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Association between the *EPHX2* p.Lys55Arg Polymorphism and Prognosis Following an Acute Coronary Syndrome

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Abstract

Inhibition of soluble epoxide hydrolase (sEH, *EPHX2*) elicits potent cardiovascular protective effects in preclinical models of ischemic cardiovascular disease (CVD), and genetic polymorphisms in *EPHX2* have been associated with developing ischemic CVD in humans. However, it remains unknown whether *EPHX2* variants are associated with prognosis following an ischemic CVD event. We evaluated the association between *EPHX2* p.Lys55Arg and p.Arg287Gln genotype with survival in 667 acute coronary syndrome (ACS) patients. No association with p.Arg287Gln genotype was observed (P=0.598). Caucasian *EPHX2* Arg55 carriers (Lys/Arg or Arg/Arg) had a significantly higher risk of 5-year mortality (adjusted hazard ratio [HR] 1.61, 95% confidence interval [CI] 1.01–2.55, P=0.045). In an independent population of 2,712 ACS patients,

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

this association was not replicated (adjusted HR 0.92, 95% CI 0.70–1.21, P=0.559). In a secondary analysis, Caucasian homozygous Arg55 allele carriers (Arg/Arg) appeared to exhibit a higher risk of cardiovascular mortality (adjusted HR 2.60, 95% CI 1.09–6.17). These results demonstrate that *EPHX2* p.Lys55Arg and p.Arg287Gln polymorphisms do not significantly modify survival after an ACS event. Investigation of other sEH metabolism biomarkers in ischemic CVD appears warranted.

Keywords

soluble epoxide hydrolase; eicosanoids; polymorphism; prognosis; cardiovascular disease; humans; ischemic; EET

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality in the United States despite recent advances in its diagnosis and treatment (1). Acute coronary syndrome (ACS: myocardial infarction (MI) and unstable angina) clinical events occur in over one million Americans annually and are a major contributor to CVD morbidity and mortality (1). Identification and characterization of key pathways that underlie the pathogenesis and progression of ischemic CVD may facilitate the development of novel therapeutic strategies that improve prognosis following ACS events.

Cytochrome P450 (CYP) epoxygenase enzymes from the CYP2C and CYP2J subfamilies metabolize arachidonic acid to form bioactive epoxyeicosatrienoic acids (EETs), which regulate numerous cellular and physiologic processes relevant to the pathogenesis of CVD (2,3). Accumulating preclinical evidence from *in vitro*, *ex vivo*, and *in vivo* models demonstrate that CYP-derived EETs exhibit potent vasodilatory, anti-inflammatory, and cellular protective effects by a variety of mechanisms (3–6). The predominant fate of EETs is through rapid metabolism by soluble epoxide hydrolase (sEH, *EPHX2*) into dihydroxyeicosatrienoic acids (DHETs), which generally have less biological activity than EETs (3). Inhibition of sEH promotes the potent cardiovascular protective effects of EETs in preclinical models of vascular inflammation, atherosclerosis, and myocardial ischemia-reperfusion injury, and has offered considerable promise as a novel therapeutic strategy for CVD in humans (3,5–8).

EPHX2 codes for human sEH (9) and has considerable genetic heterogeneity (10). Given the lack of available sEH inhibitors for clinical testing, genetic epidemiologic studies have been conducted to understand the role of sEH and EET hydrolysis in human CVD (8). The two most common nonsynonymous single nucleotide polymorphisms (SNPs) in *EPHX2* increase (p.Lys55Arg) or decrease (p.Arg287Gln) sEH activity and EET hydrolysis *in vitro* (10–12), and have been associated with the risk of developing ischemic cardiovascular and cerebrovascular disease in humans (13–19). Altogether, these data suggest that genetic variation in sEH-mediated EET hydrolysis may be important in the pathogenesis of CVD in humans. However, it remains unknown whether functional genetic variants in *EPHX2* modify prognosis in patients with existing ischemic CVD. The objective of the present study was to evaluate the relationship between two common nonsynonymous, functional *EPHX2*

SNPs (p.Lys55Arg and p.Arg287Gln) and mortality following hospitalization for an ACS clinical event.

MATERIALS AND METHODS

Study participants

The analysis was completed using DNA samples and survival data collected as part of the INvestigation oF Outcomes from acute coronary syndRoMes (INFORM) and Translational Research Investigating Underlying Disparities in Acute Myocardial Infarction Patients' Health Status (TRIUMPH) cohorts. All participating institutions obtained approval from their respective ethics committees, and all participants signed informed consent during the initial screening period, as reported previously (20,21).

The INFORM cohort, a prospective, observational study of 735 consecutive ACS patients at two Kansas City hospitals (Mid America Heart Institute and Truman Medical Center) from March 2001 to October 2002 (22–24), was utilized as a discovery cohort. Diagnosis of ACS occurred in the context of clinical care, as described (22–24). Non-ST-elevation myocardial infarction (NSTEMI) and ST-elevation myocardial infarction (STEMI) were defined by elevated troponin levels in combination with chest pain symptoms or electrocardiographic findings, and unstable angina was defined by a negative troponin level in combination with any one of the following conditions: new-onset angina (<2 months), prolonged angina (>20 minutes) at rest, recent worsening angina, or angina that occurred within 2 weeks of MI. Three physicians reviewed the charts of any patient with diagnostic uncertainty and attained consensus on the final diagnosis.

