

4. Cheema BS, Chan D, Fahey P, Atlantis E: Effect of progressive resistance training on measures of skeletal muscle hypertrophy, muscular strength and health-related quality of life in patients with chronic kidney disease: A systematic review and meta-analysis. *Sports Med* 44: 1125–1138, 2014
5. Cano NJ, Fouque D, Roth H, Aparicio M, Azar R, Canaud B, Chauveau P, Combe C, Laville M, Leveve XM; French Study Group for Nutrition in Dialysis: Intradialytic parenteral nutrition does not improve survival in malnourished hemodialysis patients: A 2-year multicenter, prospective, randomized study. *J Am Soc Nephrol* 18: 2583–2591, 2007
6. Adey D, Kumar R, McCarthy JT, Nair KS: Reduced synthesis of muscle proteins in chronic renal failure. *Am J Physiol Endocrinol Metab* 278: E219–E225, 2000
7. Bailey JL, Wang X, England BK, Price SR, Ding X, Mitch WE: The acidosis of chronic renal failure activates muscle proteolysis in rats by augmenting transcription of genes encoding proteins of the ATP-dependent ubiquitin-proteasome pathway. *J Clin Invest* 97: 1447–1453, 1996
8. Wang XH, Mitch WE: Mechanisms of muscle wasting in chronic kidney disease. *Nat Rev Nephrol* 10: 504–516, 2014
9. Cheung WW, Paik KH, Mak RH: Inflammation and cachexia in chronic kidney disease. *Pediatr Nephrol* 25: 711–724, 2010
10. Ikizler TA, Pupim LB, Brouillette JR, Levenhagen DK, Farmer K, Hakim RM, Flakoll PJ: Hemodialysis stimulates muscle and whole body protein loss and alters substrate oxidation. *Am J Physiol Endocrinol Metab* 282: E107–E116, 2002
11. Grefte S, Kuijpers-Jagtman AM, Torensma R, Von den Hoff JW: Skeletal muscle development and regeneration. *Stem Cells Dev* 16: 857–868, 2007
12. McPherron AC, Lawler AM, Lee SJ: Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature* 387: 83–90, 1997
13. Zhang L, Rajan V, Lin E, Hu Z, Han HQ, Zhou X, Song Y, Min H, Wang X, Du J, Mitch WE: Pharmacological inhibition of myostatin suppresses systemic inflammation and muscle atrophy in mice with chronic kidney disease. *FASEB J* 25: 1653–1663, 2011
14. Chen Y, Sood S, Biada J, Roth R, Rabkin R: Increased workload fully activates the blunted IRS-1/PI3-kinase/Akt signaling pathway in atrophied uremic muscle. *Kidney Int* 73: 848–855, 2008
15. Luo J, et al.: Serum glucocorticoid-regulated kinase 1 blocks CKD. *J Am Soc Nephrol* 27: 2797–2808, 2016
16. Lang F, Böhmer C, Palmada M, Seebohm G, Strutz-Seebohm N, Vallon V: (Patho)physiological significance of the serum- and glucocorticoid-inducible kinase isoforms. *Physiol Rev* 86: 1151–1178, 2006
17. Huang DY, Boini KM, Friedrich B, Metzger M, Just L, Osswald H, Wulff P, Kuhl D, Vallon V, Lang F: Blunted hypertensive effect of combined fructose and high-salt diet in gene-targeted mice lacking functional serum- and glucocorticoid-inducible kinase SGK1. *Am J Physiol Regul Integr Comp Physiol* 290: R935–R944, 2006
18. Wang J, Barbry P, Maiyar AC, Rozansky DJ, Bhargava A, Leong M, Firestone GL, Pearce D: SGK integrates insulin and mineralocorticoid regulation of epithelial sodium transport. *Am J Physiol Renal Physiol* 280: F303–F313, 2001
19. Cheng J, Truong LD, Wu X, Kuhl D, Lang F, Du J: Serum- and glucocorticoid-regulated kinase 1 is upregulated following unilateral ureteral obstruction causing epithelial-mesenchymal transition. *Kidney Int* 78: 668–678, 2010

See related article, “Serum Glucocorticoid-Regulated Kinase 1 Blocks CKD-Induced Muscle Wasting Via Inactivation of FoxO3a and Smad2/3,” on pages 2797–2808.

## Challenges in Rare Variant Association Studies for Complex Kidney Traits: *CFHR5* and IgA Nephropathy

Krzysztof Kiryluk

Department of Medicine, Division of Nephrology, College of Physicians and Surgeons, Columbia University, New York

