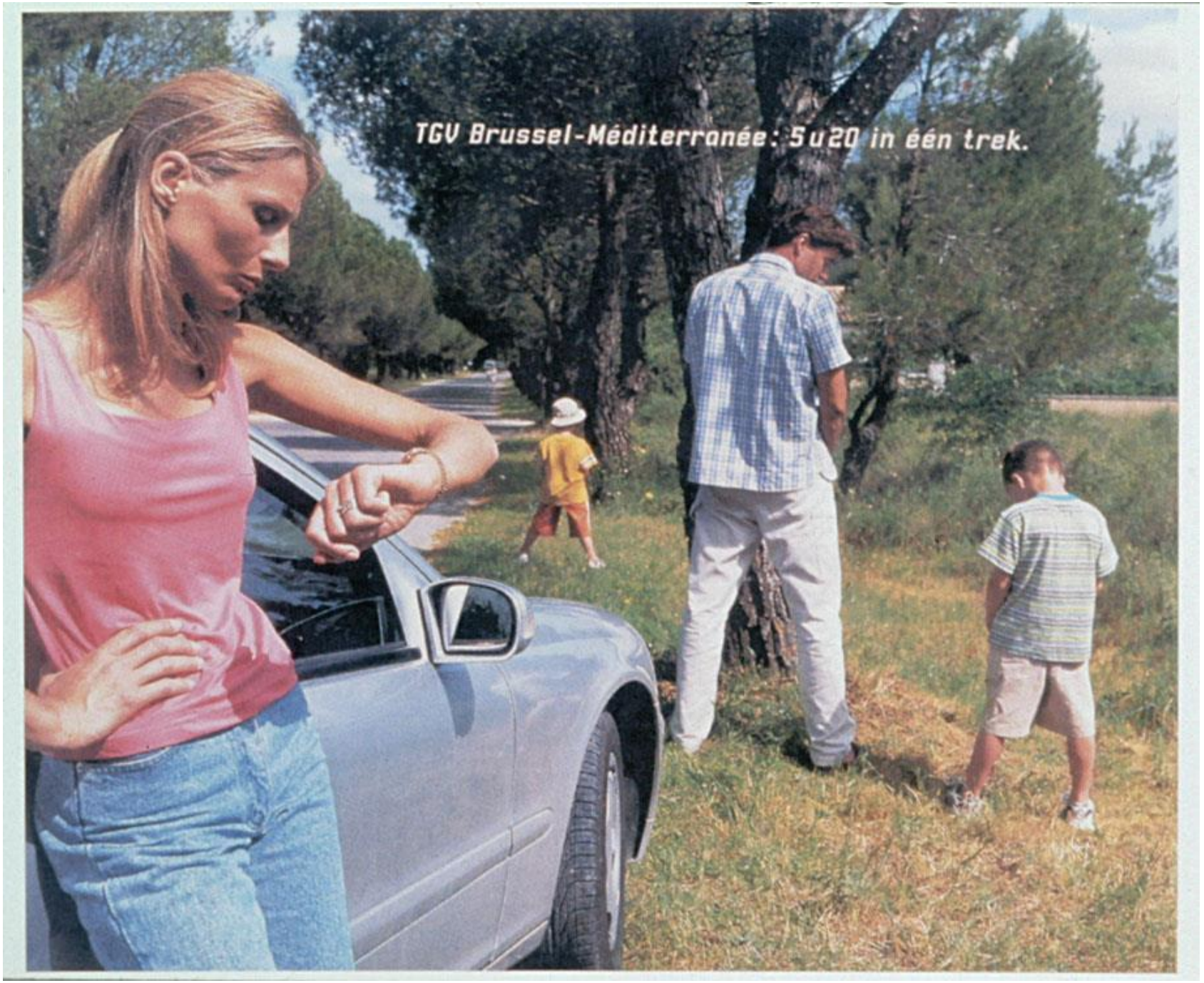
# Genetics of diabetic kidney disease and essential hypertension

# 21st Budapest Nephrology School

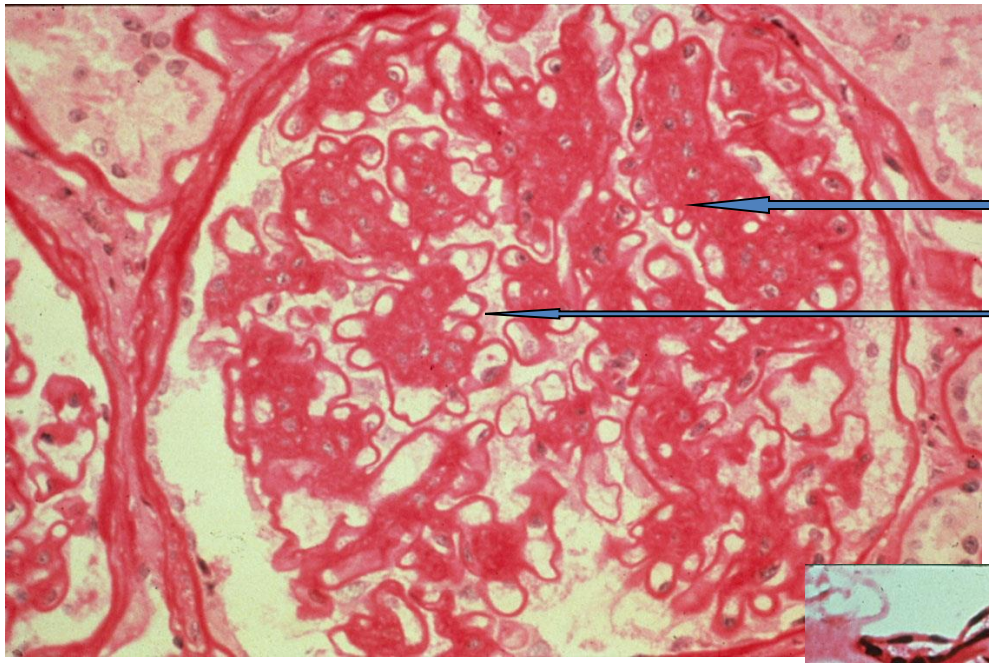
**Barry I. Freedman, MD, FACP**
**John H. Felts III Professor (Internal Medicine)**
**Chief, Section on Nephrology**


TGV Brussel-Méditerranée: 5u20 in één trek.

# Common features in essential hypertension and type 2 diabetes mellitus

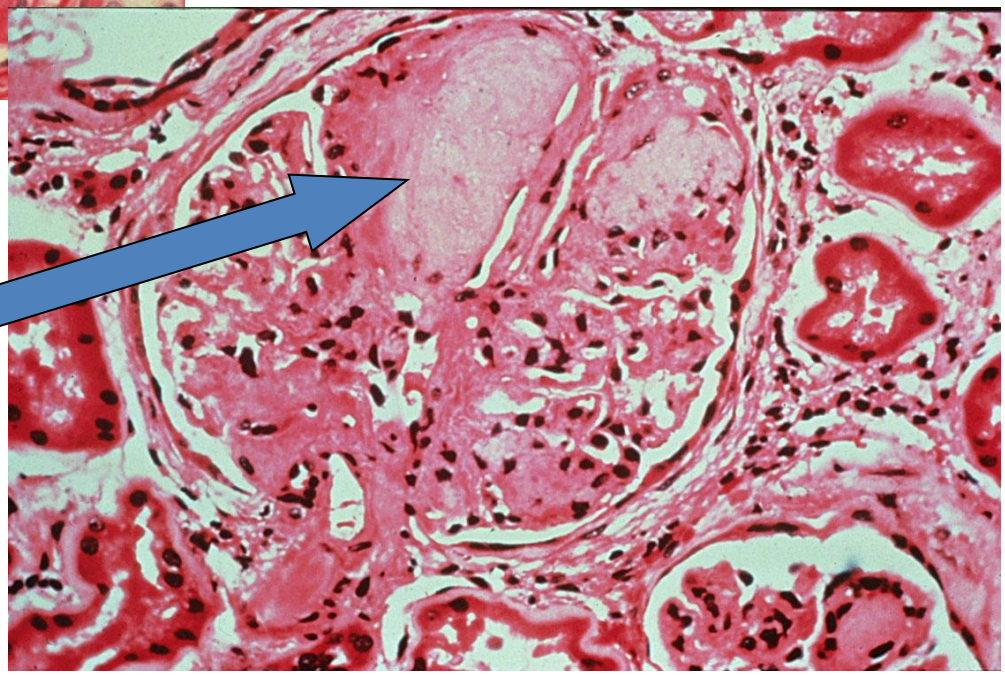
- Strong familial aggregation
- Neither is a “true disease”
  - blood pressure is a physical exam finding
  - blood glucose is a laboratory value
- Both are complex phenotypes with many causes
- Polygenic inheritance (multiple genes, each with small effect)
- Intermediate phenotypes have not proven to be of great value in gene identification

# Diabetic kidney disease (DKD)



Mesangial matrix expansion

GBM thickening



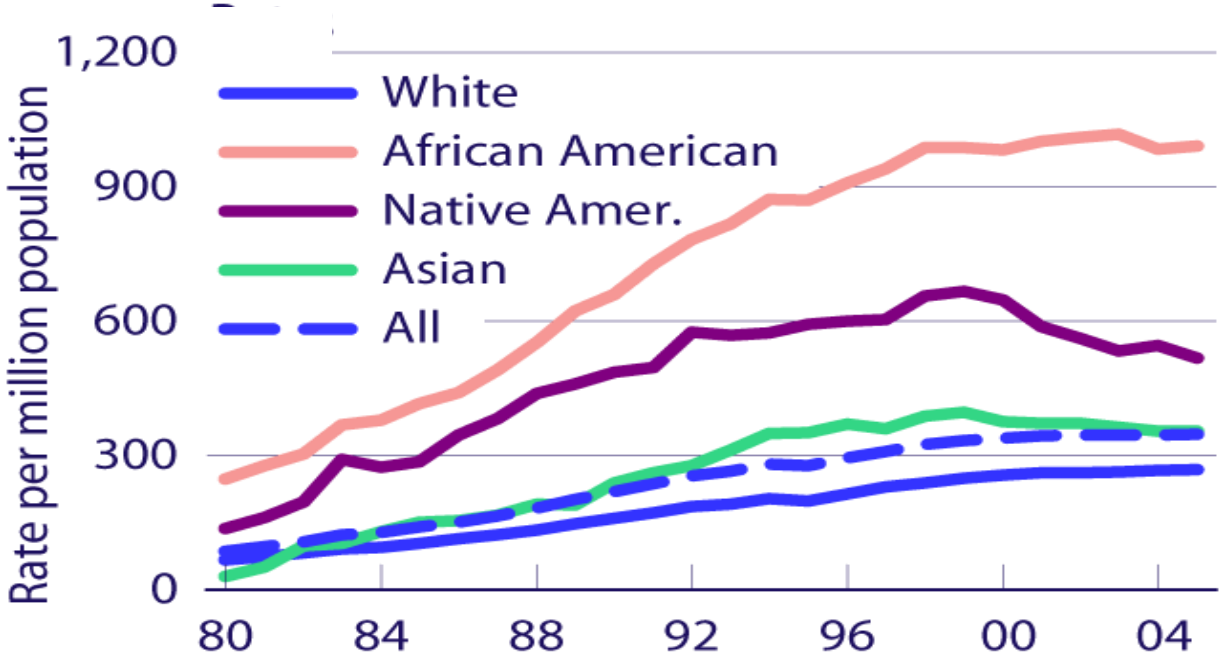
Kimmelstiel-Wilson lesion
(nodular glomerulosclerosis)

* Vascular and tubulointerstitial changes are often present

# Familial aggregation of DKD is independent from blood sugar

- Albuminuria, eGFR, ESKD and renal histology cluster in European, European American, African American, Native American, Asian, Brazilian and Indian families
- <40% of patients with diabetes develop DKD, highest rates in Native Americans and African Americans
- **Variable disease risk in groups with different population ancestry supports that evolving in different environments with disparate lifestyles contributed to differential selection of alleles and resultant different rates of DKD**

