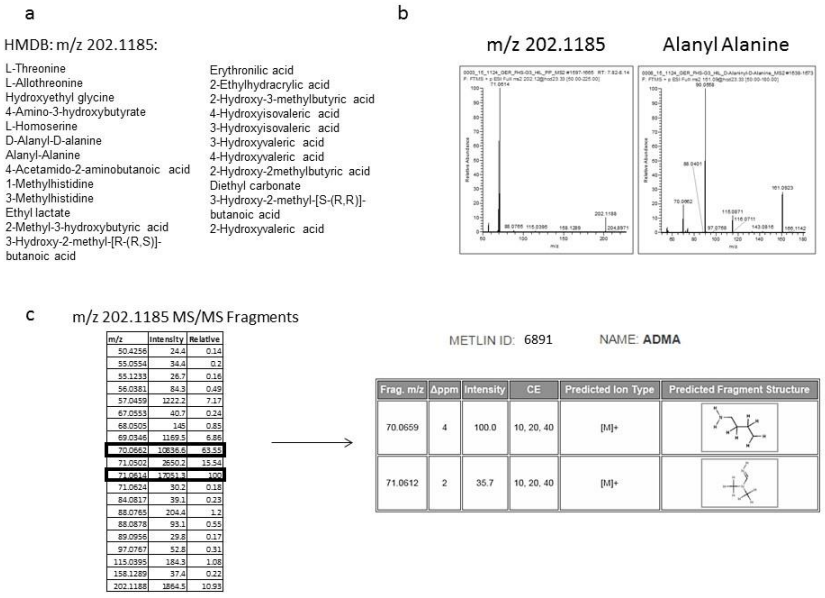
SUPPLEMENTAL DATA

Supplemental Figures with Legends

Figure S1. Database matching and representative MS/MS of putative initial ID.

Related to Figure 2.



a. We searched the Human Metabolite Database (HMDB) for candidate metabolites with a mass/charge (m/z) ratio of 202.1185, with a 5 parts-per-million (ppm) margin of error, and received 24 potential matches. **b.** Representative MS/MS spectra of m/z 202.1185 alongside MS/MS spectra of alanyl-alanine standard. **c.** The two dominant fragments in the spectra were used to search the METLIN metabolite database for metabolites containing these two fragments, and there was only one match within a 5-ppm tolerance window — ADMA.

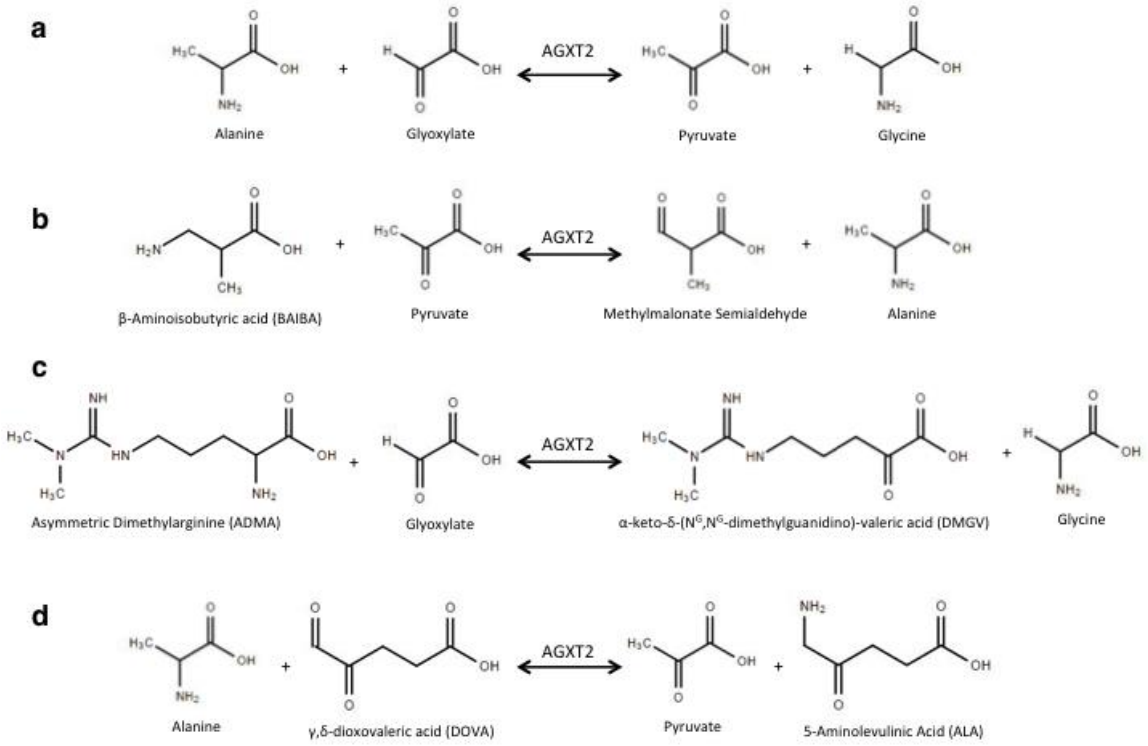
Figure S2. GWAS of all genome-wide significant SNPs for DMGV (Metabolite #5836). Related to Figure 1.

