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Polygenic overlap between kidney function and large artery atherosclerotic stroke

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Disclosures
None

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Abstract

Background and Purpose—Epidemiological studies show strong associations between kidney dysfunction and risk of ischaemic stroke, the mechanisms of which are incompletely understood. We investigated whether these associations may reflect shared heritability due to a common polygenic basis and whether this differed for ischaemic stroke subtypes.

Methods—Polygenic models were derived using GWAS meta-analysis results for three kidney traits: estimated glomerular filtration rate using serum creatinine (eGFRcrea: N=73,998), eGFR using cystatin C (eGFRcys: N=22,937) and urinary albumin to creatinine ratio (UACR: N=31,580). For each, SNPs passing ten *P*-value thresholds were used to form profile scores in 4,561 ischaemic stroke cases and 7,094 controls from the UK, Germany and Australia. Scores were tested for association with ischaemic stroke and its three aetiological subtypes: large artery atherosclerosis (LAA), cardioembolism (CE) and small vessel disease (SVD).

Results—Polygenic scores correlating with higher eGFRcrea were associated with reduced risk of LAA, with five scores reaching $P<0.05$ (peak $P=0.004$) and all showing the epidemiologically expected direction of effect. A similar pattern was observed for polygenic scores reflecting higher UACR, of which three associated with LAA (peak $P=0.01$) and all showed the expected directional association. One UACR-based score also associated with SVD ($P=0.03$). The global pattern of results was unlikely to have occurred by chance ($P=0.02$).

Conclusions—This study suggests possible polygenic correlation between renal dysfunction and ischaemic stroke. The shared genetic components may be specific to stroke subtypes, particularly large artery atherosclerotic stroke. Further study of the genetic relationships between these disorders appears merited.

Keywords

stroke; kidney; genetic epidemiology

Introduction

Epidemiological evidence supports an association between kidney dysfunction and risk of cardiovascular diseases, including stroke. In fact, the majority of individuals with chronic kidney disease (CKD) die of a cardiovascular cause before developing end-stage renal

disease [1]. In relation to stroke, kidney dysfunction is associated with multiple outcomes including incident stroke [2], recurrent stroke [3], and mortality following stroke [4]. These relationships seem related more to ischaemic, rather than haemorrhagic stroke [5, 6].

The mechanisms for these phenotypic correlations are not completely understood. Sharing of established cardiovascular risk factors – such as age, gender, blood pressure, cholesterol, smoking and diabetes – can explain some, but not all excess stroke risk in patients with CKD [3]. Sharing of pathophysiological correlates of vascular disease including carotid atherosclerosis [7], arterial stiffness [8], and cerebral white matter hyperintensity (WMH) lesions [9] also appear to explain an additional component, but not all of the excess risk.

Kidney traits and ischaemic stroke both have substantial heritable components, with about 30–50% of observed variation in glomerular filtration rate (eGFR) [10], 15–45% of variation in albuminuria [11], and 30–40% of variation in ischaemic stroke risk [12] attributable to genetic effects. Thus, associations between kidney dysfunction and ischaemic stroke may partly reflect a shared genetic component.

In recent years, genome-wide association studies (GWAS) have identified a number of single nucleotide polymorphisms (SNPs) associated with kidney traits and aetiological subtypes of ischaemic stroke. However, these variants explain only a minority (1–2%) of population variation in their respective traits. This “missing heritability” partly reflects a genetic architecture comprising numerous risk variants with effects too small to show significant association in available sample collections. However numerous SNPs aggregated into polygenic scores may show stronger association and explain more trait variation [13].

The polygenic basis of complex traits also hampers attempts to demonstrate pleiotropy for individual SNPs, since effect sizes are typically small for both traits. Using the largest available datasets, a recent study assessed individual SNPs for joint contribution to CKD and cardiovascular disease [14]. Significant cross-trait association was observed for SNPs at only one locus (*SH2B3*), suggesting that if genetic overlap between CKD and cardiovascular disease exists, the effect sizes of pleiotropic variants are likely too small to permit their individual detection.

Building on this earlier work, we hypothesised that phenotypic correlations between kidney dysfunction and ischaemic stroke may result from polygenic overlap; that is, sharing of a genetic component consisting of numerous, small-effect SNPs. This hypothesis was tested by deriving polygenic scores using GWAS results for kidney traits and testing their performance in stroke GWAS datasets. These analyses used GWAS meta-analysis results from the CKDGen Consortium and individual-level genotype data from three ischaemic stroke case-control collections.

