Association of Trypanolytic ApoL1 Variants with Kidney Disease in African Americans

Giulio Genovese,[1,2]* David J. Friedman,[1,3]* Michael D. Ross,[4] Laurence Lecordier,[5] Pierrick Uzureau,[5] Barry I. Freedman,[6] Donald W. Bowden,[7,8] Carl D. Langefeld,[8,9] Taras K. Oleksyk,[10] Andrea L. Uscinski Knob,[4] Andrea J. Bernhardy,[1] Pamela J. Hicks,[7,8] George W. Nelson,[11] Benoit Vanhollebeke,[5] Cheryl A. Winkler,[12] Jeffrey B. Kopp,[11] Etienne Pays,[5]† Martin R. Pollak[1,13]†

African Americans have higher rates of kidney disease than European Americans. Here, we show that, in African Americans, focal segmental glomerulosclerosis (FSGS) and hypertension-attributed end-stage kidney disease (H-ESKD) are associated with two independent sequence variants in the *APOL1* gene on chromosome 22 {FSGS odds ratio = 10.5 [95% confidence interval (CI) 6.0 to 18.4]; H-ESKD odds ratio = 7.3 (95% CI 5.6 to 9.5)}. The two *APOL1* variants are common in African chromosomes but absent from European chromosomes, and both reside within haplotypes that harbor signatures of positive selection. ApoL1 (apolipoprotein L-1) is a serum factor that lyses trypanosomes. In vitro assays revealed that only the kidney disease–associated ApoL1 variants lysed *Trypanosoma brucei rhodesiense*. We speculate that evolution of a critical survival factor in Africa may have contributed to the high rates of renal disease in African Americans.

African Americans suffer from kidney failure at high rates compared with individuals without recent African ancestry (*1*–*3*). Genetic variation at a locus in or near the *MYH9* gene on chromosome 22 has been associated with the increased susceptibility to focal segmental glomerulosclerosis (FSGS), HIV-associated nephropathy, and hypertension-attributed end-stage kidney disease (H-ESKD) observed in African Americans (*4*, *5*), but thus far causal mutations in *MYH9* have not been identified (*6*–*8*).

Previous genome-wide analyses have shown a strong signal of natural selection in the region containing the *MYH9* and *APOL1* genes [integrated haplotype score (iHS) data available at http://hgdp.uchicago.edu/] (*9*–*14*). This observation led us to hypothesize that the kidney disease risk alleles might be located in a larger interval than originally thought (*4*, *5*). The longer patterns of linkage disequilibrium (LD) associated with variants undergoing selection suggest that a positively selected risk variant could be in a larger interval containing the APOL genes rather than be confined to *MYH9*. Because the risk allele(s) are likely to be common in people with African ancestry, we reasoned that such alleles would be present in the data from the African individuals whose DNA was sequenced in the 1000 Genomes Project (www.1000genomes.org). We therefore used this newly available sequence data to identify polymorphisms within this expanded risk interval that showed large frequency differences between Africans and Europeans in order to test for association with renal disease.

We performed an initial association analysis comparing 205 African Americans with biopsy-proven FSGS but no family history of FSGS with 180 African-American controls. The strongest genetic associations with FSGS were clustered in a 10-kb region in the last exon of *APOL1*, the gene encoding apolipoprotein L-1 (table S1 and Fig. 1A) (*15*). The strongest signal was obtained for a two-locus allele, termed G1, consisting of the two derived nonsynonymous coding variants rs73885319 [S^{342}→G^{342} (S342G) (*15*)] and rs60910145 (I384M) in the last exon of *APOL1*. These two alleles were in perfect LD ($r^2 = 1$). The G1 allele (342G:384M) has a frequency of 52% in FSGS cases and 18% in controls (Table 1, $P = 1.07 \times 10^{-23}$).

When we performed logistic regression to control for G1, we identified a second strong signal (table S1 and Fig. 1B; $P = 4.38 \times 10^{-7}$). This second signal is a 6–base pair (bp) deletion (rs71785313, termed G2) close to G1 in *APOL1*. This deletion removes amino acids N388 and Y389 (*16*). Because of the proximity of rs73885319, rs60910145, and rs71785313, alleles G1 and G2 are mutually exclusive; recombination between them is very unlikely. Allele G2 has a frequency of 23% in FSGS cases and 15% in controls (Table 1).

After performing regressions controlling for both G1 and G2, we observed no other significant associations (table S1 and Fig. 1C). Conversely, controlling for multiple sets of variants in *MYH9* failed to eliminate the *APOL1* signal. The LD patterns in this region show that G1 and G2 are in strong LD with variants in *MYH9* (figs. S1 and S2). In particular, the *MYH9* E-1 haplotype, the best predictor of renal disease in previous studies, is present in most haplotypes containing the G1 or G2 allele. Specifically, E-1 is present in 89% of haplotypes carrying G1 and in 76% of haplotypes carrying G2, explaining the association of *MYH9* E-1 with renal disease.

