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GENETICS (AP MORRIS, SECTION EDITOR)

Progress in Defining the Genetic Basis of Diabetic Complications

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Abstract
Purpose of Review Diabetic complications affecting the kidneys, retina, nerves, and the cardiovasculature are the major causes of morbidity and mortality in diabetes. This paper aims to review the current understanding of the genetic basis of these complications, based on recent findings especially from genome-wide association studies.
Recent Findings Variants in or near *AFF3*, *RGMA-MCTP2*, *SP3-CDCA7*, *GLRA3*, *CNKSR3*, and *UMOD* have reached genome-wide significance (p value $<5 \times 10^{-8}$) for association with diabetic kidney disease, and recently, *GRB2* was reported to be associated at genome-wide significance with diabetic retinopathy. While some loci affecting cardiovascular disease in the general population have been replicated in diabetes, *GLUL* affects the risk of cardiovascular disease specifically in diabetic subjects.
Summary Genetic findings are emerging for diabetic complications, although the studies remain relatively small compared to those for type 1 and type 2 diabetes. In addition to pinpointing specific loci, the studies also reveal biological information on correlated traits and pathways.

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Keywords Genetic risk factors · Diabetic complications · Genome-wide association study · End-stage renal disease · Diabetic retinopathy · Cardiovascular disease

Introduction

Diabetic complications are a major cause of morbidity and mortality [1, 2]. While macrovascular complications affect the coronary, cerebral, and peripheral arteries, microvascular complications cause diabetic kidney disease (DKD) leading to renal failure, diabetic retinopathy (DR) leading to blindness, and peripheral and autonomic neuropathy [3].

Elevated blood glucose levels, measured as glycated hemoglobin (HbA_{1C}), are a major risk factor for both micro- and macrovascular complications [3]. In addition to clinical risk factors, genetic factors affect the risk as well. Besides improving our understanding of the mechanisms leading to disease, pinpointing the genetic risk factors also holds the promise of revealing novel biomarkers for early detection of patients at highest risk, and novel druggable target molecules for future treatment. For example, PCSK9 inhibitors, a novel group of low-density lipoprotein (LDL) cholesterol lowering drugs that reduce the risk of cardiovascular disease (CVD), are based on genetic discovery [4]. Even though the genetic background remains relatively poorly understood, genetic findings for diabetic complications are starting to emerge. The key findings, as well as the main challenges and future directions for the genetic discovery for diabetic complications, are reviewed in this article.

Renal Complications in Diabetes

DKD is a devastating complication affecting one third of the subjects with type 1 diabetes (T1D) [5]. Classically, DKD is described to commence with microalbuminuria (i.e., urinary excretion of low concentrations of albumin), leading to macroalbuminuria (urinary excretion of considerable amounts of albumin), then leading to a decrease in the (estimated) glomerular filtration rate (eGFR). However, loss of renal function can also appear without albuminuria [6]. Despite improved medical care, 2–20% of subjects with T1D develop end-stage renal disease (ESRD) that requires dialysis or a renal transplant [7, 8], and is associated with 14-fold risk of mortality compared with diabetic subjects without renal complications [2].

DKD clusters in families, with a twofold sibling recurrence risk [9–11]. Heritability estimates for albuminuria in type 2 diabetes (T2D) range from 30 to 45% [12, 13], and for eGFR they reach up to 75% [13]. Recently, genome-wide genotyping data of unrelated individuals has been utilized to estimate the narrow sense heritability h^2, i.e., the phenotypic variance explained by the additive effects of genotyped single nucleotide polymorphisms (SNPs) for albuminuria (h^2 = 27% [14••]), and for case-control phenotypes in T1D: h^2 was 35% for albuminuria-based DKD, 47% for eGFR-based chronic kidney disease (CKD; defined as eGFR <60 ml/min/1.73m^2), and 59% for the composite phenotype of both DKD and CKD [15••].

Candidate Genes for DKD

Plenty of candidate genes have been considered for DKD, with the largest number of studies investigating an insertion/deletion polymorphism rs1799752 (tagged by rs4344) in the *ACE* gene encoding the angiotensin-converting enzyme (ACE) [16, 17]. ACE inhibitors are the principal treatment for DKD and other proteinuric renal diseases, along with other medications targeting the renin-angiotensin-aldosterone system (RAAS). Despite the inclusion of over 26,000 subjects in the literature-based meta-analysis, only modest evidence of association was found [16, 17]. As it is characteristic of many candidate gene studies, the vast majority of the 63 included studies had only tens to few hundreds of participants [17]. A literature-based meta-analysis of all candidate genes for DKD found evidence of association in 17 loci. However, the analysis did not consider correction for multiple testing or apply particularly stringent significance thresholds [16]. The candidate genes were subsequently evaluated in a meta-analysis of three large cohorts from the "Genetics of Nephropathy—an International Effort" (GENIE) Consortium with up to 6366 subjects with T1D. No association remained significant after correction for multiple testing, suggesting that many previous findings were false positives, and highlighting the need for hypothesis-free genome-wide association studies (GWAS) performed in large and well-characterized study populations [18].

