We investigated the association of glycemia and 43 genetic risk variants for hyperglycemia/type 2 diabetes with amino acid levels in the population-based Metabolic Syndrome in Men (METSIM) Study, including 9,369 nondiabetic or newly diagnosed type 2 diabetic Finnish men. Plasma levels of eight amino acids were measured with proton nuclear magnetic resonance spectroscopy. Increasing fasting and 2-h plasma glucose levels were associated with increasing levels of several amino acids and decreasing levels of histidine and glutamine. Alanine, leucine, isoleucine, tyrosine, and glutamine predicted incident type 2 diabetes in a 4.7-year follow-up of the METSIM Study, and their effects were largely mediated by insulin resistance (except for glutamine). We also found significant correlations between insulin sensitivity (Matsuda insulin sensitivity index) and mRNA expression of genes regulating amino acid metabolism (1). Insulin inhibits proteolysis and associated release of amino acids and stimulates amino acid uptake and protein synthesis in skeletal muscle (2,3). Selected amino acids, however, enhance insulin secretion (4,5) or modulate insulin sensitivity (6–10), the two main mechanisms in the regulation of glucose homeostasis.

A recent study reported that three branched-chain amino acids (BCAAs), valine, leucine and isoleucine, and two aromatic amino acids, phenylalanine and tyrosine, predicted type 2 diabetes (11). The risk of diabetes was fivefold higher in individuals in the top quartile of a combination of three amino acids (isoleucine, phenylalanine, and tyrosine) compared with individuals in the lowest quartile. Although some small studies have reported that the levels of amino acids differ between individuals with normal and abnormal glucose tolerance (12,13), previous studies have not investigated the levels of amino acids across the entire range of glucose tolerance.

Amino acids modulate insulin action on glucose transport (9,14–16) and gluconeogenesis (17). High levels of BCAAs, especially leucine, have been shown to associate with insulin resistance (16,18,19) or insulin-resistant states, including diabetes (6,12,13). BCAAs have been shown to downregulate insulin action on glucose uptake by inhibiting critical steps in the postreceptor insulin signaling cascade (10), although other studies have concluded that leucine and isoleucine stimulate glucose uptake (7,8,20). Gluconeogenic amino acids (mainly alanine and glutamine) can enhance hepatic glucose production and thus lead to hyperglycemia (21,22). Finally, amino acids such as arginine, glutamine, leucine, and phenylalanine directly stimulate insulin secretion (4). Type 2 diabetes is a complex metabolic disease with a significant genetic component, and >40 gene loci associated with the risk of type 2 diabetes or hyperglycemia have been identified (29–36). The mechanisms by which these loci contribute to the risk of diabetes are only partially known. There are no previous studies on the association of these genetic variants with the levels of amino acids.

The aims of our study were (1) to investigate the relationship between the levels of amino acids and fasting and 2-h glucose across the entire range of glucose tolerance, (2) to investigate the role of insulin sensitivity and insulin secretion in this relationship, (3) to investigate the relationship between insulin sensitivity and adipose tissue...
mRNA expression of genes implicated in the catabolism of amino acids, and 4) to investigate whether any of 43 risk single nucleotide polymorphisms (SNPs) for type 2 diabetes or hyperglycemia affect serum amino acid levels.

**RESULTS**

**Gene expression analysis.**

Overall, most amino acids increased, whereas glutamine and histidine (P< 0.003) increased in isoleucine (P= 1.8×10⁻¹⁵), alanine (+17%, P= 4.3×10⁻⁴⁵), phenylalanine (+15%, P= 1.1×10⁻¹⁰), and leucine (+19%, P= 4.2×10⁻⁴⁰) levels, and a significant decrease in histidine (-9%, P= 3.3×10⁻⁴) and glutamine (-22%, P= 1.9×10⁻³⁰; Fig. 1A). Similar trends were seen across the 2hPG categories, with a significant increase in isoleucine (+38%, P= 2.7×10⁻⁵⁸), tyrosine (+30%, P= 1.1×10⁻¹⁴), alanine (+14%, P= 2.3×10⁻⁴⁵), phenylalanine (+14%, P= 1.1×10⁻¹⁵), and leucine (+19%, P= 1.0×10⁻¹⁵) levels, and a significant decrease in histidine (-5%, P= 9.1×10⁻⁴) and glutamine (-12%, P= 1.4×10⁻³⁵) levels with higher 2hPG (Fig. 1B). These effects were more significant in obese (BMI ≥27 kg/m²) than in nonobese (BMI<27 kg/m²) participants, although the trends were similar in both groups (Supplementary Fig. 2).