Haplotype frequencies for FSGS cases and controls are shown in Table 1. No difference in FSGS risk was seen when comparing participants with no risk allele to participants with one risk allele [G1 or G2, $P = 0.81$, odds ratio (OR) = 1.04, confidence interval (CI) 0.63 to 2.13]. Comparing participants with zero or one risk allele to participants with two risk alleles provided an odds ratio for FSGS of 10.5 (CI 6.0 to 18.4). This analysis supports a completely recessive pattern of inheritance.

Next, we tested association of *APOL1* variants and renal disease in a larger cohort of 1030 African-American cases with H-ESKD and 1025 geographically matched African-American controls from the southeastern United States (*7*). In this cohort, we tested 36 variants chosen on the basis of the strongest signals of positive selection in a broader genomic region. We also tested nearby coding variants, including G1, G2, and putative *MYH9* risk single-nucleotide polymorphisms (SNPs). The strongest association was again with rs73885319 (G1 tag SNP; $P = 1.1 \times 10^{-39}$) (table S2). When we controlled for rs73885319 by logistic regression, rs71785313 (G2) again emerged as the strongest association signal ($P = 8.8 \times 10^{-18}$) (table S2). The statistical significance of the combined signal ($P = 10^{-63}$) was 35 orders of magnitude stronger than for *MYH9* SNPs. When we controlled for both G1 and G2, no residual association remained after correction for multiple SNP testing (table S2). Frequencies for these alleles are shown in Table 1.

With this larger population, we were able to examine the mode of inheritance of renal disease caused by G1 and G2 with greater precision. We partitioned cases and controls by genotype. One risk allele was associated with only a small increase in renal disease risk (OR = 1.26, CI 1.01 to 1.56). Two risk alleles versus zero risk alleles yielded an OR of 7.3 (CI 5.6 to 9.5). Two risk alleles compared to one risk allele showed an OR of 5.8 (CI 4.5 to 7.5). Overall, a recessive model best explains these findings and is in agreement with our analysis of the FSGS cohort.

We compared the frequency of G1 and G2 in several HapMap populations by using 1000 Ge-

[1]Division of Nephrology, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215, USA. [2]Department of Mathematics, Dartmouth College, Hanover, NH 03755, USA. [3]Center for Vascular Biology Research, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215, USA. [4]Renal Division, Department of Medicine, Brigham and Women's Hospital (BWH), Boston, MA 02215, USA. [5]Laboratory of Molecular Parasitology, Institut de Biologie et de Médecine Moléculaires, Université Libre de Bruxelles, B-6041 Gosselies, Belgium. [6]Department of Internal Medicine/Section on Nephrology, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA. [7]Department of Biochemistry and Center for Diabetes Research, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA. [8]Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA. [9]Department of Biostatistical Sciences, Center for Public Health Genomics and Division of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA. [10]Department of Biology, University of Puerto Rico at Mayaguez, Mayaguez, Puerto Rico 00681. [11]Kidney Disease Section, National Institute of Diabetes, Digestive, and Kidney Disease, National Institutes of Health, Bethesda, MD 20892, USA. [12]Laboratory of Genomic Diversity, SAIC-Frederick, Incorporated, National Cancer Institute–Frederick, Frederick, MD 21702, USA. [13]Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, MA 02142, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: mpollak@bidmc.harvard.edu (M.R.P.); epays@ulb.ac.be (E.P.)

nomes sequence data. G1 was present in about 40% of Yoruba (from Nigeria in West Africa) chromosomes but not in any chromosomes from European, Japanese, or Chinese individuals. Similarly, G2 was detected in sequence data from three Yoruba participants but not in the other three ancestral groups. This distribution data raised the possibility that these variants were selected for in Africa but not outside of Africa. The high frequency of the disease-associated variants in Yoruba and African Americans suggests that these variants may confer selective advantage in Africa.

Given the strong evidence for selection previously described in this chromosomal region (*9*–*14*), we genotyped G1 and G2 in 180 Yoruba samples from HapMap3 to test these variants for their potential contribution to selection (table S3). The allele frequencies in Yoruba are 38% for G1 and 8% for G2. We focused on statistical tests that detect selection by evaluating differential degrees of LD surrounding a putatively selected allele compared with the LD around the alternate allele at the same locus (*13*, *14*, *17*).

