Diabetic Complications Consortium

Application Title: Gene X Environment Interaction in Diabetic Nephropathy: The Look AHEAD Trial

Principal Investigator: Jeanne McCaffery, Ph.D.

1. Project Accomplishments:

Genome-wide association studies have been successful in discovering genetic markers associated with chronic kidney disease (CKD) in the general population[1, 2] and DN in type 1 diabetes (T1D)[3] and type 2 diabetes (T2D)[4]. The prognostic value of these genetic markers for DN among individuals with T2D and the potential for gene x environment interaction involving weight loss, diet and physical activity is largely unknown. The primary goal of this pilot and feasibility study was to determine whether these human genetic discoveries in kidney disease predict the course of diabetic nephropathy among over 4,016 overweight or obese individuals with type 2 diabetes in the Look AHEAD Study[5, 6]. Using gene x environment interaction models, we further tested whether randomized lifestyle change involving weight loss through calorie restriction and physical activity can diminish genetic effects. The intensive lifestyle intervention (ILI) in Look AHEAD was previously demonstrated to reduce incidence of very high risk CKD[7] as defined by Kidney Disease Improving Global Guidelines (KDIGO) Chronic Kidney Disease Work Group classification guidelines[8], relative to Diabetes Support and Education (DSE) alone.

The aims of the pilot study focused on single nucleotide polymorphisms (SNPs) that were identified in the literature[1-4] and either directly represented on the existing genotyping platform, the Cardiometabochip[9], or having a strong proxy on the Cardiometabochip ($r^2 \geq 0.95$). We identified 13 such SNPs (Table 1) that were the focus of Aims 1 and 2.

In addition to our stated aims, we genotyped SNPs in *APOL1* associated with CKD[10, 11] and conducted preliminary analyses in the Look AHEAD African-American participants (N = 547). We also conducted Cardiometabochip-wide association studies to determine whether any additional SNPs represented on the Cardiometabochip, a genotyping platform designed to target GWAS loci associated with T2D, fasting glucose, lipids, BMI and coronary artery disease, predict incidence of very high risk CKD.

2. Specific Aims:

Aim 1. To determine the association of CKD SNPs with eGFR and UACR through the year 8 follow-up visits, and whether this association might be diminished through behavioral intervention.

Results: Thirteen SNPs were either directly represented on the Cardiometabochip or could be captured by a strong proxy on the Cardiometabochip ($r^2 \geq 0.95$). SNP characteristics are presented in **Table 1**.

Baseline eGFR. Results of the association with baseline eGFR and change in eGFR with and without interaction with treatment arm (ILI and DSE) are presented in **Table 2**. Using a Bonferroni correction for experiment-wide error (alpha = 0.05/13, or 0.0038), two SNPs were significantly associated with baseline eGFR. Each minor allele at *PRKAG2* rs10224210 was associated with -1.24 (0.34) lower eGFR. Similarly, each minor allele at *ATXN2* rs653178 was associated with -1.13 (0.32) lower eGFR. Both of these associations are consistent with the direction of effect in the prior literature[1].

Both *PRKAG2* and *ATXN2* were previously associated with eGFR in the general population. *PRKAG2*, or protein kinase, AMP-activated, gamma 2 non-catalytic subunit, is a glucose sensor important for cellular energy regulation. Variants in *ATXN2* cause spinocerebellar ataxia type 2. *ATXN2* has also been implicated in adult onset obesity[12, 13] and insulin resistance[13] in knock-out mouse models.

Change in eGFR Two different SNPS were associated with rate of change of eGFR through the year 8 follow-up visits across treatment arms after controlling for baseline eGFR. Each minor allele at *UMOD* rs1292282 was associated with 0.224 (95% CI:0.137,0.311) less of a decline in eGFR per year. Similarly, each minor allele at *SHROOM3* rs17319721 was associated with 0.136 (95% CI:0.071,0.200) less of a decline in eGFR per year. The *UMOD* rs1292282 is consistent with prior associations of the minor allele with improved eGFR[2]. However, the *SHROOM3* rs17319721 appears inconsistent with prior research finding cross-sectional association of the minor allele with lower eGFR[2]. Of note, a directionally consistent but nominally significant association of the *SHROOM3* SNP with baseline eGFR was observed (p = 0.01), potentially altering association with change in eGFR.