GWAS on DKD

GWAS have proven efficient in detecting genetic loci associated with common diseases: for example, over 80 loci have been identified for T2D to date [19]. Although the number of loci for DKD remains modest, the number of genome-wide significant findings for DKD is increasing (Table 1).

Before the currently used GWAS arrays that genotype hundreds of thousands of SNPs, a GWAS covering 80,000 polymorphisms in 188 subjects with T2D suggested an association in the *ELMO1* gene ($p = 8 \times 10^{-6}$) [20], but replication remains controversial despite evaluation in multiple cohorts [15••, 18, 32, 33]. GWAS on DKD including 1705 subjects with T1D from the US Genetics of Kidneys in Diabetes (GoKinD) collection identified four loci with a suggestive p value $<10^{-5}$ and found supporting evidence of association for variants near *FRMD3* and *CARS* genes in the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study [22]. With some subsequent studies supporting these findings and others not [18, 34, 35], replication remains inconclusive as the association has not reached genome-wide statistical significance, defined as p value $<5 \times 10^{-8}$, required for robust association due to the burden of multiple testing in GWAS.

The first genome-wide significant findings were obtained from a meta-analysis of three GWAS from the GENIE consortium, with up to 11,847 subjects with T1D including replication studies. Variants in *AFF3* ($p = 1.2 \times 10^{-8}$) and between the *RGMA* and *MCTP2* genes ($p = 2.0 \times 10^{-9}$) were associated with ESRD. Functional work on renal epithelial cells suggested that *AFF3* affects the renal tubular fibrosis, a pathological feature of severe DKD. The *RGMA/MCTP2* locus was also among the top findings in a subsequent study utilizing a data mining approach [27]. However, little is known about the flanking genes, and despite "naming" the locus based on the closest genes, the signal may target the expression or function of a gene located much further away [36]. For the DKD phenotype, suggestive association was found at the *ERBB4* gene ($p = 2.1 \times 10^{-7}$) [25]. *ERBB4* knock-out and overexpression mice show that this gene affects renal development [37]. Furthermore, pan-ErbB inhibitors protect from albuminuria in diabetic mouse models, potentially through ErbB4 [38].

Gender-stratified GWAS revealed variants on the *SP3/CDCA7* gene region associated with twofold risk of ESRD in women with T1D ($p < 5 \times 10^{-8}$). The finding was replicated in other GENIE cohorts while no association was seen in men [26]. *SP3* is a biologically plausible candidate, as the Sp3 transcription factor directly binds to the estrogen receptor α

