Short Communication

The impact of common genetic variants in the mitochondrial glycine cleavage system on relevant metabolites

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1. Introduction

The glycine cleavage system (GCS) is a crucial component of mitochondrial one-carbon metabolism (OCM). The GCS involves four distinct enzymes: glycine decarboxylase (GLDC), aminomethyltransferase (AMT), glycine cleavage system protein H (GCSH) and dihydropyrimidinase dehydrogenase (DLD). These enzymes act sequentially in the mitochondrion to cleave glycine obtained from the cytoplasm or from the breakdown of serine in the mitochondrion to produce N,N-methylene tetrahydrofolate (CH₂-THF), an active folate derivative that contributes to the transfer of one-carbon units in cellular reactions [1].

Loss-of-function mutations in two GCS genes (GLDC and AMT) cause non-ketotic hyperglycinemia (NKH), a rare recessive disease. Additionally, two NKH patients have been observed to have a complex rearrangement of GCSH [3]. Affected patients suffer from neurological impairments, seizures, and developmental delay, suggesting that the GCS is important for normal brain development and function [4]. In a study population of patients from the UK, Sweden and Japan, two of the genes encoding GCS enzymes, AMT and GLDC, were found to have missense variants associated with neural tube defects (NTDs) [5]. Similarly, variants in these genes were identified in a study of American patients with myelomeningoceles, a type of NTD [6]. Finally, GLDC rs17474391 (p.Ser951Tyr) has been observed in a patient with NKH [2] and a patient with anencephaly, a type of NTD [7].

Although genome-wide association studies (GWASs) can identify gene variants that influence traits, they are often underpowered to detect signals [8] from real but small effects of common alleles, or large effects of rare alleles. GWASs have been carried out for serum glycine, identifying a single nucleotide polymorphism (SNP) in carbamoyl-phosphate synthase 1 (rs715) [9–12]. Blood serine has been associated with a SNP in phosphoglycerate dehydrogenase (rs477992) [9, 12, 13]. To test for other genetic modifiers of OCM that may have been missed by the GWAS method, we examined whether common variants in the genes of the GCS influence relevant metabolites in a healthy population.

2. Materials and methods

2.1. The Trinity Student Study (TSS) cohort

The TSS cohort comprises a population of 2232 healthy Irish students, as described [14–16]. Informed consent and ethical approval were obtained from all participants. Briefly, blood samples were collected into EDTA and clotting tubes, processed within 3 h, and stored at −80 °C before assaying. Glycine (interassay CV = 3.3%) and serine (interassay CV = 5.7%) in serum were measured with gas-chromatography tandem mass spectrometry (GC–MS/MS) and plasma dimethylglycine (interassay CV < 10%) by liquid-chromatography tandem mass spectrometry (LC-MS/MS) by the laboratory of Bevital, Norway (www.bevital.no). Formate was measured using a
newly developed GC–MS method [19]. A single control sample was frozen in aliquots for daily analysis with formate assays of participant serum samples. The day-to-day variation for this sample was 7.4% (n = 38). Genome-wide SNP genotyping was conducted at the Center for Inherited Disease Research (CIDR, USA) using Illumina 1 M HumanOmni1-Quad_v1.0_B chips.

2.2. Metabolite association analyses

Conservative (r² < 0.9) tagging variants in the four GCS genes including 10 kb flanking regions were selected to test for association with levels of serum glycine, plasma dimethylglycine, serum serine, and serum formate. Log_{10} transformations of metabolite concentrations were used to meet normality assumptions. Linkage disequilibrium (LD) analyses were performed with Haploview; a total of 52 tag SNPs covering 93 variants were selected for association testing [20, 21]. For each SNP, association was tested using linear regression with a 1-df test (genotypic model) or a 2-df test (additive genetic model) (R statistical software). The 52 tag SNPs in the four glycine cleavage genes (GLDC, AMT, GCSH, and DLD) were tested for association with blood levels of glycine, dimethylglycine, formate, and serine in 2232 young healthy Irish adults (Table 1). Serum glycine concentrations were significantly correlated with all the other metabolites measured. The strength of these associations varied from r = 0.52; p < .001 with serine to r = 0.07; p = .004 with formate. Only one GCS SNP is significantly associated with a metabolite. GLDC rs2297442 returns a p-value of 0.0007 following 1-df association testing with log_{10} transformed glycine concentration. The 2-df test of association is also significant (p = .0031), nominally.