A recent (<10,000 years) selective sweep by a positively selected allele that rises quickly in frequency creates longer patterns of LD around the locus under selection (*18*). To determine whether this is the case for G1 and G2, we computed the extended haplotype homozygosity (EHH) (*17*) for each one of the two risk alleles and the nonrisk allele (Fig. 2A) (fig. S3). We also computed the integrated haplotype score (iHS) (*13*, *14*) and ΔiHH (integrated haplotype homozygosity) (*11*). The iHS statistic is suited to detect selective sweeps where the selected allele has reached intermediate frequency. |iHS| greater than 2 indicates unusual LD at a locus relative to the rest of the genome, a typical signature of natural selection. Because iHS is designed to have a standard normal distribution, its value is significant for the two G1 SNPs (rs73885319 and rs60910145, iHS = –2.45; Fig. 2B). ΔiHH is similar to iHS but tests absolute rather than relative differences in the length of haplotypes (*11*). ΔiHH was high for G1 SNPs (ΔiHH = 0.471 cM, more than 5 SD from the mean) and for rs71785313 (G2) (ΔiHH = 0.275 cM, 2.6 SD from the mean) compared with the genome as a whole, again showing that haplotypes carrying the derived alleles are positively selected (Fig. 2C). Results of multiple tests for selection and population differentiation for the entire region from 34,900 to 35,100 kb [National Center for Biotechnology Information (NCBI) 36] are reported in table S4. These same tests in Europeans showed no deviation from neutrality at *APOL1*.

Taken together, these data are consistent with the hypothesis that G1 rose quickly to high frequency because of positive selection in Africa. There is less power to show an effect for G2 because of its lower frequency in Yoruba (8%) and the more robust effect of G1 within the same interval, but haplotypes containing G2 show higher degrees of homozygosity than haplotypes that contain neither G1 nor G2, again suggesting positive selection for G2 in Africans (Fig. 2A).

Although statistical tests for selection are valuable for identifying haplotypes under selection, only functional tests can convincingly demonstrate the causal variant. Our selection tests indicated that G1 and G2 are on haplotypes that have been strongly selected for in Africa but not