Table 1 The main GWAS findings for DKD

Study	Population	Phenotype	*N*	Main findings
Shimazaki 2005 [20]	Japanese T2D	DKD	188 (screening), 732 (stage 2)	*ELMO1* (rs741301) $p = 8 \times 10^{-6}$
Maeda 2010 [21]	Japanese T2D	DKD	188 (screening), 1312 (stage 2)	*ACACB* (rs2268388) $p = 5.35 \times 10^{-8}$
Pezzolesi 2009 [22]; Pezzolesi 2010 [23] (US GoKinD)	European American T1D	DKD	1705	*FRMD3* (rs10868025) $p = 5.0 \times 10^{-7}$, *CARS* (rs451041) $p = 3.1 \times 10^{-6}$
McDonough 2011 [24]	African American T2D	ESRD	965 T2D-ESRD cases + 1029 non-diabetic non-DKD controls (discovery)	19 suggested loci
Sandholm 2012 [25] (GENIE)	European ancestry T1D	DKD, ESRD	6691 (discovery), 11,847 total	***AFF3* (rs7583877) $p = 1.2 \times 10^{-8}$ (ESRD)** ***RGMA/MCTP2* (rs12437854) $p = 2.0 \times 10^{-9}$ (ESRD)** *ERBB4* (rs7588550) $p = 2.1 \times 10^{-7}$ (DKD)
Sandholm 2013 [26]	European ancestry T1D	ESRD	1193 women (discovery); 2697 women in total	***SP3/CDCA7* rs4972593 $p = 3.9 \times 10^{-8}$**
Sandholm 2014 [14••]	Finnish/European T1D	albuminuria	1925 (discovery), 3750 (replication)	***GLRA3* (rs1564939/rs10011025) $p = 1.5 \times 10^{-9}$ in Finnish discovery** *PSD3/SH2D4A* (rs2410601) $p = 3.9 \times 10^{-6}$
Sambo 2014 [27]	European T1D	DKD, ESRD	3464 (discovery), 4263 (replication)	*WNT4-ZBTB40* (rs12137135), *RGMA-MCTP2* (rs17709344), *MAPRE1P2* (rs1670754), *SEMA6D-SLC24A5* (rs12917114), and *SIK1* (rs2838302) suggested with a data mining approach
Germain 2015 [28]	European ancestry T1D	DKD	1462 (discovery), 7803 total	*SORBS1* (rs1326934) $p = 0.009$ after full meta-analysis
Iyengar 2015 [29••] (FIND)	Trans-ethnic T2D	DKD	6197 (discovery), 13,736 total	***SCAF8/CNKSR3* (rs955333) $p = 1.3 \times 10^{-8}$ in all; rs12523822 $p = 5.7 \times 10^{-9}$ in American Indians**
Teumer 2016 [30]	European ancestry	Albuminuria	Up to 54,450, including 7787 diabetic	*RAB38* (rs649529) $p = 5.8 \times 10^{-7}$ *HS6ST1* (rs13427836) $p = 6.3 \times 10^{-7}$
Pattaro 2016 [31••]	Mainly European ancestry	eGFR	Up to 133,413 in discovery, 42,166 in replication, including 16,477 diabetic in total	***UMOD* (rs12917707) $p = 2.5 \times 10^{-8}$** $p < 0.05$ in diabetic subset for 19/53 eGFR loci in general population
Sandholm 2017 [15••] (SUMMIT)	European ancestry T1D	DKD, CKD, ESRD	Up to 5156 at discovery, 12,540 in total	Suggestive associations at *PTPN13, AFF3, CNTNAP2* and *NRG3*. Alleles increasing BMI ($p = 2.2 \times 10^{-5}$) and T2D ($p = 6.1 \times 10^{-4}$) associated with DKD Genome-wide correlation between DKD and smoking cessation ($p = 1.1 \times 10^{-4}$)

The associations reaching genome-wide significance, i.e., p value $<5 \times 10^{-8}$ are highlighted with bold text

forming a protein complex activated by estrogen [39]; together with the notion that *SP3* is more expressed in the glomeruli of diabetic women than men, this may explain why the association is limited to women [26].

A GWAS of albuminuria in 1925 Finnish subjects with T1D found variants in the *GLRA3* gene with $p = 1.5 \times 10^{-9}$. While nominally significant association was obtained in the meta-analysis of additional 3750 subjects with T1D and of European ancestry, the association was in the opposite direction. The authors suggest that the associated SNPs might be in linkage disequilibrium with population-specific variants, and therefore warrant replication in additional Finnish participants [26].

A trans-ethnic GWAS meta-analysis performed in the Family Investigation of Nephropathy and Diabetes (FIND) consortium identified variants associated with DKD between the *SCAF8* and *CNKSR3* genes ($p = 5.7 \times 10^{-9}$) [29••]. The association was particularly strong in American Indians. *CNKSR3* encodes for a scaffolding platform that stimulates epithelial sodium channel in response to aldosterone [40], one of the main targets of the RAAS blockers used to treat DKD. Strong association was also seen in African Americans in the *MYH9/APOL1* locus, an important genetic susceptibility locus for many non-diabetic renal diseases; the association in subjects with T2D is likely due to unrecognized non-diabetic renal disease co-occurring with T2D [29••, 35]. This highlights the challenge of phenotypic heterogeneity, particularly in subjects with T2D: while most subjects with T1D and albuminuria have pathological renal lesions characteristic of diabetic nephropathy, there is a spectrum of renal pathologies in subjects with T2D where only a third patients with albuminuria have typical diabetic nephropathy [41].

To tackle the challenge posed by phenotypic heterogeneity in DKD, seven albuminuria and/or eGFR-based phenotypic definitions were used in the GWAS meta-analysis from SUrrogate markers for Micro- and Macrovascular hard endpoints for Innovative diabetes Tools (SUMMIT) consortium, which included up to 12,540 subjects with T1D in the combined meta-analysis. Suggestive associations were found in the *AFF3, PTPN13, CNTNAP2*, and *NRG3* loci, although no locus reached genome-wide significance [15••]. Among the previously identified loci, independent evidence of association was found, after exclusion of overlapping studies, for *SIK1* [27] for ESRD. The genetic risk scores consisting of variants increasing BMI and the risk of T2D were associated with DKD, suggesting BMI and metabolic changes behind T2D as causal risk factors for DKD in T1D. This supports a previous Mendelian Randomization study reporting a causal link between BMI and DKD [42]. Furthermore, genome-wide LD-score regression analysis of the SUMMIT GWAS and other traits revealed genome-wide correlation in the genetic contribution to DKD and smoking cessation [15••], supporting epidemiological findings that smoking cessation is beneficial for avoiding DKD [43].