The TRIUMPH cohort, a prospective, observational study of 4,340 consecutive MI patients across 24 US hospitals (N=2,979 consented to genetic testing) from April 2005 to December 2008 (21,25,26), was used as a validation cohort to replicate the significant genotype-survival associations observed in INFORM. The definition of NSTEMI and STEMI was identical to INFORM; however, patients hospitalized with an unstable angina ACS event were not enrolled in TRIUMPH.

Baseline and Clinical Outcome Measures

In each cohort, extensive baseline data was determined from chart abstraction and questionnaires, race/ethnicity was based on patient self-report, and all-cause mortality was the primary prognosis outcome, as described (21,22). The Social Security Administration Death Master File was queried to determine vital status for all patients in each study over 5 years of follow-up after the baseline ACS event (https://classic.ntis.gov/products/ssa-dmf/). This query was performed prior to new restrictions and expunging of some records from the database. In the TRIUMPH cohort, death due to cardiovascular causes was evaluated as a secondary outcome. Cardiovascular mortality data were obtained through query of the Centers for Disease Control and Prevention National Death Index (NDI), as described (27).

Genotyping

Genomic DNA was isolated and purified from whole blood using QIAamp DNA purification kit (Qiagen, Germantown, MD, USA), as described (25,26). In the INFORM cohort, the *EPHX2* p.Lys55Arg (rs41507953) and p.Arg287Gln (rs751141) polymorphisms were genotyped by pyrosequencing assays (25). In the TRIUMPH cohort, the p.Lys55Arg polymorphism was genotyped using a TaqMan allelic discrimination assay (C_32297897_10), and performed according to manufacturer's directions (Applied Biosystems, Foster City, CA), as described (26,28). PCR and allelic discrimination were performed using the ABI 7500 real-time PCR platform. For all variants, genotype call rates were greater than 95% and in Hardy-Weinberg equilibrium.

Among the genotyped samples in each cohort, the study population was limited to those self-identified as Caucasian or African American to minimize the impact of population stratification on the results. The p.Lys55Arg genotype data was available in 667 ACS patients in the INFORM cohort (545 Caucasians, 122 African Americans) and 2,712 patients in the TRIUMPH cohort (2,049 Caucasians, 663 African Americans), which served as the primary study populations for analysis.

Statistical analysis

Data are presented as mean ± standard deviation or count (proportion) unless otherwise indicated. Baseline characteristics were compared across genotype groups using a Student's t-test (dominant model) or one-way ANOVA (additive model) for continuous variables, or chi-squared test or Fisher's exact test, as appropriate, for categorical variables. Deviations from Hardy-Weinberg equilibrium (race-stratified) were evaluated by chi-squared test.

In each cohort, genotype-survival associations were evaluated using Cox proportional hazards models and log-rank tests to compare Kaplan-Meier survival curves across genotypes, as described (25,26). In the INFORM (discovery) cohort, the association between p.Lys55Arg and p.Arg287Gln genotype with survival was first investigated using a dominant model of inheritance (Arg55 carriers versus Lys/Lys; and Gln287 carriers versus Arg/Arg, respectively). In addition to conducting this analysis in the full cohort, race-stratified analyses were performed. However, due to the small sample size of African American participants in INFORM (N=122), the race-stratified analysis was performed in the Caucasian strata only.

In the TRIUMPH cohort, the primary analysis used a dominant model of inheritance to validate the p.Lys55Arg genotype-survival association observed in INFORM (Arg55 carriers versus Lys/Lys). Genotype-mortality associations were also evaluated using an additive model of inheritance (Arg/Arg versus Lys/Arg versus Lys/Lys) as a secondary analysis. All analyses were conducted in the full cohort followed by race-stratified analyses in the Caucasian and African American strata, and included both all-cause mortality and cardiovascular mortality as endpoints.

In both INFORM and TRIUMPH, adjusted models included pre-specified baseline covariates known to predict adverse outcomes in ACS patients including age, race, sex, ACS type (STEMI, NSTEMI, unstable angina), diabetes, and revascularization treatment strategy

(medical management, PCI, CABG). Analyses were performed using SAS 9.2 (SAS Institute, Cary, NC). Since this was a candidate polymorphism study with a validation cohort, a P<0.05 was considered to be statistically significant.

RESULTS

INFORM baseline characteristics and *EPHX2* genotype frequencies

In INFORM, the mean age was 60.6 years, 82% were Caucasian, 36% were female, 29% experienced a STEMI as their index event, and 63% underwent coronary artery revascularization during the index hospitalization (Table 1). The minor allele frequency (MAF) for p.Lys55Arg was 11.7% in Caucasians and 21.7% in African Americans. The MAF for p.Arg287Gln was 10.2% in Caucasians and 14.6% in African Americans. Genotype frequencies for p.Lys55Arg (Table 2) and p.Arg287Gln (Supplemental Table 1) were consistent with Hardy-Weinberg equilibrium (P<0.05), and previous study populations (13). No significant differences in baseline characteristics across p.Lys55Arg genotype were observed (Supplemental Table 2).

EPHX2 genotype and mortality in the INFORM cohort

In INFORM, the overall 5-year mortality rate was 17.1%. An increase in 5-year mortality rates was observed across *EPHX2* p.Lys55Arg genotype groups, and Arg55 variant allele carriers exhibited a significantly higher risk of death (22.4% in Arg55 carriers versus 15.3% for Lys/Lys; unadjusted HR 1.50, 95% confidence interval [CI] 1.02–2.22, P=0.042) (Figure 1A, Supplemental Table 3). This association was similar, but not statistically significant in the adjusted model (adjusted HR 1.40, 95% CI 0.93–2.09, P=0.103).