Locus	Trait	SNP	Ch	Major/Minor Allele	MAF	Beta	s.e.	P Value
AGXT2	5836	rs468327	5	G/A	0.11	0.40	0.07	3.79E-09
AGXT2	5836	rs28305	5	G/C	0.08	0.47	0.08	4.55E-09
AGXT2	5836	rs37370	5	T/C	0.07	0.46	0.08	1.36E-08
AGXT2	5836	rs37376	5	C/G	0.08	0.46	0.08	1.51E-08
AGXT2	5836	rs37377	5	T/G	0.08	0.46	0.08	1.53E-08
AGXT2	5836	rs40200	5	G/A	0.08	0.46	0.08	1.55E-08
AGXT2	5836	rs37379	5	G/A	0.08	0.46	0.08	1.55E-08
AGXT2	5836	rs37369	5	C/T	0.05	0.54	0.10	1.60E-08
AGXT2	5836	rs37371	5	G/A	0.08	0.46	0.08	1.61E-08
AGXT2	5836	rs40199	5	A/G	0.07	0.47	0.09	2.96E-08

SNP = single nucleotide polymorphism, Ch= chromosome, MAF = minor allele frequency, s.e. = standard error.

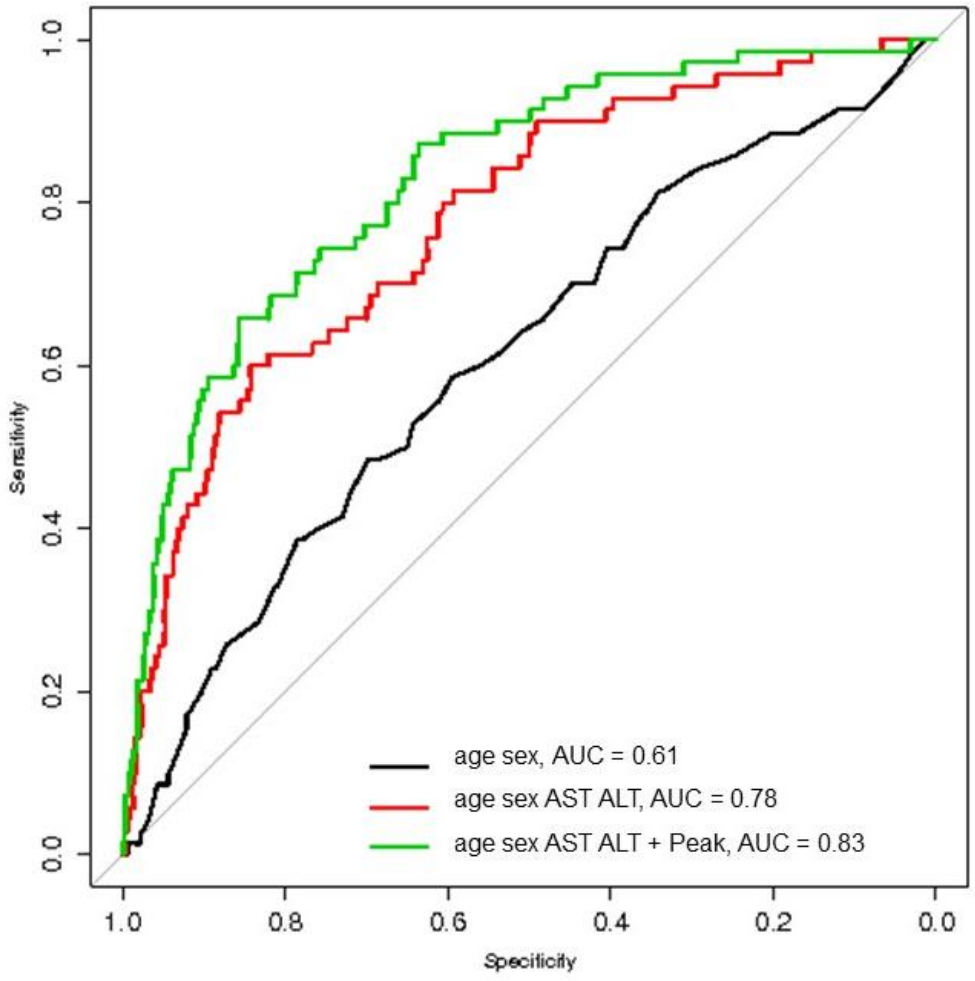