Methods

Data sources and study samples

For the derivation stage we used genome-wide association meta-analysis results for three established kidney function traits: 1) estimated glomerular filtration rate (eGFR) based on

serum creatinine (eGFRcrea: N=73,998) [15]; 2) eGFR based on Cystatin C (eGFRcys: N=22,937) [15] and; 3) urinary albumin to creatinine ratio (UACR: N=31,580) [16]. Higher GFR describes better kidney function, while elevated UACR suggests kidney disease. Details of individual studies are provided in the Data Supplement (Tables I–II).

Testing of polygenic scores were performed using individual-level genotype data for ischaemic stroke cases and controls from the Wellcome Trust Case Control Consortium 2 (WTCCC2) Ischaemic Stroke Study [17] and the Australian Stroke Genetics Collaborative (ASGC) [18]. Three major aetiological subtypes of ischaemic stroke were determined: 1) large artery atherosclerosis (LAA); 2) cardioembolism (CE) and; 3) small vessel disease (SVD). Stroke subtyping was performed using the TOAST system [19]. All studies were approved by appropriate ethics committees, and participants provided written informed consent.

Genotyping, imputation and quality control

For ischaemic stroke (IS) studies, genotyping was performed using Illumina arrays followed by quality control and imputation to the HapMap Phase II CEU reference. Principal components analysis (PCA) was performed using Eigenstrat [20], based on 95,016 approximately independent, directly genotyped SNPs. Principal component covariates of ancestry were calculated following three iterations of PCA with outlier removal.

SNP enrichment assessment, polygenic scoring and association analyses

Using GWAS meta-analysis results for the three kidney traits, sets of SNPs passing 10 graded *P*-values ($P_{threshold}$=0.0001, 0.001, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and 1) were extracted. SNP sets were pruned by removing correlated SNPs (r^2>0.2) within 1Mb, preferentially retaining the most significantly associated SNP [21]. Pruned SNP sets were used to form polygenic scores for ischaemic stroke cases and controls as the sum of reference alleles for each SNP weighted by the summary regression coefficient for the relevant kidney trait. Polygenic scores were tested for association with ischaemic stroke and its subtypes using mixed effects logistic regression adjusted for three ancestry principal components, incorporating study site as a random effect. The proportion of stroke case-control variation explained by polygenic scores (R^2) was estimated as previously described for mixed effects logistic models [22]. Polygenic score tests were one-sided, since *a priori*, we sought to identify effects consistent with established epidemiological evidence. For eGFRcrea and eGFRcys, the pre-specified effect direction was negative, since higher eGFR correlates with reduced stroke risk [2]. For UACR a positive effect was pre-specified [23]. The study-wise significance threshold was derived using the method proposed by Galwey [24] (Data Supplement). A flowchart describing the polygenic analysis is in the Online Data Supplement (Figure I).

At a significance threshold of 0.05, we had 98% power to detect polygenic scores explaining ≥0.2% of variance in case/control status for any validation subtype, 81–82% power to detect scores explaining ≥0.1% of variance (varying by subtype) and 51–53% power for scores explaining ≥0.05% of variance [13]. At a significance threshold of 0.001, the corresponding power estimates were 76–78%, 32–34% and 10–11%, respectively.

Lookup for individual SNPs associated with kidney or stroke traits

As a secondary analysis, we conducted targeted, cross-trait analyses for individual SNPs previously associated with kidney traits or ischaemic stroke subtypes. These analyses are described in the Online Data Supplement.

Results

SNP Enrichment

SNP set enrichment across *P*-value thresholds for the three kidney traits is shown in Figure 1. Enrichment was strongest for eGFR based on serum creatinine (eGFRcrea). Modest SNP enrichment was also observed for eGFR based on Cystatin C (eGFRcys). Results for urinary albumin to creatinine ratio (UACR) showed less marked evidence for enrichment.

Polygenic scoring results

A total of 4,561 ischaemic stroke cases and 7,094 controls were used for polygenic score association testing (Table 1). Based on observed correlation among traits (Online Data Supplement), the adjusted study-wise significance threshold was α=0.001.