In the race-stratified analysis, the p.Lys55Arg genotype-survival association was evident in the Caucasian strata, and persisted after adjustment for demographic and clinical covariates (22.7% in Arg55 carriers versus 14.3% for Lys/Lys; adjusted HR 1.61, 95% CI 1.01–2.55, P=0.045; Figure 1B). Although a stepwise increase in 5-year mortality rates were also observed across Caucasian subjects with zero (Lys/Lys: 14.3%), one (Lys/Arg: 21.8%), or two (Arg/Arg: 33.3%) copies of the Arg55 variant allele, these differences were not statistically significant (additive model: log-rank P=0.064; Supplemental Table 3).

In contrast, no association was observed between 5-year mortality and *EPHX2* p.Arg287Gln genotype (Supplemental Figure 1). Similar results were observed in the full study population (unadjusted HR 0.88, 95% CI 0.55–1.41, P=0.598) and the Caucasian strata (unadjusted HR 0.91, 95% CI 0.52–1.59, P=0.745).

TRIUMPH baseline characteristics and *EPHX2* genotype frequencies

The TRIUMPH cohort was used as a validation cohort. Similar to the INFORM cohort, the mean age in TRIUMPH was 59.2 years, 76% were Caucasian, 32% were female, 44% experienced a STEMI as their index event, and 76% underwent coronary artery revascularization during the index hospitalization (Table 1). Consistent with the INFORM cohort genotyping results, the *EPHX2* p.Lys55Arg MAF was 11.2% in Caucasians and 22.4% in African Americans (Table 2). Within the Caucasian strata, there were no

significant differences in baseline characteristics across p.Lys55Arg genotype (Supplemental Table 4). In African Americans, history of chronic heart failure, treatment strategy during the index visit, and beta-blocker use at discharge differed across genotype groups (Supplemental Table 5).

EPHX2 p.Lys55Arg genotype and mortality in the TRIUMPH cohort

Consistent with the INFORM cohort, the 5-year all-cause mortality rate in TRIUMPH was 17.8% (15.8% in Caucasians, 24.3% in African Americans). The rate of death due to cardiovascular causes was 9.3% (8.2% in Caucasians, 13.0% in African Americans).

In contrast to INFORM, *EPHX2* Arg55 carriers did not exhibit a significantly higher risk of death in the overall TRIUMPH study population when using a dominant model (19.7% in Arg55 carriers versus 17.2% for Lys/Lys, adjusted HR 1.01, 95% CI 0.82–1.23, P=0.950; Supplemental Table 6). After stratifying by race, no association was observed in either Caucasians (15.4% in Arg55 carriers versus 15.9% for Lys/Lys, adjusted HR 0.92, 95% CI 0.70–1.21, P=0.559) or African Americans (26.6% in Arg55 carriers versus 22.8% for Lys/Lys, adjusted HR 1.14, 95% CI 0.84–1.57, P=0.402; Supplemental Table 6). Furthermore, Arg55 carrier status was not significantly associated with death due to cardiovascular causes in the overall study population, or the Caucasian and African American strata (Supplemental Table 7).

Using an additive mode of inheritance, a stepwise increase in 5-year all-cause mortality was observed across *EPHX2* p.Lys55Arg genotype groups in the overall population (Figure 2A); these differences were less pronounced than observed in the INFORM cohort and not statistically significant (log-rank P=0.142). In the adjusted analysis, subjects homozygous for the Arg55 variant allele (Arg/Arg, N=63) exhibited a 1.22-fold (95% CI 0.94–2.57) higher risk of mortality relative to wild-type (Lys/Lys) subjects; however, this relationship was not statistically significant (Figure 2A). Similar non-significant relationships between Arg/Arg genotype and allcause mortality were observed in Caucasians (adjusted HR 1.28, 95% CI 0.53–3.11) and African Americans (adjusted HR 1.30, 95% CI 0.69–2.45) (Supplemental Table 6).

Similar to the all-cause mortality results, subjects homozygous for the Arg55 variant allele (Arg/Arg) had a nominally greater 5-year risk of death due to cardiovascular causes relative to wild-type (Lys/Lys) subjects (adjusted HR 1.26, 95% CI 0.60–2.67) that was not statistically significant (Figure 2B). Race-stratified analyses revealed that Caucasian subjects carrying the Arg/Arg genotype exhibited a significantly higher risk of cardiovascular mortality compared to wild-type (Lys/Lys) subjects (17.2% versus 8.2%, respectively; adjusted HR 2.60, 95% CI 1.09–6.17, Figure 3A). In contrast, no association between Arg/Arg genotype and cardiovascular mortality was observed in African Americans (Figure 3B).