Overall, most amino acids increased, whereas glutamine and histidine decreased with higher FPG and 2hPG.

**Gene expression analysis.**

Table 1 shows that all amino acids that were increased in hyperglycemia negatively correlated with Matsuda ISI (r ≤−0.3, P ≤ 1×10⁻¹⁵) and positively (r = 0.2, P = 3×10⁻⁸⁵) with the InsAUC₀–⁹⁰/GluAUC₀–⁹⁰ index of early-phase insulin secretion. For glutamine, which was decreased in hyperglycemia, the correlations were weaker and in the opposite direction (r = 0.17 for Matsuda ISI and −0.12 for InsAUC₀–⁹⁰/GluAUC₀–⁹⁰). Correlations of amino acid levels (except for histidine) with InsAUC₀–⁹⁰/GluAUC₀–⁹⁰ were weaker than correlations with Matsuda ISI and were largely attenuated after the adjustment of InsAUC₀–⁹⁰/GluAUC₀–⁹⁰ for Matsuda ISI. Furthermore, additional adjustment for Matsuda ISI attenuated or abolished most of the associations between glucose categories and amino acid levels (with the exception of valine and histidine), whereas adjustment for InsAUC₀–⁹⁰/GluAUC₀–⁹⁰ attenuated only the associations for histidine (Supplementary Table 2). Thus, insulin sensitivity seemed to at least partly explain the relationship between glucose and amino acid levels.
Association of eight amino acid levels with risk of type 2 diabetes. We investigated the relationship between amino acid levels and the development of type 2 diabetes during a 4.7-year follow-up, including 526 re-examined METSIM participants (375 with NGT at baseline and follow-up examinations, and 151 with type 2 diabetes). Of eight amino acids measured at baseline, high levels of alanine ($P = 6.7 \times 10^{-5}$), leucine ($P = 0.005$), isoleucine ($P = 0.006$), phenylalanine ($P = 0.009$), tyrosine ($P = 0.045$), valine ($P = 0.025$), histidine ($P = 0.025$), and glutamine ($P = 10^{-5}$) were associated with the development of type 2 diabetes.

FIG. 1. Amino acid levels in categories of FPG (A) and 2hPG (B) levels across the entire range of glucose tolerance. Points represent unadjusted means of amino acid levels in each glucose category. $P$ values were calculated using general linear model adjusted for age and BMI. *$P < 0.05$. **$P < 10^{-10}$. ***$P < 10^{-30}$.

TABLE 1 Pearson correlations between the levels of eight amino acids and indices of insulin sensitivity (Matsuda ISI) and early-phase insulin secretion (InsAUC$_{0-30}$/GluAUC$_{0-30}$) in nondiabetic METSIM participants

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Matsuda ISI</th>
<th>InsAUC$<em>{0-30}$/GluAUC$</em>{0-30}$</th>
<th>InsAUC$<em>{0-30}$/GluAUC$</em>{0-30}$ adjusted for ISI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N$</td>
<td>$r$</td>
<td>$P$</td>
</tr>
<tr>
<td>Alanine</td>
<td>8,672</td>
<td>−0.379</td>
<td>1E-294</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>8,661</td>
<td>−0.340</td>
<td>1E-232</td>
</tr>
<tr>
<td>Leucine</td>
<td>8,661</td>
<td>−0.279</td>
<td>1E-154</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>8,666</td>
<td>−0.485</td>
<td>&lt;1E-294</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>8,582</td>
<td>−0.400</td>
<td>&lt;1E-294</td>
</tr>
<tr>
<td>Valine</td>
<td>8,661</td>
<td>−0.296</td>
<td>2E-174</td>
</tr>
<tr>
<td>Histidine</td>
<td>8,672</td>
<td>−0.024</td>
<td>0.026</td>
</tr>
<tr>
<td>Glutamine</td>
<td>7,871</td>
<td>0.172</td>
<td>2E-53</td>
</tr>
</tbody>
</table>
HYPERGLYCEMIA, SNPS, AND AMINO ACIDS