# ESRD incidence rates by population ancestry: USRDS




**African American : European American ~ 4**

**Native American : European American = 1.9**

**Asian : European American = 1.3**

**DKD is the reported cause of ESKD in 45% of incident cases**

# Natural history of DKD

- Progressive loss of eGFR can occur independently from albuminuria (10% with progressive T1 DKD lack excessive albuminuria or microalbuminuria). Caution using albuminuria as surrogate for DKD
- Impact of ACE inhibitors/ARBs on renal phenotypes (no effect on DKD initiation; some effect on DKD progression)
- Competing effect of CVD mortality as CKD progresses
- DKD phenotypes in genetic studies may differ: (Overt proteinuria/ESKD (severe disease) vs. microalbuminuria)

# Proteinuria in patients with T2D

- Up to 50% lack DKD on kidney biopsy:

Richards NT et al, Nephrol Dial Transplant 2002

Pham TT et al, Am J Nephrol 2007

Mou S et al, Diabetes Research Clinical Practice 2010

Shah V et al, J Am Soc Nephrol 2003 – Native Americans

Zhou J et al, Nephrol Dial Transplant 2007 – Chinese population

- **Predicting non-DKD in proteinuric T2D** **(Zhou J NDT '07)**
- **Shorter diabetes duration**
- **Lower systolic blood pressure**
- **Absence of diabetic retinopathy**
- **Lower HbA1c**

# New paradigms of susceptibility to diabetic kidney disease

- ESRD / advanced nephropathy is the phenotype of greatest interest (severely reduced eGFR)
- Albuminuria and kidney function (eGFR) are under independent genetic control
- Epidemiology of ESKD (and risk allele frequencies) can differ between groups with different population ancestry
- Search for diabetic kidney disease susceptibility genes suggests multiple interactive genes, each with weak effect

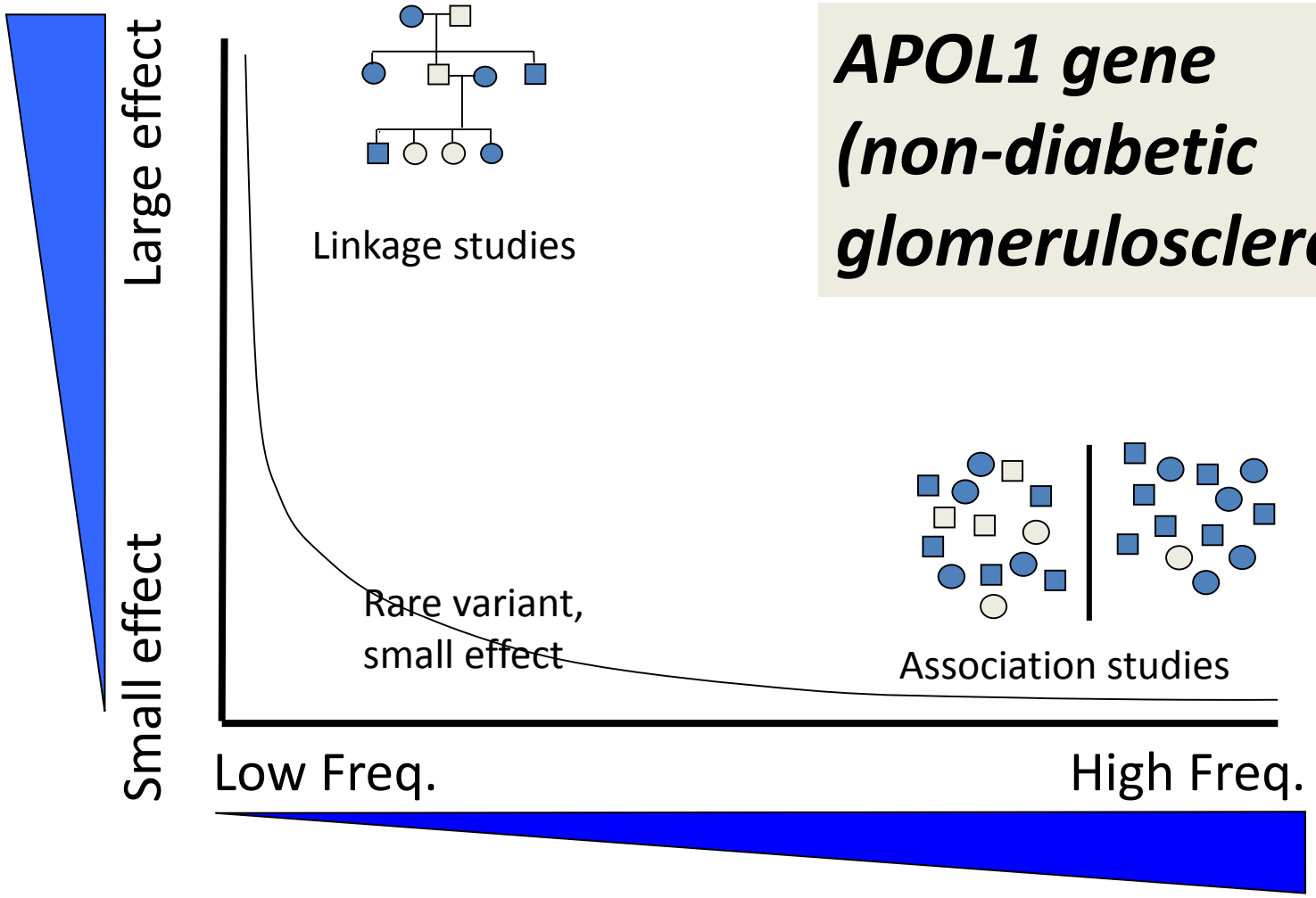
# Genetic analysis approaches

**DNA homology:**

- **~99.1% between Africans, Europeans, Asians**

- **Linkage analysis**
- **Candidate gene analyses**
- **Genome-wide association study**
- Admixture mapping (no DKD findings in African Americans)
- Deep sequencing of genes and genomic regions
- Rare exonic (coding) variants
- Expression QTL mapping
- Copy number variation (CNV)
- microRNAs

# Properties of DNA variants that have an impact on gene discovery




***APOL1 gene (non-diabetic glomerulosclerosis)***

# Replicated Diabetic Kidney Disease Susceptibility Genes

Angiotension converting enzyme (ACE, candidate gene)

Endothelial nitric oxide synthase (NOS3, candidate gene)

Erythropoietin (EPO, candidate gene)

Protein kinase C beta (PRKCB, candidate gene)

Carnosinase 1 (CNDP1, linkage)

Acetyl-coenzyme A carboxylase beta (ACACB, GWAS)

Engulfment and cell motility 1 gene (ELMO1, GWAS)

4.1 FERM domain containing 3 (FRMD3, GWAS)

Neurocalcin delta (NCALD, GWAS)

Plasmacytoma variant translocation 1 (PVT1, GWAS)

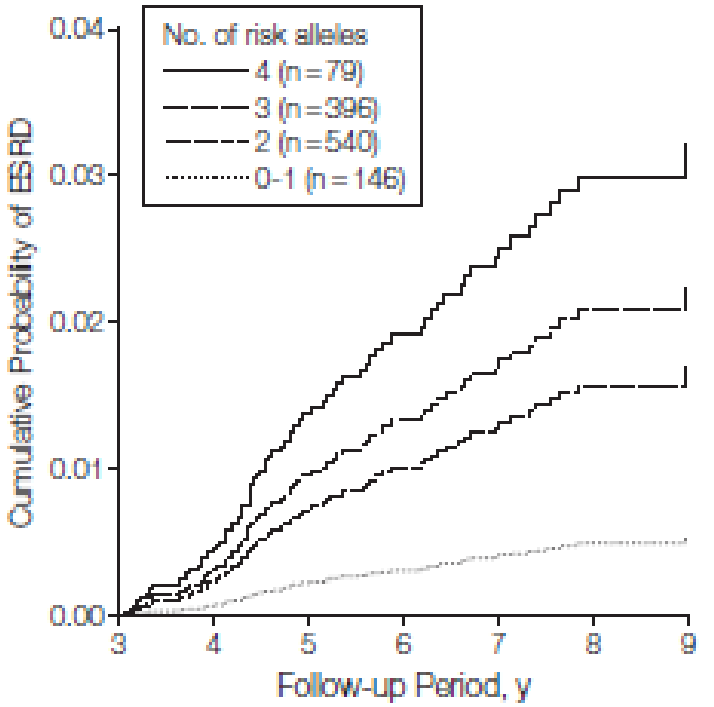
AF4/FMR2 family, member 3 (AFF3, GENIE GWAS)

# Genetic Variants of the Protein Kinase C-β 1 Gene and Development of End-Stage Renal Disease in Patients With Type 2 Diabetes


Ronald C. W. Ma, MB BChir
Claudia H. T. Tam, MPhil
Ying Wang, PhD
Andrea O. Luk, MB ChB
Cheng Hu, PhD
Xilin Yang, PhD
Vincent Lam, BSc
Alfred W. H. Chan, MPhil
Janice S. K. Ho, MPhil
Chun-Chung Chow, MBBS
Peter C. Y. Tong, PhD
Weiping Jia, MD, PhD
Maggie C. Y. Ng, PhD
Wing-Yee So, MD
Juliana C. N. Chan, MD


ASIA IS IN THE MIDST OF AN EPIdemic of type 2 diabetes.[1-3] A recent large-scale epidemiology survey estimated that there are 94.2 million adults affected by diabetes in China, with another 148.2 million with prediabetes.[4] Renal failure is an important cause of mortality among patients with type 2 diabetes.[5,6] Asian populations appear to be particularly at risk of diabetic kidney disease (DKD). In the Microalbuminuria Prevalence (MAP) Study, microalbuminuria was present in

**Figure**. Cumulative Probability of New-Onset ESRD According to Number of Risk Alleles, Adjusted for Conventional Risk Factors




No. at risk by allele count

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 4 | 79 | 79 | 74 | 63 | 54 | 46 | 34 |
| 3 | 396 | 394 | 376 | 340 | 309 | 265 | 182 |
| 2 | 540 | 535 | 510 | 468 | 415 | 349 | 245 |
| 0-1 | 146 | 144 | 138 | 124 | 109 | 94 | 66 |

# GWAS in advanced diabetic kidney disease

- **Japanese T2 DKD** Shimazaki A & Maeda S Diabetes 2005;54:1171-1178
  - 80K gene-based SNPs. *ELMO1*, *ACACB*, and *NCALD*
- **EA T1 DKD (Joslin GoKinD)** Pezzolesi M et al. Diabetes 2009; 58:1403-10. 360K SNPs
  - *FRMD3, CARS* approached genome-wide significance
  - Replication seen with time to onset of DKD in EDIC-DCCT
- **AA T2D-ESKD (Wake Forest)** McDonough C et al. Kidney Int 2011; 79:563-72.