Two scores derived from eGFRcrea showed nominal association with broad IS, reaching *P*=0.02 and *P*=0.05 (for $P_{threshold}$=0.001 and 0.01, respectively, see Table 2). Five eGFRcrea-based scores showed nominal association with LAA, with peak association (*P*=0.004) observed for $P_{threshold}$=0.05. This score explained an estimated 0.26% of LAA case-control variation (Data Supplement, Table III). For both IS and LAA, scores for all ten *P*-value thresholds demonstrated the expected negative direction of effect, with genetic scores indicative of higher eGFRcrea correlating with reduced (negative) stroke risk. No eGFRcrea-based score showed association with either cardioembolism (CE) or small vessel disease (SVD) and effect directions were also inconsistent within these subtypes.

Polygenic scores derived from eGFRcys showed no association with broad ischaemic stroke or any of its subtypes (all P>0.05, see Data Supplement, Table IV). Further, effect directions were inconsistent across tests within each stroke type.

For UACR, one score ($P_{threshold}$=0.01) showed nominal association with broad IS (*P*=0.04) (Table 3). Three scores were also nominally associated with LAA (peak *P*=0.01 at $P_{threshold}$=0.001, R^2=0.11%) and one score showed nominal association with SVD (*P*=0.03 at $P_{threshold}$=0.0001, R^2=0.14%, see Data Supplement Table III). For the three traits (IS, LAA and SVD), all effects were in the expected direction, with scores indicative of higher UACR (microalbumuria) correlating with positive stroke risk.

None of the polygenic associations quite passed the study-wide significance threshold of 0.001. However within each of the five trait combinations showing nominal association (eGFRcrea-IS, eGFRcrea-LAA, UACR-IS, UACR-LAA, UACR-SVD), the direction of effect across all ten tests was in accordance with prior evidence. We conducted simulations to empirically estimate the probability of this pattern occurring by chance for any given kidney-stroke trait combination (Online Data Supplement). For eGFRcrea, the probability of a set of ten tests showing a negative effect was *P*=0.080, based on 10,000 simulations. For

eGFRcys and UACR, the corresponding one-sided probabilities were 0.067 and 0.069, respectively. Thus, the observed pattern was unlikely to have arisen by chance in any given set of ten tests.

If we consider all kidney-stroke combinations where such consistency was observed, the results appear even less likely to have occurred by chance. Excluding results for broad IS, we conducted three sets of approximately independent stroke subtype tests for each of the three kidney traits. Among these nine sets of tests, we observed three in which consistent effects in the expected direction were uniformly observed (eGFRcrea-LAA, UACR-LAA, and UACR-SVD). The probability of this occurring by chance is approximately 0.023 (Online Data Supplement), or about one in every 43 studies such as ours. This suggests our nominal associations likely to represent true polygenic correlations of small effect.

Lookup for individually associated SNPs

Of 40 SNPs previously associated with kidney traits, one (rs653178) was significantly associated (one-sided $P=20\times10^{-4}$) with large artery atherosclerotic stroke (LAA) [25] after multiple testing adjustment. Of seven stroke-associated SNPs, none were significantly associated with kidney traits (Tables V–VI, Data Supplement).

Discussion

This study suggests that reported epidemiological associations between renal disease and stroke may be partly explained by shared genetic factors, and that this association may differ for ischaemic stroke subtypes, in this study being most marked for large artery stroke. However, the causal genetic variants are likely of very small individual effect, detectable in the current study only when aggregated into highly polygenic scores and even then, achieving only nominal significance. Independent replication will be necessary to confirm the validity of these results.

An important factor affecting the significance of our results was power for individual stroke subtypes. For nominally significant associations, the kidney trait profile scores explained from 0.1 to 0.26% of case-control variation for different stroke subtypes. Power analyses indicated larger samples would be necessary to identify the observed effects at more stringent significance levels.

Although the proportion of stroke subtype variance explained by kidney-based scores was low, this does not mean the true genetic overlap is small. Profile scoring combines errors in effect estimates across all SNPs in the score, which usually produces estimates of explained variance markedly lower than true values [26].

We observed the strongest polygenic correlations between eGFR defined based on serum creatinine and large artery atherosclerotic stroke (LAA). Using eGFRcrea as the derivation trait, nominal significance of polygenic scores with LAA was sustained across nearly the full range of *P*-value derivation thresholds. If these results reflect a true genetic correlation, the pattern of results is consistent with a complex genetic model involving numerous small-effect variants influencing diverse biological processes [13].