DISCUSSION

We investigated the impact of *EPHX2* p.Lys55Arg and p.Arg287Gln polymorphisms on prognosis in patients with CVD following hospitalization for an ACS event. An association

between *EPHX2* p.Lys55Arg, but not p.Arg287Gln, genotype and 5-year mortality was observed in the INFORM cohort. Arg55 variant allele carriers had a significantly higher risk of death compared to those with a wild-type Lys/Lys genotype, and this association was most pronounced in Caucasians. However, the significantly higher risk of death observed in Arg55 allele carriers was not validated in the TRIUMPH cohort, including race-stratified analyses in Caucasian and African American patients. In Caucasians, Arg/Arg homozygotes did exhibit a significantly higher risk of cardiovascular death compared to Lys/Lys individuals; however, since a higher risk of cardiovascular mortality was not observed in heterozygous Lys/Arg individuals compared to Lys/Lys, this secondary analysis should be interpreted with caution until confirmed in future studies. Collectively, these results demonstrate that *EPHX2* p.Lys55Arg and p.Arg287Gln polymorphisms do not significantly modify 5-year survival after an ACS event, and suggest that common *EPHX2* genetic variants may not be useful biomarkers to risk stratify and guide identification of ACS patients with enriched potential to benefit from EET-targeted therapeutics in development. Future studies are necessary to determine whether other biomarkers of the EET metabolic pathway are associated with prognosis in ACS patients.

It is well established that CYP-derived EETs, which are rapidly hydrolyzed by sEH, elicit potent anti-inflammatory, vasodilatory, fibrinolytic, anti-apoptotic and mitochondrial preservation effects in the cardiovascular system (6,7), and that promoting the effects of EETs elicits myocardial and vascular protection in multiple preclinical models of CVD (8). Notably, early studies in an *ex vivo* mouse model of myocardial ischemia-reperfusion injury demonstrated that global disruption of murine *Ephx2* (*Ephx2*$^{-/-}$) and cardiomyocyte-specific overexpression of the human *CYP2J2* epoxygenase promote cardioprotection in an EET-dependent manner via a variety of mechanisms (29,30). *In vivo* studies have further demonstrated that *Ephx2*$^{-/-}$ mice and wild-type mice receiving a pharmacologic inhibitor of sEH have reduced infarct size and improved cardiac remodeling in both models of myocardial ischemia-reperfusion injury elicited by temporary ligation of the left anterior descending (LAD) coronary artery (31,32) and models of sustained ischemic injury following permanent occlusion of the LAD artery (33). Although the cardioprotective effects were attenuated relative to young mice, aged *Ephx2*$^{-/-}$ mice also exhibited reduced cardiac remodeling following permanent LAD occlusion, demonstrating that inhibition of sEH-mediated EET hydrolysis is cardioprotective in the setting of aging (34). Altogether, these preclinical studies suggest that therapeutic interventions that promote the cardioprotective effects of EETs offer considerable promise as a novel therapeutic strategy to reduce pathologic sequelae and improve prognosis following an ischemic ACS event.

Consistent with the cardiovascular protective effects of EETs in preclinical models, several epidemiologic studies have shown that *EPHX2* p.Lys55Arg polymorphism, which confers increased sEH activity and EET hydrolysis (10), is associated with a higher risk of developing coronary artery disease (16), ischemic stroke (19), and impaired forearm vasodilator responses (28) in Caucasian populations. The observed association between the Arg55 allele and higher risk of mortality following ACS in Caucasians in the INFORM cohort is consistent with these prior studies and the hypothesis that higher sEH activity results in lower EET levels and poorer cardiovascular outcomes. However, it is important to note that the association between p.Lys55Arg genotype and higher CVD risk has not been

consistently described in the literature. This includes a lack of association between the Arg55 allele and risk of ischemic stroke in Caucasians (18), risk of restenosis after PCI in Caucasians (35), risk of atrial fibrillation recurrence after catheter ablation in Caucasians (36), and risk of developing coronary artery disease in African Americans (16).

In the current study, the observed association between *EPHX2* Arg55 carrier status (Lys/Arg or Arg/Arg) and higher risk of death in the discovery cohort was not replicated in the larger TRIUMPH cohort. Although we did observe that Caucasian patients homozygous for p.Lys55Arg variant (Arg/Arg) had significantly higher risk of cardiovascular death compared to those with the Lys/Lys genotype, it is important to note that this was a secondary objective and the study was not powered to detect genotype-prognosis associations in the small number of homozygous patients. Thus, this result must be interpreted with caution until validated in an independent population. However, if validated, the low frequency of Arg/Arg patients in Caucasian populations (1–2%) limits the potential clinical utility of *EPHX2* p.Lys55Arg genotype as a prognostic biomarker in ACS patients that identifies subsets of the population with enriched potential to derive benefit from novel therapeutic strategies that promote the cardioprotective effects of EETs.

In contrast to p.Lys55Arg, the *EPHX2* p.Arg287Gln polymorphism significantly decreases sEH enzyme activity and EET hydrolysis (10). Consistent with the cardiovascular and metabolic protective effects elicited with sEH inhibition in preclinical models, the Gln287 variant allele was associated with lower risk of ischemic stroke in a Chinese population (37) and improved insulin sensitivity in an American population (38). In contrast, Gln287 carrier status has been associated with higher risk of CVD, including a higher risk of coronary artery calcification in African Americans (14), insulin resistance in Japanese type 2 diabetics (39), ischemic stroke in Europeans (18), and atrial fibrillation after catheter ablation (36). The current study, which did not observe an association between p.Arg287Gln genotype and mortality following ACS, is consistent with multiple prior studies reporting no association between the Gln287 variant and CVD risk (15,16,19,40,41).

