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Family-based studies to the rescue of genome-wide association studies in renal function

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Contrary to the apparent impossibility of replicating linkage results across studies on renal outcomes, and denying the general difficulty of identifying meaningful association signals under previously identified linkage peaks, a new study on an isolated Mongolian population could replicate two previously reported linkage peaks and corroborate them by significant associations at multiple single-nucleotide polymorphisms. Although the two genetic loci are not novel, the study sheds light on key aspects of the genetic analysis of kidney function in the general population.

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Linkage analysis is performed to assess whether a phenotype of interest—a disease outcome or any measured biomarker level—is transmitted through generations along with a chromosomal segment. Linkage scans are often based on microsatellite markers located at an average distance of a few centimorgans (cMs) from each other, with 1 cM corresponding to approximately one million DNA base pairs. Consequently, causal loci can only be mapped to large regions. The analysis is generally performed by a multipoint approach; that is, several consecutive markers are combined together into long-range haplotypes. Thus, the identified loci may represent a single gene with a relatively large effect or several closely linked genes, each with a moderate effect, that together have a large effect. The identified linkage regions are usually refined by association analysis, which aims to detect whether a particular phenotype is more likely to happen when a specific allele is present. Association is performed on very dense maps of biallelic single-nucleotide polymorphisms, tested one by one. In this way, the candidate region is narrowed down and is defined by all single-nucleotide polymorphisms that are in strong linkage disequilibrium (physical correlation) with the one more strongly associated with the investigated phenotype. Park and colleagues[1] (this issue) performed a linkage analysis of the glomerular filtration rate estimated from serum creatinine ($eGFR_{creat}$), followed by family-based association testing, conditional to the presence of linkage. This approach has been successfully applied in several other contexts. The family-based association testing prevents spurious association due to population stratification,[2] while the association analysis within predefined linkage regions reduces the risk of false positives by reducing the number of tests performed.

Of the two identified loci, the one on chromosome 9q21 concerns the FERM domain-containing protein 3 (*FRMD3*) gene. Variants in or near this gene have been previously associated with type 1 diabetic nephropathy (DN) in people of European ancestry,[3] with DN defined by either persistent proteinuria or end-stage renal disease. In a study on African Americans with type 2 diabetes,[4] the association between *FRMD3* and DN was confirmed, but only when accounting for the genetic makeup of two other genes: non-muscle myosin heavy chain 9 (*MYH9*) and apolipoprotein L1 (*APOL1*) on chromosome 22, which are known to confer high risk of nondiabetic nephropathy. In particular, the strong association was observed only among people lacking specific haplotypes in the *MYH9* or *APOL1* gene. Additionally, the authors demonstrated that the association between *FRMD3* and type 2 DN was related to nephropathy itself and not to diabetes, which could explain why the association was found both in type 1 and in type 2 DN. Now, Park *et al.*[1] show that the association with *FRMD3* is not limited to DN but extends to $eGFR_{creat}$ in a general population, unselected for diabetes or other diseases. The association becomes stronger with adjustment for other renal function determinants such as glucose level, body mass index, and hypertension. These results match very well with previous linkage analyses, in which linkage between the 9q21 locus and $eGFR_{creat}$ was observed to be independent of diabetes status[5] and was replicated in the general population.[6] Taken together, these results suggest that the *FRMD3*