A GWAS with up to 54,450 subjects from the general population, including a subset of 7787 subjects with diabetes (mainly T2D), suggested *HS6ST1* and *RAB38/CTSC* ($p < 10^{-6}$) as novel loci for albuminuria in subjects with, but not without diabetes [30]. The largest GWAS meta-analysis on eGFR and CKD in the general population included 133,413 individuals at the discovery stage, and identified 53 loci (p value $<5 \times 10^{-8}$) for kidney traits [31••]. In the subset of 16,477 subjects with diabetes, significant association (p value $= 2.5 \times 10^{-8}$) was found for eGFR at rs12917707 at *UMOD*, a major susceptibility locus in the general population, and nominal associations ($p < 0.05$) were found at 19 of the 53 loci [31••].

Whole Exome Sequencing

GWAS findings feature mainly common variants, as GWAS genotyping arrays are designed to capture common variation, and genotype imputation does not sufficiently capture rare variation. Therefore, sequencing-based methods are needed to investigate low-frequency (minor allele frequency [MAF] 1–5%) and rare variants (MAF <1%) not detected on GWAS genotyping chip platforms. While whole genome sequencing is already possible, targeting only the protein coding sequence (representing less than 2% of the genome) with whole exome sequencing (WES) significantly reduces the costs, thus allowing higher sequencing depth resulting in better quality genotypes, and higher numbers of samples to be sequenced to increase the statistical power. Based on WES data for T2D, a candidate gene evaluation of the *RREB1* gene, an upstream regulator of RAAS, was undertaken in 529 African American cases with T2D and ESRD, and 535 controls without DKD and diabetes. Replication and trait segregation in diabetic subjects without ESRD and ESRD subjects without diabetes suggested that variants in *RREB1* may modulate the risk of T2D, ESRD, and non-diabetic renal disease ($p = 3.5 \times 10^{-7}$ for rs9379084 for the combined T2D-ESRD phenotype), but further replication is warranted to confirm the findings, especially as variants in this gene were previously associated with T2D in a large GWAS meta-analysis [44].

WES efforts are emerging for DKD. WES of 997 subjects with T1D from the SUMMIT consortium did not reveal any variants, or genes enriched for rare or low-frequency variants with stringent statistical significance ($p < 5 \times 10^{-7}$) [15••]. Common variants in *ERBB4* were among the strongest associations for ESRD; variants in the same gene were suggestively associated with DKD in the GENIE GWAS, albeit a subset of the included subjects is overlapping [15••].

DNA Methylation

DNA methylation has been suggested as a potential explanation for "metabolic memory," a sustained effect of improved blood glucose levels [45•]. An early genome-wide DNA methylation study covering 25,578 CpG sites in 192 participants with T1D identified 19 loci differentially methylated in blood of DKD cases versus controls without renal complications [46]. As the currently used DNA methylation chips can address hundreds of thousands of CpG sites [47•], a larger study could reveal more genes related to DKD methylation.

Diabetic Retinopathy

DR is one of the leading causes of blindness worldwide [48], affecting 30% of subjects with T2D [49] and 50–80% of subjects with T1D [49, 50]; among the T1D subjects with severe renal complications, nearly all have at least some level of diabetic retinal changes [2]. DR has the highest sibling recurrence risk of the microvascular diabetic complications [51], and the heritability estimates for DR range from 25 to 52% [52–56].

As for DKD, many candidate gene studies have been performed on DR, including meta-analyses, e.g., for *ACE* [57], *AKR1B1* (encoding for aldose reductase) [58], and *VEGF* (encoding for vascular endothelial growth factor) [59]. A systematic meta-analysis of 20 candidate genes for DR found the strongest evidence of association for the z-2 microsatellite and rs759853 on *AKR1B1* (p value = 1×10^{-4}) and nominally significant evidence for polymorphisms in *NOS3, VEGF, ITGA2,* and *ICAM1* genes [60]. A large-scale multi-ethnic candidate gene study testing 49,320 SNPs from ~2000 candidate genes found suggestive associations with p value below 10^{-6}, but the findings did not replicate [61]. In a meta-analysis of 1907 subjects with T1D, none of the previously reported loci remained significant after adjustment for multiple testing, suggesting that many of the previous reports may represent false positive findings [62].