*J Am Soc Nephrol* 27: 2547–2551, 2016.  
doi: 10.1681/ASN.2016040383

With the first successful genome-wide application of linkage disequilibrium mapping in 2005,<sup>1</sup> genome-wide association studies (GWASs) have now surpassed their 10th anniversary. GWASs have been applied extensively to dissect contributions of common variants to complex disease, and thousands of robust disease associations have been identified using this approach.<sup>2</sup> Important discoveries have also been made in nephrology, where GWASs provided new insights into the regulation of BP,<sup>3</sup> renal function,<sup>4</sup> and albuminuria.<sup>5</sup> In addition, several landmark studies have shown strong contribution of common variants to the risk of glomerular disease, providing novel clues about human biology of these disorders. For example, the discovery of African *APOL1* risk alleles explained a large fraction of racial disparities in kidney disease and pointed to a completely new disease mechanism for FSGS.<sup>6</sup> GWAS findings for IgA nephropathy (IgAN) established the pathogenic role of the intestinal network for IgA production and the alternative complement pathway.<sup>7–9</sup> These findings led to a significant refinement of the disease pathogenesis model and provided novel clues about the disease geoepidemiology.<sup>10–12</sup> Similarly, the genetic interaction between variants in *PLA2R1* and *HLA* arising from GWAS solidified the pathogenesis model for membranous nephropathy, highlighting the antigen-HLA interplay as central to the disease process.<sup>13</sup>

Despite this progress, however, a large portion of the genetic contribution to many complex traits remains unexplained, including traits for which very large GWAS meta-analyses have already been performed. This issue has been identified as the missing heritability problem. The missing heritability has many potential explanations, including the possibility that low-frequency variants substantially contribute to the inherited risk of disease. Such rare alleles are not well captured on popular microarray genotyping platforms and thus, have been

Published online ahead of print. Publication date available at www.jasn.org.

**Correspondence:** Dr. Krzysztof Kiryluk, Department of Medicine, Division of Nephrology, College of Physicians and Surgeons, Columbia University, 1150 St. Nicholas Avenue, Russ Berrie Pavilion 412, New York, NY 10032. Email: kk473@columbia.edu

Copyright © 2016 by the American Society of Nephrology

largely ignored by traditional GWASs. However, rare alleles can be accurately detected by direct DNA sequencing. Rapid progress in sequencing technology now enables investigations of the role of rare genetic variants in complex traits through sequence-based association studies. Although the jury is still out on whether such studies can account for the missing heritability, this design has already been deployed for several complex disorders, and we can certainly expect its broader application to kidney traits in the near future.

Sequence-based association studies present substantial challenges that relate to the detection, analysis, and interpretation of rare variants. Unless sample sizes or variant effect sizes are very large, these studies are usually limited by low statistical power. Moreover, the requisite multiple test corrections are poorly understood, creating difficulties in the interpretation of findings. In GWASs, the standard approach involves a single-variant test with the significance threshold of  $5 \times 10^{-8}$  that accounts for the estimated number of common haplotype blocks in the genome.<sup>14</sup> Given sufficient sample size, a similar approach can also be applied to rare variants, but its power is inversely related to allelic frequency. For example, to achieve 80% power to detect a disease association for a rare variant with a frequency of 1:1000 (0.1%) and an odds ratio of 2.0, one would require >60,000 patients (and an equal number of controls) to detect a statistically significant association for a disease with a population prevalence of 5%. In addition, single-variant tests on the basis of standard regression methods might not be accurate if the number of subjects with the variant is very small. Numerous alternative strategies have, therefore, been proposed to circumvent these issues. Most strategies aggregate variants over genomic regions or genes and evaluate their cumulative effects, increasing power when multiple variants in the group are associated with a trait.

The burden tests represent the simplest class of aggregation tests; they collapse information for multiple variants into a single genetic score, which is then regressed against the disease status. The genetic score captures genotype information by counting the number of minor alleles across all variants in a gene or region. These scores can also be weighted by allelic frequency (to up weight rare variants) or on the basis of functional predictions (to up weight potentially deleterious variants). One intuitive way to consider the significance for gene-based burden tests is by the application of a simple Bonferroni correction for the number of independent tests. In this case, the number of independent tests corresponds to the number of genes in the human genome; assuming 20,000 genes,  $P < 0.05/20,000$  or  $2.5 \times 10^{-6}$  can be considered as statistically significant. Therefore, collapsing rare variants over genes provides a clear power advantage, because one pays a smaller penalty for multiple tests compared with a single-variant analysis.

The burden tests perform reasonably well if a region of interest harbors a large fraction of causal variants with the same direction of effect. However, the tests lose power if both risk and protective variants coexist in a region or when the majority of variants have no effect. To handle these more complex

situations, a number of alternatives have been developed, including adaptive burden tests and variance component tests (refer to the work by Lee *et al.*<sup>15</sup> for a recent review). One of the most popular tests in this category is the sequence kernel association test (SKAT), which uses a weighted sum of squares of single-variant score statistics, thus accommodating variants with opposed directions of association.<sup>16</sup>

Given the complexities of rare variant tests, independent replication of new associations remains one of the most critical aspects of a good study design. However, the best practices for proper validation of rare variant associations are not as well developed as for traditional GWAS. Effective strategies for replication depend on the characteristics of the discovered variants, including their frequencies and effect sizes, and may involve targeted genotyping of selected variants or preferably, resequencing studies of genes or regions of interest. One must also bear in mind that successful replication of rare variant associations is not equivalent to showing causality, and experimental validation is usually needed to confirm the biologic relevance of an implicated gene.