GWAS of all significant genome-wide association SNPs for DMGV (Metabolite #5836). The gene locus, SNP, chromosomal location, major/minor alleles, minor allele frequency, beta coefficient, standard error, and p-values are provided for each metabolite.

Figure S3. AGXT2 Enzymatic Reactions. Related to Figure 2.



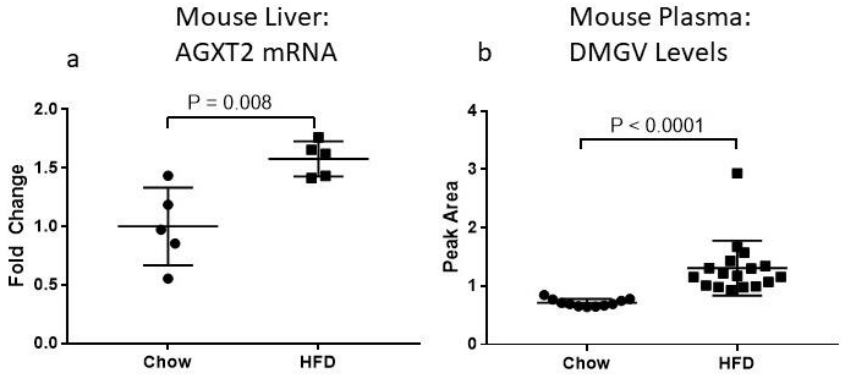
a. The reaction from which alanine-glyoxylate aminotransferase 2 (AGXT2) derives its name, in which it catalyzes the transamination of alanine and glyoxylate to form pyruvate and glycine. AGXT2 also catalyzes transamination of: β-aminoisobutyric acid (BAIBA) and pyruvate to form methylmalonate semialdehyde and alanine (**b**); asymmetric dimethylarginine (ADMA) and glyoxylate to form α-keto-δ-(N^G,N^G-dimethylguanidino)-valeric acid (DMGV) and glycine (**c**); and alanine and γ,δ-dioxovaleric acid (DOVA) to form pyruvate and 5-Aminolevulinic Acid (ALA) (**d**).