The primary pathophysiological mechanism for LAA is atherosclerosis of the large cerebral arteries [19], a surrogate marker of which is carotid intima media thickness (cIMT). Various epidemiological studies have reported inverse associations between eGFR and cIMT. The majority show that this association can be explained by traditional cardiovascular risk factors including age, smoking, hypertension, obesity, diabetes and dyslipidaemia [27, 28]. Thus, polygenic correlations between eGFR and LAA – if confirmed – may reflect genetic variants influencing atherosclerosis or its heritable risk factors.

We observed no co-trait association for eGFR scores derived from cystatin C at $P<0.05$. The lack of similar results between eGFRcrea and eGFRcys may reflect the smaller sample size for the latter; the larger discovery set for eGFRcrea will increase polygenic score precision [13]. Greater discriminatory power of eGFRcrea-based scores was also supported by stronger SNP enrichment across *P*-value thresholds (Figure 1). Previous GWAS meta-analyses have also identified considerably more SNPs associated with eGFRcrea than eGFRcys. Given that both are measures of GFR and have similar heritability, the different results may largely reflect differences in sample size and power to identify variants of modest effect.

Polygenic scores derived from microalbuminuria (UACR) showed nominal associations with LAA across various *P*-value thresholds. This is consistent with epidemiological associations between UACR, cIMT and cardiovascular disease, although the pathophysiological basis of these relationships is less clear. In contrast to GFR, microalbuminuria seems not to reflect generalised atherosclerosis [23], but may represent another common pathophysiologic process such as endothelial dysfunction or low-grade inflammation [29].

We observed no evidence for polygenic overlap between renal function and cardioembolic stroke (CE), in spite of epidemiological associations between CKD and atrial fibrillation (AF), the major CE risk factor. However, increased AF prevalence has mainly been shown in patients with advanced kidney disease [30, 31]. Furthermore, factors associated with AF in the general population seem not to be associated with AF in CKD [32], suggesting pathophysiological differences.

Our results suggested possible polygenic overlap between microalbuminuria and small vessel disease (SVD). These results are consistent with epidemiological associations between renal function and cerebral small vessel disease [9], which have been interpreted as suggesting a systemic generalised microvascular disease underlies both pathologies [33]. Given the epidemiological evidence, more significant genetic associations might have been expected. Our modest results may reflect two factors. Firstly, accuracy of diagnosis of small vessel stroke is greatly improved by the routine use of MRI, which was only used for about 50% of stroke patients in the current study. Secondly pathological and imaging data suggest that small vessel disease is phenotypically heterogeneous, incorporating two distinct subtypes. One is characterised by single larger lacunar infarcts and thought to primarily relate to atherosclerosis; the other is characterised by multiple small lacunar infarcts and leukoaraiosis, and is related to a diffuse small vessel arteriopathy [34, 35]. The latter subtype has been particularly associated with microalbuminuria. If renal disease is genetically

correlated with only one SVD subtype, the heterogeneity of broadly defined SVD will have reduced our ability to detect any genetic overlap. The study of SVD samples with finer-scale phenotyping will likely provide better insights into genetic pleiotropy for SVD.

We observed largely negative evidence for cross-trait association for *individual* SNPs strongly associated with either kidney function or stroke. Only one SNP previously associated with eGFR was associated with both large artery and small vessel stroke; this SNP has been recently associated with broad ischaemic stroke [36] and was also the only variant showing cross-trait association in the previous CKDGen analysis [14].

An important limitation of this study was modest sample sizes for stroke subtypes. Sample size is a challenge for genetic studies of stroke, reflecting the technical nature of case ascertainment and the presence of multiple aetiological types. These are among the largest current IS samples with GWAS but our results should be validated in larger, well-phenotyped stroke GWAS datasets as they become available.