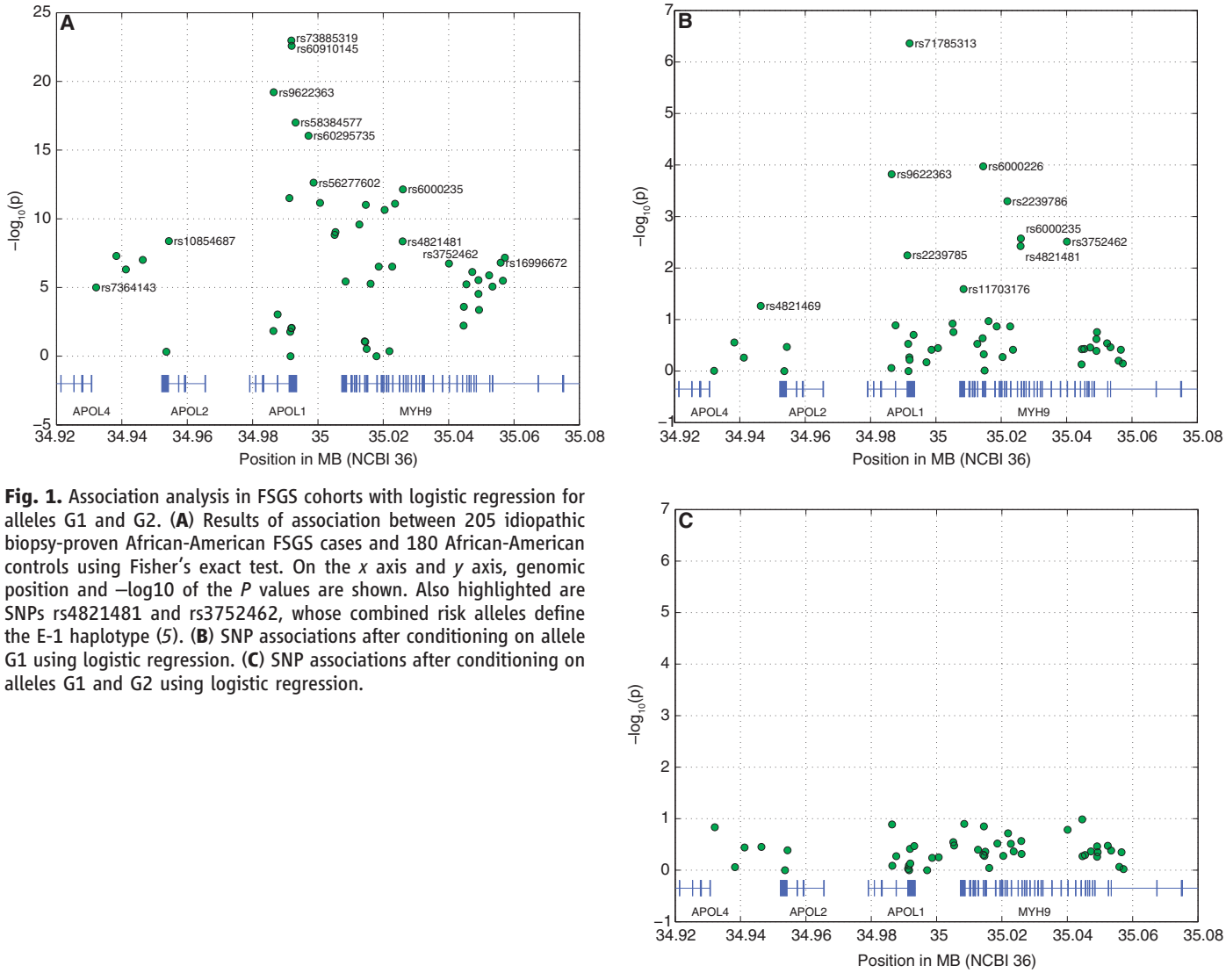

Fig. 1. Association analysis in FSGS cohorts with logistic regression for alleles G1 and G2. (**A**) Results of association between 205 idiopathic biopsy-proven African-American FSGS cases and 180 African-American controls using Fisher's exact test. On the *x* axis and *y* axis, genomic position and –log10 of the *P* values are shown. Also highlighted are SNPs rs4821481 and rs3752462, whose combined risk alleles define the E-1 haplotype (*5*). (**B**) SNP associations after conditioning on allele G1 using logistic regression. (**C**) SNP associations after conditioning on alleles G1 and G2 using logistic regression.

in Europe or Asia. G1 and G2 are plausible candidates for the causal variants because they alter the sequence of the encoded protein.

ApoL1 is the trypanolytic factor of human serum that confers resistance to the *Trypanosoma brucei brucei* (*T. b. brucei*) parasite (*19*, *20*). *T. b. brucei* has evolved into two additional subspecies, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*, which have both acquired the ability to infect humans (*21*, *22*). *T. b. rhodesiense* is predominantly found in Eastern and Southeastern Africa, whereas *T. b. gambiense* is typically found in Western Africa, although some overlap exists (*23*). Because these parasites exist only in sub-Saharan Africa, we hypothesized that the *APOL1* gene may have undergone natural selective pressure to counteract these trypanosoma adaptations. As an initial test of this hypothesis, we performed in vitro assays to compare the trypanolytic potential of the variant, disease-associated forms of ApoL1 proteins with that of the "wild-type" form of ApoL1 protein that is not associated with renal disease.

T. b. rhodesiense can infect humans because of a serum resistance–associated protein (SRA) that interacts with the C-terminal helix of ApoL1 and inhibits its antitrypanosomal activity (*20*, *24*). A recent study showed that mutations and deletions engineered into this helix prevent SRA from binding to ApoL1 (*25*). Intriguingly, one of the G1 sequence variants (I384M) and the 6-bp deletion (G2) are located exactly at the SRA binding site in the ApoL1 C-terminal helix.

We conducted an analysis of the in vitro lytic potential of 75 human plasma samples with different combinations of G1 and G2 genotypes on

Table 1. Number and frequencies of APOL1 genotypes and alleles in FSGS and H-ESKD cases and controls.

	BWH FSGS cases	NIH FSGS cases	Total cases	Controls	H-ESKD cases	Controls
			Genotype			
WT + WT	3	26	29	77	239	409
WT + G1	6	21	27	41	173	250
WT + G2	0	9	9	36	124	155
G1 + G1	25	35	60	9	219	41
G1 + G2	15	38	53	8	203	50
G2 + G2	3	11	14	5	44	18
Total	52	140	192	176	1002	923
			Allele			
G1 Freq.	0.68	0.47	0.52	0.18	0.41	0.21
G2 Freq.	0.19	0.25	0.23	0.15	0.21	0.13