In addition to partially shared pathophysiological mechanisms, there is evidence of a shared genetic background between DR and DKD [62]. Two candidate gene studies identified loci with genome-wide significance for a combined ESRD and DR phenotype after meta-analysis with replication cohorts; subsequent replication at the erythropoietin (*EPO*) gene [63] has been inconclusive [18, 62, 64], whereas replication of a recent association ($p = 7.1 \times 10^{-9}$) in *SLC19A3*, a thiamin (vitamin B_1) transporter, has not yet been attempted in other studies [65•].

GWAS on DR

Multiple GWAS on DR have been published including studies in Mexican Americans [66], Taiwanese [67], Chinese [68], Japanese [69], and White Australian [70••] subjects with T2D, and in European American subjects with T1D [71] (Table 2). However, the numbers of subjects were small (a couple of hundreds up to 3000) and the results mostly suggestive. Four loci (*HS6ST3, ARHGAP22, PLXDC2*, and *KIAA0825*) reached genome-wide significance in a GWAS including 749 subjects with T2D, but no replication of the findings was attempted [67]. Subsequently, one study provided nominally significant associations ($p < 0.05$) at the *PLXDC2* and *ARHGAP22* loci [62]. While other replication efforts of the early GWAS findings supported associations in or near *API5* ($p = 0.0005$) [73], *CEP135* ($p = 2 \times 10^{-5}$) [74], *NPY2R* ($p = 3 \times 10^{-5}$) [74], and *INSR* ($p = 9 \times 10^{-4}$) [75], the findings were mainly inconclusive [62, 70••, 73–76], and no systematic effort has been published to pool together the collective evidence for these and other suggested loci.

A recent GWAS in 844 white Australians with T2D found variants associated with severe non-proliferative DR near the *GRB2* gene, with directionally consistent replication in all three replication cohorts (both T2D and T1D, and of European and Indian ancestry), resulting in a p value of 4.2×10^{-8} at the combined meta-analysis [70••]. *GRB2* activates the MAPK pathway in response to insulin by binding the major insulin receptor substrate IRS-1, and *GRB2* expression was found upregulated in the retina of the mouse model for retinopathy [70••].

Whole Exome Sequencing

A small WES study of 43 Saudi subjects with diabetes without DR and 64 with DR identified three genes, *NME3, LOC728699,* and *FASTK*, with an excess of rare variants in subjects without DR (all p values $<5 \times 10^{-8}$) [72•]. As for the more recent observations from GWAS, validation in other cohorts is still required to confirm the findings.

DNA Methylation

A recent study investigated the genome-wide methylation patterns in 28 cases with T1D and DR, in 30 diabetic controls, and in 7 converters who developed DR during the 6.3 years follow-up. Among the 485,577 CpG sites evaluated in the study, 349 sites at 233 unique loci were differentially methylated between the cases and controls. Among the 349 sites, 28 CpG sites were differentially methylated also between the DR controls and DR convertors [47•]. In addition to proposing novel genes for the pathology of DR, the authors suggested that DNA methylation patterns could be used as biomarkers to

Table 2 Main GWAS, WES, and genome-wide methylation studies for DR

Study	Population	*N*	Main findings
GWAS			
Fu 2010 [66]	Mexican American T2D	286	13 loci with $p < 0.0001$
Huang 2011 [67]	Taiwanese T2D	174 T2D-DKD cases + 575 T2D controls; 100 non-diabetic controls	***KIAA0825*** **(rs17376456)** $\boldsymbol{p = 3.0 \times 10^{-9}}$ ***HS6ST3*** **(rs2038823)** $\boldsymbol{p = 4.7 \times 10^{-11}}$ ***ARHGAP22*** **(rs4838605)** $\boldsymbol{p = 1.9 \times 10^{-9}}$ ***PLXDC2*** **(rs12219125)** $\boldsymbol{p = 9.3 \times 10^{-9}}$ *p* values are for best genetic models
Grassi 2011 [71] (EDIC + GoKinD US)	European American T1D	2829	3 loci with $p < 10^{-6}$ (*AKT3, A2BP1, LEKR1-VEPH1*)
Sobrin 2011 [61] (CARe)[a]	Multi-ethnic T2D, incl. T1D in replication	2691 (1254 European American) in discovery, 8041 in replication	2 loci with $p < 10^{-6}$ (*SELP, IDUA*), did not replicate
Sheu 2013 [68]	Chinese T2D	1007	3 loci with $p < 10^{-5}$, not replicated in Hispanics
Awata 2014 [69]	Japanese T2D	446 (discovery), 1986 in total	*RP1-90L14.1/CEP162* (rs9362054) $p = 4.4 \times 10^{-8}$ in stage 1 + 2, $p = 1.4 \times 10^{-7}$ in total
Burdon 2015 [70••]	White Australian T2D; multi-ethnic replication in T1D + T2D	844 (discovery), 2494 total	***GRB2*** **(rs9896052)** $\boldsymbol{p = 4.2 \times 10^{-8}}$
Candidate gene studies for combined ESRD + DR phenotype			
Tong 2008 [63]	European Ancestry T1D and T2D	2572 in total from 3 cohorts	***EPO*** **(rs1617640)** $\boldsymbol{p = 3 \times 10^{-11}}$
Porta 2016 [65•]	European Ancestry T1D	753 in total from 2 studies	***SLC19A3*** **(rs12694743)** $\boldsymbol{p = 7.1 \times 10^{-9}}$
Whole exome sequencing (WES)			
Shtir 2016 [72•]	Saudi	107	Excess of rare variants in *NME3, LOC728699,* and *FASTK* ($p < 5 \times 10^{-8}$)
Genome-wide DNA methylation			
Agardh 2015 [47•]	Swedish T1D	28 cases, 30 controls, 7 converters	349 CpG sites at 233 loci differentially methylated (FDR < 0.05)