New Supplemental Figure S4. Fatty liver discrimination.



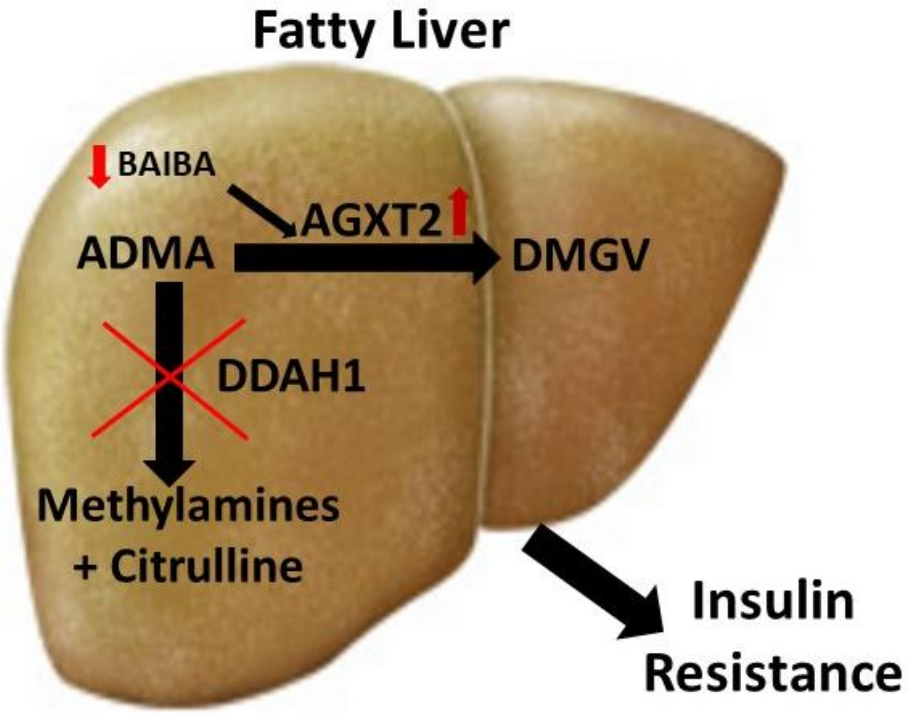
Receiver-operator characteristic curves (ROCs) of ALT + AST (red line) vs. ALT + AST + metabolite Peak (DMGV) (green line) showing a clear improvement in discrimination for liver fat of DMGV on top of AST and ALT (P = 0.014).

New Supplemental Figure S5. AGXT2 Expression and Plasma DMGV Levels.



a. AGXT2 mRNA expression levels are significantly higher in the livers of 14-week-old mice fed a high-fat diet (with confirmed liver fat) for 6 weeks compared to chow-fed mice (with no liver fat) (Mann-Whitney, n= 5 per group, 1±0.15 vs. 1.58±0.07 a.u., P = 0.008). **b.** Plasma DMGV levels in high-fat diet mice (with confirmed liver fat) were significantly higher than chow mice (with no liver fat) (Mann-Whitney, n = 11 chow mice, n= 17 HFD mice, 0.72±0.07 a.u. vs 1.31±0.47 a.u., P < 0.0001).

New Supplemental Figure S6. Schematic of working model.



Schematic illustrating working model: decreased DDAH1 enzymatic and decreased BAIBA levels in fatty liver disease lead to an increased concentration of ADMA that binds AGXT2 more freely (due to decreased levels of BAIBA, the major substrate of AGXT2) and thereby leads to increased conversion to DMGV by AGXT2. Furthermore, (as per our mouse liver AGXT2 mRNA expression data), there is increased AGXT2 expression, which could result in accelerated conversion of increased ADMA resulting in greater levels of DMGV.