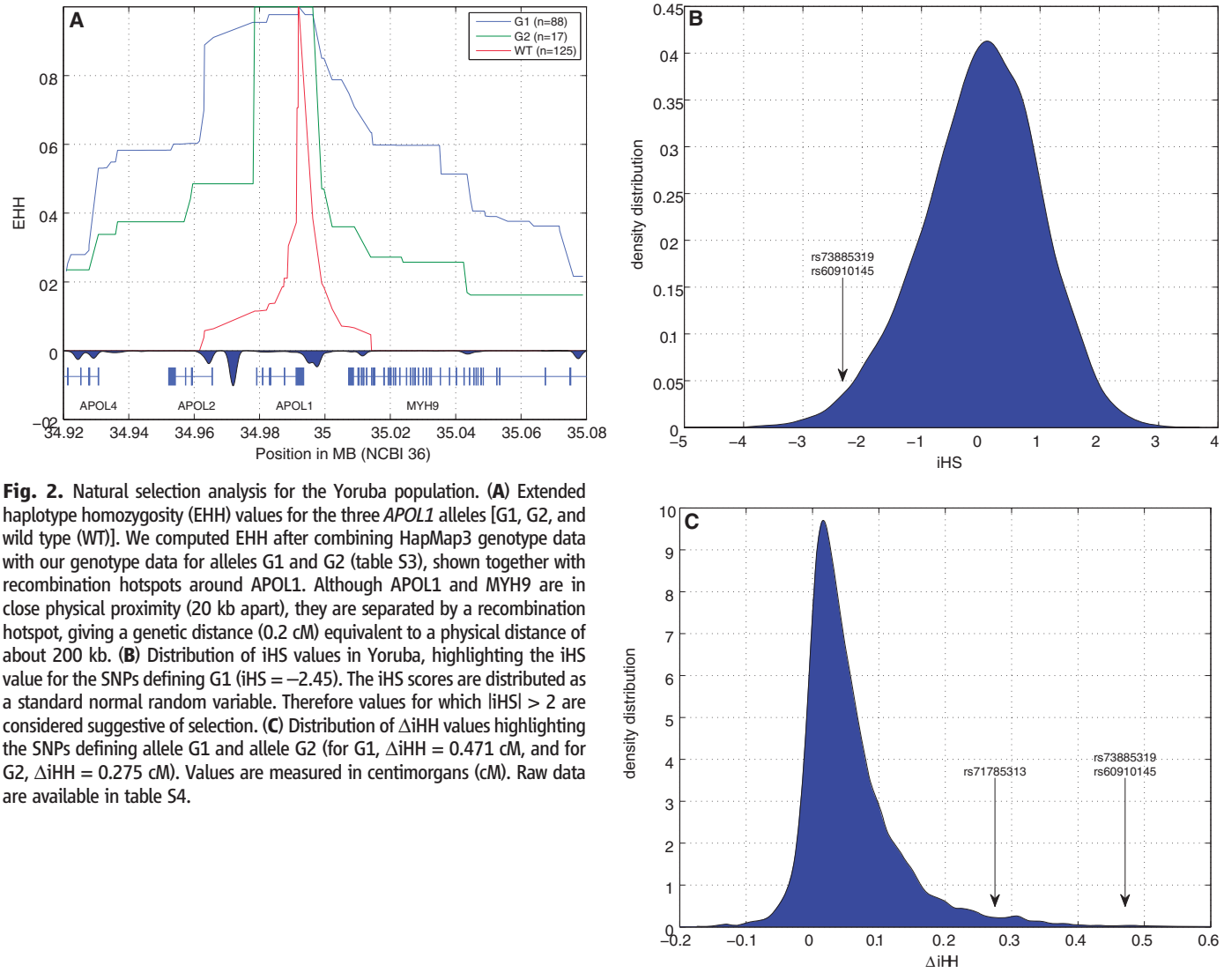

Fig. 2. Natural selection analysis for the Yoruba population. (**A**) Extended haplotype homozygosity (EHH) values for the three *APOL1* alleles [G1, G2, and wild type (WT)]. We computed EHH after combining HapMap3 genotype data with our genotype data for alleles G1 and G2 (table S3), shown together with recombination hotspots around APOL1. Although APOL1 and MYH9 are in close physical proximity (20 kb apart), they are separated by a recombination hotspot, giving a genetic distance (0.2 cM) equivalent to a physical distance of about 200 kb. (**B**) Distribution of iHS values in Yoruba, highlighting the iHS value for the SNPs defining G1 (iHS = –2.45). The iHS scores are distributed as a standard normal random variable. Therefore values for which |iHS| > 2 are considered suggestive of selection. (**C**) Distribution of ΔiHH values highlighting the SNPs defining allele G1 and allele G2 (for G1, ΔiHH = 0.471 cM, and for G2, ΔiHH = 0.275 cM). Values are measured in centimorgans (cM). Raw data are available in table S4.