  1 million SNPs (Affy 6.0)
  - Genome-wide significant association detected in several regions, including 22q
  - *LIMK2* (near *APOL1* but not in LD), *RPS12* and *SFI1* were associated
  - *FRMD3* was associated…. when accounting for *APOL1*
- **GENIE T1 DKD (ESKD)** Sandholm N et al. PLoS Genet 2012. 2.4 m SNPs imputed
  - UK ROI, FinnDiane, and GoKinD US cohorts meta-analyzed
  - *AFF3* and an intergenic locus on 15q26 residing between *RGMA* and *MCTP2* were associated with T1D-ESKD with genome wide significance
- **FIND DM-ESKD AA, EA, MA, AI ethnicities** 1 million SNPs (Affy 6.0) completed

  Meta-analysis across 4 ethnic groups (combined Discovery + Replication sets) revealed genome-wide significant association with SNPs between 2 genes with potential roles in DKD

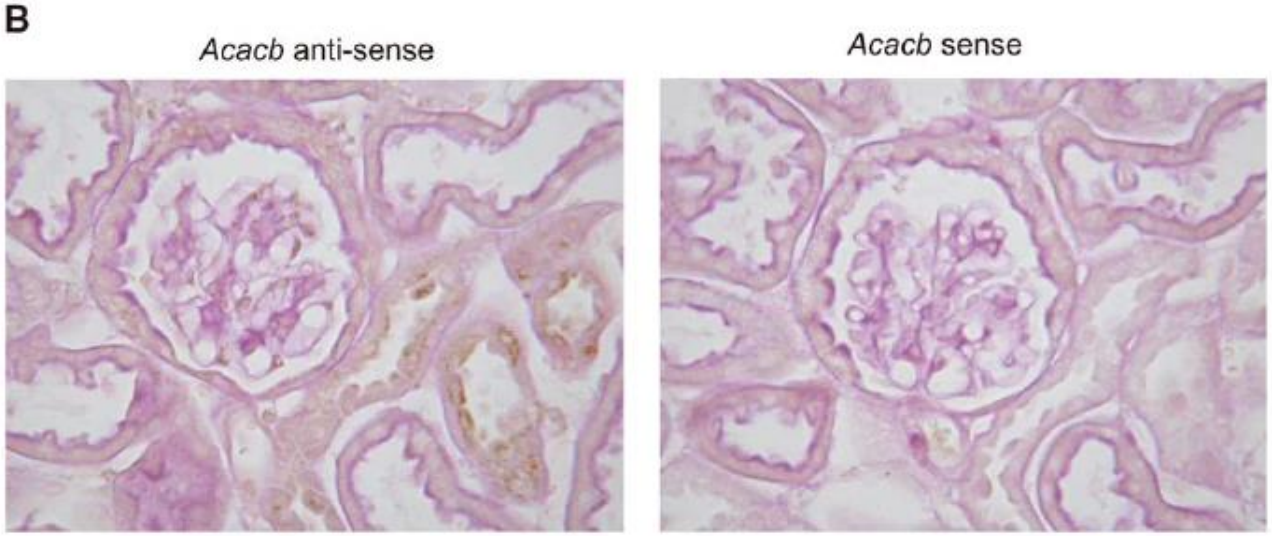
  *These genes have differential expression in Pima and European kidney tissue*

  Prior association p-value $10^{-7}$ with kidney function in East Asians

# A Single Nucleotide Polymorphism within the Acetyl-Coenzyme A Carboxylase Beta Gene Is Associated with Proteinuria in Patients with Type 2 Diabetes

Shiro Maeda[1*], Masa-aki Kobayashi[1,2], Shin-ichi Araki[3], Tetsuya Babazono[4], Barry I. Freedman[5], Meredith A. Bostrom[5], Jessica N. Cooke[5], Masao Toyoda[6], Tomoya Umezono[6], Lise Tarnow[7], Torben Hansen[7,8], Peter Gaede[7], Anders Jorsal[7], Daniel P. K. Ng[9], Minoru Ikeda[10], Toru Yanagimoto[10], Tatsuhiko Tsunoda[11], Hiroyuki Unoki[1], Koichi Kawai[12], Masahito Imanishi[13], Daisuke Suzuki[6], Hyoung Doo Shin[14], Kyong Soo Park[15,16], Atsunori Kashiwagi[3], Yasuhiko Iwamoto[4], Kohei Kaku[17], Ryuzo Kawamori[18], Hans-Henrik Parving[19,20], Donald W. Bowden[5], Oluf Pedersen[7,19,21], Yusuke Nakamura[22]

- **GWAS for diabetic kidney disease in Japanese**
- **80,000 gene-based SNPs**
- ***ACACB* determines rate-limiting step in fatty acid oxidation**
- **Widely replicated, including in European Americans**
- ***NCALD* and *ELMO1* also identified in this sample**




*ACACB* expressed in human proximal tubule epithelial cells

**Figure 2. Expression profiles of *ACACB*.** (A) Expression profiles of *ACACB* in various human tissues evaluated by real-time PCR. (B) Results of *in situ* hybridization for 20-week-old normal mouse kidneys using mouse *Acacb* anti-sense (left) and sense (right) probes.

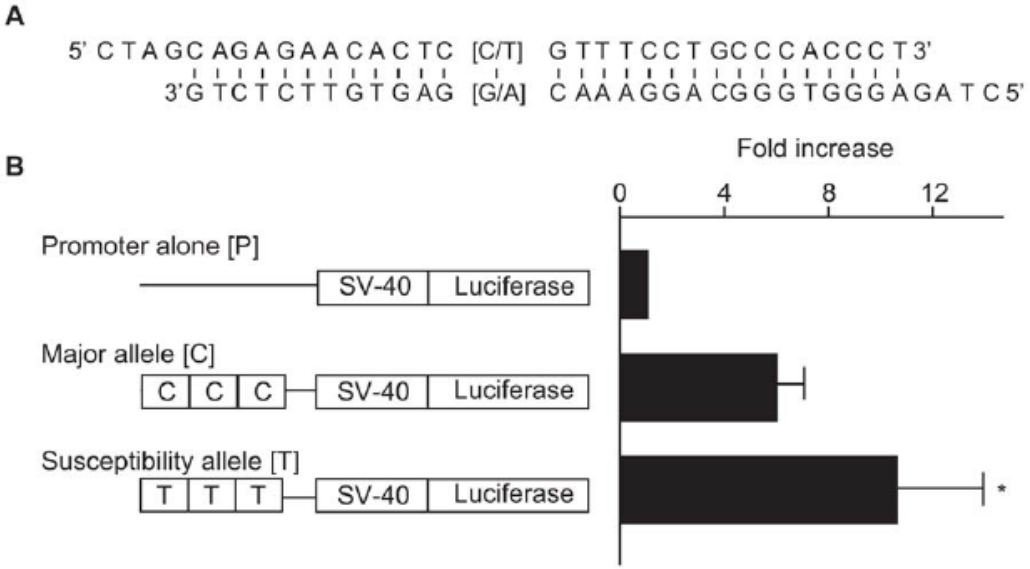



**Figure 3. Effect of a 29-bp DNA fragment containing the associated SNP (rs2268388) on transcriptional activity in cultured hRPTECs.** (A) A 29-bp sequence including SNP rs2268338. Overhung sequences are compatible ends for concatenation. (B) Enhancer activities of the DNA fragments corresponding to each allele in human RPTECs. Results are shown as mean ± SD of the ratio of activity to that of the promoter alone obtained from 4 independent experiments. * $p = 0.045$ vs. major allele.
doi:10.1371/journal.pgen.1000842.g003

# Genetic Variations in the Gene Encoding ELMO1 Are Associated With Susceptibility to Diabetic Nephropathy

Atsuyuki Shimazaki,[1] Yoshihiro Kawamura,[1] Akio Kanazawa,[1] Akihiro Sekine,[1] Susumu Saito,[1] Tatsuhiko Tsunoda,[1] Daisuke Koya,[2] Tetsuya Babazono,[3] Yasushi Tanaka,[4] Masafumi Matsuda,[5] Koichi Kawai,[6] Tomohiro Iizumi,[7] Masahito Imanishi,[8] Toshihiro Shinosaki,[9] Toru Yanagimoto,[9] Minoru Ikeda,[9] Shigeki Omachi,[9] Atsunori Kashiwagi,[2] Kohei Kaku,[5] Yasuhiko Iwamoto,[3] Ryuzou Kawamori,[4] Ryuichi Kikkawa,[2] Masatoshi Nakajima,[9] Yusuke Nakamura,[1,10] and Shiro Maeda[1]

*ELMO1* association with T2 DKD was first identified in the Japanese

Replicated in African Americans (T2 DKD from Wake Forest)

Replicated in European Americans (T1 DKD from GoKinD)

**Different intronic *ELMO1* markers associate in each population**

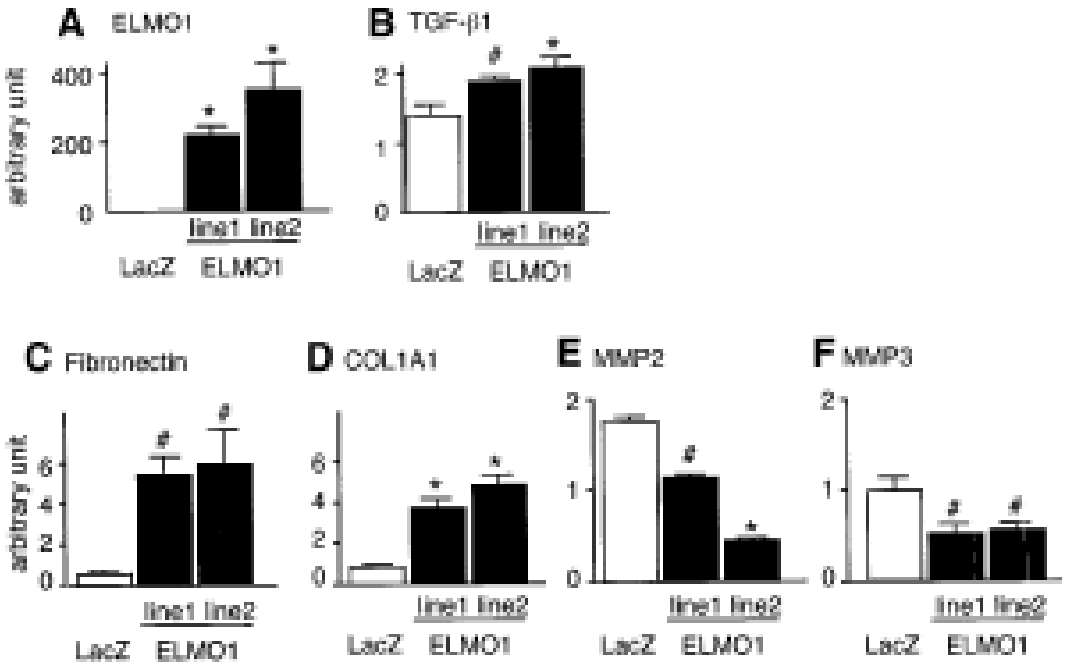



FIG. 5. The effects of *ELMO1* overexpression in COS cells. The expression of each gene was analyzed by real-time quantitative RT-PCR. *A*: ELMO1. *B*: TGF-β1. *C*: Fibronectin. *D*: Collagen type I (α1). *E*: MMP-2. *F*: MMP-3. □, cells transfected with pcDNA-LacZ; ■, cells transfected with *ELMO1*. *$P < 0.0001$ vs. LacZ; #$P < 0.005$ vs. LacZ.