The associations reaching genome-wide significance, i.e., *p* value $<5 \times 10^{-8}$ are highlighted with bold text

FDR false discovery rate

[a] Large-scale candidate gene study testing 49,320 SNPs from ~2000 candidate genes

predict DR, but acknowledged that independent replication is first required to confirm the findings [47•].

Diabetic Neuropathy

Diabetic neuropathy is a microvascular complication affecting the neurons, resulting in a spectrum of clinical manifestations such as neuropathic pain (peripheral neuropathy) and cardiac and gastrointestinal autonomic neuropathy. Familial clustering has been found for diabetic neuropathy, but more modest than for other microvascular complications [51]. A recent study utilized genome-wide genotype data to estimate 11% narrow sense heritability for diabetic neuropathic pain [77•]; when stratified by gender, narrow sense heritability was higher in males (30%) than in females (15%) [78].

Maybe because of the challenges to define the phenotype, only a few genetic studies have been performed on diabetic neuropathy. Although no systematic literature-based meta-analyses have been published on all candidate genes for diabetic neuropathy, meta-analyses have been performed for the insertion/deletion variant in *ACE* [79, 80] and a polymorphism in the methylenetetrahydrofolate reductase (*MTHFR*) gene [81], all showing nominal evidence of association with diabetic neuropathy.

Despite the small number of candidate gene studies, two GWAS have been published for neuropathic pain in diabetes based on 3063 subjects with T2D from the Genetics of Diabetes and Audit Research Tayside Study (GoDARTS)

[77•, 78]. Cases were defined based on prescription of medicines, and positive monofilament test; 8% of the subjects filled these criteria of neuropathic pain [77•]. The GWAS found suggestive evidence of association at rs17428041 near the *GFRA2* gene ($p = 1.8 \times 10^{-7}$) [77•]. Subsequent analysis of the same study with somewhat relaxed participant inclusion criteria, including 4221 subjects, suggested association at rs71647933 between the *ZSCAN20* and *TLR12P* genes (p value = 4.9×10^{-7}, OR = 1.65, 95% confidence interval [CI] 1.36–2.02). The same locus yielded also the strongest association in the female only dataset (p value = 2.7×10^{-7}, OR = 2.31, 95% CI 1.68–3.17; association was not significant in men). Among men, the strongest association was obtained at rs6986153, near to *HMGB1P46* (p value 8.0×10^{-7}, OR = 1.67, 95% CI 1.34–2.08) [78]. As no replication was attempted, and the associations did not reach genome-wide significance, further evaluation of these loci in other cohorts is required to replicate these findings.

Cardiovascular Complications in Diabetes

CVD is the most common cause of death among patients with diabetes and atherosclerosis is central to its development [1]. Atherosclerosis occurs when a plaque consisting of substances found in the blood is built up in the arteries, resulting in narrowing of the arteries. Thus, blood flow is compromised to organs such as the heart muscle, the brain, and the extremities, causing the devastating cardiovascular complications known as coronary artery disease (CAD), cerebrovascular disease, and peripheral arterial disease (PAD).