Both *UMOD* and *SHROOM3* were previously associated with eGFR in the general population. *UMOD*, or uromodulin, codes for the most abundant protein in human urine. Variants in *SHROOM3* have been linked to impairment of the glomerular filtration barrier in animal models[14].

The only significant interaction with treatment arm in predicting change in eGFR indicated that the minor allele at *ATXN2* rs653178 predicted a slower decline in eGFR in DSE (Beta, 95% CI = 0.15, (0.06 - 0.24)) but not ILI (Beta, 95% CI = -0.50, (-0.13 - 0.04)) resulting in a SNP x treatment arm interaction passing statistical significance for experiment-wide error (p = 0.002).

UACR. Results for association with logUACR are presented in **Table 3**. None of the SNPs were significantly associated with logUACR at baseline, whereas one SNP, *PVT1* rs2608054, predicted change in ACR through the year 8 follow-up visit. Each minor allele at rs2608054 was associated with ~1% increase (1.009 95% CI:1.004,1.015) to the per-year rate of change for logUACR (because UACR levels generally increase over time, this effect represents a faster increase in UACR levels). No significant interactions with treatment arm were observed.

PVT1, a non-coding RNA, was previously associated with end-stage renal disease (ERSD) in type 2 diabetes among Pima Indians[4] and ESRD in type 1 diabetes in Caucasians[15]. When over expressed, it relates to cellular proliferation and inhibits cellular apoptosis and has been implicated in several cancers. *PVT1* is also expressed in the kidney[15] and appears to contribute to diabetic nephropathy through accumulation of extracellular matrix proteins in the glomeruli[16, 17].

Aim 2. To determine the association of CKD SNPs with incidence of very-high-risk CKD through a median of 8 years of follow-up, and whether this association might be diminished through behavioral intervention.

None of the 13 SNPs were associated with incidence of Very High Risk CKD, either independent of or in interaction with treatment arm at the level of experiment-wide statistical significance (**Table 4**). The strongest association was observed for rs1719246, an intergenic SNPs in the *SLC28A2/GATM* region, with each minor allele associated with 1.29 (95% CI:1.06-1.57) greater risk of Very High Risk CKD (p = 0.009), consistent with the prior association of this allele with reduced eGFR.

Additional data:

1. *APOL1.* In the African-American subsample of Look AHEAD (N=547), we genotyped two loci in *APOL1* previously shown to increase risk for kidney disease in African-Americans: a two-allele haplotype termed "G1" consisting of the two nonsynonymous coding variants rs73885319 (S342G) and rs60910145 (I384M) and a 6 base pair deletion (rs71785313, termed "G2") close to G1 (N=479 0/1 risk variants; N=68 2 risk variants). This region is under positive selection in African-Americans, is protective against Tyrpanosomiasis but increases risk for kidney disease and potentially cardiovascular disease[10, 11, 18]. Results for Very High Risk CKD and cardiovascular morbidity and mortality are presented in **Figure 1**. Little association was observed between these variants and prospective CKD. Although the eyeball test suggests some association with CVD morbidity and mortality, this association

did not approach statistical significance with the sample size and number of events in Look AHEAD. Given the relatively small sample sizes within ILI and DSE, we were unable to further differentiate the effect of these *APOL1* variants in relation to treatment arm. *APOL1* variants did not predict baseline or decline in eGFR or ACR in this African-American subsample.