\[ (P = 3.3 \times 10^{-5}) \], tyrosine \((P = 0.001)\), and phenylalanine \((P = 0.048)\) were significantly associated with incident diabetes (adjusted for age and BMI; Table 2). Additional adjustment for Matsuda ISI alone, or Matsuda ISI and InsAUC_{0-30/GluAUC_{0-30}}, but not for InsAUC_{0-30/GluAUC_{0-30}} alone, abolished statistical significance, suggesting that the association of these amino acids with incident diabetes was mostly explained by insulin resistance. Further adjustment for fasting hyperglycemia did not essentially change the association, but adjustment for 2-h glucose made the association for alanine statistically significant \((P = 0.027)\). The elevated baseline glutamine level was significantly associated with a decreased risk of newly developed type 2 diabetes \((P = 4.1 \times 10^{-5})\), and this association persisted after the adjustment for Matsuda ISI \((P = 1.5 \times 10^{-3})\) or InsAUC_{0-30/GluAUC_{0-30}} \((P = 6.7 \times 10^{-5})\) or both \((P = 0.048)\). Additional adjustment for fasting glucose abolished this association \((P = 0.051)\). The levels of glutamine were significantly lower at baseline in 440 participants who developed abnormal glucose tolerance (IGT and/or IFG, diabetes) at follow-up, compared with 575 participants who remained normoglycemic \((155.6 \pm 159.6; \ P = 0.019)\), adjusted for age and BMI (Supplementary Table 3). We also performed statistical analyses in a larger sample, including all 1,624 re-examined participants who did not develop diabetes during the follow-up and 151 participants who developed type 2 diabetes. The results remained essentially similar, although \(P\) values were less significant.

Gene expression of genes involved in amino acid metabolism in relation to insulin sensitivity. Analysis of microarray data from subcutaneous adipose tissue samples of 200 METSIM participants showed that Matsuda ISI correlated significantly with mRNA levels of several genes involved in the metabolism of alanine, including a key enzyme, alanine aminotransferase \((r = 0.46, \text{FDR} = 9.7 \times 10^{-3})\); glutamine, including a key enzyme, glutamine synthetase \((r = 0.44, \text{FDR} = 4.0 \times 10^{-3})\); BCAA, including key enzymes of BCAA degradation, branched chain amino-acid transaminase 2 \((r = 0.36, \text{FDR} = 2.5 \times 10^{-3})\) and branched-chain \(\alpha\)-keto acid dehydrogenase \((\text{BCKDH})\ \text{A and B} \ (r = 0.45 \text{ and } 0.35, \text{FDR} = 1.3 \times 10^{-3} \text{ and } 3.0 \times 10^{-3})\); and phenylalanine, tyrosine, and histidine (Supplementary Table 4). The most consistent results were observed for the BCAA degradation, as enzyme-mRNAs correlated positively with Matsuda ISI at almost all steps of the metabolic pathway (Supplementary Fig. 3). Figure 2 shows that the mRNA levels of three key enzymes in BCAA degradation also correlated negatively with ISI, FPG, and 2hFP levels. However, these associations disappeared when controlled for Matsuda ISI. These results indicate that the association between insulin sensitivity and amino acid catabolism (especially BCAA catabolism) also exists at the mRNA level and add further evidence that insulin sensitivity is likely contributing to higher BCAA levels in hyperglycemia.

Risk variants for type 2 diabetes and/or hyperglycemia and the levels of eight amino acids. Of the 43 SNPs investigated, only GCKR rs780094 was significantly associated with the levels of several amino acids after Bonferroni correction for multiple testing \((P < 1.45 \times 10^{-4})\) (Fig. 3 and Supplementary Table 1). The glucose-increasing C (major) allele was associated with lower levels of alanine (effect size \(-1.9\% \text{ per C allele, } P = 1.6 \times 10^{-11}\) and isoleucine \((-2.3\%, \text{FDR} = 3.1 \times 10^{-6})\) and higher levels of glutamine \((+1.2\%, \text{FDR} = 1.0 \times 10^{-6})\) adjusted for age and BMI). The C allele also had nominally significant effects on leucine \((-1.2\%, \text{FDR} = 0.001)\), tyrosine \((+1.1\%, \text{FDR} = 0.001)\), and histidine \((+0.8\%, \text{FDR} = 0.003)\). These associations did not change significantly after additional adjustment for FPG, 2hPG, or Matsuda ISI (data not shown), traits known to be modulated by SNPs of GCKR. Furthermore, the association of amino acids with newly developed type 2 diabetes was not affected by further adjustment for rs780094.