Although the current study was the first to prospectively assess associations between *EPHX2* variants and prognosis following an ACS clinical event, other recent studies have prospectively investigated clinical outcomes in patients with existing cerebrovascular and renal disease. Martini et al. demonstrated that *EPHX2* p.Lys55Arg, but not p.Arg287Gln, was associated with higher risk of new stroke events and death in patients with aneurysmal subarachnoid hemorrhage (42). Similarly, Arg55, but not Gln287, variant allele carriers were at higher risk of acute kidney injury following cardiac surgery (43). In contrast, p.Arg287Gln was associated with improved kidney survival in Korean patients with IgA nephropathy (44), lower early neurologic deterioration in Chinese patients admitted for minor ischemic stroke (45), and favorable outcomes in aneurysmal subarachnoid hemorrhage patients (46). The lack of consistent findings across studies suggests that the relationship between genetic variation in *EPHX2* and the risk of CVD outcomes is likely complex and may only be present in certain populations.

In addition to its well-characterized epoxide hydrolase effects, the sEH enzyme also posseses phosphatase activity; however, the biological relevance of the phosphatase domain

is less clear (6,7). There is evidence that the *EPHX2* p.Lys55Arg and p.Arg287Gln variants alter the phosphatase activity of sEH. The *EPHX2* p.Lys55Arg variant has been reported to increase both hydrolase and phosphatase activity *in vitro* (12). In contrast, a prior study demonstrated that the p.Lys55Arg variant decreased phosphatase activity *in vitro* (47). These contradictory findings appear to be related to substrate-specific effects (12). The functional implications of *EPHX2* variants on phosphatase activity *in vivo* and CVD risk remain unclear and require further investigation.

Ultimately, the identification of biomarkers predictive of dysfunction in the EET metabolic pathway and poor prognosis following ACS could guide the development of novel therapeutics that promote the cardiovascular protective effects of EETs. Beyond genetic variants in *EPHX2*, quantification of epoxyeicosanoid metabolite concentrations may provide a more precise characterization of inter-individual variability in sEH activity, and greater utility as a biomarker predictive of prognosis and treatment response, since clinical and demographic factors significantly contribute to inter-individual variation in circulating EET levels and EET:DHET ratios (biomarkers of sEH activity) in humans (48). Moreover, we recently reported that multiple clinical factors, most notably the presence of obstructive atherosclerotic CVD, were associated with low circulating EET levels in patients referred for coronary angiography (49). Future studies investigating the relationship between baseline EET levels and prognosis in patients with established CVD are needed to provide further insight into the therapeutic potential of novel drugs in development, including sEH inhibitors and structural analogs of EETs, in patients with CVD (7,8).

There are limitations to this study. First, although TRIUMPH is one of the largest registries to provide detailed information on clinical, genetic, and metabolic characteristics in a racially diverse population, with a primary goal of investigating racial disparities in post-MI outcomes (21), the current investigation had limited power to detect genotype-prognosis associations in the African American subset. Secondly, since modest effect sizes (HR<1.5) further limit power to detect significant associations, even in the larger Caucasian strata and when using a dominant model of inheritance, evaluating associations in homozygote variant allele carriers with adequate power would require much larger sample sizes; thus, the observed associations with Arg/Arg genotype in the additive model should be interpreted with caution and considered hypothesis-generating. Third, mortality was the only prognosis outcome adjudicated in the INFORM and TRIUMPH cohorts over the 5-year follow-up period, and was the focus of our analysis. Assessment of major adverse cardiovascular events (MACE), which include CV mortality, nonfatal MI, nonfatal stroke, and hospitalization for unstable angina, would have increased the number of events and enhanced statistical power. However, these outcomes were not available for analysis. A future study that evaluates associations between functional genetic variants in *EPHX2* and MACE outcomes in ACS patients is warranted. Fourth, circulating levels of EETs were not quantified in TRIUMPH or INFORM cohort participants; thus, it is unknown whether the presence of *EPHX2* genetic variants influenced EET levels. Lastly, although p.Lys55Arg and p.Arg287Gln are common functionally relevant polymorphisms, we cannot rule out whether associations between other genetic variants in *EPHX2* and prognosis exist.

Conclusions

In summary, *EPHX2* p.Lys55Arg, but not p.Arg287Gln, genotype was associated with 5-year mortality following ACS in a discovery cohort (INFORM). However, this association was not replicated in an independent population (TRIUMPH). Although secondary analyses revealed that Caucasians homozygous for the Arg55 variant allele may be at higher risk of cardiovascular mortality following ACS, this observation should be interpreted with caution. Altogether, these results suggest that *EPHX2* p.Lys55Arg and p.Arg287Gln polymorphisms do not significantly modify prognosis after an ACS event in humans. Future studies are necessary to determine whether other biomarkers of the EET metabolic pathway are associated with prognosis in CVD patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- *EPHX2* p.Lys55Arg genotype and prognosis were evaluated in ischemic CVD patients
- Arg55 carriers exhibited higher 5-year mortality than Lys/Lys in a discovery cohort
- Higher mortality risk in Arg55 carriers was not replicated in an independent cohort
- Higher risk of cardiovascular death was observed in Caucasian Arg55 homozygotes