2. Cardiometabochip-wide analysis for Very High Risk CKD. We further determined whether any SNPs present on the Cardiometabochip were associated with Very High Risk CKD at a level of chip-wide significance. No SNPs exceed this level of significance for either main effects (**Figure 2**) or interaction with treatment arm (**Figure 3**). Nonetheless, the strongest associations with Very High Risk CVD across treatment arms were: *ZFPM2*, or *FOG2*, previously implicated in glomerular hypertrophy[19] and *LRRC16A*, previously associated with uric acid[20] and gout[21]. Among the strongest SNP x treatment arm interactions were *LXM1B* and *ELOVL6*, involved in the development of the glomerular basement membrane and fatty acid elongation respectively. We will look forward to working with other cohorts with strong representation of participants with diabetes to attempt to replicate these results.

3. Publications:

The primary Look AHEAD paper on diabetic nephropathy was recently published. We anticipate submitting a publication on our primary aims this fall or early next spring.

4. Data sharing:

This ancillary study is governed by the data sharing policies of the parent Look AHEAD grant. Data from baseline to year 4 are already available through the NIDDK repository. We do not have sufficient consent to share the genetic data from these participants with investigators outside of Look AHEAD.

Table 1. Single nucleotide polymorphisms with prior evidence for association with chronic kidney disease available on the Cardiometabochip

	Annotation Information							
SNP	**Proxy_For**	**Outcome**	**Sample**	**CHR**	**Modeled Allele**	**Other Allele**	**Location**	**Closest gene**
rs10170838 [3]	rs7588550	T1DN	T1D	2	C	T	Intronic	*ERBB4*
rs10224210[1]	rs7805747	CKD	General	7	C	T	Intronic	*PRKAG2*
rs12593921[3]	rs12437854	ESRD	T1D	15	G	A	Intergenic	*RGMA*
rs1260326[1]		eGFR	General	2	C	T	exonic;splicing	*GCKR*
rs12922822[1]	rs12917707	eGFR	General	16	T	C	Intergenic	*UMOD;PDILT*
rs1719246[1]	rs2453533	eGFR	General	15	A	T	Intergenic	*SLC28A2;GATM*
rs17319721[1]		eGFR	General	4	A	G	Intronic	*SHROOM3*
rs1880670[1]	rs1933182	eGFR	General	1	C	T	Intergenic	*SORT1;PSMA5*
rs2608054 [4]	rs2648875	ESRD	T2D	8	G	A	ncRNA_intronic	*PVT1*
rs267734[1]		eGFR	General	1	A	G	Intergenic	*CERS2;ANXA9*
rs4744712[1]		eGFR	General	9	A	C	Intronic	*PIP5K1B*
rs653178[1]		eGFR	General	12	G	A	Intronic	*ATXN2*
rs8101881[1]	rs12460876	eGFR	General	19	C	T	Intergenic	*SLC7A9;CEP89*

Table 2. SNP associations with estimated glomerular filtration rate through year 8 follow-up visits

