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Metabolic Heritability at Birth: Implications for Chronic Disease Research

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Abstract

Recent genome-wide association studies of the adult human metabolome have identified genetic variants associated with relative levels of several acylcarnitines, which are important clinical correlates for chronic conditions such as type 2 diabetes and obesity. We have previously shown that these same metabolite levels are highly heritable at birth; however, no studies to our knowledge have examined genetic associations with these metabolites measured at birth. Here, we examine, in 743 newborns, 58 single nucleotide polymorphisms (SNPs) in 11 candidate genes previously associated with differing relative levels of short-chain acylcarnitines in adults. Six SNPs (rs2066938, rs3916, rs3794215, rs555404, rs558314, rs1799958) in the short chain acyl-CoA dehydrogenase gene (*ACADS*) were associated with neonatal C4 levels. Most significant was the G allele of rs2066938, which was associated with significantly higher levels of C4 ($P=1.5\times10^{-29}$). This SNP explains 25% of the variation in neonatal C4 levels, which is similar to the variation previously reported in adult C4 levels. There were also significant ($P<1\times10^{-4}$) associations between neonatal levels of C5-OH and SNPs in the solute carrier family 22 genes (*SLC22A4* and *SLC22A5*) and the 3-methylcrotonyl-CoA carboxylase 1 gene (*MCCC1*). We have replicated, in newborns, SNP associations between metabolic traits and the *ACADS* and *SLC22A4* genes observed in adults. This research has important implications not only for the identification of rare inborn errors of metabolism but also for personalized medicine and early detection of later life risks for chronic conditions.

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Supplementary Data: Two tables of additional results are included in the supplementary data word document files.

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Keywords

short chain acylcarnitines; chronic disease; newborn screening; metabolic heritability

INTRODUCTION

Recent genome-wide association studies of the adult human metabolome have revealed strong genetic associations with normal variation in many metabolites involved in β oxidation(Gieger *et al*, 2008; Illig *et al*, 2010; Suhre *et al*, 2011). Unsurprisingly, these common single nucleotide polymorphisms (SNPs) reside in genes where rare variants cause rare fatty acid oxidation disorders that are routinely detected by newborn screening programs(Pasquali *et al*, 2006). While extreme abnormalities in β oxidation result in rare conditions such as shortchain or medium chain acylcarnitine dehydrogenase deficiency, less severe disruptions in β oxidation are implicated in a host of adult chronic conditions such as type 2 diabetes mellitus, obesity, cancer, ulcerative colitis and neurodegenerative diseases(Bene *et al*, 2013; De Preter *et al*, 2012; Kelley *et al*, 1999; Lowe and Bain, 2013; Schooneman *et al*, 2013; Yeh *et al*, 2006).

The heritability of metabolites in β oxidation, specifically short, medium and long chain acylcarnitines has been investigated with findings of high heritability, particularly for short-chain acylcarnitines in adults(Shah *et al*, 2009). We replicated these findings in neonates showing genetic heritabilitybetween 44-66% for the short-chain acylcarnitines C2, C3, C4 and C5(Alul *et al*, 2013). Investigating genetic regulators of these metabolites at one of the earliest time points possible, i.e. birth, is of paramount importance for personalized medicine and preventative strategies targeted to those at the greatest risk of developing chronic metabolic conditions later in life.

It is also well established that preterm infants are more likely to exhibit elevations in the metabolites measured during newborn screening and are more likely to have a false positive newborn screen for various β oxidation disorders(Atzori *et al*, 2011; Ryckman *et al*, 2013). While these metabolic abnormalities in preterm infants are often dismissed as a consequence of developmental immaturity or total parenteral nutrition, there is compelling evidence suggesting that individuals born preterm have permanent vascular and metabolic disturbances that track through childhood and adulthood (Clark *et al*, 2007; Kelleher *et al*, 2008; Lewandowski *et al*, 2013; Mathai *et al*, 2013; Parkinson *et al*, 2013).A current hypothesis is that the substantive burden of risk for later life chronic disease in low birth weight and preterm infants is due to accelerated postnatal catch-up growth (Eriksson *et al*, 2000a; Eriksson *et al*, 2000b; Lapillonne and Griffin, 2013).However, this phenomenon alone, does not explain all of the increased risk in those infants and in fact, some of this burden of risk may be a result of metabolic‘disturbances’present at birth(Lapillonne and Griffin, 2013). Common genetic polymorphisms could enhance these metabolic disturbances in that some infants may have polymorphisms that result in higher (or lower) levels of a given metabolite and in a preterm infant these perturbations may be further enhanced by the degree of prematurity and resulting complications.

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In this study we sought to examine, in newborns, the common SNPs previously shown to strongly associate with acylcarnitine levels in adults. The goal was to determine if the same alleles associate with acylcarnitine levels measured routinely as part of neonatal screening. Wefocus on the shortchain acylcarnitines (C0, C2, C3, C4 and C5) as these were shown to have the highest genetic heritability in our previous study(Alul *et al*, 2013). We investigate candidate SNP and metabolite associations in 743 newborns. Identifying, at birth, common polymorphisms associated with intermediate phenotypes, which in adults are biomarkers for chronic conditions such as type 2 diabetes, may aid in the prediction of later life chronic metabolic conditions.