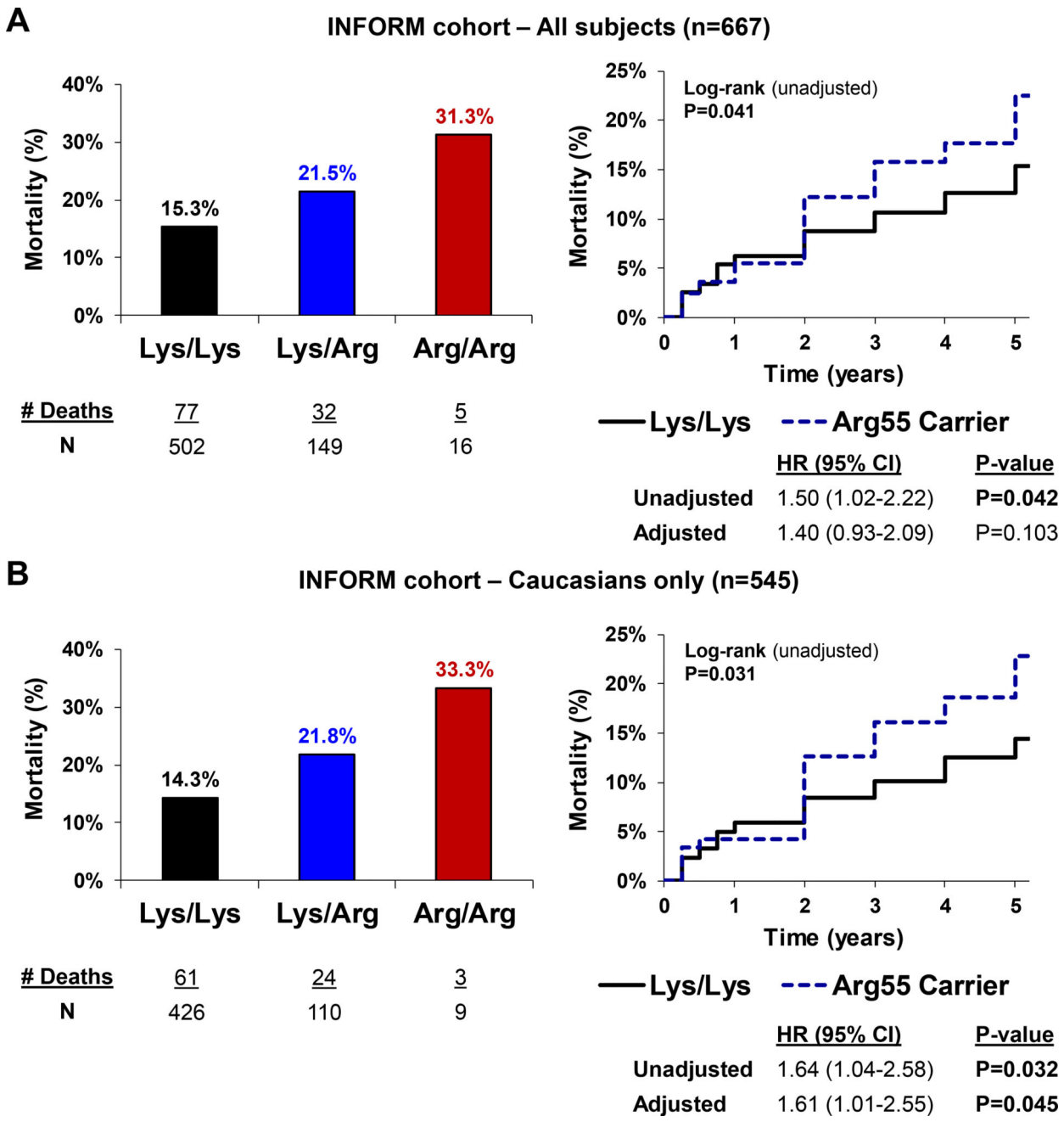

Figure 1. Mortality in INFORM cohort participants by *EPHX2* p.Lys55Arg genotype

Five-year mortality rates and Kaplan-Meier curves in (A) Caucasian + African-American (n=667) and (B) Caucasian only (n=545) participants from the INFORM cohort following hospitalization for an acute coronary syndrome event. Data are presented by *EPHX2* p.Lys55Arg genotype, and the number of deaths and N in each genotype group are provided. The Kaplan-Meier curves used a dominant model and compared Arg55 carriers to wild-type (Lys/Lys). The unadjusted and adjusted hazard ratio (HR), 95% confidence interval (CI) and P-value are provided.