	No Interaction with Arm				Interaction with Arm		
	Effect on baseline value (Int)		Effect on slope (per-year, additive)		Change in slope (DSE)*	Change in slope (ILI)*	Interaction
rsID	Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	Beta (95% CI)	p-value
rs10170838	0.019 (-1.336,1.374)	9.7851E-01	0.080 (-0.070,0.230)	2.9634E-01	0.051 (-0.163,0.265)	0.107 (-0.104,0.317)	7.1450E-01
rs10224210	**-1.235 (-1.904,-0.566)**	**2.9813E-04**	-0.057 (-0.130,0.016)	1.2466E-01	-0.003 (-0.107,0.100)	-0.116 (-0.218,-0.013)	1.3141E-01
rs12593921	0.797 (-0.312,1.907)	1.5904E-01	0.045 (-0.076,0.167)	4.6447E-01	0.007 (-0.165,0.178)	0.094 (-0.079,0.267)	4.8017E-01
rs1260326	-0.869 (-1.479,-0.259)	5.2319E-03	-0.034 (-0.099,0.031)	3.0407E-01	-0.031 (-0.123,0.061)	-0.041 (-0.132,0.050)	8.7510E-01
rs12922822	0.746 (-0.038,1.530)	6.2207E-02	**0.224 (0.137,0.311)**	**4.5780E-07**	0.264 (0.143,0.384)	0.179 (0.054,0.304)	3.3983E-01
rs1719246	-0.289 (-0.905,0.327)	3.5773E-01	-0.051 (-0.111,0.010)	1.0132E-01	-0.04 (-0.128,0.048)	-0.053 (-0.137,0.031)	8.3804E-01
rs17319721	-0.745 (-1.334,-0.155)	1.3363E-02	**0.136 (0.071,0.200)**	**3.7918E-05**	0.108 (0.016,0.201)	0.162 (0.072,0.252)	4.1217E-01
rs1880670	0.471 (-0.174,1.115)	1.5241E-01	-0.069 (-0.134,-0.003)	4.1036E-02	-0.006 (-0.100,0.088)	-0.129 (-0.221,-0.038)	6.5656E-02
rs2608054	-0.003 (-0.624,0.618)	9.9190E-01	0.043 (-0.024,0.109)	2.0750E-01	0.067 (-0.027,0.161)	0.015 (-0.078,0.108)	4.3453E-01
rs267734	-1.051 (-1.824,-0.278)	7.6766E-03	0.007 (-0.077,0.091)	8.6518E-01	-0.034 (-0.153,0.085)	0.057 (-0.061,0.176)	2.8524E-01
rs4744712	0.156 (-0.432,0.744)	6.0297E-01	-0.006 (-0.071,0.059)	8.5195E-01	-0.068 (-0.161,0.025)	0.048 (-0.042,0.138)	8.0614E-02
rs653178	**-1.128 (-1.750,-0.506)**	**3.7933E-04**	0.045 (-0.016,0.107)	1.4598E-01	**0.149 (0.061,0.237)**	**-0.049 (-0.134,0.036)**	**1.5111E-03**
rs8101881	0.722 (0.144,1.301)	1.4374E-02	0.041 (-0.023,0.104)	2.1083E-01	0.027 (-0.065,0.118)	0.049 (-0.039,0.136)	7.3465E-01

In no interaction models, effects adjusted for genetic ancestry (top 3 multidimensional scaling vectors), sex, age, randomization arm, and time, including interactions between time and arm, and time and age.