T. b. brucei, *T. b. rhodesiense*, and *T. b. gambiense*. All 75 plasma samples efficiently lysed *T. b. brucei*, but none of them lysed *T. b. gambiense*. Of the 75 samples, 46 lysed SRA-positive *T. b. rhodesiense* clones, which are typically resistant to lysis by human serum, and all 46 originated from individuals harboring at least one G1 or G2 allele (table S5). As measured by titration using serial dilution, the lytic potency of these plasmas against SRA-positive *T. b. rhodesiense* was higher for G2 than for G1, whereas it was similar for both genotypes against SRA-negative parasites (Fig. 3A). Although lysis of *T. b. rhodesiense* by G2 could be explained by the inability of SRA to bind to this mutant, this conclusion did not hold for G1 ApoL1 variants, which SRA could still efficiently bind (Fig. 3B).

We confirmed these results with recombinant ApoL1 proteins. The S342G/I384M (G1) and delN388/Y389 (G2) (*16*) variants lysed both SRA-negative and SRA-positive *T. b. rhodesiense* parasites (Fig. 3C) but not *T. b. gambiense*. Although G2 was more potent than G1 against SRA-positive *T. b. rhodesiense*, the reverse was true on SRA-negative parasites. Recombinant ApoL1 variants with either S342G alone or I384M alone were less lytic against *T. b. rhodesiense* than when present together, whereas recombinant ApoL1 engineered to have both G1 and G2 mutations was not more active than mutants with G2 alone (Fig. 3C). As shown in Fig. 3, D and E, all measured features of the *T. b. rhodesiense* lytic process (kinetics, transient inhibition by chloroquine, typical swelling of the lysosome) were similar to those observed on *T. b. brucei* with either normal human serum or recombinant ApoL1 (*19*). Therefore, deletion of N388/Y389 was necessary and sufficient to prevent interaction with SRA and to confer on ApoL1 the ability to lyse *T. b. rhodesiense* in vitro, whereas the combination of S342G and I384M was required for maximal ability to lyse *T. b. rhodesiense* despite remaining bound by SRA. None of the variant forms of ApoL1 lysed *T. b. gambiense*.

In summary, we have shown that sequence variation in *APOL1* contributes to the increased risk of renal disease in African Americans. Two lines of evidence support this conclusion: (i) the nonsynonymous variants coded by G1 and the coding region deletion G2 in *APOL1* are the sequence variants showing the strongest association with FSGS and H-ESKD, and (ii) association of renal disease with the *MYH9* sequence variants disappears after controlling for the *APOL1* risk variants. An important question to be addressed in future studies is how sequence variation in ApoL1 mechanistically contributes to the pathogenesis of kidney disease. The recessive model that best fits