The study by Zhai *et al.*<sup>17</sup> in this issue of the *Journal of the American Society of Nephrology* illustrates some of the challenges in the execution and interpretation of sequence-based rare variant associations. The study is motivated by the initial GWAS findings for IgAN, which established a disease association within the *complement factor H (CFH)* locus on chromosome 1q32 encompassing the *CFH* gene and five *CFH*-related genes (*complement factor H-related protein 1 [CFHR1]* through *CFHR5*).<sup>7</sup> Subsequently, the GWAS signal at this locus has been replicated across diverse cohorts of different ethnicities<sup>8,9</sup> and recently, fine mapped to a single most likely causal variant—a common combined deletion of *CFHR1* and *CFHR3* genes that is protective against IgAN.<sup>18</sup>

In parallel, rare internal duplication within *CFHR5* has been shown to cause a familial form of C3GN.<sup>19</sup> This disease, also known as *CFHR5* nephropathy, is endemic to Cyprus, where the founder mutation likely arose in a common ancestor approximately 16 generations ago. The disease shares some clinical features of IgAN, including microscopic hematuria and synpharyngitic flares with characteristic episodes of macrohematuria. Similar to IgAN, the risk of progressive renal impairment is more common in men, and the disease has been reported to recur after kidney transplantation.<sup>20</sup> *CFHR5* is a universal component of complement deposits *in vivo*, suggesting its function in complement regulation.<sup>21</sup> Indeed, recent studies show that *CFHR5*, *CFHR2*, and *CFHR1* share a dimerization motif that enables the formation of homo- and heterodimers enhancing the avidity of these proteins for C3b, allowing them to function as competitive antagonists of factor H.<sup>22</sup>

Given these findings, Zhai *et al.*<sup>17</sup> hypothesized that rare genetic variation in *CFHR5* may also be contributing to the risk of IgAN. By applying SKAT to *CFHR5* sequence data from 500 patients with IgAN and 576 controls, Zhai *et al.*<sup>17</sup> observed a difference in rare variant distributions with  $P = 2 \times 10^{-3}$ . Careful analysis of the 28 detected rare variants identified nine as potentially functional, including three variants altering protein length and three variants increasing C3b binding to

recombinant CFHR5 by *in vitro* assays. On the basis of these promising data, Zhai *et al.*<sup>17</sup> conclude that these rare variants contribute to the genetic risk of IgAN and suggest CFHR5 as a new “IgAN susceptibility gene.”

Considering multiple challenges with the design and interpretation of rare variant association studies, one must critically assess the provided evidence in support of this claim. Certainly, CFHR5 represents an excellent candidate gene for IgAN on the basis of its established involvement in C3GN. However, the SKAT *P* value for rare variants in CFHR5 is only suggestive, falling short of the conservative genome-wide significance for gene-based tests. Moreover, population stratification may still be confounding this association, because rare variant tests are particularly sensitive to even subtle ancestry differences between patients and controls. Finally, it is not yet clear if the detected differences in the distribution of rare variants are truly independent or merely shadowing the effects of the nearby CFHR3 and CFHR1 deletion. Additional studies that combine sequencing and copy number variant typing to jointly analyze the full spectrum of genetic variation across this region will be needed to establish the precise haplotype relationships between protective and risk alleles. In this regard, important lessons can be learned from the recent studies of age-related macular degeneration, where carefully conducted conditional and haplotype analyses of the CFH locus defined several independent common and rare alleles with opposing effects on the disease risk.<sup>23,24</sup>

In summary, although CFHR5 represents a biologically plausible candidate, more evidence is still needed before unequivocally declaring CFHR5 as a new IgAN susceptibility gene. As noted by Zhai *et al.*,<sup>17</sup> independent replication is needed to confirm that these findings are not caused by chance or an artifact because of uncontrolled biases. Moreover, validation studies in non-Asian cohorts would also help to solidify the evidence and provide more information on the generalizability of these intriguing findings to other populations.

## ACKNOWLEDGMENTS

K.K. is supported, in part, by National Institutes of Health (NIH) grant R01DK105124 from the National Institute of Diabetes and Digestive and Kidney Diseases and NIH grant U01HG008680 from the National Human Genome Research Institute.

The content is solely the responsibility of the author and does not represent the official views of the NIH.

## DISCLOSURES

None.

