GATM gene variants and statin myopathy risk

ARISING FROM L. M. Mangravite *et al*. *Nature* **502,** 377–380 (2013); doi:10.1038/nature12508

Mangravite *et al.*[1] identified six expression quantitative loci (eQTLs) that interacted with simvastatin exposure by using 480 lymphoblastoid cell lines exposed to β-hydroxy simvastatin acid *in vitro*. One of these SNPs (rs9806699) within the glycine amidinotransferase (*GATM*) gene was shown to have an association with statin-induced myopathy in two independent cohorts ($n = 172$ myopathy cases), conferring a protective effect (odds ratio = 0.61, 95% confidence interval = 0.39–0.95, $P = 0.03$). Our genotyping results from statin myopathy patients do not appear to replicate this finding. There is a Reply to this Brief Communication Arising by Mangravite, L. M. *et al. Nature* **513,** http://dx.doi.org/10.1038/nature13630 (2014).

Using primarily the UK Clinical Practice Research Datalink, an electronic healthcare record database, we recruited 145 cases with statin-induced myopathy and 537 statin-exposed control patients[2]. In addition, five patients meeting our case inclusion criteria were identified prospectively through a tertiary adult muscle clinic. Our myopathy phenotype was defined as serum creatine kinase levels of greater than 4 × upper limit of normal (ULN) or clinical record of rhabdomyolysis concurrent with statin prescription. In a proof-of-concept study, using a subset of patients (78 cases, 372 controls)[3] we were able to show an association between the *SLCO1B1*5* allele (rs4149056) and both statin-induced myopathy (odds ratio = 2.1, 95% confidence interval = 1.3–3.1) and severe myopathy ($n = 23$, odds ratio = 4.1, 95% confidence interval = 2.1–8.2), consistent with the genome-wide association study (GWAS) findings from the SEARCH collaborative[4].

We have undertaken genotyping for the rs9806699 *GATM* single-nucleotide polymorphism (SNP) in our cases and drug-exposed control patients ($n = 150$ and 587, respectively, after quality control) in order to attempt replication of the association shown by Mangravite *et al.*[1]. However, we were unable to show a significant difference in the minor allele frequency of rs9806699 between myopathy cases (MAF = 0.28) and controls (MAF = 0.30) (odds ratio = 0.94, $P = 0.68$). The MAF in our cases was similar to that identified in controls in the paper by Mangravite *et al.*[1]. By limiting cases to just those with 'severe' myopathy (creatine kinase > 10 × ULN or rhabdomyolysis) ($n = 37$), we again failed to show a significant difference in MAF between cases and controls (odds ratio = 0.94, $P = 0.83$). Further analysis restricted to patients only receiving simvastatin (99 cases, 344 controls) also did not demonstrate an association between rs9806699 and risk of either myopathy (odds ratio = 1.12, $P = 0.49$) or severe myopathy ($n = 26$, odds ratio = 1.42, $P = 0.24$). Analysis restricted to the 120 cases that were not on drugs known to interact with statins also did not change the result. We have also undertaken genome wide analysis of 128 myopathy cases (Illumina Human OmniExpress Exome 8v1), and comparison with the WTCCC2 (Wellcome Trust Case Control Consortium 2) genotype data also did not show any association between statin myopathy (generalized or severe) and any of 90 typed or imputed SNPs within the *GATM* gene locus.

In conclusion, we have not been able to replicate the association between the rs9806699 *GATM* SNP and statin myopathy reported by Mangravite *et al.*[1] in an independent sample set despite the fact that all patients were of European ancestry and had similar statin-myopathy phenotypes. This association will need to be assessed in more patients, and through an individual patient-data meta-analysis to determine, first, whether the SNP is relevant to a sub-phenotype of statin myopathy, and second, its clinical and mechanistic relevance.

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GATM locus does not replicate in rhabdomyolysis study

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All HMG-CoA reductase inhibitors (statins) can cause muscle injury ranging from asymptomatic elevations in creatine kinase levels to severe muscle breakdown (rhabdomyolysis) leading to kidney failure and death[1], and the genetic variants responsible for this uncommon adverse drug reaction remain largely undiscovered. Mangravite *et al.* reported a new locus in the gene *GATM* (rs9806699) that was associated with a decreased risk of muscle injury in two case-control studies of myopathy (odds ratio, 0.60)[2]. In a larger case-control study of statin-related rhabdomyolysis, a more severe form of muscle injury, we were unable to replicate this finding. This failure to replicate raises questions about the role of *GATM* in statin-related muscle injury. There is a Reply to this Brief Communication Arising by Mangravite, L. M. *et al. Nature* **513,** http://dx.doi.org/10.1038/nature13630 (2014).