A number of nominally significant associations of other SNPs with amino acids were found; the top ranking was rs8042680 (PRC1). The type 2 diabetes risk allele (A) was nominally associated with higher levels of leucine \((+1.2\%, \text{FDR} = 0.002)\), isoleucine \((-1.7\%, \text{FDR} = 0.001)\), and valine \((-1.2\%, \text{FDR} = 0.001)\) (Supplementary Table 1). The most consistent nominally significant associations were seen for rs75789326 \((IRS1)\). The nonrisk allele (G) was associated with lower leucine \((-0.8\%, \text{FDR} = 0.019)\), isoleucine \((-1.5\%, \text{FDR} = 0.003)\), tyrosine \((-0.7\%, \text{FDR} = 0.037)\), valine \((-0.7\%, \text{FDR} = 0.047)\), and histidine \((-0.5\%, \text{FDR} = 0.039)\) levels.

### DISCUSSION

This is the first large, population-based study aiming to investigate the relationship between hyperglycemia and 43 risk SNPs for type 2 diabetes/hyperglycemia and amino acid levels. We observed that with increasing FPG and/or 2hPG, the levels of alanine, valine, leucine, isoleucine, phenylalanine, and tyrosine increased, whereas the levels of histidine and glutamine decreased. Significant correlations between

### TABLE 2

Association between the baseline levels of eight amino acids and newly developed type 2 diabetes during the follow-up of the METSIM study participants (logistic regression adjusted for age, BMI, and additional covariates)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Odds Ratio (95% CI)</th>
<th>(P^a)</th>
<th>(P^b)</th>
<th>(P^c)</th>
<th>(P^d)</th>
<th>(P^e)</th>
<th>(P^f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>1.024 (1.012–1.037)</td>
<td>6.7E-05</td>
<td>0.154</td>
<td>6.0E-07</td>
<td>0.895</td>
<td>0.191</td>
<td>0.027</td>
</tr>
<tr>
<td>Glutamine</td>
<td>0.973 (0.962–0.985)</td>
<td>4.1E-06</td>
<td>1.5E-04</td>
<td>6.7E-06</td>
<td>0.048</td>
<td>0.051</td>
<td>0.007</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.958 (0.885–1.038)</td>
<td>0.296</td>
<td>0.132</td>
<td>0.409</td>
<td>0.187</td>
<td>0.794</td>
<td>0.127</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.101 (1.052–1.152)</td>
<td>3.3E-05</td>
<td>0.293</td>
<td>5.5E-08</td>
<td>0.649</td>
<td>0.013</td>
<td>0.064</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.047 (1.014–1.081)</td>
<td>0.065</td>
<td>0.246</td>
<td>0.002</td>
<td>0.379</td>
<td>0.253</td>
<td>0.197</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.062 (1.001–1.128)</td>
<td>0.048</td>
<td>0.879</td>
<td>0.003</td>
<td>0.292</td>
<td>0.146</td>
<td>0.630</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.118 (1.048–1.183)</td>
<td>0.001</td>
<td>0.466</td>
<td>1.2E-05</td>
<td>0.528</td>
<td>0.142</td>
<td>0.139</td>
</tr>
<tr>
<td>Valine</td>
<td>1.013 (0.955–1.071)</td>
<td>0.149</td>
<td>0.657</td>
<td>0.050</td>
<td>0.264</td>
<td>0.408</td>
<td>0.940</td>
</tr>
</tbody>
</table>

Subjects with newly diagnosed type 2 diabetes at follow-up \((N = 151)\) were compared against those with NGT/NFG at the baseline and follow-up examinations \((N = 375)\). Bold type indicates statistical significance. *Adjustment for age and BMI. †Adjustment for age, BMI, and Matsuda ISI. ‡Adjustment for age, BMI, and InsAUC_{0-30/GluAUC_{0-30}}. §Adjustment for age, BMI, InsAUC_{0-30/GluAUC_{0-30}} and Matsuda ISI. ¶Adjustment for age, BMI, InsAUC_{0-30/GluAUC_{0-30}} Matsuda ISI, and FPG. #Adjustment for age, BMI, InsAUC_{0-30/GluAUC_{0-30}} Matsuda ISI, and 2hPG.
insulin sensitivity and mRNA expression of genes regulating amino acid metabolism were found. Only 1 SNP (rs780094 in \(GCKR\)) of 43 risk SNPs for type 2 diabetes or hyperglycemia was significantly associated with the levels of several amino acids.