## REFERENCES

1. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J: Complement factor H polymorphism in age-related macular degeneration. *Science* 308: 385–389, 2005
2. Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, Klemm A, Flicek P, Manolio T, Hindorf L, Parkinson H: The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res* 42: D1001–D1006, 2014
3. Kato N, Loh M, Takeuchi F, Verweij N, Wang X, Zhang W, Kelly TN, Saleheen D, Lehne B, Mateo Leach I, Drong AW, Abbott J, Wahl S, Tan ST, Scott WR, Campanella G, Chadeau-Hyam M, Afzal U, Ahluwalia TS, Bonder MJ, Chen P, Dehghan A, Edwards TL, Esko T, Go MJ, Harris SE, Hartiala J, Kasela S, Kasturiratne A, Khor CC, Kleber ME, Li H, Mok ZY, Nakatochi M, Sapari NS, Saxena R, Stewart AF, Stolk L, Tabara Y, Teh AL, Wu Y, Wu JY, Zhang Y, Aits I, Da Silva Couto Alves A, Das S, Dorajoo R, Hopewell JC, Kim YK, Koivula RW, Luan J, Lytytikäinen LP, Nguyen QN, Pereira MA, Postmus I, Raitakari OT, Bryan MS, Scott RA, Sorice R, Tragante V, Traglia M, White J, Yamamoto K, Zhang Y, Adair LS, Ahmed A, Akiyama K, Asif R, Aung T, Barroso I, Bjornnes A, Braun TR, Cai H, Chang LC, Chen CH, Cheng CY, Chong YS, Collins R, Courtney R, Davies G, Delgado G, Do LD, Doevendans PA, Gansevoort RT, Gao YT, Grammer TB, Grarup N, Grewal J, Gu D, Wander GS, Hartikainen AL, Hazen SL, He J, Heng CK, Hixson JE, Hofman A, Hsu C, Huang W, Husemoen LL, Hwang JY, Ichihara S, Igase M, Isono M, Justesen JM, Katsuya T, Kibriya MG, Kim YJ, Kishimoto M, Koh WP, Kohara K, Kumari M, Kwek K, Lee NR, Lee J, Liao J, Lieb W, Liewald DC, Matsubara T, Matsushita Y, Meitinger T, Mihailov E, Milani L, Mills R, Mononen N, Müller-Nurasyid M, Nabika T, Nakashima E, Ng HK, Nikus K, Nutile T, Ohkubo T, Ohnaka K, Parish S, Paternoster L, Peng H, Peters A, Pham ST, Pinidiyapathirage MJ, Rahman M, Rakugi H, Rolandsson O, Rozario MA, Ruggiero D, Sala CF, Sarju R, Shimokawa K, Snieder H, Sparsø T, Spiering W, Starr JM, Stott DJ, Stram DO, Sugiyama T, Szymczak S, Tang WH, Tong L, Trompet S, Turjanmaa V, Ueshima H, Uitterlinden AG, Umemura S, Vaarasmaki M, van Dam RM, van Gilst WH, van Veldhuisen DJ, Viikari JS, Waldenberger M, Wang Y, Wang A, Wilson R, Wong TY, Xiang YB, Yamaguchi S, Ye X, Young RD, Young TL, Yuan JM, Zhou X, Asselbergs FW, Ciullo M, Clarke R, Deloukas P, Franke A, Franks PW, Franks S, Friedlander Y, Gross MD, Guo Z, Hansen T, Jarvelin MR, Jørgensen T, Jukema JW, Kähönen M, Kajio H, Kivimaki M, Lee JY, Lehtimäki T, Linneberg A, Miki T, Pedersen O, Samani NJ, Sørensen TI, Takayanagi R, Toniolo D, Ahsan H, Allayee H, Chen YT, Danesh J, Deary IJ, Franco OH, Franke L, Heijman BT, Holbrook JD, Isaacs A, Kim BJ, Lin X, Liu J, März W, Metspalu A, Mohlke KL, Sanghera DK, Shu XO, van Meurs JB, Vithana E, Wickremasinghe AR, Wijmenga C, Wolfenbutter BH, Yokota M, Zheng W, Zhu D, Vineis P, Kyrtopoulos SA, Kleinjans JC, McCarthy MI, Soong R, Gieger C, Scott J, Teo YY, He J, Elliott P, Tai ES, van der Harst P, Kooner JS, Chambers JC; BIOS-consortium; CARDIo GRAMplusCD; LifeLines Cohort Study; InterAct Consortium: Trans-ancestry genome-wide association study identifies 12 genetic loci influencing blood pressure and implicates a role for DNA methylation. *Nat Genet* 47: 1282–1293, 2015
4. Pattaro C, Teumer A, Gorski M, Chu AY, Li M, Mijatovic V, Garnaas M, Tin A, Sorice R, Li Y, Taliun D, Olden M, Foster M, Yang Q, Chen MH, Pers TH, Johnson AD, Ko YA, Fuchsberger C, Tayo B, Nalls M, Feitosa MF, Isaacs A, Dehghan A, d'Adamo P, Adeyemo A, Dieffenbach AK, Zonderman AB, Nolte IM, van der Most PJ, Wright AF, Shuldiner AR, Morrison AC, Hofman A, Smith AV, Dreisbach AW, Franke A, Uitterlinden AG, Metspalu A, Tonjes A, Lupo A, Robino A, Johansson Å, Demirkan A, Kollerits B, Freedman BI, Ponte B, Oostra BA, Paulweber B, Krämer BK, Mitchell BD, Buckley BM, Peralta CA, Hayward C, Helmer C, Rotimi CN, Shaffer CM, Müller C, Sala C, van Duijn CM, Saint-Pierre A, Ackermann D, Shriner D, Ruggiero D, Toniolo D, Lu Y, Cusi D, Czamara D, Ellinghaus D, Siscovick DS, Ruderfer D, Gieger C, Grallert H, Rohtchina E, Atkinson EJ, Holliday EG, Boerwinkle E, Salvi E, Bottinger EP, Murgia F, Rivadeneira F, Ernst F, Kronenberg F, Hu FB, Navis GJ, Curhan GC, Ehret GB, Homuth G, Coassin S, Thun GA, Pistis G, Gambaro G, Malerba G, Montgomery GW, Eiriksdottir G, Jacobs G, Li G, Wichmann HE, Campbell H, Schmidt H, Wallaschofski H, Völzke H, Brenner H, Kroemer HK, Kramer H, Lin H, Mateo Leach I, Ford I, Guessous I, Rudan I, Prokopenko I, Borecki I, Heid IM, Kolcic I, Persico I, Jukema JW, Wilson JF, Felix JF, Divers J, Lambert