Summary

This study suggests a potential polygenic basis for epidemiological associations between renal dysfunction and ischaemic stroke. The effects of the putative shared genetic components appear small and potentially specific to distinct stroke types.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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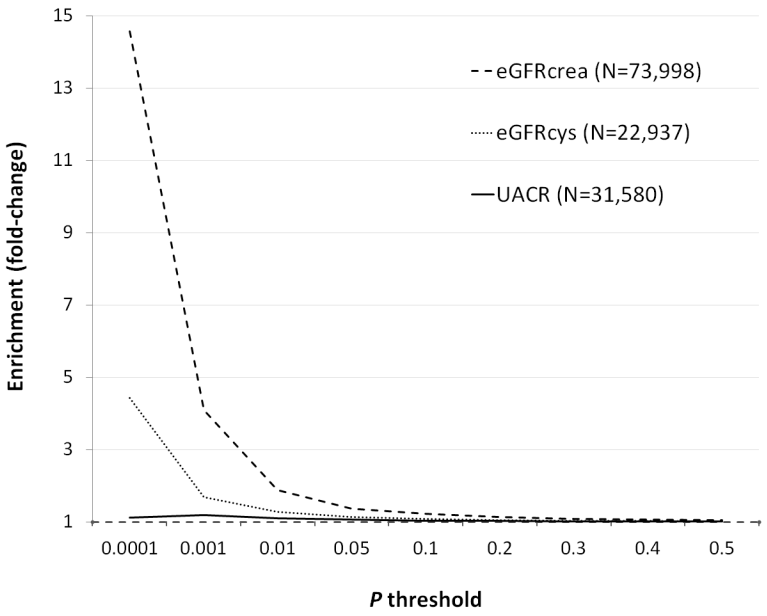

Figure 1.

Observed SNP enrichment across *P*-value thresholds in GWAS results for kidney traits. N denotes total sample size. Enrichment reflects the fold-change increase in SNPs reaching each threshold, relative to the value expected by chance.

Table 1

Ischaemic stroke samples used for polygenic score testing

Study	IS	LAA	CE	SVD	Controls
ASGC	1,071	375	226	287	1,212
WTCCC2-Munich	1,140	338	322	104	775†
WTCCC2-UK	2,350	494	450	471	5,107
Total	**4,561**	**1,207**	**998**	**862**	**7,094**

*LAA, large artery atherosclerosis; CE, cardioembolism; SVD, small vessel disease.

†WTCCC2-Munich controls were selected from the German KORA study, which also contributed to kidney trait meta-analyses.

Table 2

Association of eGFRcrea-based polygenic scores with ischaemic stroke

		Ischaemic stroke		LAA*		CE*		SVD*	
$P_{threshold}$†	N_{SNPs}≠	*P*‖	Direction	*P*‖	Direction§	*P*	Direction	*P*	Direction
0.0001	280	0.13	−	0.29	−	0.69	+	0.20	−
0.001	1,193	**0.02**	−	**0.03**	−	0.31	−	0.06	−
0.01	6,769	**0.05**	−	0.07	−	0.36	−	0.26	−
0.05	24,621	0.07	−	**0.004**	−	0.32	−	0.57	+
0.1	42,861	0.18	−	**0.04**	−	0.37	−	0.59	+
0.2	74,252	0.16	−	0.06	−	0.36	−	0.41	−
0.3	101,719	0.21	−	0.07	−	0.33	−	0.41	−
0.4	125,887	0.16	−	0.06	−	0.24	−	0.33	−
0.5	147,541	0.14	−	**0.04**	−	0.28	−	0.35	−
1	220,045	0.10	−	**0.04**	−	0.21	−	0.27	−

*LAA, large artery atherosclerosis; CE, cardioembolism; SVD, small vessel disease.

†Threshold for selecting SNPs into the score.

≠Number of SNPs forming the score.

§Indicates whether a score predicting higher values of the kidney trait predicts increased (+) or decreased (−) stroke risk.

‖Results reaching $P<0.05$ are underlined and in bold font.

Table 3

Association of UACR-based polygenic scores with ischaemic stroke

		Ischaemic stroke		LAA		CE		SVD	
$P_{threshold}$	N_{SNPs}	*P*	Direction	*P*	Direction	*P*	Direction	*P*	Direction
0.0001	89	0.29	+	0.08	+	0.84	−	**0.03**	+
0.001	722	0.27	+	**0.01**	+	0.88	−	0.47	+
0.01	5,541	**0.04**	+	**0.03**	+	0.73	−	0.12	+
0.05	22,476	0.15	+	**0.04**	+	0.57	−	0.24	+
0.1	40,818	0.38	+	0.11	+	0.65	−	0.19	+
0.2	72,518	0.35	+	0.07	+	0.54	−	0.24	+
0.3	99,798	0.28	+	0.09	+	0.36	+	0.25	+
0.4	124,037	0.26	+	0.09	+	0.40	+	0.21	+
0.5	145,753	0.27	+	0.07	+	0.39	+	0.18	+
1	218,171	0.33	+	0.08	+	0.40	+	0.23	+