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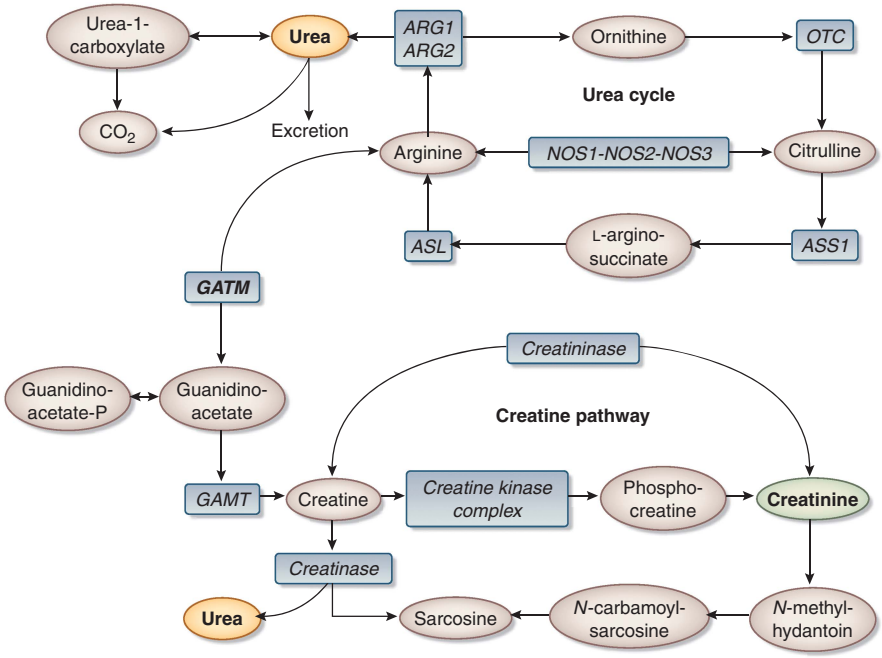

Figure 1 | Involvement of the *GATM* gene in the biosynthesis of creatinine and urea levels. Rectangular boxes represent gene products (mostly proteins); ovals represent compounds. Gene name abbreviations: *ARG1* and *ARG2*, arginase types I and II; *ASL*, argininosuccinate lyase; *ASS1*, argininosuccinate synthase 1; *GAMT*, guanidinoacetate *N*-methyltransferase; *GATM*, glycine amidinotransferase (L-arginine:glycine amidinotransferase); *NOS1-NOS2-NOS3*, nitric oxide synthase 1, 2, and 3; *OTC*, ornithine carbamoyltransferase. (Adapted from the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database, http://www.genome.jp/kegg/.)

locus is related to kidney function independently of the major sources of renal insufficiency, that is, diabetes and hypertension. However, some issues await clarification. First, no association between $eGFR_{creat}$ and *FRMD3* has been reported so far by any of the large meta-analyses of genome-wide association studies in people of European, African, and Asian ancestry.[7–9] Is it because stratification for the *MYH9*/*APOL1* risk haplotypes was not considered in those studies? If that is the case, the next hypothesis is that the *MYH9*/*APOL1* risk haplotypes are absent or at least particularly rare in the small Mongolian isolate considered by Park *et al.*[1] Answering these questions would help clarify the role of both *FRMD3* and *MYH9*/*APOL1* gene variants in renal function regulation.

The locus identified on chromosome 15q21 was previously reported to be in linkage with creatinine levels in West Africans with type 2 diabetes[10] and in a European isolated-population study.[11] Within this region, the strongest signal was observed in a locus where many different genes are in strong linkage disequilibrium with each other. *A priori*, none of them can be excluded from being responsible for the changes in $eGFR_{creat}$ levels. Nevertheless, the obvious candidate seems to be the L-arginine:glycine amidinotransferase (*GATM* or *AGAT*) gene. *GATM* is highly expressed in the kidney and pancreas and is involved in creatine biosynthesis from the beginning of embryonic development. This could explain the large extent of replication across ethnicities.[8,12] Interestingly, the gene is located in a bridge position between the creatine pathway and the urea cycle (Figure 1). From this perspective, it is not surprising that the *P* values at this gene reported by Park *et al.*[1] are so much smaller than the *P* values reported before from studies of similar size. In fact, Park *et al.* estimated the $eGFR_{creat}$ using the six-parameter Modification of Diet in Renal Disease Study equation, which includes both serum creatinine and blood urea nitrogen. Blood urea nitrogen is likely to have played an important role in the reported association, because it not only derives from the urea cycle but is also a sub-product of the creatine pathway, when creatinase synthesizes sarcosine. Thus, the reported result probably reflects the pleiotropic nature of *GATM*, which is involved in the regulation of both creatinine and blood urea nitrogen. Whether *GATM* plays a role in renal function regulation seems unlikely, because the association with $eGFR_{creat}$ could not be replicated when glomerular filtration rate was estimated with cystatin C.[12]

