Taqman Assay for Genotyping CKD-Associated APOL1 SNP rs60910145: A Cautionary Note

To the Editor: There are 2 APOL1 variant haplotypes, G1 and G2, that predispose individuals of African descent to a diverse spectrum of chronic kidney disease in the homozygous (G1/G1 or G2/G2) or compound heterozygous state (G1/G2).[1,2] The G1 haplotype (approximately 23% of African Americans) carries 2 nonsynonymous variants: rs73885319 (p.S342G) and rs60910145 (p.I384M). Genotyping rs73885319 is generally sufficient, as the 2 single-nucleotide polymorphisms (SNPs) are in near-absolute linkage disequilibrium; however, researchers often genotype both G1 variants for quality control purposes and to distinguish between 2 G1 sub-haplotypes: $G1^{GM}$ (has both S242G and I384M amino acid substitutions) and the rare $G1^{G+}$ (only the S242G substitution).[2] The G2 haplotype harbors a 6–base pair in-frame deletion (rs71785313, also known as rs143830837, approximate 13% frequency in African Americans). We use custom Taqman assays for these SNPs: rs73885319 (Assay ID-AH20SD1), rs60910145 (Assay ID-AHWR1JA), and RS71785313 (Assay ID-AH1RT7T). Many laboratories type the *APOL1* SNPs using Taqman assays that are available from ThermoFisher Scientific (Waltham, MA) as predesigned assays. The ThermoFisher Web site notes that one of the polymerase chain reaction primers for rs60910145 (Assay ID-C__89555688_10) spans the 6–base pair deletion of the G2 SNP. We found that the presence of the 6–base pair deletion results in a failure of amplification of the rs60910145 allele on the G2 haplotype. Thus, the G2/G2 haplotype will fail to amplify for rs60910145, and $G1^{GM}$/G2 individuals will fail to amplify the rs60910145 allele on the G2 haplotype and be scored as homozygous for the variant nucleotide instead of heterozygous (Table 1). Although the G2 SNP is absent or rare in Asian and white individuals, and thus will not affect RS60910145 typing in those populations, most investigators are typing RS60910145 in populations of African descent in which G2 is much more common. We have typed a set of 1110 African American samples with the 2 different versions of the rs60910145 assay, as well as rs73885319 and rs143830837, and confirmed this result (7.3% of the rs60910145 genotypes obtained with the predesigned assay failed or gave the incorrect genotype). Specifically, with the predesigned assay, 1.4% of the samples tested had a G2/G2 genotype, so the RS60910145 amplification failed, and 5.9% of the samples had the $G1^{GM}$,G2 genotype, and they were incorrectly typed as G instead of GT at RS60910145. When the incorrect RS60910145 result for the $G1^{GM}$,G2 genotype samples is combined with results from the rs73885319 and rs71785313 SNP assays, it will result in an inferred haplotype never actually observed on African chromosomes. (Table 2)

It is important to avoid the rs71785313 deletion while designing genotyping probes or primers for the APOL1 region. ThermoFisher has been made aware of this problem and plans to replace the commercial assay for rs60910145 with the custom-designed assay (Assay ID-AHWR1JA). Investigators who have used the ThermoFisher predesigned assay are cautioned that rs60910145 results using the predesigned ThermoFisher assay are not reliable. It is difficult to ascertain from the literature which Taqman assay investigators use, because they do not typically report the part number for the RS60910145 assay. Although the custom-designed assay for rs60910145 is not listed on the ThermoFisher Web site, it can be purchased by specifying RS60910145 Assay ID-AHWR1JA.

Table 1. Taqman SNP typing with the predesigned rs60910145 (C__89555688_10) assay results in errors in some individuals with the G2 allele: haplotype[a]

	RS73885319 (p.S242G)	RS60910145 (p.I384M)	RS71785313 (p.N388_Y389del)	Amplification of RS60910145 with predesigned primer
Haplotype				
G0	A	T	I	Yes
$G1^{GM}$	**G**	**G**	I	Yes
$G1^{G+}$	**G**	T	I	Yes
G2	A	T	**D**	No

[a]Derived allele in bold.

Table 2. Taqman SNP typing with the predesigned rs60910145 (C__89555688_10) assay results in errors in some individuals with the G2 allele: genotypes observed in a survey population of 1110 African American samples

Genotype	rs60910145 assay		Frequency observed, %
	Custom assay	Predesigned assay	
G0,G0	T	T	47.5
G0,$G1^{GM}$	GT	GT	25.7
G0,$G1^{G+}$	T	T	0.3
$G1^{GM}$,$G1^{GM}$	G	G	4.1
$G1^{GM}$,$G1^{G+}$	GT	GT	0.5
G0,G2	T	T	14.5
$G1^{GM}$,G2	GT	G[a]	5.9
G2,G2	T	No amplification	1.4

[a]When combined with results from the rs73885319 and rs71785313 SNP assays, will result in an inferred haplotype not observed on African chromosomes.

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Hydroxocobalamin as a Cause of Oxalate Nephropathy

To the Editor: I read with interest the recently published article by Lumlertgul *et al*. regarding secondary oxalate nephropathy.[1] In this systematic review, the authors describe causes and outcomes of oxalate nephropathy by gathering data from case reports and case series. Unfortunately, the authors chose to restrict the literature search to exposure of more than 30 days before the diagnosis. In doing so, the authors missed the impact of short-term drug exposure. We recently described that hydroxocobalamin (Cyanokit, Merck Santé SAS) is associated with oxaluria and oxalate nephropathy.[2] Cyanokit is approved by the Food and Drug Administration for the treatment of cyanide poisoning. Hydroxocobalamin chelates cyanide to form cyanocobalamin, which is excreted by the kidneys. My colleagues and I described two cases of biopsy-proven oxalate nephropathy after exposure to Cyanokit.[2] Both patients presented with severe acute kidney injury (AKI). Furthermore, in a cohort of burn patients, the use of Cyanokit was significantly associated with the risk of AKI, even after adjustment for potential confounding factors. Oxaluria was also reported in healthy volunteers and animals receiving hydroxocobalamin.[3] To conclude, nephrologists and intensivists caring for patients with AKI should be aware of the risk of oxalate nephropathy even after short-term exposure to drugs such as hydroxocobalamin. Detection of oxaluria can easily orient the diagnosis.

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The Authors Reply: We thank Dr. Legrand for his comments regarding hydroxocobalamin as a cause of oxalate nephropathy. We opted to exclude patients with short-term exposure (<30 days) to hyperoxaluria-enabling conditions, as we assumed that these would be mostly accidental or intentional intoxications with short duration of follow-up (<1 month in most cases), and as a result might have a different prognosis compared to those in patients with longer