Diabetes has been considered a "CVD equivalent," i.e., subjects with diabetes have the same risk of a cardiovascular event as subjects with pre-existing CVD [82]. The connection between DKD and CVD is particularly strong in T1D: 40% of patients with DKD develop CVD by the age of 40, compared to 7% in patients without DKD [83]. CVD also occurs much earlier in life in T1D than in T2D, with a more rapid disease progression, and with women and men equally affected [83, 84]. However, among subjects with T2D, an adverse cardiovascular risk profile can be observed, even before diabetes diagnosis [85], whereas CVD is a long-term complication in T1D [83].

Genetics Basis of Coronary Artery Disease

Family history of CAD is an important risk factor for CAD both in the general population [86], and among insulin-treated subjects with diabetes [87], suggesting a genetic influence. In family studies in T2D, a heritability of 41% was attained for intima media thickness, a marker of subclinical atherosclerosis [88]. Whereas many candidate gene studies for CAD have been undertaken in T2D subjects, e.g., for the *HP* gene encoding haptoglobin [89, 90] with a milder effect also seen in subjects with T1D [91], relatively few have studied the genes involved in the development of CAD in T1D.

GWAS on CAD

To date, only one GWAS has been performed with the aim to identify genetic determinants of CAD specific to diabetes [92]. In the GWAS, which included 4188 participants with T2D from five cohorts, one variant (rs10911021) near *GLUL,* encoding a glutamine synthase, reached genome-wide significance (OR = 1.36, p value = 2×10^{-8}). Additional analyses in a subset of 2374 non-diabetic participants found no association with CAD, suggesting rs10911021 is specific to subjects with diabetes. Furthermore, in the CARDIoGRAM consortium including 86,995 participants (22,233 cases, 64,762 controls) from the general population, rs10911021 showed only a nominally significant association with CAD, which was likely to be driven by the proportion (15%) of T2D participants within CARDIoGRAM [92]. Subsequent studies have replicated this finding for both incident CVD morbidity and mortality in T2D [93, 94].

Recently, analyses from a GWAS in the ACCORD trial revealed new risk loci that affect CVD mortality specifically in intensively treated participants with T2D [95•]. In the ACCORD trial, a decreased risk of nonfatal CVD events among intensively treated participants with T2D (HbA1c <6.0%) was accompanied by a paradoxical rise in cardiovascular mortality. With a sample size of 2667 participants with T2D from the intensively treated arm of the ACCORD study, two loci, at 10q26 (rs9299870) and 5q13 (rs57922), attained genome-wide significance and both polymorphisms increased the risk of cardiovascular death threefold. Neither of the two loci were associated with CVD mortality in the standard treatment arm. However, the specific design and setting of the ACCORD study made replication of these findings particularly challenging.

In the general population, GWAS have successfully revealed ~60 distinct risk loci for CAD [96, 97•]. While some of these loci also associate with CAD in T2D (Fig. 1), the direction of effect is not always in line with that in the general population [98]. The joint effects of these CAD susceptibility polymorphisms have also been inconclusive [98–100], and larger studies are needed in diabetes. One locus that convincingly replicates in the presence of diabetes is a polymorphism on chromosome 9p21 [101], near *CDKN2A* and *CDKN2B.* It is associated with a ~20% increased risk of CAD in