As demonstrated by the several independent hits reported, and as expected on the basis of the multipoint nature of the linkage analysis, it is likely that the linkage signal on chromosome 15 is due to the joint contribution of several other genes. Among those reported, some attention should be given to the gliomedin (*GLDN*) gene, which was also associated with serum creatinine in a genome-wide association study on people of Asian ancestry.[9] Another important gene under the linkage peak is the WD repeat-containing protein 72 (*WDR72*) gene, which was already associated with creatinine, $eGFR_{creat}$, and blood urea nitrogen in the cited Asian meta-analysis[9] as well as in other ethnicities. *WDR72* is not mentioned by Park *et al.*[1] possibly because it did not reach the strict significance threshold or because of some special genetic structure of this isolated population at that locus.

Going beyond these very interesting results, it is worth reasoning about the role of pedigree-based, isolated-population studies in the search for genetic determinants of complex phenotypes, such as renal function. One advantage of isolated-population studies is the homogeneous environment shared by all the individuals. This homogeneity, which is translated into more homogeneous environmental exposures, can enhance the phenotypic differences that are purely due to a genetic component, thus boosting the association signals by reducing the standard error estimates. In addition, it is anticipated that specific

variants that are rare in the general population can be overrepresented in population isolates, because of the lower genetic admixture. Together with the availability of next-generation sequencing technology, which allows the uncovering of millions of rare and very rare mutations, a new paradigm has taken hold in genetics: the 'common disease, multiple rare variants' hypothesis, which is contrasted with the old 'common disease, common variant' hypothesis. The new hypothesis suggests that the same or very similar phenotype manifestations can occur due to rare mutations on different nucleotides. Within this framework, large and expensive studies, based on tens of thousands of unrelated individuals, may be inefficient to detect the causal variants, while pedigree-based studies provide a powerful design, where a few individuals may be enough to identify the causal mutations.[13] Whether the results from population isolates can then be generalized to larger populations is a matter of debate. However, results such as those presented by Park *et al.*[1] are reassuring. The possibility to follow the transmission of the genetic pattern across generations in an extended pedigree is a unique way to track genetic variants that could be responsible for the observed phenotypic variations. With the advent of next-generation sequencing technology, pedigree-based studies will become increasingly important in identifying pathways involved in regulation of biological markers.

DISCLOSURE
The authors declared no competing interests.

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Shining light on vitamin D trials in chronic kidney disease

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Vitamin D compounds may have extraskeletal functions. Chronic kidney disease (CKD) offers an opportunity to investigate these actions, as vitamin D deficiency is prevalent in this population and actions of vitamin D such as those on the heart and glucose metabolism are highly relevant. However, recent randomized controlled trials have tempered enthusiasm. We appraise a trial by de Boer *et al.* that addresses effects of paricalcitol on glucose metabolism in CKD, and offer perspectives on future trials.

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Chronic kidney disease (CKD) continues to be a modern-day epidemic, and it is associated with significant morbidity and mortality.[1] Although there is an abundance of experimental animal studies as well as clinical cross-sectional and longitudinal studies in this field, well-designed and adequately powered randomized controlled trials (RCTs) evaluating specific interventions remain relatively scarce.[2] In this regard, the RCT reported by de Boer *et al.*[3] (this issue) is certainly a step in the right direction.

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CKD and metabolic syndrome

Metabolic syndrome and its individual components, including insulin resistance, are now recognized as independent risk factors for CKD development in diabetic as well as in nondiabetic patients.[4] CKD independently increases insulin resistance, creating a vicious cycle that increases cardiovascular risk.[5] Altered glucose metabolism and reduced tissue sensitivity to insulin have been reported for more than two decades in both early-stage and advanced