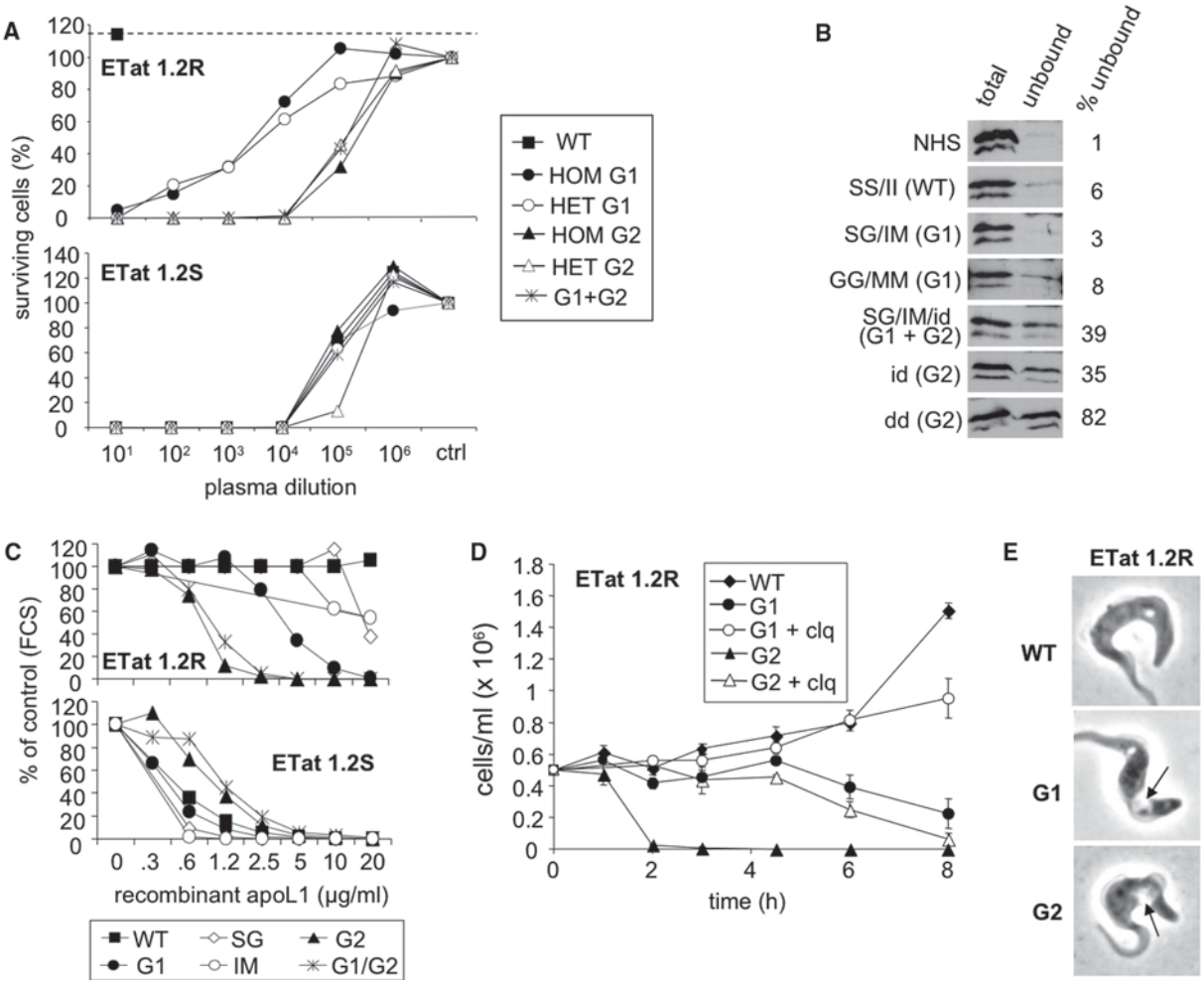

Fig. 3. G1 and G2 alleles of ApoL1 kill *T. b. rhodesiense*. Trypanolytic potential of ApoL1 variants on normal human serum–resistant (SRA+) and normal human serum–sensitive (SRA–) *T. b. rhodesiense* ETat 1.2 clones. ETat 1.2R is resistant to normal human serum, and ETat 1.2S is sensitive to normal human serum. (**A**) Titration of trypanolytic activity in human plasma samples after overnight incubation, expressed as % survival compared with fetal calf serum (FCS) control. hom and het, homozygous and heterozygous mutations, respectively. (**B**) ApoL1 content of various plasma samples before and after affinity chromatography through SRA column (NHS, normal human serum; WT, wild-type ApoL1; S, Ser^{342}; G, Gly^{342}; I, Ile^{384}; M, Met^{384}; i, insertion of N388/Y389; d, deletion of N388/Y389). (**C**) Trypanolytic activity of various recombinant ApoL1 variants after overnight incubation, expressed as % survival compared with fetal calf serum (FCS) control. (**D**) Kinetics of trypanolysis by 20 μg/ml recombinant ApoL1 variants in the presence or absence of 25 μM chloroquine (clq). Error bars indicate SD (n = 3). (**E**) Phenotype of ETat1.2R trypanosomes incubated with various recombinant ApoL1 (20 μg/ml; 1-hour and 30-min and 6-hours incubation, for G1 and G2 respectively; the arrows point to the swelling lysosome).

our genetic data suggests that ApoL1 is performing a critical role in the kidney that is impaired in the setting of the ApoL1 variants, although toxicity of the ApoL1 variants remains a possibility.

We have shown that both ApoL1 variants lyse a deadly subspecies of *Trypanosoma* that is normally completely resistant to ApoL1 lytic activity. The G2 mutation prevents the SRA virulence factor produced by *T. b. rhodesiense* from binding to and inactivating ApoL1. Even 10,000-fold dilutions of plasma containing these mutations (particularly G2) are active against the parasite. This raises the possibility that transfusion of small volumes of plasma, ApoL1-containing HDL particles, or recombinant protein might be effective treatment for trypanosomiasis caused by *T. b. rhodesiense*.

The kidney disease–associated variants are located on haplotypes that show statistical evidence of natural selection. The lytic activity of the variant proteins against *Trypanosoma* provides a plausible—albeit still speculative—biological explanation for natural selection. The results are consistent with a heterozygous advantage model because the protective effect against *T. b. rhodesiense* is dominant whereas the association with renal disease is recessive. Sickle cell disease is a well-established precedent for a model in which mutations conferring heterozygote advantage against a parasitic infection can confer a strong biological disadvantage for homozygotes (*26*). When present in heterozygous form, certain hemoglobin mutations confer protection against malaria but when homozygous cause severe diseases of the red blood cell (for example, sickle cell disease and thalassemia).