- JC, Stafford JM, Gaspoz JM, Smith JA, Faul JD, Wang JJ, Ding J, Hirschhorn JN, Attia J, Whitfield JB, Chalmers J, Viikari J, Coresh J, Denny JC, Karjalainen J, Fernandes JK, Endlich K, Butterbach K, Keene KL, Lohman K, Portas L, Launer LJ, Lyytikäinen LP, Yengo L, Franke L, Ferrucci L, Rose LM, Kedenko L, Rao M, Struchalin M, Kleber ME, Cavalieri M, Haun M, Cornelis MC, Ciullo M, Pirastu M, de Andrade M, McEvoy MA, Woodward M, Adam M, Cocca M, Nauck M, Imboden M, Waldenberger M, Pruijm M, Metzger M, Stumvoll M, Evans MK, Sale MM, Kähönen M, Boban M, Bochud M, Rheinberger M, Verweij N, Bouatia-Naji N, Martin NG, Hastie N, Probst-Hensch N, Soranzo N, Devuyst O, Raitakari O, Gottesman O, Franco OH, Polasek O, Gasparini P, Munroe PB, Ridker PM, Mitchell P, Muntner P, Meisinger C, Smit JH, Kovacs P, Wild PS, Froguel P, Rettig R, Mägi R, Biffar R, Schmidt R, Middelberg RP, Carroll RJ, Penninx BW, Scott RJ, Katz R, Sedaghat S, Wild SH, Kardia SL, Ulivi S, Hwang SJ, Enroth S, Kloiber S, Trompet S, Stengel B, Hancock SJ, Turner ST, Rosas SE, Stracke S, Harris TB, Zeller T, Zemunik T, Lehtimäki T, Illig T, Aspelund T, Nikopous T, Esko T, Tanaka T, Gyllensten U, Völker U, Emilsson V, Vitart V, Aalto V, Gudnason V, Chouraki V, Chen WM, Igl W, März W, Koenig W, Lieb W, Loos RJ, Liu Y, Snieder H, Pramstaller PP, Parsa A, O'Connell JR, Susztak K, Hamet P, Tremblay J, de Boer IH, Böger CA, Goessling W, Chasman DI, Köttgen A, Kao WH, Fox CS; ICBP Consortium; AGEN Consortium; CARDIOGRAM; CHARGE-Heart Failure Group; ECHOGen Consortium: Genetic associations at 53 loci highlight cell types and biological pathways relevant for kidney function. *Nat Commun* 7: 10023, 2016
5. Böger CA, Chen MH, Tin A, Olden M, Köttgen A, de Boer IH, Fuchsberger C, O'Seaghdha CM, Pattaro C, Teumer A, Liu CT, Glazer NL, Li M, O'Connell JR, Tanaka T, Peralta CA, Kutalik Z, Luan J, Zhao JH, Hwang SJ, Akyzbekova E, Kramer H, van der Harst P, Smith AV, Lohman K, de Andrade M, Hayward C, Kollerits B, Tönjes A, Aspelund T, Ingelsson E, Eiriksdottir G, Launer LJ, Harris TB, Shuldiner AR, Mitchell BD, Arking DE, Franceschini N, Boerwinkle E, Egan J, Hernandez D, Reilly M, Townsend RR, Lumley T, Siscovick DS, Psaty BM, Kestenbaum B, Haritunians T, Bergmann S, Vollenweider P, Waeber G, Mooser V, Waterworth D, Johnson AD, Florez JC, Meigs JB, Lu X, Turner ST, Atkinson EJ, Leak TS, Aasærd K, Skorpen F, Syvänen AC, Illig T, Baumert J, Koenig W, Krämer BK, Devuyst O, Mychaleckyj JC, Minelli C, Bakker SJ, Kedenko L, Paulweber B, Coassin S, Endlich K, Kroemer HK, Biffar R, Stracke S, Völzke H, Stumvoll M, Mägi R, Campbell H, Vitart V, Hastie ND, Gudnason V, Kardia SL, Liu Y, Polasek O, Curhan G, Kronenberg F, Prokopenko I, Rudan I, Arnlöv J, Hallan S, Navis G, Parsa A, Ferrucci L, Coresh J, Shlipak MG, Bull SB, Paterson NJ, Wichmann HE, Wareham NJ, Loos RJ, Rotter JI, Pramstaller PP, Cupples LA, Beckmann JS, Yang Q, Heid IM, Rettig R, Dreisbach AW, Bochud M, Fox CS, Kao WH; CKDGen Consortium: CUBN is a gene locus for albuminuria. *J Am Soc Nephrol* 22: 555–570, 2011
  6. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, Bowden DW, Langefeld CD, Oleksyk TK, Uscinski Knob AL, Bernhardt AJ, Hicks PJ, Nelson GW, Vanhollenbeke B, Winkler CA, Kopp JB, Pays E, Pollak MR: Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science* 329: 841–845, 2010
  7. Gharavi AG, Kiryluk K, Choi M, Li Y, Hou P, Xie J, Sanna-Cherchi S, Men CJ, Julian BA, Wyatt RJ, Novak J, He JC, Wang H, Lv J, Zhu L, Wang W, Wang Z, Yasuno K, Gunel M, Mane S, Umlauf S, Tikhonova I, Beeran I, Savoldi S, Magistroni R, Ghiggeri GM, Bodria M, Lugani F, Ravani P, Ponticelli C, Allegri L, Boscutti G, Frasca G, Amore A, Peruzzi L, Coppo R, Izzi C, Viola BF, Prati E, Salvadori M, Mignani R, Gesualdo L, Bertinetto F, Mesiano P, Amoroso A, Scolari F, Chen N, Zhang H, Lifton RP: Genome-wide association study identifies susceptibility loci for IgA nephropathy. *Nat Genet* 43: 321–327, 2011
  8. Kiryluk K, Li Y, Sanna-Cherchi S, Rohanizadegan M, Suzuki H, Eitner F, Snyder HJ, Choi M, Hou P, Scolari F, Izzi C, Gigante M, Gesualdo L, Savoldi S, Amoroso A, Cusi D, Zamboli P, Julian BA, Novak J, Wyatt RJ, Mucha K, Perola M, Kristiansson K, Viktorin A, Magnusson PK, Thorleifsson G, Thorsteinsdottir U, Stefansson K, Boland A, Metzger M, Thibaudin L, Wanner C, Jager KJ, Goto S, Maixnerova D, Karnib HH, Nagy J, Panzer U, Xie J, Chen N, Tesar V, Narita I, Berthoux F, Floege J, Stengel B, Zhang H, Lifton RP, Gharavi AG: Geographic differences in genetic susceptibility to IgA nephropathy: GWAS replication study and geospatial risk analysis. *PLoS Genet* 8: e1002765, 2012