*** *ELMO1* overexpression drives TGFβ1, fibronectin and COL1A1 (and reduces production of MMP2 and MMP3) in COS cells**

# *FRMD3* associations in DKD (and other forms of CKD)

## Genome-Wide Association Scan for Diabetic Nephropathy Susceptibility Genes in Type 1 Diabetes

Marcus G. Pezzolesi,[1] G. David Poznik,[1] Josyf C. Mychaleckyj,[2] Andrew D. Paterson,[3,4] Michelle T. Barati,[5] Jon B. Klein,[5] Daniel P.K. Ng,[1,8] Grzegorz Placha,[1,7] Luis H. Canani,[1,8] Jacek Bochenski,[1] Daryl Waggott,[9] Michael L. Merchant,[5] Bozena Krolewski,[1] Lucia Mirea,[4,9] Krzysztof Wanic,[1] Pisut Katavetin,[1] Masahiko Kure,[1] Pawel Wolkow,[1,10] Jonathon S. Dunn,[1] Adam Smiles,[1] William H. Walker,[1] Andrew P. Boright,[11] Shelley B. Bull,[4,9] the DCCT/EDIC Research Group,* Alessandro Doria,[1] John J. Rogus,[1] Stephen S. Rich,[2] James H. Warram,[1] and Andrzej S. Krolewski[1]

Diabetes 2009

**GoKinD and EDIC-DCCT with T1D**

## Differential Effects of *MYH9* and *APOL1* Risk Variants on *FRMD3* Association with Diabetic ESRD in African Americans

PLoS Genet 2011

**African Americans with T2D**

Barry I. Freedman[1,9]*, Carl D. Langefeld[2,9]*, Lingyi Lu[2], Jasmin Divers[2], Mary E. Comeau[2], Jeffrey B. Kopp[3], Cheryl A. Winkler[4], George W. Nelson[5], Randall C. Johnson[5,6], Nicholette D. Palmer[7,8,9], Pamela J. Hicks[7,8,9], Meredith A. Bostrom[7,8,9], Jessica N. Cooke[8,9,10], Caitrin W. McDonough[8,9,10], Donald W. Bowden[7,8,9,11]*

## A family-based association study after genome-wide linkage analysis identified two genetic loci for renal function in a Mongolian population

**Asian families**

Hansoo Park[1,2,10], Hyun-Jin Kim[1,3,10], Seungbok Lee[1,3,10], Yun Joo Yoo[4], Young Seok Ju[1,5], Jung Eun Lee[6], Sung-Il Cho[7], Joohon Sung[7], Jong-Il Kim[1,3,8,9,11] and Jeong-Sun Seo[1,3,5,8,9,11]

Kidney Int 2012

**Family-based association analysis confirms the role of the chromosome 9q21.32 locus in the susceptibility of diabetic nephropathy.** PLoS One 2013

**Pezzolesi MG, Jeong J, Smiles AM, Skupien J, Mychaleckyj JC, Rich SS, Warram JH, Krolewski AS**

**European American families with T2D**

# *FRMD3* in nephropathy

- Non-coding *FRMD3* SNP rs1888747 was repeatedly associated with DKD
- Transcriptional regulation modeling revealed overlap with bone morphogenetic protein (BMP) pathway gene regulation (via a shared transcription factor binding site)
- Potential role for BMP signaling pathway in susceptibility to DKD?

- Martini S and Kretzler M. Diabetes 2013

OPEN ACCESS Freely available online

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# New Susceptibility Loci Associated with Kidney Disease in Type 1 Diabetes

Niina Sandholm[1,2,3☯], Rany M. Salem[4,5,6☯], Amy Jayne McKnight[7☯], Eoin P. Brennan[8,9☯], Carol Forsblom[1,2], Tamara Isakova[10], Gareth J. McKay[7], Winfred W. Williams[6,11], Denise M. Sadlier[8,9], Ville-Petteri Mäkinen[1,2,12], Elizabeth J. Swan[7], Cameron Palmer[4,5], Andrew P. Boright[13], Emma Ahlqvist[14], Harshal A. Deshmukh[15], Benjamin J. Keller[16], Huateng Huang[17], Aila J. Ahola[1,2], Emma Fagerholm[1,2], Daniel Gordin[1,2], Valma Harjutsalo[1,2,18], Bing He[19], Outi Heikkilä[1,2], Kustaa Hietala[1,20], Janne Kytö[1,20], Päivi Lahermo[21], Markku Lehto[1,2], Raija Lithovius[1,2], Anne-May Österholm[19], Maija Parkkonen[1,2], Janne Pitkäniemi[22], Milla Rosengård-Bärlund[1,2], Markku Saraheimo[1,2], Cinzia Sarti[22], Jenny Söderlund[1,2], Aino Soro-Paavonen[1,2], Anna Syreeni[1,2], Lena M. Thorn[1,2], Heikki Tikkanen[23], Nina Tolonen[1,2], Karl Tryggvason[19], Jaakko Tuomilehto[18,24,25,26], Johan Wadén[1,2], Geoffrey V. Gill[27], Sarah Prior[28], Candace Guiducci[4], Daniel B. Mirel[4], Andrew Taylor[4,11], S. Mohsen Hosseini[29,30], DCCT/EDIC Research Group[31,32¶], Hans-Henrik Parving[33,34], Peter Rossing[34,35], Lise Tarnow[34,35], Claes Ladenvall[14], François Alhenc-Gelas[36], Pierre Lefebvre[37], Vincent Rigalleau[38], Ronan Roussel[39,40,41], David-Alexandre Tregouet[42], Anna Maestroni[43], Silvia Maestroni[43], Henrik Falhammar[44,45], Tianwei Gu[44], Anna Möllsten[46], Danut Cimponeriu[47], Mihai Ioana[48], Maria Mota[48], Eugen Mota[48], Cristian Serafinceanu[49], Monica Stavarachi[47], Robert L. Hanson[50], Robert G. Nelson[50], Matthias Kretzler[51], Helen M. Colhoun[15], Nicolae Mircea Panduru[49], Harvest F. Gu[44], Kerstin Brismar[44,45], Gianpaolo Zerbini[43], Samy Hadjadj[52,53], Michel Marre[39,40,54], Leif Groop[14], Maria Lajer[35], Shelley B. Bull[55,56], Daryl Waggott[55], Andrew D. Paterson[30,56], David A. Savage[7], Stephen C. Bain[28], Finian Martin[8,9], Joel N. Hirschhorn[4,5,6*], Catherine Godson[8,9*], Jose C. Florez[4,6,11*], Per-Henrik Groop[1,2,57*], Alexander P. Maxwell[7,58*]

In the GEnetics of Nephropathy: an International Effort (GENIE) consortium, we have undertaken a meta-analysis of genome-wide association studies (GWAS) of T1D DN comprising ~2.4 million single nucleotide polymorphisms (SNPs) imputed in 6,691 individuals. After additional genotyping of 41 top ranked SNPs representing 24 independent signals in 5,873 individuals, combined meta-analysis revealed association of two SNPs with ESRD: rs7583877 in the *AFF3* gene ($P = 1.2 \times 10^{-8}$) and an intergenic SNP on chromosome 15q26 between the genes *RGMA* and *MCTP2*, rs12437854 ($P = 2.0 \times 10^{-9}$). Functional data suggest that *AFF3* influences renal tubule fibrosis via the transforming growth factor-beta (TGF-β1) pathway. The

Genome-wide significance reached only when T1D ESKD was the phenotype…….

# Lack of consistent results in DKD genetics?

- Candidate gene analyses were limited in scope
  - Incomplete candidate gene characterization
  - Small sample sizes
- Different phenotypes; albuminuria vs. eGFR
- Survival effect in ESKD (CVD death with CKD)
- Initiation vs. progression genes
- Effects of different population ancestries
- Inclusion of cases w/ non-diabetic kidney disease

# Conclusions in DKD genetics

- There is no single major DKD gene
- Multiple genes, each likely with small effect
- Different variants in same gene associate in distinct population ancestries (***ELMO1***)
- Shared risk for T1DN and T2DN?
  - ***FRMD3****:* EA T1DN and AA T2DN
  - ***ELMO1***: Japanese and AA T2DN, EA T1DN

# Genetics in disorders of blood pressure (hypertension and hypotension)

- Monogenic disorders well known
- NIH Family Blood Pressure Program (FBPP):
  - GenNet - AA, EA
  - Genetic Epidemiology Network of Atherosclerosis (GENOA) - AA, EA, MA
  - HyperGEN - AA, EA
  - Standford Asia Pacific Program in Hypertension and Insulin Resistance (SAPPHIRe) – Chinese and Japanese origin

# Advances in Blood Pressure Genomics

Patricia B. Munroe, Michael R. Barnes, Mark J. Caulfield

***Abstract:*** **The elucidation of genes implicated in Mendelian forms of hypertension demonstrates rare variants with substantial effects are responsible, and often these genes lie within pathways managing sodium homeostasis. More recently with advances in affordable high-throughput genotyping strategies, multiple common genetic variants with modest effects on blood pressure (<1 mm Hg systolic) have been discovered in the population. In aggregate, these common variants explain <3% of the variance of blood pressure. Although these findings may offer new mechanistic insights into the biology of blood pressure, a key question is can these findings translate into patient benefit? It is timely to reflect on recent advances in genomics, and the use of new resources, such as the 1000 Genomes Project and the Encyclopedia of DNA Elements, to annotate likely causal variants, and their relevance to cardiovascular disease. In this review, we discuss the advances in relation to our knowledge of the genetic architecture of blood pressure, and whether gene discoveries might influence cardiovascular risk assessment, help to stratify patient response to medicine, or identify new biological pathways for novel therapeutic targets. (*Circ Res*. 2013:112:1365-1379.)**

Following 3 Tables from this reference

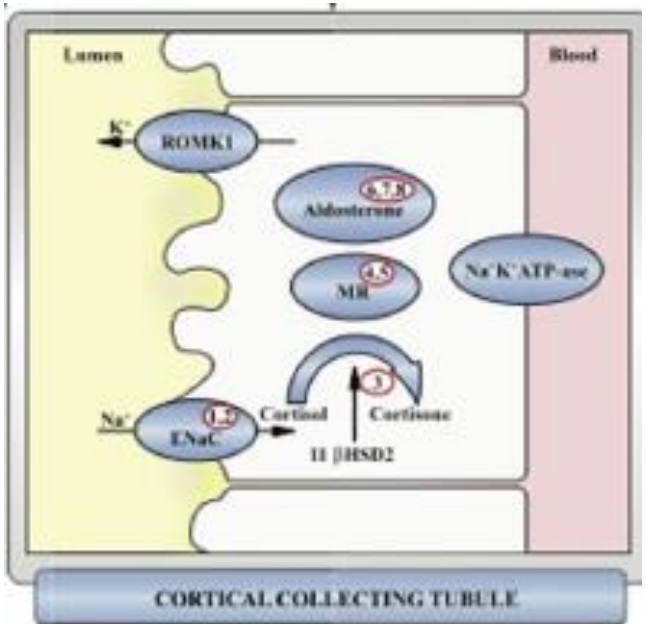
## Monogenic Forms of Hypertension and Hypotension

| Condition | Mode of Inheritance | Phenotype | Chromosome(s) | Gene, Biological Effect, and Reference |
|---|---|---|---|---|
| Hypertension | | | | |
| Pseudohypoaldosteronism type 2; familial hyperkalemic hypertension; Gordons syndrome | Autosomal dominant and autosomal recessive | Hypertension<br>↑K+, metabolic acidosis | 2q36, 5q31, 12p13, 17q21 | Protein kinase, lysine deficient 1 and 4 (WNK 1, WNK 4)[23]<br>Kelch-like protein 3 (KLHL3) and Cullin 3 (Cullin).[24,25]<br>Overactivity of sodium chloride cotransporter, via different regulatory mechanisms |
| Hypertension associated with PPARγ mutations | Autosomal dominant | Hypertension<br>Insulin resistance, type 2 diabetes mellitus | 3p25 | Peroxisome proliferator-activated receptor gamma (PPARγ)[97]<br>Malfunctioning nuclear receptor PPARγ |
| Hypertension exacerbated by pregnancy | Autosomal dominant | Early hypertension, exacerbated by pregnancy | 4q31.23 | Mineralocorticoid receptor (NR3C2)[98] |
| Glucocorticoid remediable aldosteronism | Autosomal dominant | Hypertension<br>with other variable features | 8q24.3 | 11 β-hydroxylase/aldosterone synthase (CYP11B1/CYP11B2) chimera[99] |
| Congenital adrenal hyperplasia with 11β-hydroxylase deficiency | Autosomal recessive | Hypertension<br>with other variable features | 8q | 11 β-hydroxylase (CYP11B1)[100]<br>↓11β hydroxylase |
| Congenital adrenal hyperplasia with 17α-hydroxylase deficiency | Autosomal recessive | Hypertension<br>absent sexual maturation, ↓K+ | 10q24.32 | 17 α-hydroxylase (CYP17A1)[101]<br>↓17α hydroxylase |
| Hypertension and brachydactyly | Autosomal dominant | Hypertension<br>brachydactyly | 12p | Unknown[102] |
| Apparent mineralocorticoid excess | Autosomal recessive | Hypertension<br>↓K+, metabolic alkalosis, ↓renin, ↓↓ aldosterone | 16q | 11 β-hydroxysteroid dehydrogenase (HSD11B2).[103]<br>↓11 β-hydroxysteroid dehydrogenase |
| Liddle syndrome | Autosomal dominant | Hypertension<br>↓K+, metabolic alkalosis, ↓renin, ↓ aldosterone | 12p13.31, 16p12.2 | Sodium channel nonvoltage-gated 1, β and γ subunits (SCCN1A, SCNN1B, SCNN1G).[104]<br>Abnormal ENaC: reduced |