**Hyperglycemia and levels of eight amino acids.** We demonstrated that the levels of alanine, phenylalanine, valine, leucine, isoleucine, and tyrosine increased and the levels of histidine and glutamine decreased in hyperglycemia, indicating that the changes in amino acid levels parallel closely the changes in FPG and 2hPG levels. Similar trends were also observed in normoglycemia, suggesting that even mild elevations of glucose levels result in changes in amino acid levels. The largest and most significant increase across the FPG and 2hPG categories was observed for isoleucine (increased up to 38 and 30%, respectively, compared with the reference category), and alanine (increased up to 17 and 14%), and the largest decrease was seen for glutamine (by 22 and 12%, respectively). We also showed that obesity did not have a major effect on these associations, although the effects of hyperglycemia on the levels of amino acids tended to be more pronounced in obese individuals.

The levels of all amino acids correlated inversely (except for glutamine correlating positively) with insulin sensitivity (Matsuda ISI). The strongest correlation was found for isoleucine \((r = -0.49)\), a BCAA previously shown to be a potent activator of glucose uptake in skeletal muscle \((7,8)\). Insulin resistance is likely to be an important mechanism explaining these associations because the adjustment for Matsuda ISI attenuated or abolished most of the associations between glucose (especially 2hPG) and amino acid levels. Further evidence for the role of insulin resistance mediating the associations of amino acids with hyperglycemia comes from our 4.7-year prospective follow-up of the METSIM cohort. We demonstrated that alanine, leucine, isoleucine, tyrosine, and glutamine were associated with incident type 2 diabetes. Adjustment for insulin sensitivity abolished significant associations, with the exception of glutamine, indicating that insulin resistance is likely to play an important role in the risk of type 2 diabetes induced by amino acids. Insulin secretion or hyperglycemia did not significantly modify these associations. Our study not only supports the recent observations of a relationship between amino acid levels with the risk of diabetes \((11)\) but also offers a possible underlying mechanism to explain this relationship.

Our study found that the levels of BCAAs (particularly isoleucine) were strongly correlated with mRNA levels of genes involved in BCAA catabolism. BCAAs are thought to be early indicators of insulin resistance \((6,10,18,35)\). High levels of BCAAs promote insulin resistance by interfering with the insulin-signaling pathway \((10)\) or by directly inhibiting muscle glucose transport and/or phosphorylation in skeletal muscle \((9)\), whereas insulin resistance in turn may contribute to high levels of amino acids by increased release of BCAAs (and other amino acids) due to impaired ability of insulin to suppress proteolysis in skeletal muscle \((36)\). BCAAs are oxidized in skeletal muscle and adipose tissue \((37)\) to form alanine and glutamine \((38)\). Oxidation of BCAAs was increased in individuals with type 2 diabetes \((21)\). In animal models of type 2 diabetes, BCAA catabolism is downregulated due to low activity of BCKDH complex \((39)\), the key enzyme of BCAA oxidation. In our study, adipose tissue mRNA expression of \(BCKDHA\)
**BCKDHB** (encoding α and β polypeptides of BCKDH E1) as well as of **BCAT2** (branched chain amino-acid transaminase 2 mitochondrial, the first enzyme in BCAA catabolism) and other genes involved in BCAA catabolism correlated positively with Matsuda ISI and negatively with glucose levels. This suggests that the insulin resistance-related decrease in degradation of BCAAs could be one of the mechanisms leading to the elevation of BCAA levels in hyperglycemia in humans. Other possible mechanisms for the elevation of BCAAs in hyperglycemia could be a decreased uptake and increased release of BCAAs from skeletal muscle due to increased protein catabolism in insulin resistance.

Alanine is a nonessential amino acid synthesized from pyruvate and amino acids (mainly BCAAs) primarily in skeletal muscle and gut and used for gluconeogenesis in the liver. Alanine is the main precursor for gluconeogenesis (38,40) and a stimulator of glucagon secretion (41). Therefore, high plasma levels of alanine may contribute especially to fasting hyperglycemia by enhancing gluconeogenesis. Our study shows that elevated levels of alanine in hyperglycemia, both in obese and nonobese individuals, could be mediated by insulin resistance, which is supported by a significant positive correlation between insulin sensitivity and mRNA level of alanine aminotransferase, the key enzyme in alanine metabolism.