  9. Kiryluk K, Li Y, Scolari F, Sanna-Cherchi S, Choi M, Verbitsky M, Fasel D, Lata S, Prakash S, Shapiro S, Fischman C, Snyder HJ, Appel G, Izzi C, Viola BF, Dallera N, Del Vecchio L, Barlassina C, Salvi E, Bertinetto FE, Amoroso A, Savoldi S, Rocchietti M, Amore A, Peruzzi L, Coppo R, Salvadori M, Ravani P, Magistroni R, Ghiggeri GM, Caridi G, Bodria M, Lugani F, Allegri L, Delsante M, Maiorana M, Magnano A, Frasca G, Boer E, Boscutti G, Ponticelli C, Mignani R, Marcantoni C, Di Landro D, Santoro D, Pani A, Polci R, Feriozzi S, Chicca S, Galliani M, Gigante M, Gesualdo L, Zamboli P, Battaglia GG, Garozzo M, Maixnerová D, Tesar V, Eitner F, Rauen T, Floege J, Kovacs T, Nagy J, Mucha K, Pączek L, Zaniew M, Mizerska-Wasiak M, Roszkowska-Blaim M, Pawlaczyk K, Gale D, Barratt J, Thibaudin L, Berthoux F, Canaud G, Boland A, Metzger M, Panzer U, Suzuki H, Goto S, Narita I, Caliskan Y, Xie J, Hou P, Chen N, Zhang H, Wyatt RJ, Novak J, Julian BA, Feehally J, Stengel B, Cusi D, Lifton RP, Gharavi AG: Discovery of new risk loci for IgA nephropathy implicates genes involved in immunity against intestinal pathogens. *Nat Genet* 46: 1187–1196, 2014
  10. Kiryluk K, Novak J, Gharavi AG: Pathogenesis of immunoglobulin A nephropathy: Recent insight from genetic studies. *Annu Rev Med* 64: 339–356, 2013
  11. Kiryluk K, Novak J: The genetics and immunobiology of IgA nephropathy. *J Clin Invest* 124: 2325–2332, 2014
  12. Magistroni R, D'Agati VD, Appel GB, Kiryluk K: New developments in the genetics, pathogenesis, and therapy of IgA nephropathy. *Kidney Int* 88: 974–989, 2015
  13. Stanescu HC, Arcos-Burgos M, Medlar A, Bockenbauer D, Kottgen A, Dragomirescu L, Voinescu C, Patel N, Pearce K, Hubank M, Stephens HA, Laundry V, Padmanabhan S, Zawadzka A, Hofstra JM, Coenen MJ, den Heijer M, Kiemeny LA, Bacq-Daika D, Stengel B, Powis SH, Brenchley P, Feehally J, Rees AJ, Debiec H, Wetzels JF, Ronco P, Mathieson PW, Kleta R: Risk HLA-DQA1 and PLA(2)R1 alleles in idiopathic membranous nephropathy. *N Engl J Med* 364: 616–626, 2011
  14. Dudbridge F, Gusnanto A: Estimation of significance thresholds for genomewide association scans. *Genet Epidemiol* 32: 227–234, 2008
  15. Lee S, Abecasis GR, Boehnke M, Lin X: Rare-variant association analysis: Study designs and statistical tests. *Am J Hum Genet* 95: 5–23, 2014
  16. Wu MC, Lee S, Cai T, Li Y, Boehnke M, Lin X: Rare-variant association testing for sequencing data with the sequence kernel association test. *Am J Hum Genet* 89: 82–93, 2011
  17. Zhai YL, Meng SJ, Zhu L, Shi SF, Wang SX, Liu LJ, Lv JC, Yu F, Zhao MH, Zhang H: Rare variants in the complement factor h-related protein 5 gene contribute to genetic susceptibility to IgA Nephropathy. *J Am Soc Nephrol* 27: 2894–2905, 2016
  18. Xie J, Kiryluk K, Li Y, Mladkova N, Zhu L, Hou P, Ren H, Wang W, Zhang H, Chen N, Gharavi AG: Fine mapping implicates a deletion of CFHR1 and CFHR3 in protection from IgA nephropathy in Han Chinese [published online ahead of print March 3, 2016]. *J Am Soc Nephrol* doi:ASN.2015111210
  19. Gale DP, de Jorge EG, Cook HT, Martinez-Barricarte R, Hadjisavvas A, McLean AG, Pusey CD, Pierides A, Kyriacou K, Athanasiou Y, Voskarides K, Deltas C, Palmer A, Frémeaux-Bacchi V, de Cordoba SR, Maxwell PH, Pickering MC: Identification of a mutation in complement factor H-related protein 5 in patients of Cypriot origin with glomerulonephritis. *Lancet* 376: 794–801, 2010
  20. Vernon KA, Gale DP, de Jorge EG, McLean AG, Galliford J, Pierides A, Maxwell PH, Taube D, Pickering MC, Cook HT: Recurrence of complement factor H-related protein 5 nephropathy in a renal transplant. *Am J Transplant* 11: 152–155, 2011
  21. McRae JL, Cowan PJ, Power DA, Mitchelhill KI, Kemp BE, Morgan BP, Murphy BF: Human factor H-related protein 5 (FHR-5). A new complement-associated protein. *J Biol Chem* 276: 6747–6754, 2001
  22. Goicoechea de Jorge E, Caesar JJ, Malik TH, Patel M, Colledge M, Johnson S, Hakobyan S, Morgan BP, Harris CL, Pickering MC, Lea SM: Dimerization of complement factor H-related proteins modulates complement activation in vivo. *Proc Natl Acad Sci USA* 110: 4685–4690, 2013