Mangravite *et al.* used differential gene expression profiling of lymphoblastoid cell lines exposed to simvastatin to identify *cis*-expression quantitative trait loci (eQTLs) for the gene *GATM* as candidate loci for

Table 1 | Association of *GATM* loci with the risk of cerivastatin-related rhabdomyolysis

	All subjects					Excluding fibrate users				
SNP	Cases (n = 175), MAF	Controls (n = 645), MAF	OR	95% CI	P value	Cases (n = 76), MAF	Controls (n = 643), MAF	OR	95% CI	P value
rs9806699	0.27	0.28	1.01	0.70–1.45	0.96	0.24	0.28	0.84	0.52–1.36	0.49
rs1719247	0.29	0.25	1.37	0.98–1.90	0.07	0.24	0.25	1.00	0.64–1.57	0.99
rs1346268	0.29	0.27	1.25	0.90–1.73	0.18	0.24	0.27	0.88	0.52–1.36	0.57

Rhabdomyolysis case subjects had creatine kinase levels >10 × the upper limit of normal and used cerivastatin at the time of onset of symptoms of muscle pain or weakness. Control subjects did not experience rhabdomyolysis and used the following statins: lovastatin (44%), simvastatin (19%), pravastatin (18%), atorvastatin (13%), fluvastatin (6%) or cerivastatin (1%). CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism.

pharmacogenomic associations with muscle injury, which they evaluated in two case-control studies of myopathy. Variation at their most significant *cis*-eQTL for *GATM*, rs9806699, was associated with a decreased risk of muscle injury (odds ratio = 0.60, 95% confidence interval = 0.39–0.95) in a study with 72 mild myopathy cases (blood creatine kinase levels > 3 × the upper limit of normal (ULN) with muscle symptoms) recruited from a healthcare organization (Marshfield). In a second study with 39 mild and 61 severe myopathy cases (creatine kinase > 10 × ULN with muscle symptoms) using simvastatin during the SEARCH clinical trial, variation at two single-nucleotide polymorphisms (SNPs) in linkage disequilibrium with rs9806699 ($r^2 \geq 0.7$) was also associated with a decreased risk of muscle injury (rs1719247, odds ratio = 0.61, 95% confidence interval = 0.42–0.88; rs1346268, odds ratio = 0.62, 95% confidence interval = 0.43–0.90). On the basis of these epidemiologic findings and the results of functional studies in hepatocyte-derived cell lines, the authors identified *GATM* as a new genetic locus for statin-induced myopathy.

We attempted to replicate these findings in a case-control study of rhabdomyolysis (creatine kinase > 10 × ULN and muscle symptoms) related to the use of cerivastatin[3,4], which was removed from the market in 2001 because of a high incidence of this adverse drug reaction[5]. Rhabdomyolysis cases (175; 94.9% with European ancestry) were compared with statin-using control subjects from the Cardiovascular Health Study without rhabdomyolysis (645; 99.7% with European ancestry). Variation at rs9806699 was not associated with the risk of rhabdomyolysis (odds ratio = 1.01, 95% confidence interval = 0.70–1.45), and variation at the other two SNPs was weakly associated with an increased risk (rs1719247, odds ratio = 1.37, 95% confidence interval = 0.98–1.90; rs1346268, odds ratio = 1.25, 95% confidence interval = 0.90–1.73). Ninety-nine rhabdomyolysis cases used fibrates, which can cause drug–drug interactions with statins, and excluding fibrate users also resulted in null associations (Table 1). Combining our results (all subjects) with the results of Carr *et al.*[6] and Mangravite *et al.*[2] in a fixed-effects meta-analysis resulted in null associations at rs9806699 (odds ratio = 0.88, 95% confidence interval 0.72–1.08, $P = 0.22$), rs1719247 (odds ratio = 0.86, 95% confidence interval = 0.69–1.07, $P = 0.17$) and rs1346268 (odds ratio = 0.85, 95% confidence interval = 0.68–1.05, $P = 0.12$). There was statistical heterogeneity at rs1719247 ($\tau^2 = 0.22$, $P = 0.001$) and rs1346268 ($\tau^2 = 0.14$, $P = 0.009$).