## Monogenic Forms of Hypertension and Hypotension

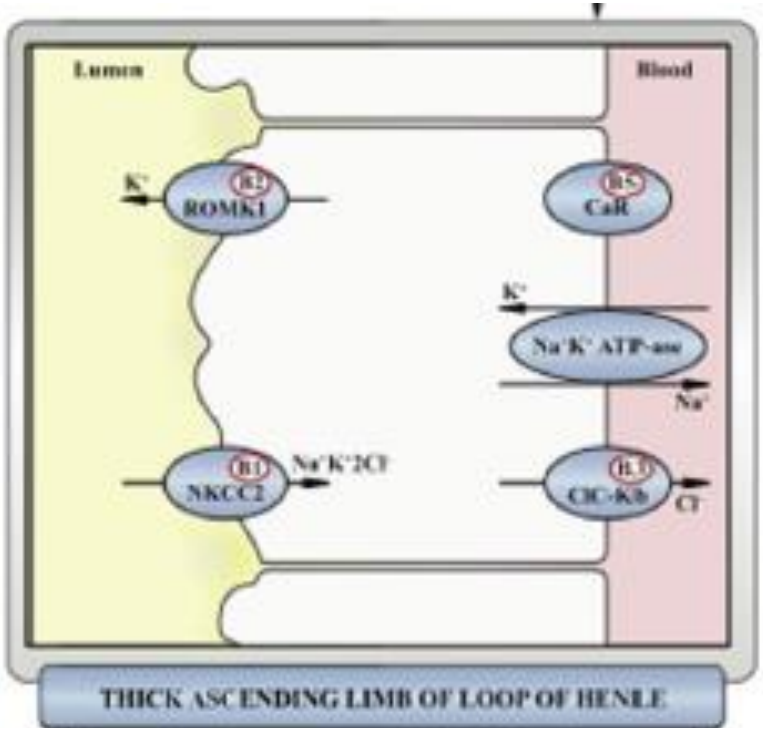
| | | | | |
|---|---|---|---|---|
| Hypotension | | | | |
| Bartter syndrome type 3 | Autosomal recessive | Birth at term, nephrocalcinosis, ↓K+, increased renin and aldosterone | 1p36.13 | CLCNKB,[105] kidney chloride channel B gene. Chloride channel current is compromised in renal tubule. |
| Bartter syndrome type 2 | Autosomal recessive | Prematurity, nephrocalcinosis, postnatal transient ↑K+ hypotension | 11q24.3 | KCNJ1.[106] The ATP-sensitive inwardly-rectifying potassium channel, ROMK. Dysregulation of potassium secretion and NaCl reabsorption in the kidney |
| Barter syndrome type 1 | Autosomal recessive | Prematurity, nephrocalcinosis, hypotension | 15q21.1 | SLC12A1,[107] sodium-potassium-chloride cotransporter-2 (NKCC2). Defect in chloride transport cross the medullary thick ascending limb |
| Gitelman syndrome | Autosomal recessive | Salt-losing tubulopathy, ↓$Ca^{+}$, ↓↓$Mg^{2+}$, Hypotension | 16q13 | SLC12A3[108] thiazide-sensitive sodium-chloride (Na-Cl) cotransporter (NCCT). Loss of function of NCCT |

# Cortical Collecting Tubule




1. Liddle's syndrome: gain in function mutation of ENaC ↑BP
2. Pseudohypoaldosteronism type 1: autosomal recessive loss of function mutation of ENaC ↓BP
3. Syndrome apparent mineralocorticoid excess: deficient 11 β HSD2 w/ inadequate conversion of cortisol to cortisone; excess cortisol has equal affinity for mineralocorticoid receptor (MR) as aldosterone = apparent mineralocorticoid excess ↑BP
4. Pseudohypoaldosteronism type 1: autosomal dominant loss of function of the mineralocorticoid receptor ↓BP
5. Hypertension exacerbated by pregnancy: mutation leads to MR activation by non-aldosterone hormones (progesterone) ↑BP
6. Glucocorticoid remediable aldosteronism: genetic defect leads to ACTH-mediated excess of aldosterone ↑BP
7. Congenital isolated hypoaldosteronism: hereditary defect in adrenal gland synthesis of aldosterone ↓BP
8. Salt wasting congenital adrenal hyperplasis: secondary to 21 OHD deficiency (leads to deficiency of aldosterone and glucocorticoids, with androgen excess) ↓BP

# Bartter's syndrome – typically hypotensive



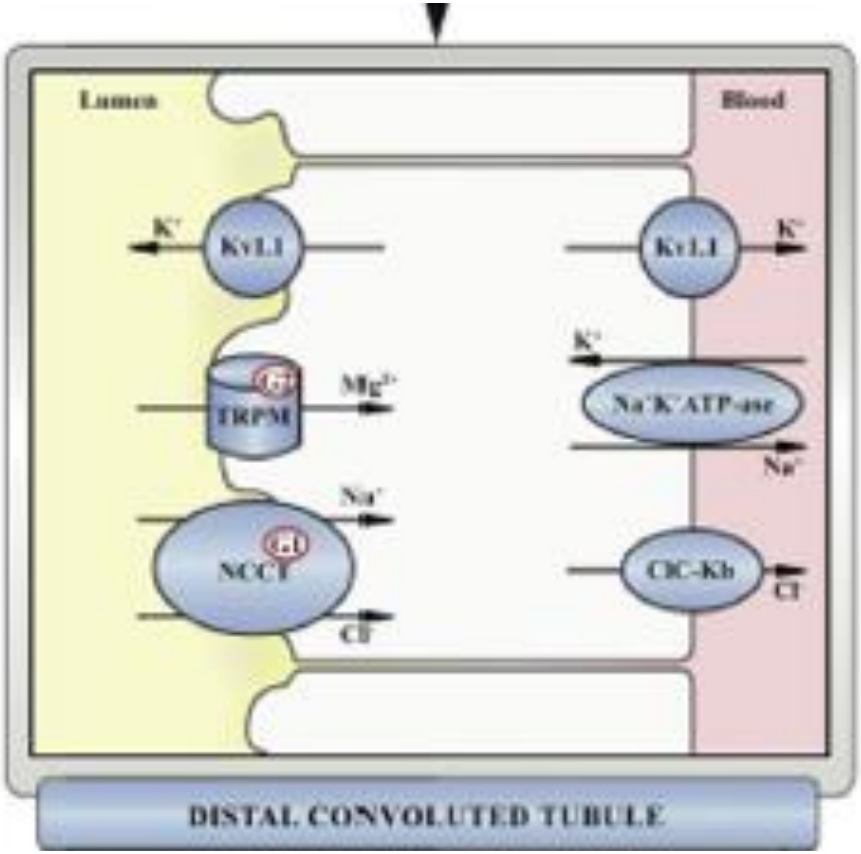

Loss of function mutations in:

- SLC1A1, gene for NKCC2 co-transporter (Bartter's Syndrome type 1),
- KCNJ1, gene for ROMK (Bartter's Syn. type 2),
- CLCNKB, gene for chloride channel ClC-Kb (Bartter's Syn. Type 3)

Gain in function mutations of:

- CaR, gene for calcium sensing receptor (Bartter's Syn. type 5)

# Gitelman's syndrome – typically hypotensive




- Gitelman's Syndrome caused by inactivating mutations in SLC12A3 for the thiazide sensitive sodium chloride (NCCT) co-transporter.
- Hypomagnesemia caused by decreased expression of TRPM6

# Effects of Rare and Common Blood Pressure Gene Variants on Essential Hypertension

## Results From the Family Blood Pressure Program, CLUE, and Atherosclerosis Risk in Communities Studies

Khanh-Dung H. Nguyen, Vasyl Pihur, Santhi K. Ganesh, Ankit Rakha, Richard S. Cooper, Steven C. Hunt, Barry I. Freedman, Joe Coresh, W.H. Linda Kao, Alanna C. Morrison, Eric Boerwinkle, Georg B. Ehret, Aravinda Chakravarti

***Rationale*:** Hypertension affects ≈30% of adults in industrialized countries and is the major risk factor for cardiovascular disease.

***Objective*:** We sought to study the genetic effect of coding and conserved noncoding variants in syndromic hypertension genes on systolic blood pressure (BP) and diastolic BP to assess their overall impact on essential hypertension.

***Methods and Results*:** We resequenced 11 genes (*AGT, CYP11B1, CYP17A1, HSD11B2, NR3C1, NR3C2, SCNN1A, SCNN1B, SCNN1G, WNK1,* and *WNK4*) in 560 European American (EA) and African American ancestry GenNet participants with extreme systolic BP. We investigated genetic associations of 2535 variants with BP in 19 997 EAs and in 6069 African Americans in 3 types of analyses. First, we studied the combined effects of all variants in GenNet. Second, we studied 1000 Genomes imputed polymorphic variants in 9747 EA and 3207 African American Atherosclerosis Risk in Communities subjects. Finally, we genotyped 37 missense and common noncoding variants in 6591 EAs and in 6521 individuals (3659 EA/2862 African American) from the CLUE and Family Blood Pressure Program studies, respectively. None of the variants individually reached significant false-discovery rates ≤0.05 for systolic BP and diastolic BP. However, on pooling all coding and noncoding variants, we identified at least 5 loci (*AGT, CYP11B1, NR3C2, SCNN1G,* and *WNK1)* with higher association at evolutionary conserved sites.

***Conclusions*:** Both rare and common variants at these genes affect BP in the general population with modest effects sizes (<0.05 standard deviation units), and much larger sample sizes are required to assess the impact of individual genes. Collectively, conserved noncoding variants affect BP to a greater extent than missense mutations. (*Circ Res*. 2013;112:318-326.)

## Novelty and Significance

### What Is Known?

- Essential hypertension affects ≈30% of adults in industrialized countries but its molecular pathogenesis is largely unknown.
- Blood pressure (BP) is a heritable trait with a heritability estimate of ≈40% to 60%. It also is influenced by age, body mass index, diet, stress level, and sympathetic tone.
- Mendelian forms of hypotension and hypertension are caused by rare DNA variants that have large impacts on BP.
- In addition, a number of common DNA variants have been discovered to impact BP, explaining 1% to 2% of systolic BP and diastolic BP variations in the general population.

### What New Information Does This Article Contribute?

- Rare variants in genes known to be involved in hypotension or hypertension do not have large effects in the general population.
- Conserved noncoding sequences, at these same genes, although lacking precise functional information, contribute significantly to BP variation.
- If all genetic effects are small, then large studies (with a minimum of 50 000 subjects) are required to obtain meaningful results.

We resequenced the coding and conserved noncoding regions of 11 genes known to be involved in hypertension by focusing on individuals at the extremes of systolic BP. Analyses of common and rare variants at these 11 genes, individually, did not yield significant association with systolic BP or diastolic BP. However, by pooling coding and conserved noncoding elements, and by weighting their genetic contribution by allele frequency and nucleotide conservation, we detected a strong association with BP in at least 5 loci. This analysis suggests that both common and rare variants have small effects (≈0.05 standard deviation unit) on BP. The findings of this study reveal a significant contribution of conserved noncoding elements in genes known to be involved in hypotension or hypertension to BP traits in the general population. Consequently, both the exome and the conserved genomic segments in the human genome need to be comprehensively examined in large samples to allow full elucidation of the genetic contributions to BP.