23. Fritsche LG, Igl W, Bailey JN, Grassmann F, Sengupta S, Bragg-Gresham JL, Burdon KP, Hebring SJ, Wen C, Gorski M, Kim IK, Cho D, Zack D, Souied E, Scholl HP, Bala E, Lee KE, Hunter DJ, Sardell RJ, Mitchell P, Merriam JE, Cipriani V, Hoffman JD, Schick T, Lechanteur YT, Guymer RH, Johnson MP, Jiang Y, Stanton CM, Buitendijk GH, Zhan X, Kwong AM, Boleda A, Brooks M, Gieser L, Ratnapriya R, Branham KE, Foerster JR, Heckenlively JR, Othman MI, Vote BJ, Liang HH, Souzeau E, McAllister IL, Isaacs T, Hall J, Lake S, Mackey DA, Constable IJ, Craig JE, Kitchner TE, Yang Z, Su Z, Luo H, Chen D, Ouyang H, Flagg K, Lin D, Mao G, Ferreyra H, Stark K, von Strachwitz CN, Wolf A, Brandl C, Rudolph G, Olden M, Morrison MA, Morgan DJ, Schu M, Ahn J, Silvestri G, Tsironi EE, Park KH, Farrer LA, Orlin A, Brucker A, Li M, Curcio CA, Mohand-Said S, Sahel JA, Audo I, Benchaboune M, Cree AJ, Rennie CA, Goverdhan SV, Grunin M, Hagbi-Levi S, Campochiaro P, Katsanis N, Holz FG, Blond F, Blanché H, Deleuze JF, Igo RP Jr, Truitt B, Peachey NS, Meuer SM, Myers CE, Moore EL, Klein R, Hauser MA, Postel EA, Courtenay MD, Schwartz SG, Kovach JL, Scott WK, Liew G, Tan AG, Gopinath B, Merriam JC, Smith RT, Khan JC, Shahid H, Moore AT, McGrath JA, Laux R, Brantley MA Jr, Agarwal A, Ersoy L, Caramoy A, Langmann T, Saksens NT, de Jong EK, Hoyng CB, Cain MS, Richardson AJ, Martin TM, Blangero J, Weeks DE, Dhillon B, van Duijn CM, Doheny KF, Romm J, Klaver CC, Hayward C, Gorin MB, Klein ML, Baird PN, den Hollander AI, Fauser S, Yates JR, Allikmets R, Wang JJ, Schaumberg DA, Klein BE, Hagstrom SA, Chowers I, Lotery AJ, Léveillard T, Zhang K, Brilliant MH, Hewitt AW, Swaroop A, Chew EY, Pericak-Vance MA, DeAngelis M, Stambolian D, Haines JL, Iyengar SK, Weber BH, Abecasis GR, Heid IM: A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet* 48: 134–143, 2016
24. Raychaudhuri S, Iartchouk O, Chin K, Tan PL, Tai AK, Ripke S, Gowrisankar S, Vemuri S, Montgomery K, Yu Y, Reynolds R, Zack DJ, Campochiaro B, Campochiaro P, Katsanis N, Daly MJ, Seddon JM: A rare penetrant mutation in CFH confers high risk of age-related macular degeneration. *Nat Genet* 43: 1232–1236, 2011