In interaction models, models contain same covariates, however also involve three-way interactions between time, arm, and age as well as time, arm, and SNP genotype

*Change in slope per modeled allele (MA), assuming an additive effect

Table 3. SNP associations with albumin:creatinine ratio (UACR) through year 8 follow-up visits

	No Interaction with Arm						
	Effect on baseline value (Int)		Effect on slope (per-year, additive)		Change in slope (DSE)*	Change in slope (ILI)*	Interaction
rsID	Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	Beta (95% CI)	p-value
rs10170838	0.869 (0.777,0.972)	1.4085E-02	1.011 (0.999,1.023)	6.8751E-02	1.014 (0.997,1.031)	1.008 (0.992,1.025)	6.4079E-01
rs10224210	0.994 (0.941,1.051)	8.3692E-01	1.003 (0.997,1.008)	3.8492E-01	1.002 (0.994,1.011)	1.003 (0.995,1.011)	9.3522E-01
rs12593921	1.015 (0.926,1.113)	7.5165E-01	1.005 (0.995,1.015)	3.1519E-01	1.008 (0.995,1.022)	1.001 (0.988,1.015)	4.7049E-01
rs1260326	0.952 (0.905,1.001)	5.5927E-02	1.007 (1.001,1.012)	1.1813E-02	1.007 (1.000,1.014)	1.006 (0.999,1.013)	8.6153E-01
rs12922822	0.969 (0.908,1.034)	3.4235E-01	0.999 (0.993,1.006)	8.7251E-01	0.993 (0.983,1.002)	1.007 (0.997,1.017)	4.3652E-02
rs1719246	1.039 (0.987,1.093)	1.4332E-01	1.003 (0.999,1.008)	1.6037E-01	1.001 (0.994,1.008)	1.006 (0.999,1.012)	2.9847E-01
rs17319721	0.953 (0.908,1.001)	5.5802E-02	0.998 (0.993,1.003)	4.0425E-01	0.994 (0.987,1.002)	1.001 (0.994,1.008)	2.0478E-01
rs1880670	1.037 (0.983,1.094)	1.8372E-01	1.001 (0.996,1.007)	5.9255E-01	1.001 (0.993,1.008)	1.002 (0.995,1.009)	8.0119E-01
rs2608054	0.964 (0.916,1.015)	1.6026E-01	**1.009 (1.004,1.015)**	**4.1273E-04**	1.010 (1.003,1.018)	1.009 (1.001,1.016)	7.9733E-01
rs267734	1.019 (0.956,1.087)	5.5582E-01	1.002 (0.995,1.009)	5.4395E-01	1.006 (0.996,1.015)	0.998 (0.989,1.008)	2.8565E-01
rs4744712	0.940 (0.895,0.987)	1.2642E-02	0.998 (0.993,1.003)	4.8722E-01	0.998 (0.990,1.005)	0.999 (0.992,1.006)	8.5230E-01
rs653178	0.982 (0.933,1.034)	4.9132E-01	0.995 (0.991,1.000)	6.1786E-02	0.995 (0.988,1.002)	0.996 (0.989,1.003)	8.2131E-01
rs8101881	0.993 (0.946,1.042)	7.7159E-01	0.999 (0.994,1.004)	6.7044E-01	0.993 (0.986,1.001)	1.004 (0.997,1.011)	3.4589E-02