Although most cases from the SEARCH trial involved severe myopathy, it is possible that the *GATM* variants identified by Mangravite *et al.* protect against mild but not severe statin-related muscle injury. Other differences in the study populations could also result in heterogeneity of the effects of these variants. An alternative explanation for the discrepant findings is that *GATM* is not related to this adverse drug reaction. By contrast, a non-synonymous variant in the drug transporter gene *SLCO1B1* (rs4149056) that decreases the clearance of statins[7,8] has been associated with statin-related muscle injury of various severity and statin types[9–12]. The odds ratio for the rs4149056 minor allele in our rhabdomyolysis study (2.0) (ref. 3) was similar to the odds ratios in a study of less-severe myopathy cases related to simvastatin use (2.1) and in a recent meta-analysis (2.2) (ref. 11). In other words, the drug transporter encoded by *SLCO1B1* is a widely replicated finding[7].

The approach by Mangravite *et al.*[2] of identifying potential new pharmacogenomic interactions through differential gene expression profiling is innovative. However, the failure to replicate their findings in a large study of rhabdomyolysis raises questions about whether *GATM* represents a genuine genetic locus for this adverse drug reaction.

Methods

Case subjects were recruited through attorneys representing cerivastatin users who developed rhabdomyolysis. Trained abstractors reviewed medical records to validate rhabdomyolysis events. As cerivastatin comprised a small fraction of statin use during its market life (March 1998 to August 2001), it was not practicable to assemble a broad sample of cerivastatin users who did not develop rhabdomyolysis. Instead, the control group comprised statin-using participants of the Cardiovascular Health Study, a prospective cohort study of older adults[13,14]. This work was funded by a grant from the NHLBI, HL078888.

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Mangravite *et al.* reply

REPLYING TO D. F. Carr *et al. Nature* **513,** http://dx.doi.org/10.1038/nature13628 (2014); J. S. Floyd *et al. Nature* **513,** http://dx.doi.org/10.1038/nature13629 (2014)

Our study[1] tested for associations of single-nucleotide polymorphisms (SNPs) at the *GATM* loci with statin-induced myopathy based on the finding that one of these SNPs (rs986699) was associated with statin-induced expression of *GATM* in a panel of human lymphoblastoid cell lines, and the fact that *GATM* encodes the enzyme responsible for synthesis of creatine, a major source of energy in skeletal muscle[1]. Significant associations with incidence of myopathy were found for rs9806699 in statin users from the Marshfield Clinic cohort. Furthermore, significant association was reported in both the Marshfield cohort and in the SEARCH clinical trial of simvastatin treatment for two SNPs in linkage disequilibrium with the index SNP (rs1719247 and rs1346268, $r^2 > 0.7$) that were genotyped in each of these groups. We have extended our meta-analysis to include the study data reported in the accompanying Comments by Carr *et al.*[2] and Floyd *et al.*[3], two studies that individually failed to replicate this association.

The original analysis was performed on data from patients who were not on fibrates in the Marshfield and SEARCH populations. This was done to mitigate the risk that a possible modest protective effect of the SNPs would be masked by the known pharmacokinetic confounding caused by concomitant use of fibrates or other drugs that promote myopathy by altering statin pharmacokinetics[4]. We have done the same for the results of Floyd *et al.* in the meta-analyses presented below, although it is notable that, based on clinical presentation and creatine kinase levels, the majority of the myopathy cases of Floyd *et al.* were considerably more severe than in the originally reported cohorts[5]. This analytical approach was not possible for the study of Carr *et al.*, since data for this subgroup were not provided. In this regard we note that because pharmacokinetic effects are major determinants of statin toxicity, the confirmation by both Carr *et al.* and Floyd *et al.* of an association of myopathy with a functional variant of the transporter gene *SLC01B1* is not representative of the power of their analyses to detect a SNP association with a modest pharmacodynamic effect.

A fixed-effects meta-analysis yielded the following *P* values: rs9806699 (Marshfield, Carr *et al.* and Floyd *et al.*), $P = 0.085$; rs1719247 (Marshfield, SEARCH and Floyd *et al.*), $P = 0.0042$; rs1346268 (Marshfield, SEARCH and Floyd *et al.*), $P = 0.0035$. Thus, the statistical significance of the initially reported association is weakened but not eliminated by the inclusion of the additional cohorts. Future efforts to replicate these findings should give consideration to heterogeneity of patient characteristics, matching of statin exposure in cases and controls, avoidance of concomitant drug use and other confounding factors, and the statistical power to detect an association of modest effect size. We agree with Carr *et al.* that the association should be assessed in more patients and hope that a larger meta-analysis will be performed. In addition, further studies will be required to determine a mechanistic basis for a contribution of *GATM* genetic variation to the risk of statin-related myopathy. This Reply is written by the subset of authors that designed and led these analyses.

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