**Table 3. Genetic Association Results of Pooled Variants by Gene, Population, and Type**

| | EA | | | | | | African American | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | All Variants | | | Missense | | | All Variants | | | Missense | | |
| Gene | SBP | DBP | k | SBP | DBP | k* | SBP | DBP | k | SBP | DBP | k* |
| All | **0.008** | 0.073 | 1,205 | 0.157 | 0.460 | 39 | **0.004** | 0.189 | 1,842 | 0.124 | 0.259 | 70 |
| *AGT* | **0.009** | 0.183 | 64 | 0.035 | 0.501 | 3 | 0.698 | 0.763 | 122 | 0.462 | 0.775 | 6 |
| *CYP11B1* | **0.005** | 0.047 | 66 | 0.181 | 0.115 | 4 | 0.388 | 0.434 | 113 | 0.520 | 0.624 | 9 |
| *CYP17A1* | 0.401 | 0.350 | 25 | 0.996 | 0.943 | 4 | 0.115 | 0.598 | 37 | 0.567 | 0.616 | 4 |
| *HSD11B2* | 0.072 | 0.113 | 57 | 0.972 | 0.938 | 2 | 0.111 | 0.766 | 71 | 0.168 | 0.221 | 3 |
| *NR3C1* | 0.476 | 0.790 | 97 | 0.835 | 0.756 | 2 | 0.014 | 0.104 | 174 | 0.879 | 0.778 | 3 |
| *NR3C2* | **$4.0\times10^{-5}$** | 0.024 | 333 | 0.006 | 0.096 | 3 | 0.013 | 0.629 | 507 | 0.742 | 0.790 | 8 |
| *SCNN1A* | 0.106 | 0.286 | 71 | 0.016 | 0.164 | 6 | 0.085 | 0.380 | 152 | 0.021 | 0.285 | 12 |
| *SCNN1B* | 0.071 | 0.078 | 72 | 0.290 | 0.165 | 5 | 0.110 | 0.560 | 118 | 0.033 | 0.017 | 7 |
| *SCNN1G* | **$3.5\times10^{-4}$** | 0.073 | 142 | 0.081 | 0.067 | 5 | 0.122 | 0.455 | 129 | 0.278 | 0.728 | 3 |
| *WNK1* | 0.297 | 0.181 | 234 | 0.020 | 0.218 | 2 | **0.004** | **0.003** | 322 | 0.688 | 0.360 | 12 |
| *WNK4* | 0.870 | 0.812 | 44 | 0.999 | 0.990 | 3 | 0.022 | 0.400 | 97 | 0.080 | 0.089 | 3 |

DBP, diastolic blood pressure; EA indicates European American; FDR false-discovery rates; and SBP, systolic blood pressure.

We assessed association by pooling (collapsing) all variants, missense, and noncoding variants within each gene and across all genes within EAs and African Americans. In addition, missense variants were weighted by PolyPhen2 scores (*note that PolyPhen2 scores are not available for all missense variants), whereas phyloP scores weighted all and noncoding variants. The numbers of variants (k) involved are provided; probability values with FDR ≤0.05 are in bold.

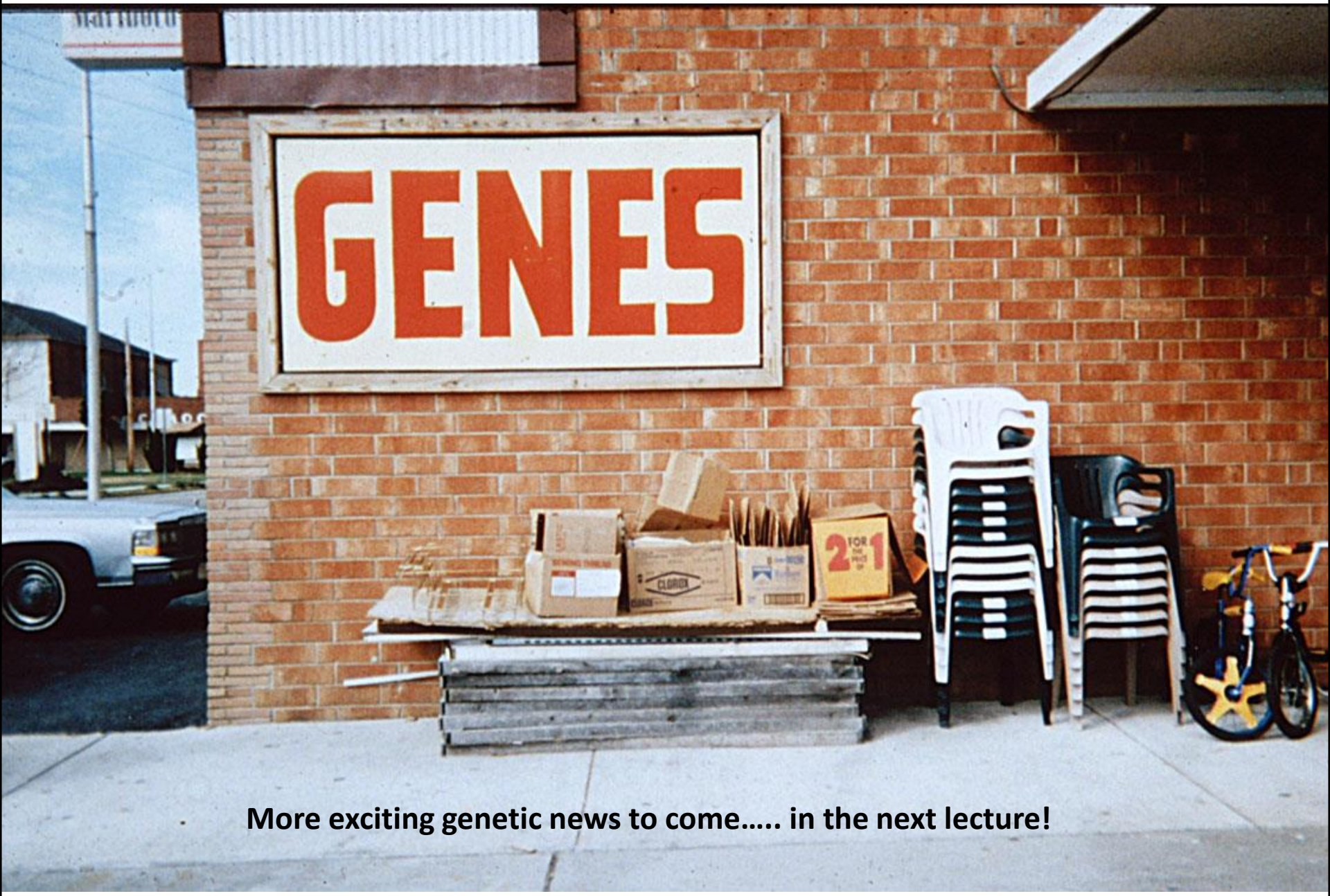
***AGT*** - angiotensinogen

***CYP11B1*** - 11 β hydroxylase (Congenital adrenal hyperplasia and GRA)

***NR3C2*** - hypertension exacerbated by pregnancy (mineralocorticoid receptor)

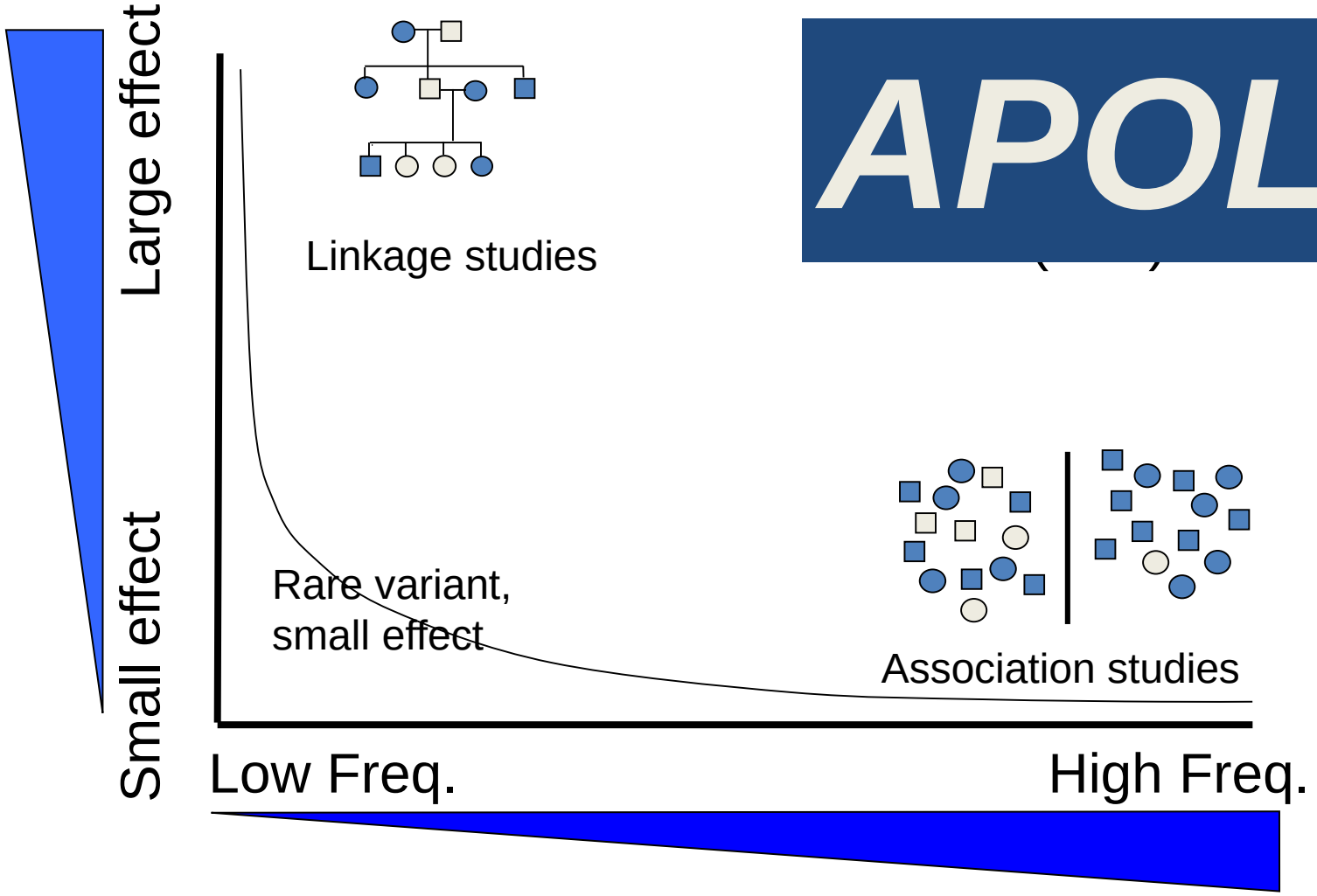
***SCNN1G*** - ENaC (Liddle's syndrome)