UACR was transformed and modeled on the $\log_e$ scale. Effects have been exponentiated back onto the original scale and so represent multiplicative effects on the mean, i.e. 1.05 denotes a 5% increase.

In no interaction models, effects adjusted for genetic ancestry (top 3 multidimensional scaling vectors), sex, age, randomization arm, and time, including interactions between time and arm, and time and age.

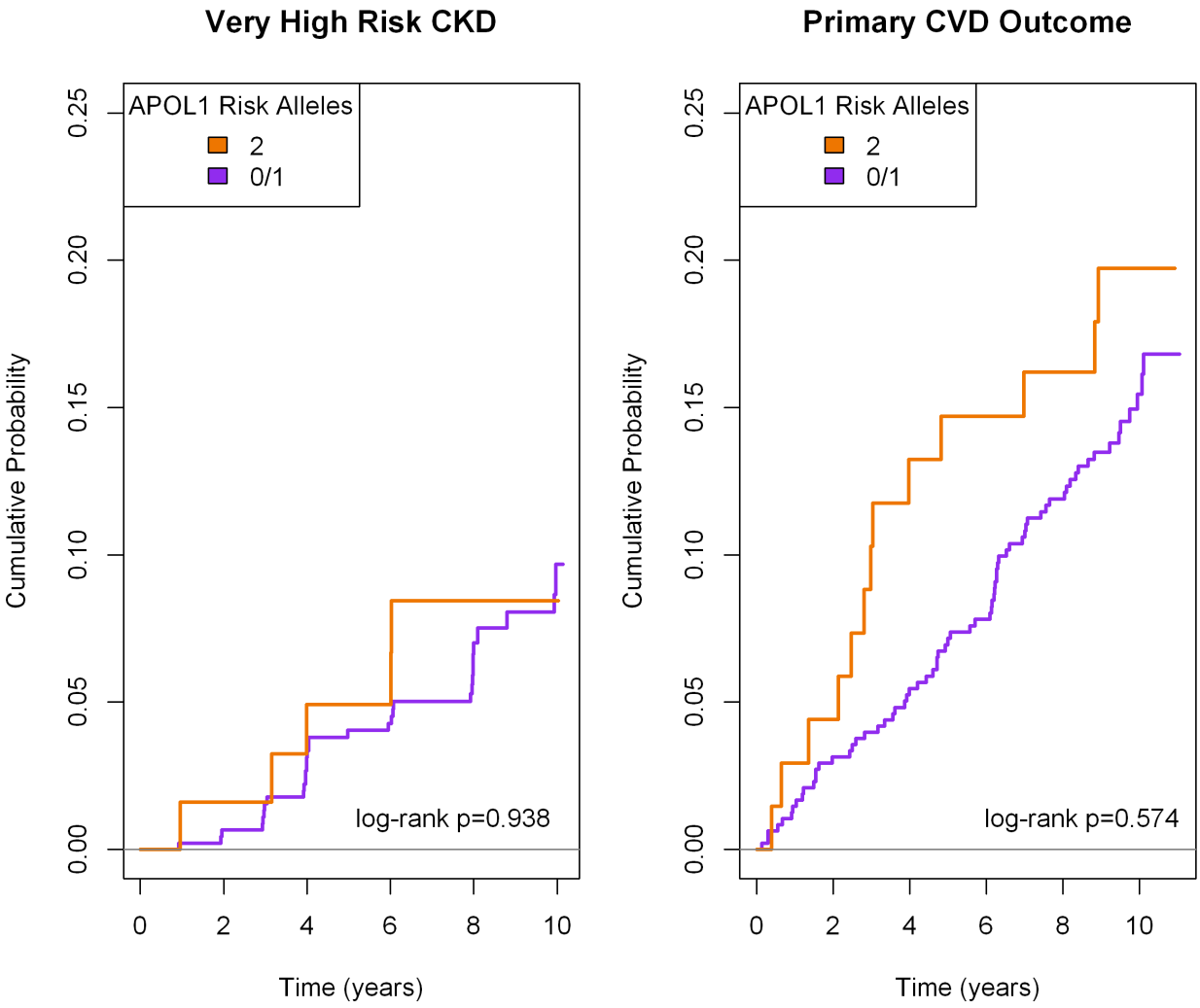
In interaction models, models contain same covariates, however also involve three-way interactions between time, arm, and age as well as time, arm, and SNP genotype