We then sought to replicate the association between rs2297442-G and decreased levels of circulating glycine by examining results from two recent GWASs in which glycine was measured. Our observation was replicated in two studies of Finnish individuals (n = 16,506, β = −0.039, p = .0014 [24]; n = 8545, β = −0.039, p = .028 [25]).

We also asked if GLDC mRNA expression levels are influenced by rs2297442 genotype. A Gene-Tissue Expression (GTEx) query of rs2297442 did not reveal a strong or consistent effect on GLDC mRNA levels across multiple tissues. In contrast, several other GLDC SNPs strongly influenced GLDC mRNA levels in several tissues (p < 1 × 10^{-46}). For example, in thyroid tissue, GLDC rs35374927 is estimated to have a large impact on GLDC mRNA levels compared to GLDC rs2297442 (Fig. 1). These eQTLs of strong effect reside in LD blocks near the 5’ end of the gene, as opposed to the SNPs in the LD block at the 3’ end of the gene where rs2297442 is located.

2.3. GLDC expression association analyses

The public Gene-Tissue Expression resource (https://www.gtexportal.org/home/) was used to search for associations between SNPs and GLDC mRNA levels (mRNA expression quantitative trait locus, eQTLs).

3. Results

The 52 tag SNPs in the four glycine cleavage genes (GLDC, AMT, GCSH, and DLD) were tested for association with blood levels of glycine, dimethylglycine, formate, and serine in 2232 young healthy Irish adults (Table 1). Serum glycine concentrations were significantly correlated with all the other metabolites measured. The strength of these associations varied from r = 0.52; p < .001 with serine to r = 0.07; p = .004 with formate. Only one GCS SNP is significantly associated with a metabolite. GLDC rs2297442 returns a p-value of 0.0007 following 1-df association testing with log_{10} transformed glycine concentration. The 2-df test of association is also significant (p = .0031), nominally.

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4. Discussion

The results of these association tests confirm that variation in GLDC can influence serum glycine concentrations. The variant reported here, GLDC rs2297442, is a noncoding polymorphism found in the seventh of the ten introns in this gene. The GTEx data revealed that other, unlinked SNPs in the gene have a larger impact on GLDC mRNA levels, albeit in limited, specific tissues. We conclude that rs2297442 does not influence serum glycine levels via modulation of GLDC transcript levels. It is possible that rs2297442 is simply linked to the causal SNP. A search for a linked, exonic SNP in a population of European ancestry [26] failed to identify any strongly related variants (r² < 0.2).

These results are consistent with the presence of a common SNP in GLDC that may increase the activity or alter the tissue distributions of this enzyme, causing reduced levels of circulating glycine. We predict that this effect on serum glycine concentrations may not be clinically relevant in this healthy population; however, it may contribute to diseases with a complex mode of inheritance, where combinations of many environmental and genetic factors of small individual effect must converge. The known link between folic acid supplementation and neural tube defect (NTD) prevention implicates perturbations of OCM as a key risk factor. In a study of 258 NTD patients, 27 single-base substitutions were discovered in GLDC, six of which influenced enzyme activity in an in vitro system [5]. The association between GLDC rs2297442 and serum glycine levels in the healthy populations in this study and others identifies a candidate locus for studies investigating the genetic basis of NKH, NTDs or any pathophysiology involving OCM.

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Data statement

The data upon which these analyses are based have been deposited in and are available from the dbGaP database under dbGaP accession phs000789.v1.p1.
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Conflict of interest statement
The authors have no conflicts of interest to declare.

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