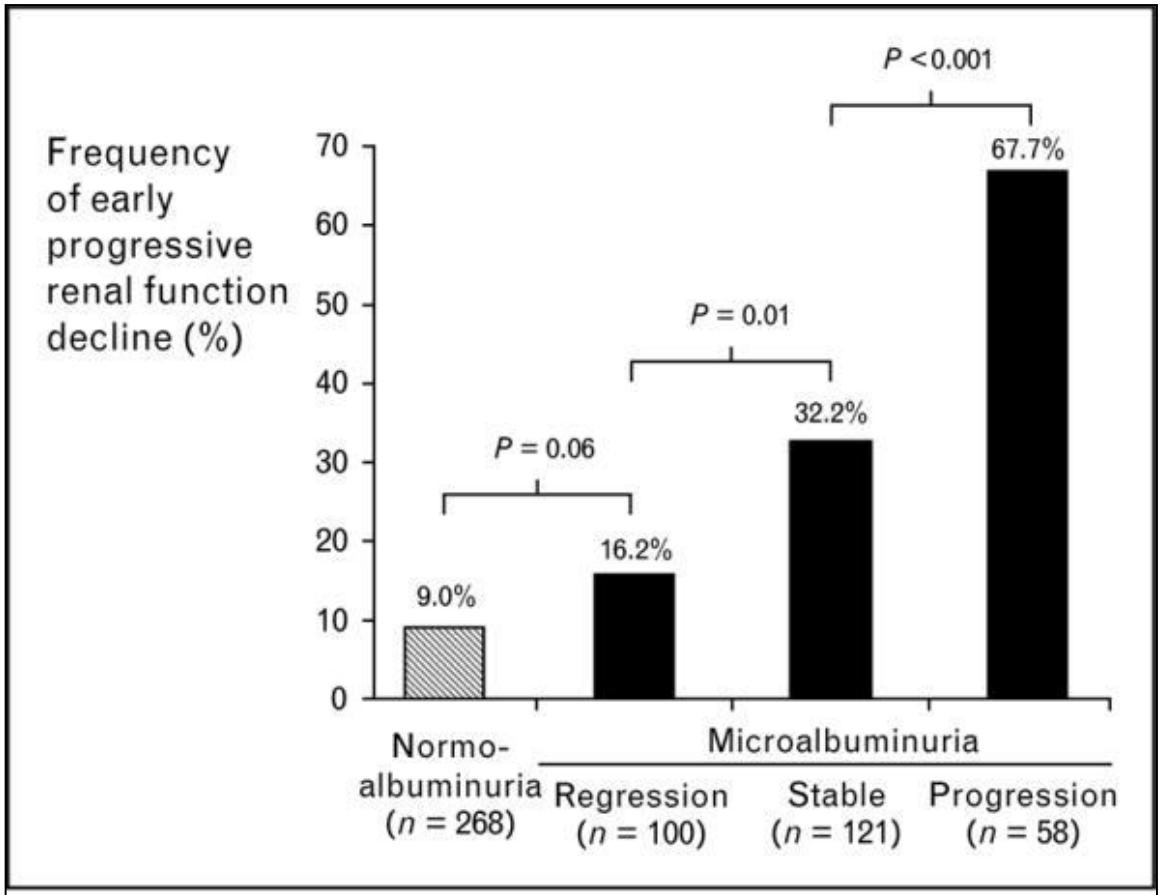
***WNK1*** – pseudohypoaldosteronism type 2 & Gordon's syndrome




**More exciting genetic news to come….. in the next lecture!**

# Properties of DNA variants that have an impact on gene discovery






Regression is defined as a halving (decrease by 50% or more) of the urinary albumin excretion rate (in μg/min) ($N = 100$). Stable is defined as no change greater than a halving or doubling of the urinary albumin excretion rate ($N = 121$). Progression is defined as a doubling (increase by 100% or more) of the urinary albumin excretion rate ($N = 58$). Twenty-two patients had insufficient urine samples in the second follow-up interval to determine the course of their microalbuminuria. Adapted from [18••].

**Early nephropathy in type 1 diabetes: the importance of early renal function decline.**
Perkins, Bruce; Krolewski, Andrzej

Current Opinion in Nephrology & Hypertension.
18(3):233-240, May 2009.
DOI: 10.1097/MNH.0b013e3283293db1

Figure 3 Frequency (in percentage) of early progressive renal function decline in patients with normoalbuminuria and microalbuminuria divided according to the 4-year course of microalbuminuria in the first Joslin Kidney Study

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The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

# Renal and Retinal Effects of Enalapril and Losartan in Type 1 Diabetes

Michael Mauer, M.D., Bernard Zinman, M.D., Robert Gardiner, M.D., Samy Suissa, Ph.D., Alan Sinaiko, M.D., Trudy Strand, R.N., Keith Drummond, M.D., Sandra Donnelly, M.D., Paul Goodyer, M.D., Marie Claire Gubler, M.D., and Ronald Klein, M.D., M.P.H.

Renal interstitial and glomerular changes in normoalbuminuric T1D subjects were not impacted by early use of ACE inhibitor or ARB versus placebo (retinopathy improved)

# UKPDS-Type 2 DM

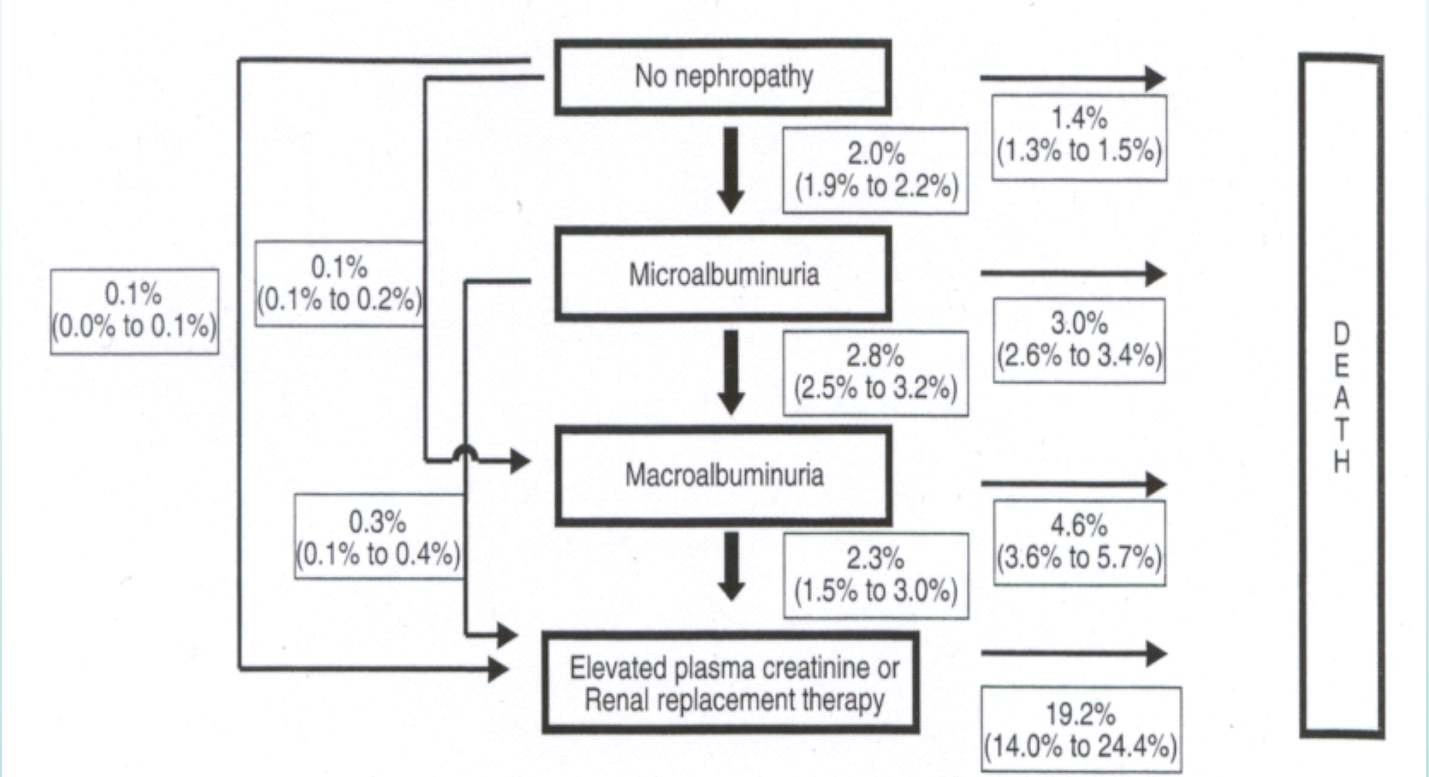



Fig. 1. Annual transition rates with 95% confidence intervals through the stages of nephropathy and to death from any cause.

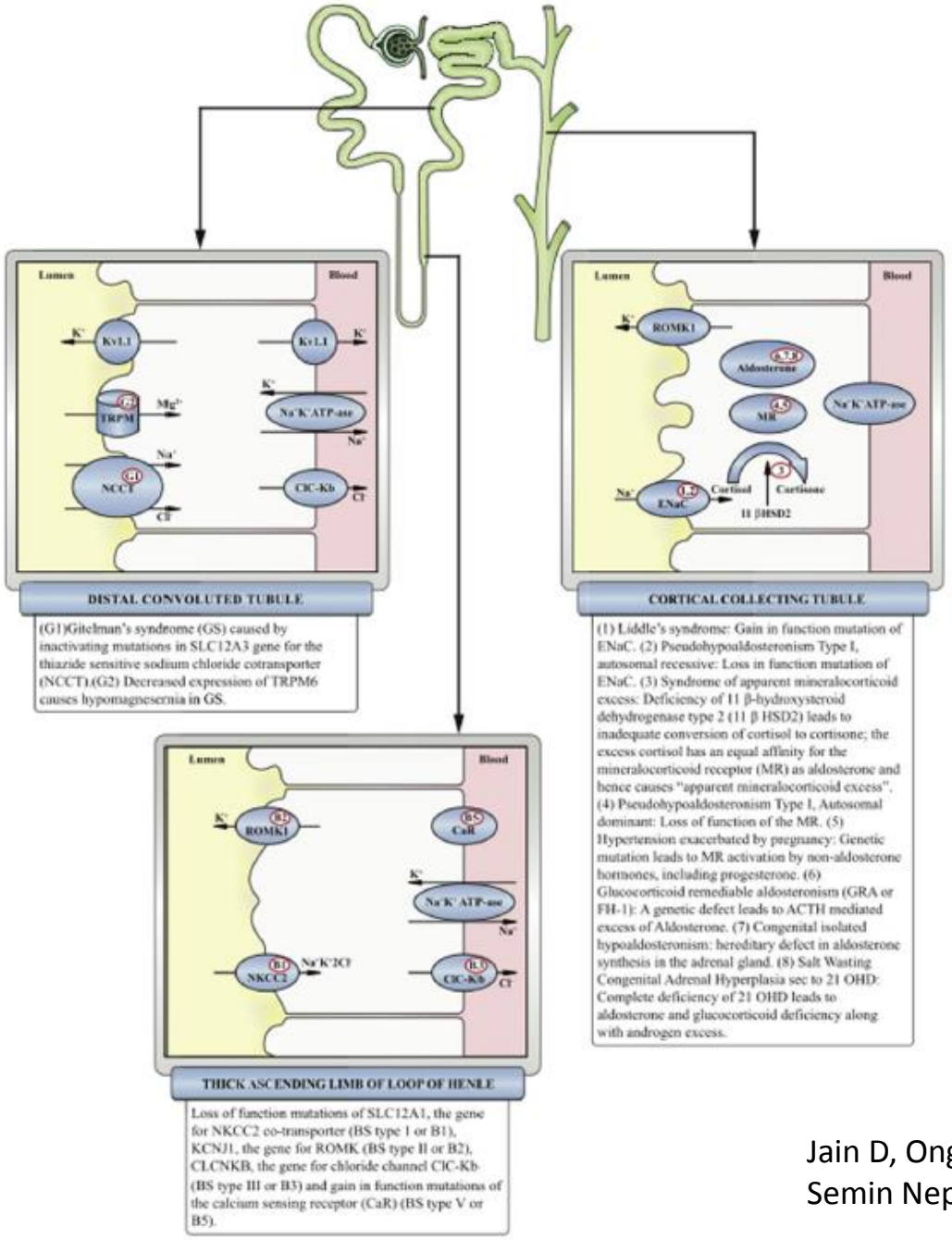
Survival effect when studying populations with ESKD

| Potassium Wasting Disorders | Potassium Retaining Disorders |
|---|---|
| Low Plasma Renin Levels<br>• Liddle's syndrome<br>• Hypertension exacerbated by pregnancy<br>• Glucocorticoid remediable aldosteronism (GRA or FH-1)<br>• Non-glucocorticoid Remediable Aldosteronism<br>• Syndrome of Apparent Mineralocorticoid Excess<br><br>High Plasma Renin Levels<br>• Bartter's syndrome<br>• Gitelman's syndrome | • Pseudohypoaldosteronism Type I<br>• Pseudohypoaldosteronism Type II (Gordon's syndrome or Familial Hyperkalemic Hypertension)<br>• Congenital Isolated Hypoaldosteronism<br>• Salt wasting (SW) form of Congenital Adrenal Hyperplasia (CAH) |