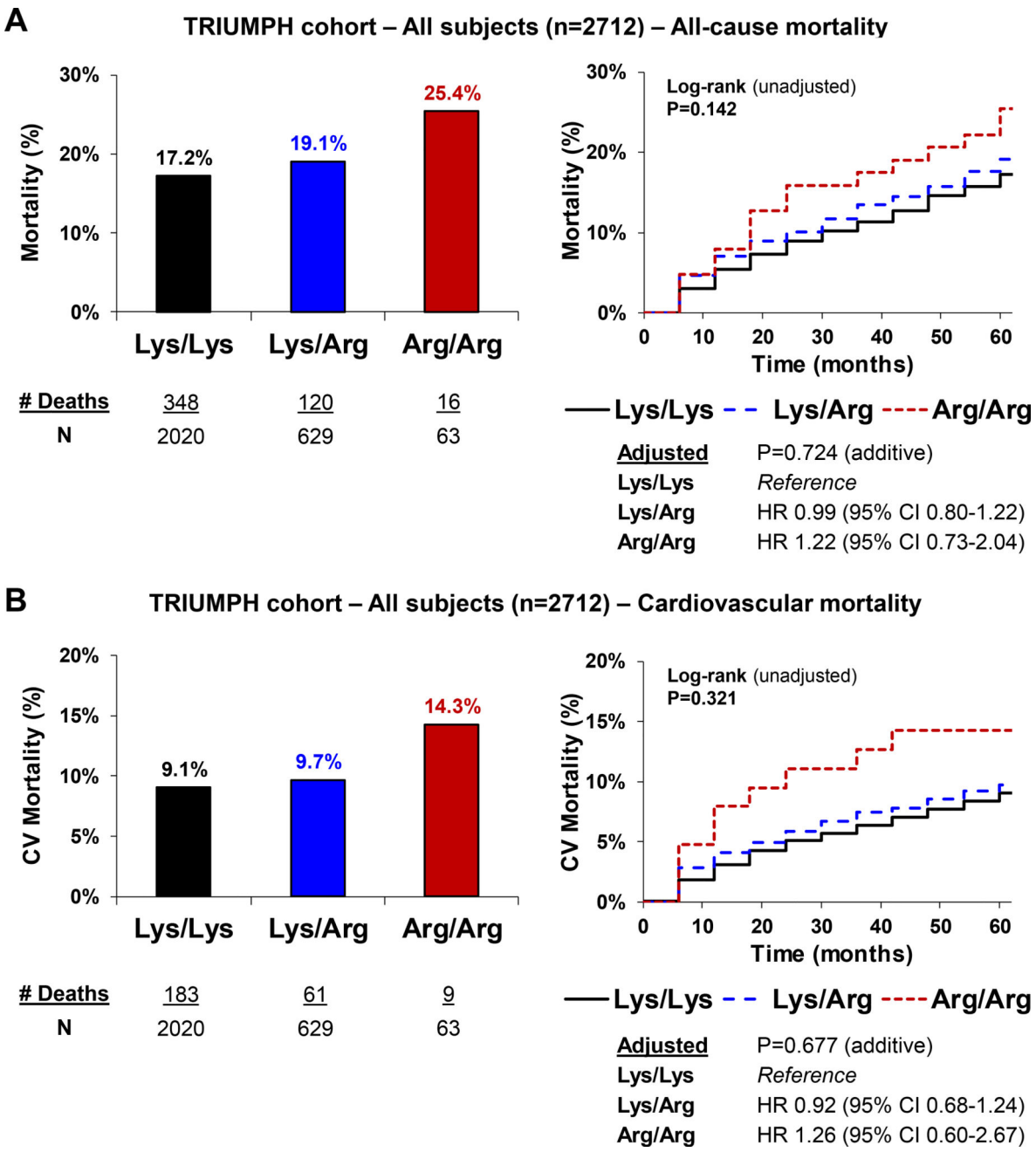

Figure 2. Mortality in all TRIUMPH cohort participants by *EPHX2* p.Lys55Arg genotype

Five-year mortality rates and Kaplan-Meier curves in Caucasian + African-American (n=2712) participants from the TRIUMPH cohort following hospitalization for an acute myocardial event. (A) Death due to any cause. (B) Death due to cardiovascular causes. Data are presented by *EPHX2* p.Lys55Arg genotype, and, the number of deaths and N in each genotype group are provided. The Kaplan-Meier curves used an additive model, and compared Arg55 heterozygotes (Lys/Arg) and Arg55 homozygotes (Arg/Arg) to wild-type (Lys/Lys). The adjusted hazard ratio (HR), 95% confidence interval (CI) and P-value are provided.