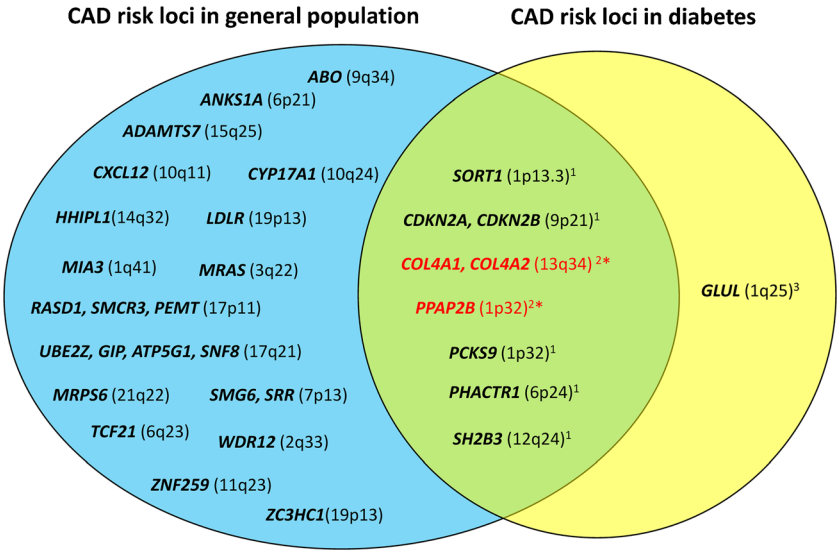

Fig. 1 CAD risk loci identified in GWAS in the general population and in subjects with diabetes. So far, only a subset of CAD risk loci from the general population have been found associated with CAD in the context of diabetes, and some of these associations go in the opposite direction* (13q34 and 1p24). Evaluation in larger studies with diabetic subjects may reveal more overlapping genes involved in the development of CAD in diabetes. The figure includes only genes that have been studied in subjects with diabetes. [1]Associations with $p < 0.05$ for CAD in Nurses Health Study (NHS), Health Professional Follow-up Study, Joslin Heart Study (JHS) as reported in Qi et al. 2011 [98]. [2]Associations with $p < 0.05$ for CAD in Diabetes Heart Study (DHS) as reported in supplement of Raffield et al. 2015 [99]. [3]GWAS locus for CAD in T2D reported from Qi et al. 2013 [92]

T2D [101], which is comparable to that in the general population [98, 102], and in the presence of poor glycemic control, the CAD risk for this locus is magnified in T2D [102]. However, it is currently not known whether 9p21 or any of the other CAD risk loci identified in the general population also replicate in T1D.

Genetic Basis of Cerebrovascular Disease

The incidence of cerebrovascular disease, or stroke, is elevated among subjects with diabetes, particularly in the presence of other complications [83, 103]. Stroke consists mainly of ischemic strokes, but also hemorrhagic strokes [103]. Ischemic stroke can be further divided into large vessel disease, cardioembolic stroke, and small vessel disease, and recent GWAS in the general population on these subtypes suggest that different genetic factors are involved in different subtypes [104]. Genetic studies on stroke in diabetes are currently lacking; some of the genetic studies on cardiovascular complications in diabetes have included stroke in the composite CVD phenotype [90, 93, 94, 95•], but have not separately considered stroke or its subtypes.

Genetic Basis of Peripheral Arterial Disease

PAD is clinically observed as an aberrant ankle-brachial index, claudication, or critical limb ischemia, ultimately requiring amputation, particularly in subjects with diabetes [105]. Relatively few genetic risk loci that affect the development of PAD have been discovered, even in the general population. However, the 9p21 region associates with PAD in the general population [106] and preliminary findings from ongoing PAD GWAS suggest 9p21 also as a risk locus in diabetes, and highlight other polymorphisms that are only associated with PAD in diabetes [107].

Conclusions

There has been progress in defining the genetic basis of diabetic complications in the last 5 years, with the number of robustly identified susceptibility loci increasing. However, many challenges remain in the investigation of the genetic contribution to these complications.

A key challenge in genetic studies of all microvascular complications is the definition of the phenotype. Unprecise intermediate markers such as albuminuria or eGFR are used to assess renal function as renal biopsies cannot be taken for research purposes. Furthermore, various measures (e.g., albuminuria vs. eGFR), symptoms (neuropathic pain vs. peripheral neuropathy), and severity of symptoms complicate the study design and meta-analysis between studies. While heritability estimates may inform definition of sub-phenotypes [15••], there must be a balance between homogenizing the phenotype and reduction in number of subjects.

The overlap between the genetic basis of CVD in the general population vs. in the diabetic subset remains unknown. As macrovascular complications (CAD, stroke) also exist in the general population, the same genetic risk factors may affect subjects with and without diabetes, with some additional diabetes-specific susceptibility loci (e.g., *GLUL* [92]). By contrast, diabetic nephropathy and retinopathy differ pathologically from kidney and retinal diseases in non-diabetic subjects; Even though the genetic risk factors for general kidney disease seem to also affect subjects with T2D to some extent [31••], this may be due to a considerable proportion of subjects with T2D having non-diabetic kidney disease (i.e., not diabetic nephropathy) [29••, 35], and the genetic risk factors for DKD seem to substantially differ from the ones affecting the general population.

For DKD, GWAS meta-analyses of multiple cohorts already exist, and larger efforts are underway in subjects with T2D [108], and in >20,000 subjects with T1D [109]. By contrast, for DR, the participant numbers remain small and all but one published GWAS [71] are based on a single GWAS at the discovery stage; only one GWAS on CVD has been published in subjects with diabetes, and none in T1D. Larger data sets and international collaboration to combine genome-wide data across studies will be essential for discovery of novel loci and to clarify the role of previously reported signals. While whole exome and genome sequencing may reveal novel low-frequency and rare variants for diabetic complications, the exome chip provides an alternative approach to targeting low-frequency variants at lower cost, making it feasible in larger cohorts [109]. Preliminary results from family-based approaches suggest novel loci as well [110].

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Compliance with Ethical Standards

Conflict of Interest Emma Dahlström and Niina Sandholm declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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- •• Of major importance

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