See related article, "Rare Variants in the Complement Factor H-Related Protein 5 Gene Contribute to Genetic Susceptibility to IgA Nephropathy," on pages 2894–2905.

## The Search for a Biomarker of Relapse in ANCA-Associated Vasculitis

Meghan E. Free and Ronald J. Falk

University of North Carolina Kidney Center, University of North Carolina, Chapel Hill, Chapel Hill, North Carolina

*J Am Soc Nephrol* 27: 2551–2553, 2016.  
doi: 10.1681/ASN.2016030347

Biomarkers are invaluable tools in clinical medicine that aid in disease diagnosis, natural history, prognosis, and response to

Published online ahead of print. Publication date available at www.jasn.org.

**Correspondence:** Dr. Meghan E. Free, University of North Carolina Kidney Center, 5020 Burnett Womack/CB#7155, University of North Carolina, Chapel Hill, NC 27599-7155. Email: meghan\_free@med.unc.edu

Copyright © 2016 by the American Society of Nephrology

therapy. Because of their importance in patient care, potential biomarkers must undergo analytic validation (determination of the analytic performance of the assay itself) and clinical validation (determination of the ability of the test to predict the clinical condition that it is intended to detect). After analytic validation is established, clinical validation of biomarkers requires testing in three different populations: (1) the targeted disease population, (2) disease controls who are difficult to distinguish from the targeted disease population by means other than the candidate biomarker, and (3) demographically matched healthy controls.

ANCA have proven to be useful biomarkers of small vessel vasculitis,<sup>1</sup> in that they have repetitively gone through all of the steps required for validation. These autoantibodies participate in the pathogenesis of the disease<sup>2</sup> and are a B lymphocyte target of therapy using rituximab.<sup>3–8</sup> In fact, the disorder is now termed ANCA-associated or ANCA vasculitis.

The field of ANCA-associated vasculitis, like so many autoimmune diseases, is one in which cycles of relapse and remission are common. The ability to detect relapsing disease before overt clinical manifestations and organ damage with a biomarker of relapse or remission has been a focus of much investigation. The study by O'Reilly *et al.*<sup>9</sup> in this issue of the *Journal of the American Society of Nephrology* examines the biomarker potential of urinary soluble CD163 (sCD163) to predict active renal vasculitis. In very carefully performed studies, elevated urinary sCD163 seems to distinguish active renal vasculitis from patients in remission and those with active vasculitis characterized by nonrenal symptoms. The test was similarly applied to patients with other forms of glomerular disease. Rigorous testing of sCD163 is evident, starting from a proof of principle experiment in a rat model of vasculitis through detection of CD163 expression in kidney biopsy specimens and finally, through urinary detection of sCD163 by ELISA in both an inception cohort and two validation cohorts (one internal and one external). A limitation of this study, however, is evident in the validation cohorts.<sup>9</sup> There seems to be some patients with SLE and antiglomerular basement membrane disease who also had elevated levels of CD163 on kidney biopsy, which poses the question as to whether sCD163 can distinguish active ANCA-associated vasculitis from disease controls. A disease control that was not examined is the patient with vasculitis and an increasing creatinine in whom the etiology of kidney injury is not overtly apparent. In these patients, in whom a decline in GFR is not attributable to active vasculitis, the gold standard would be a kidney biopsy (for example, AKI caused by a variety of other causes, with the renal biopsy being the only distinguishing definitive diagnosis). A prime example of this would be a patient with ANCA-associated vasculitis and AKI who has an infection. As shown in this study,<sup>9</sup> elevated sCD163 is noted in some patients with sepsis, therefore limiting the value of sCD163 to discern causes of renal injury. Taken together, although urinary sCD163 is certainly elevated in renal vasculitic flares, there are confounding elevations in other patient groups that remain unresolved.