Glutamine is a nonessential gluconeogenic amino acid synthesized from glucose or amino acids or released by proteolysis from skeletal muscle (42). Unlike alanine, glutamine is primarily taken up by the gut and kidneys, where it is processed to glucose via the gluconeogenesis pathway. Glucagon, but not insulin sensitivity, seems to play a role in the uptake of glutamine by the gut (43). Differential regulation of glutamine metabolism, compared with other amino acids, could possibly explain the opposite associations between glutamine and glucose metabolism. We found that plasma levels of glutamine decreased with elevated levels of FPG and 2hPG and that a low level of glutamine was significantly associated with future diabetes, independently of obesity and insulin resistance. Baseline glutamine levels were already decreased in normoglycemic participants who developed abnormal glucose tolerance at follow-up compared with those who remained normoglycemic. Our results are in agreement with previous studies showing that patients with type 2 diabetes (12) and lean insulin-resistant normoglycemic offspring of patients with type 2 diabetes have lower levels of glutamine than normoglycemic individuals (44).

**Risk variants for type 2 diabetes and/or hyperglycemia and the levels of eight amino acids.** A common variant in **GCKR** was the only SNP among 43 loci associated with the levels of several amino acids. **GCKR** encodes the glucokinase regulatory protein, which inhibits the effects of glucokinase (GCK) on glycogen synthesis and glycolysis in the liver (45). SNPs at the **GCKR** locus have been associated with fasting glycemia (24,46), risk of type 2 diabetes (46–48), insulin resistance (46–48), and elevated hepatic glucose uptake (49). In our study, the glucose-increasing major C allele of the intronic SNP rs780094 was significantly and unexpectedly associated with decreased levels of alanine and isoleucine and elevated levels of glutamine.
Mechanisms explaining the relationship of rs780094 of GCKR with amino acids are unknown, but the association with low levels of alanine could be a consequence of reduced glycolysis induced by GCKR resulting in decreased production of pyruvate and its conversion to alanine. On the basis of our study, it remains unclear whether the effects of rs780094 of GCKR on amino acid metabolism are primary or secondary. Although in our study the C allele of rs780094 was associated with both FPG and 2hPG, as well as with Matsuda ISI, the association of GCKR with the levels of amino acids was independent of these variables (data not shown). The GCKR variant did not affect the association of amino acids with newly developed type 2 diabetes, which agrees with its modest effect on amino acid levels.

Although several type 2 diabetes/hyperglycemia risk SNPs showed nominally significant associations with the levels of selected amino acids, the most consistent results were found for rs7578362 near IRS1. The major A major allele previously linked to type 2 diabetes risk in a genome-wide association study (26) was nominally associated with elevated levels of several amino acids (valine, leucine, isoleucine, tyrosine, histidine). IRS1 encodes the insulin receptor substrate 1, an important component of the insulin-signaling pathway, and its gene variant affects insulin resistance and adiposity (50). The association of IRS1 with amino acid levels, if confirmed, could contribute to evidence for a causal relationship between insulin resistance and amino acid metabolism. The type 2 diabetes risk allele of the rs8042680 in PRC1, encoding protein regulator of cytokinesis 1, was associated specifically but nominally with higher levels of all three BCAAs. PRC1 is not known to have a function in amino acid metabolism. These results suggest that the relationship between the levels of glucose and amino acids could be determined, at least in part, by genes regulating hyperglycemia or the risk of diabetes.

This study has limitations. Only Finnish men were included, and therefore, we do not know whether our results are applicable to women and to different ethnic or racial groups. We had only a modest statistical power to demonstrate statistically significant associations of gene variants with amino acids.

In conclusion, our large, population-based study shows that levels of branched-chain, aromatic amino acids and alanine increase whereas glutamine and histidine decrease with increasing FPG and/or 2hPG levels. These associations seemed to be mediated by insulin resistance, at least in part, supported by correlations of expression of genes involved in amino acid metabolism with the Matsuda ISI. Among the 43 loci associated with risk for hyperglycemia or type 2 diabetes, only GCKR rs780094 was significantly associated with several amino acids, especially with the levels of alanine.

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A.S. wrote the manuscript and researched the data. M.C., N.K.S., P.P., and A.J.L. performed the mRNA experiments, analyzed the data, and reviewed and edited the manuscript. P.S. conceived, designed, and performed the NMR experiments, analyzed the data, and reviewed and edited the manuscript. A.J.K. analyzed the NMR data, contributed analysis tools, and reviewed and edited the manuscript. H.C., J.P., and J.P. researched the data and reviewed and edited the manuscript. L.L.B., M.A.M., and F.S.C. designed and performed genotyping and reviewed and edited the manuscript. M.B. contributed analysis tools and reviewed and edited the manuscript. J.K. designed the study and reviewed the manuscript. M.A.-K. conceived and designed the NMR experiments, analyzed the data, and reviewed and edited the manuscript. M.L. designed the study, contributed to discussion, and reviewed and edited the manuscript. M.L. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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