*Change in slope per modeled allele (MA), assuming an additive effect

Table 4. SNP associations with very high risk chronic kidney disease through a median of 8 years of follow-up

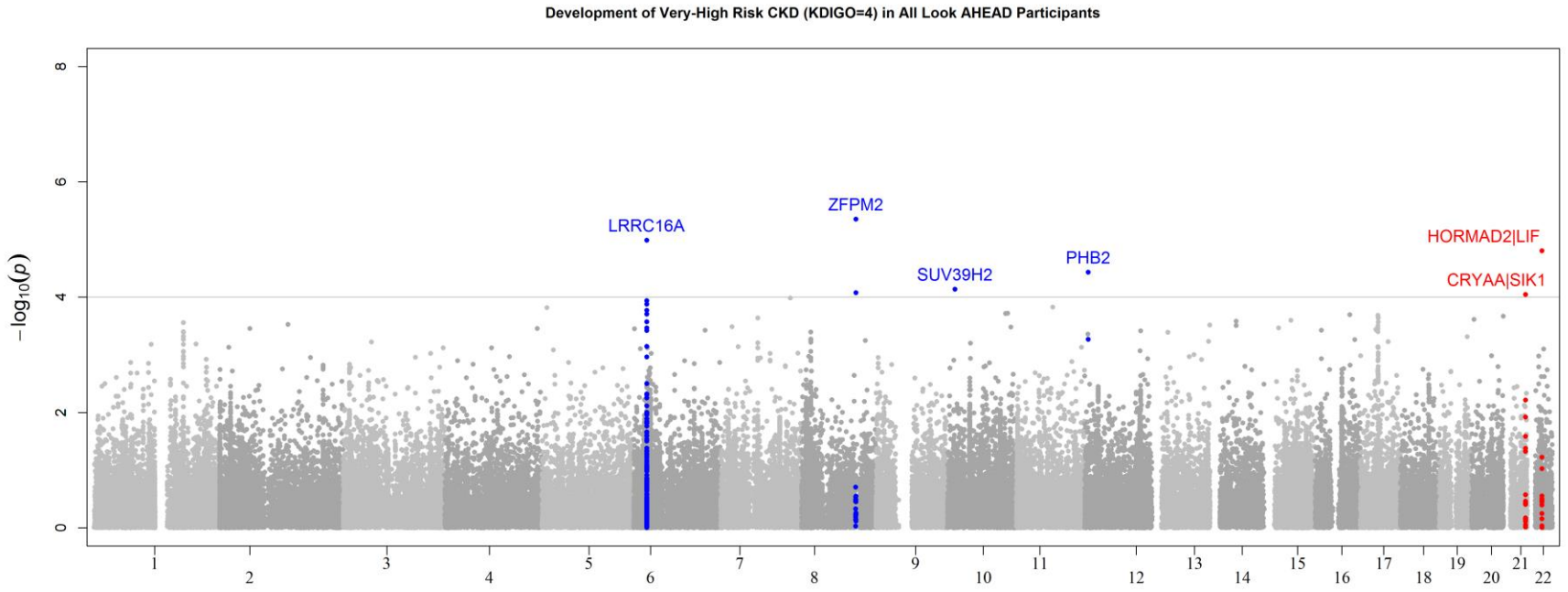
	Very High Risk CKD		Very High Risk CKD Interaction with Arm		
	No Interaction with Arm		DSE	ILI	Interaction
SNP	HR (95% CI)	p-value	HR (95% CI)	HR (95% CI)	p-value
rs10170838	1.122 (0.754-1.67)	5.6986E-01	1.114 (0.664-1.87)	1.133 (0.619-2.074)	0.966109947
rs10224210	1.179 (0.96-1.447)	1.1545E-01	1.097 (0.838-1.437)	1.295 (0.958-1.75)	0.412660716
rs12593921	0.781 (0.537-1.137)	1.9665E-01	0.994 (0.644-1.533)	0.473 (0.224-0.996)	0.088927474
rs1260326	0.881 (0.725-1.069)	1.9961E-01	0.948 (0.741-1.213)	0.793 (0.589-1.067)	0.351686634
rs12922822	0.755 (0.575-0.991)	4.2770E-02	0.812 (0.578-1.14)	0.67 (0.428-1.05)	0.502393082
rs1719246	1.29 (1.064-1.565)	9.6829E-03	1.373 (1.076-1.752)	1.189 (0.904-1.562)	0.409582988
rs17319721	0.858 (0.71-1.037)	1.1379E-01	0.76 (0.59-0.979)	1 (0.758-1.321)	0.146589855
rs1880670	0.882 (0.719-1.083)	2.3011E-01	0.836 (0.644-1.085)	0.949 (0.706-1.274)	0.506885041
rs2608054	1.039 (0.858-1.258)	6.9828E-01	1.176 (0.928-1.491)	0.859 (0.636-1.159)	0.094737638
rs267734	1.057 (0.834-1.34)	6.4461E-01	0.981 (0.718-1.342)	1.168 (0.824-1.655)	0.459352199
rs4744712	0.895 (0.743-1.079)	2.4438E-01	0.873 (0.683-1.117)	0.924 (0.699-1.222)	0.763540764
rs653178	1.104 (0.907-1.344)	3.2144E-01	1.052 (0.822-1.345)	1.177 (0.896-1.548)	0.51302941
rs8101881	0.976 (0.815-1.17)	7.9592E-01	0.955 (0.752-1.212)	1.005 (0.767-1.317)	0.780837256

FIGURE 1. Kaplan-Meier curves for the incidence of very high risk CKD (KDIGO=4) and the primary CVD composite outcome by APOL1 genotype for African-Americans in the Look AHEAD trial