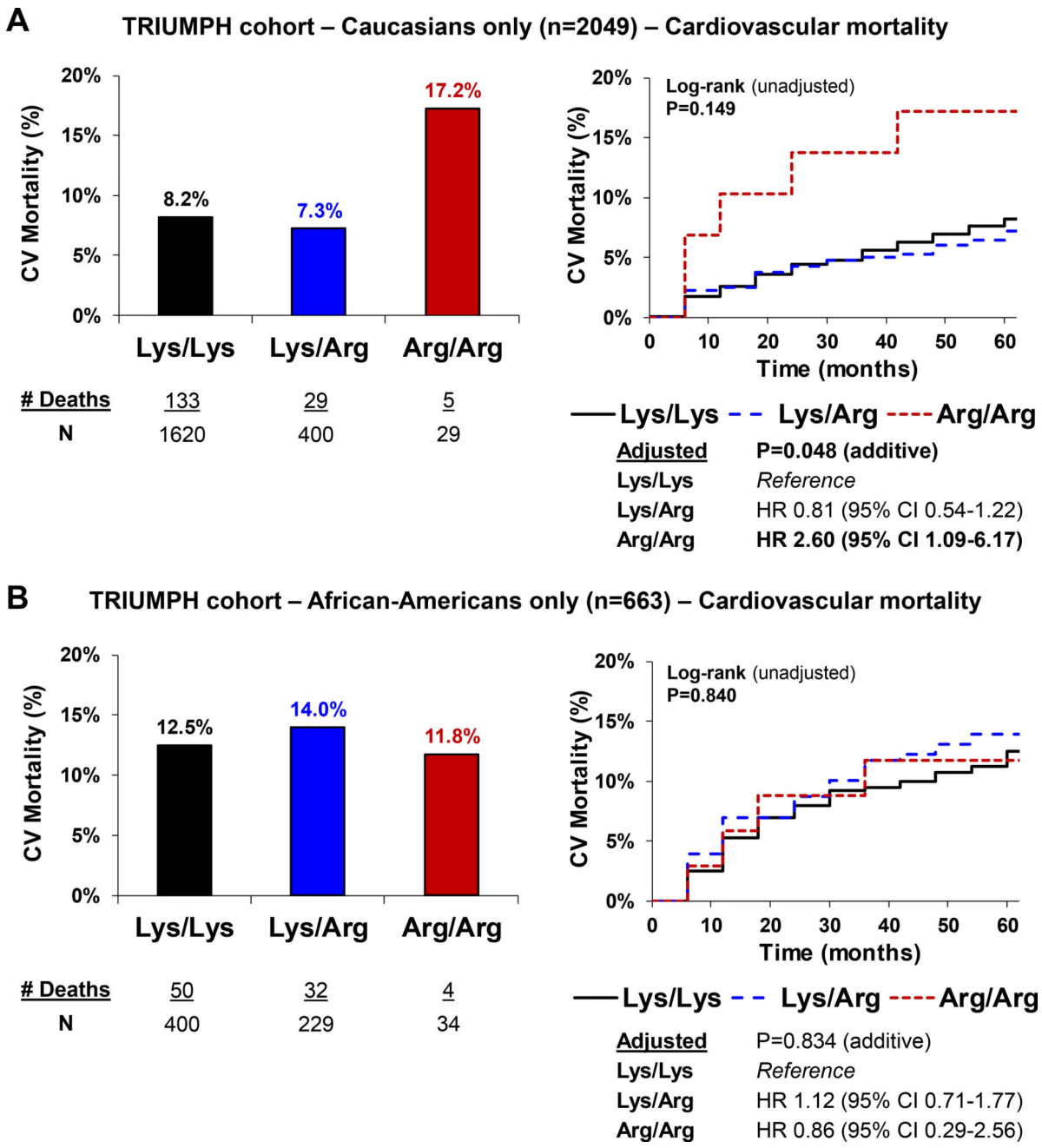

Figure 3. Cardiovascular mortality in Caucasian and African-American TRIUMPH cohort participants by *EPHX2* p.Lys55Arg genotype

Five-year rates of death due to cardiovascular causes and Kaplan-Meier curves in (A) Caucasian + African-American (n=2049) and (B) Caucasian only (n=663) participants from the TRIUMPH cohort following hospitalization for an acute myocardial event. Data are presented by *EPHX2* p.Lys55Arg genotype, and the number of deaths and N in each genotype group are provided. The Kaplan-Meier curves used an additive model, and compared Arg55 heterozygotes (Lys/Arg) and Arg55 homozygotes (Arg/Arg) to wild-type

(Lys/Lys). The adjusted hazard ratio (HR), 95% confidence interval (CI) and P-value are provided.

Table 1

Characteristics of the study populations.

Characteristic		INFORM	TRIUMPH
N		667	2712
Age (years)		60.6 ± 12.4	59.2 ± 12.2
Gender			
	Male	426 (63.9%)	1855 (68.4%)
	Female	241 (36.1%)	857 (31.6%)
Race			
	Caucasian	545 (81.7%)	2049 (75.6%)
	African-American	122 (18.3%)	663 (24.4%)
ACS Type			
	Unstable angina	273 (40.9%)	0 (0%) *
	Non ST elevation MI	204 (30.6%)	1526 (56.3%)
	ST elevation MI	190 (28.5%)	1186 (43.7%)
Past medical history			
	Current smoker	240 (36.0%)	1062 (39.2%)
	Hypertension	437 (65.5%)	1775 (65.4%)
	Diabetes	187 (28.0%)	795 (29.3%)
	Hyperlipidemia	404 (60.6%)	1326 (48.9%)
	Chronic heart failure	52 (7.8%)	217 (8.0%)
	Prior MI	217 (32.5%)	532 (19.6%)
Treatment Strategy during index visit			
	Medical management	250 (37.5%)	674 (24.9%)
	Revascularization procedure#	417 (62.5%)	2056 (75.8%)
Discharge Medications			
	Beta-blocker	536 (80.4%)	2459 (90.7%)
	Statin	503 (75.4%)	2398 (88.4%)
	Aspirin	620 (93.0%)	2579 (95.1%)

Acute coronary syndrome, ACS; MI, myocardial infarction.

Data presented as mean ± standard deviation or count (%).

*The TRIUMPH cohort did not enroll participants with a diagnosis of ACS/unstable angina

#Percutaneous coronary intervention procedure or coronary artery bypass grafting surgery

Table 2

EPHX2 p.Lys55Arg genotype frequencies in the INFORM and TRIUMPH cohorts.

p.Lys55Arg Genotype		INFORM	TRIUMPH
All subjects		*n=667*	*n=2712*
	Lys/Lys	502 (75.3%)	2020 (74.5%)
	Lys/Arg	149 (22.3%)	629 (23.2%)
	Arg/Arg	16 (2.4%)	63 (2.3%)
	MAF	13.6%	13.9%
Caucasians		*n=545*	*n=2049*
	Lys/Lys	426 (78.1%)	1620 (79.1%)
	Lys/Arg	110 (20.2%)	400 (19.5%)
	Arg/Arg	9 (1.7%)	29 (1.4%)
	MAF	11.7%	11.2%
African Americans		*n=122*	*n=663*
	Lys/Lys	76 (62.3%)	400 (60.3%)
	Lys/Arg	39 (32.0%)	229 (34.5%)
	Arg/Arg	7 (5.7%)	34 (5.1%)
	MAF	21.7%	22.4%

Data presented as count (%) or allele frequency.

Hardy-Weinberg P-values (race-stratified) in each cohort were: INFORM (Caucasians: P=0.540; African-Americans: P=0.508), TRIUMPH (Caucasians: P=0.448; African-Americans: P=0.869)