It will be interesting to determine the distribution of these mutations throughout sub-Saharan Africa. In present-day Africa, *T. b. rhodesiense* is found in the eastern part of the continent, whereas we noted high frequency of the trypanolytic variants and the signal of positive selection in a West African population. Changes in trypanosome biology and distribution and/or human migration may explain this discrepancy, or resistance to *T. b. rhodesiense* could have favored the spreading of *T. b. gambiense* in West Africa. Alternatively, ApoL1 variants may provide immunity to a broader array of pathogens beyond just *T. b. rhodesiense*, as a recent report linking ApoL1 with anti-Leishmania activity may suggest (*27*). Thus, resistance to *T. b. rhodesiense* may not be the only factor causing these variants to be selected.

The APOL1 risk alleles for renal disease occur in more than 30% of African-American chromosomes. Given their high frequency and their strong effect on disease risk, unraveling the molecular mechanisms by which they contribute to renal injury will be of great importance in understanding and potentially preventing renal disease in individuals of recent African ancestry.

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28. We thank members of the Broad Institute Medical and Population Genetics Program for helpful advice and review of the manuscript. We thank the study participants for their participation and J. Robinson for the help provided accessing the 1000 Genomes project data. We thank X. C. Zhou [National Cancer Institute (NCI)] and E. Binns-Roemer (NCI), P. Poelvoorde [Université Libre de Bruxelles (ULB)], P. Tebabi (ULB) and A. Pays (ULB) for excellent technical assistance. We thank the investigators in the BWH and NIH FSGS genetic studies: W. Briggs, R. Dart, S. Korbet, M. Mokrzycki, P. Kimmel, T. Ahuja, J. Berns, E. Simon, M. Smith, H. Trachtman, M. Michel, J. R. Schelling, G. Appel, and A. Katz. The work at the University of Brussels was supported by the "CIBLES" program of the Walloon Region, the Fonds National de la Recherche Scientifique, and the Interuniversity Attraction Poles program of the Belgian Science Policy. This work was also supported by grants from the NIH (R01 DK54931 to M.R.P; K08-DK076868 to D.J.F.; R01 DK066358 and R01 DK053591 to D.W.B.; and R01 HL56266, R01 DK 070941, and R01 DK 084149 to B.I.F.), the National Institute of Diabetes, Digestive, and Kidney Disease Intramural research program (Z01 DK043308 to J.B.K.), the Intramural Research Program of NCI, Center for Cancer Research (Project ZIA BC 010022; C.A.W. and G.W.N.), and the NephCure Foundation (to M.R.P). The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. The publisher or recipient acknowledges right of the U.S. Government to retain a nonexclusive, royalty-free license in and to any copyright covering the article. This project has been funded in part with federal funds from the NCI, NIH, under contract HHSN261200800001E. All participants gave informed consent for genetic studies, and research was conducted under protocols approved by the appropriate institutional review boards. J.B.K., G.W.N., C.A.W., and the NCI have a patent application pending for chromosome 22 polymorphisms and haplotypes for genetic screening and clinical applications. M.R.P., G.G., the Brigham and Women's Hospital, and Beth Israel Deaconess Medical Center have been added as co-inventors to an amendment to this application naming the specific ApoL1 SNPs described in this manuscript. E.P., L.L., P.U., and ULB have applied for a patent related to the use of plasma from ESKD patients to treat human sleeping sickness by plasma transfer.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1193032/DC1
Materials and Methods
Figs. S1 to S3
Tables S1 to S5
References

4 March 2010; accepted 1 July 2010
Published online 15 July 2010;
10.1126/science.1193032
Include this information when citing this paper.

An Allosteric Self-Splicing Ribozyme Triggered by a Bacterial Second Messenger

Elaine R. Lee,[1]* Jenny L. Baker,[2]* Zasha Weinberg,[1,3] Narasimhan Sudarsan,[1,3] Ronald R. Breaker[1,3,4]†

Group I self-splicing ribozymes commonly function as components of selfish mobile genetic elements. We identified an allosteric group I ribozyme, wherein self-splicing is regulated by a distinct riboswitch class that senses the bacterial second messenger c-di-GMP. The tandem RNA sensory system resides in the 5′ untranslated region of the messenger RNA for a putative virulence gene in the pathogenic bacterium *Clostridium difficile*. c-di-GMP binding by the riboswitch induces folding changes at atypical splice site junctions to modulate alternative RNA processing. Our findings indicate that some self-splicing ribozymes are not selfish elements but are harnessed by cells as metabolite sensors and genetic regulators.

Metabolite-sensing riboswitches control gene expression in most bacteria, plants, and fungi (*1*–*3*). Computer-assisted searches (*4*) have recently been used to identify additional riboswitches (*5*), including an RNA motif (Fig. 1A) that associates with genes involved in c-di-GMP (cyclic di-guanosyl-5′-monophosphate) production, degradation, and signaling (fig. S1)