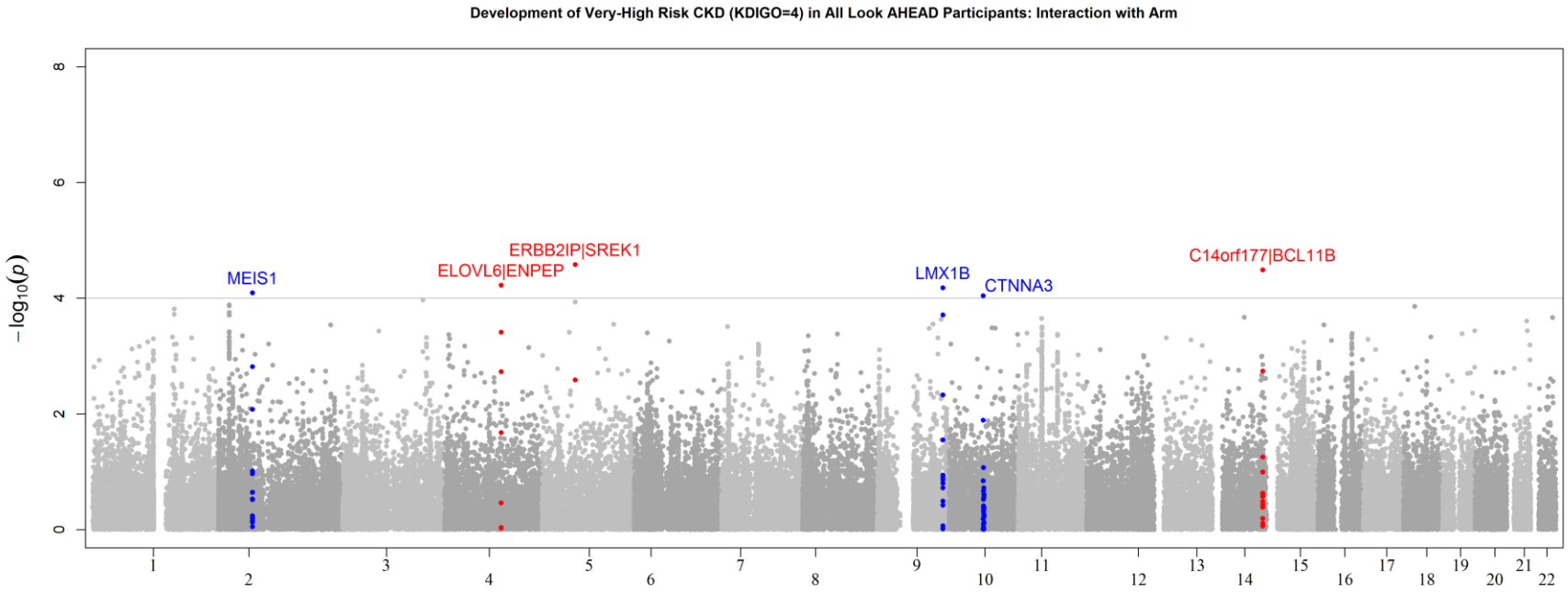
Primary composite CVD outcome includes death from cardiovascular causes, nonfatal myocardial infarction, nonfatal stroke, or hospitalization for angina.

FIGURE 2. Metabochip-wide analysis of main effect associations (independent of randomization arm) with the development of very-high risk CKD (KDIGO=4) in all Look AHEAD participants



Analyses included only common SNPs (MAF>5% pooled across all ethnicities) with no strong evidence of deviation from Hardy-Weinberg equilibrium (p>0.0001 in European ancestry subgroup). P-values from Cox regression model with adjustments for age, sex, randomization arm, and genetic ancestry (top 3 MDS vectors). Note that the gray line just reflects the threshold I used to highlight SNPs on the plot, it does not reflect any sort of meaningful multiple testing correction. Red points indicate situations where the index SNP is intergenic, while blue points indicate index SNPs that fall within a gene.

FIGURE 3. Metabochip-wide analysis of SNP interactions with randomization arm with the development of very-high risk CKD (KDIGO=4) in all Look AHEAD participants



Analyses included only common SNPs (MAF>5% pooled across all ethnicities) with no strong evidence of deviation from Hardy-Weinberg equilibrium (p>0.0001 in European ancestry subgroup). P-values from Cox regression model with adjustments for age, sex, and genetic ancestry (top 3 MDS vectors). Note that the gray line just reflects the threshold I used to highlight SNPs on the plot, it does not reflect any sort of meaningful multiple testing correction. Red points indicate situations where the index SNP is intergenic, while blue points indicate index SNPs that fall within a gene.

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