**Figure 2.** Classification of genetic disorders of potassium homeostasis.

**Summary of Blood Pressure Loci**

| SNP | Locus Nickname | Chr | BP Position (Build 37) | CEU RAF | Potential Blood Pressure Candidates | Novel Drug Network, ENCODE or nsSNP Candidates | Reference |
|---|---|---|---|---|---|---|---|
| rs880315 | CASZ1 | 1 | 10 796 866 | 0.65§ | CASZ1† | … | 39 |
| rs5068‡ | NPPA/NPPB | 1 | 11 905 974 | 0.06 | MTHFR*†, CLCN6†, NPPA*, NPPB* | PLOD1*, AGTRAP* | 73 |
| rs17030613 | ST7L-CAPZA1 | 1 | 113 190 807 | 0.49§ | SLC16A1 | CAPZA1*†, ST7L†, MOV10† | 19 |
| rs2004776‡ | AGT | 1 | 230 848 702 | 0.24 | AGT*† | … | 109 |
| rs16849225‡ | FIGN-GRB14 | 2 | 164 906 820 | 0.61§ | FIGN | … | 19 |
| rs13082711 | SLC4A7 | 3 | 27 537 909 | 0.78 | SLC4A7† | EOMES* | 20 |
| rs9815354 | ULK4 | 3 | 41 912 651 | 0.17 | ULK4†, CTNNB1* | … | 18 |
| rs319690 | MAP4 | 3 | 47 927 484 | 0.51 | MAP4†, SMARCC1 | CDC25A* | 19, 21 |
| rs419076 | MECOM | 3 | 169 100 886 | 0.47 | MECOM† | … | 20 |
| rs871606 | CHIC2 | 4 | 54 799 245 | 0.85 | PDGFRA* | … | 21 |
| rs16998073 | FGF5 | 4 | 81 184 341 | 0.21 | FGF5† | | 17 |
| rs13107325 | SL | 4 | 103 188 709 | 0.05 | SLC39A8† | | 20 |
| rs6825911 | | | 111 381 638 | 0.51§ | ENPEP*† | | 19 |
| rs13139571 | | | 156 645 513 | 0.76 | GUC | | 20 |
| rs1799945 | | | | 0.14 | | | 20 |
| rs805303 | | | | | | | 20 |
| rs17477177 | PIK3C | | | | | | 21 |
| rs3918226 | NOS3 | | | | | , SLC4A2* | 47 |
| rs2898290 | BLK-GATA4 | | | | | CTSB*, SOX7*, NEIL2*, BLK†, LINC00208† | 110 |
| rs2071518 | NOV | | | | | | 21 |
| rs11014166‡ | CACNB2 | | | | | | 18 |
| rs1530440 | c10 | | | | | | 17 |
| rs932764 | | | | | | | 20 |
| rs11191548 | | | | | | | 17 |
| rs180125 | | | | 0.6 | | | 109 |
| rs661348 | | | 92 | 0.43 | | | 47 |
| rs7129220 | | | 70 350 538 | 0.89 | ADM | | 20 |
| rs2014408 | | | 16 365 282 | 0.21 | SOX6*† | | 47 |
| rs381815 | PL | 11 | 16 902 268 | 0.30 | PLEKHA7† | | 18 |
| rs633185 | FLJ3 TMEM | 11 | 11 538 410 | 0.28 | PGR*, TRPC6* | | 20 |
| rs11222084 | ADAMTS8 | 11 | 130 273 230 | 0.38 | … | ADAMTS8† | 21 |
| rs17249754‡ | ATP2B1 | 12 | 90 060 586 | 0.37 | ATP2B1*† | DUSP6* | 16 |
| rs653178‡ | SH2B3 | 12 | 112 007 756 | 0.53 | ALDH2*, PTPN11*, SH2B3*† | ACAD10*, RPL6*, CUX2* | 17 |
| rs2384550‡ | TBX3-TBX5 | 12 | 115 352 731 | 0.35 | TBX5*, TBX3 | … | 18 |
| rs1378942‡ | CYP1A1-CSK | 15 | 75 077 367 | 0.36 | CYP1A1*, CYP1A2*†, CSK*†, ULK3, MPI | LMAN1L*, SCAMP2*, hsa-mir-4513† | 17 |
| rs2521501 | FES-FURIN | 15 | 91 437 388 | 0.31 | FES*†, FURIN*† | PRC1* | 20 |
| rs13333226 | UMOD | 16 | 20 365 654 | 0.81 | UMOD† | ACVR2A†, ORC4† | 44 |
| rs17608766 | GOSR2 | 17 | 45 013 271 | 0.86 | GOSR2† | WNT3*, NSF*, RPRML† | 20 |
| rs16948048 | ZNF652 | 17 | 47 440 466 | 0.39 | ZNF652†, NGFR | PHB*, IGF2BP1* | 17 |
| rs805303 | CNAS-EDN3 | 20 | 57 727 777 | 0.45 | EDN3* CNAS*, ZNF831*† | | 20 |
| rs1378942 | JAG1 | 20 | 10 888 030 | 0.48 | JAG1 | | 20 |




Jain D, Ong S, Warnock DG
Semin Nephrol 2013; 33:300-309

. Mechanisms of disease in different segments of the nephron. ATPase, adenosine triphosphatase.

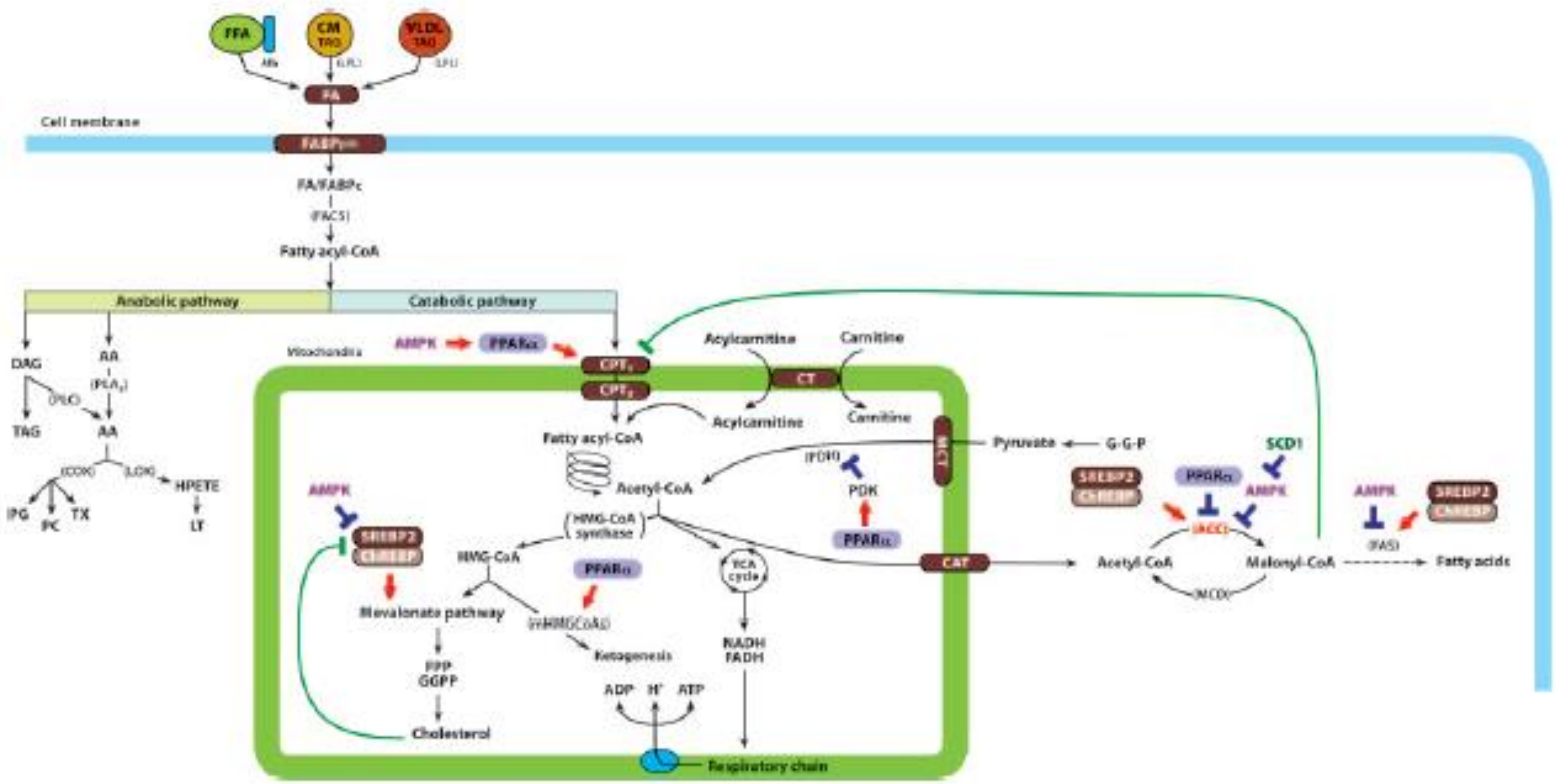
# Association Testing of Previously Reported Variants in a Large Case-Control Meta-analysis of Diabetic Nephropathy

*Diabetes* 61:2187–2194, 2012

Winfred W. Williams,[1,2,3,4] Rany M. Salem,[2,5] Amy Jayne McKnight,[6] Niina Sandholm,[7,8,9] Carol Forsblom,[7,8] Andrew Taylor,[1,2,10] Candace Guiducci,[2] Jarred B. McAteer,[1,2,10] Gareth J. McKay,[6] Tamara Isakova,[11] Eoin P. Brennan,[12,13] Denise M. Sadlier,[12,13,14] Cameron Palmer,[2,5] Jenny Söderlund,[7,8] Emma Fagerholm,[7,8] Valma Harjutsalo,[7,8,15] Raija Lithovius,[7,8] Daniel Gordin,[7,8] Kustaa Hietala,[7,16] Janne Kytö,[7,16] Maija Parkkonen,[7,8] Milla Rosengård-Bärlund,[7,8] Lena Thorn,[7,8] Anna Syreeni,[7,8] Nina Tolonen,[7,8] Markku Saraheimo,[7,8] Johan Wadén,[7,8] Janne Pitkäniemi,[17] Cinzia Sarti,[17] Jaakko Tuomilehto,[15,17,18] Karl Tryggvason,[19] Anne-May Österholm,[19] Bing He,[19] Steve Bain,[20] Finian Martin,[12,21] Catherine Godson,[12,13] Joel N. Hirschhorn,[2,5] Alexander P. Maxwell,[6] Per-Henrik Groop,[7,8] and Jose C. Florez,[1,2,4,10] for the GENIE Consortium

In summary, we have presented evidence that several previously reported genetic associations with DN in type 1 diabetes could not be replicated in a large, homogeneous sample of subjects with type 1 diabetes. Our failure to replicate these associations underscores the need to apply stringent statistical thresholds of significance, maximize power through meta-analysis of all available data, and seek replication in independent samples, as has been proposed by a number of different authors (31,32). Finally, the ap-

Meta Analysis




*** *ACACB* encodes enzyme for rate-limiting step in mitochondrial fatty acid oxidation**