METHODS

Study Population

Study samples were drawn from newborn dried blood spot (DBS) cards that were obtained through the State Hygienic Laboratory (SHL) at the University of Iowa and previously processed into DNA (Alul *et al*, 2013). Briefly, DNA was extracted into solution via the AutoGen (Holliston, MA) QuickGene-810 nucleic acid extraction machine with the DNA Tissue Kit (AutoGen), following manufacturer's recommendations. Carnitine levels were quantified by tandem mass spectrometry according to standard SHL protocol. Approval for use of the de-identified data and DBS cards was granted by the Congenital Inherited Disease Advisory Committee and the Iowa Department of Public Health and a waiver of consent was obtained from the Institutional Review Board of the University of Iowa (IRB no. 200908793). Information on race and ethnicity was not available for this sample population; however, roughly 87% of births in Iowa during the time our samples were obtained were Caucasian (Alul *et al*, 2013).

The newborn samples were collected between 24 and 72 hours of life as part of the routine newborn screening program and tested at the State Hygienic Laboratory for the conditions included on the Iowa newborn screening panel. We only included for analysis those neonates that had a normal newborn screen and werenot on total parenteral nutrition if they were born term (gestation = 40 weeks).

Marker Selection

A total of 72 SNPs covering11 candidategenes were selected for genotyping (Table 1). The table shows the strongest SNP-analyte associations in the literature, however, there were significant associations with theother short-chain acylcarnitines and the medium and long-chain acylcarnitines for SNPs of interest (http://metabolomics.helmholtz-muenchen.de/gwa/). Gene coverage ranged from 75-90%, with the exception of *SLC16A9*, for which only one marker was analyzed. Five SNPs were chosen based on previously reported associations with carnitines of interest (Suhre *et al*, 2011). The remaining SNPs were selected from genes known to be associated with short-chain acylcarnitines. SNP selection was accomplished by searching candidate genes in the HapMap Database (http://hapmap.ncbi.nlm.nih.gov/) Genome Browser, release #28, and the resulting SNP genotype data for CEU was viewed using HaploView software (Broad Institute). SNP sets for each gene were selected based on a combination of having aminimum minor-allele frequency (MAF) of 0.01 with a minimum

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total MAF for the gene of 0.8 and high linkage disequilibrium between SNPs.TagSNP coverage was determined in HaploView (Broad Institute) using the CEU data release #28 and represents the amount of captured variation in the Hapmap CEU data for each gene region.

Genotyping

TaqMan assays (Applied Biosystems, Foster City, CA) for the 72 markers were tested on control DNA prior to genotyping samples on the EP1 SNP Genotyping System and GT 192.24 Dynamic Array Integrated Fluidic Circuits (Fluidigm, San Francisco, CA). 70 SNP genotyping assays were available and ordered using the Assay-On-Demand service from Applied Biosystems; two assays were custom designed from Applied Biosystems. An additional marker (rs273909)identified in the literature was run using Applied BiosystemsTaqMan technology. These genotyping assays contain primers to amplify the region containing the SNP of interest and two TaqMan Minor Groove Binder probes that are specific to the polymorphic variant alleles at the site labeled with different fluorescent reporter dyes, VIC and FAM. All reactions were performed using the standard protocol specified by Fluidigm. Two CEPH individuals served as positive controls and double-distilled water was used as a negative control. After thermocycling, fluorescence levels of the VIC and FAM dyes were measured for each sample-SNP combination using the EP1 Reader (Fluidigm), and genotypes were called using the Fluidigm SNP Genotyping Analysis software (Fluidigm) and manually inspected for unusual patterns suggesting artifacts or detection of multiple SNPs by a single assay.. Genotypes were uploaded into the Progeny database (Progeny Software, South Bend, IN) containing the phenotypic data for subsequent statistical analysis. Markers with <95% genotyping efficiency or deviations from Hardy-Weinberg analysis ($p<0.01$) were excluded from analysis.

Statistical Analysis

Primary metabolite-SNP analysis focused on the short-chain acylcarnitines: C0 (free carnitine), C2 (acetylcarnitine), C3 (propionylcarnitine), C3-DC (malonylcarnitine), C4 (butyrylcarnitine+isobutyrylcarnitine), C4-DC (methylmalonylcarnitine), C5 (isovalerylcarnitine+methylbutyrylcarnitine), C5-DC (glutarylcarnitine) and C5-OH (3-hydroxyisovalerylcarnitine), as these were the most highly heritable based on previous studies and there were well defined candidate genes and SNPs associated with their levels (Alul *et al*, 2013; Gieger *et al*, 2008; Illig *et al*, 2010; Suhre *et al*, 2011) in adults (Table 1 and 2). Each SNP-analyte level combination was initially screened for association using non-parametric Kruskal-Wallis tests, as most analytes were not normally distributed. A total of 522 SNP-analyte tests were performed independently in all infants combined (N=743). Correction for multiple testing was achieved with Bonferroni (0.05/522 tests = $p<1\times10^{-4}$).Analysis was also performed in preterm and term infants separately to determine consistency of findings among term and preterm infants. Further analysis was performed using linear regression for analyte-SNP combinations that met significance by Bonferroni; analytes for these analyses were transformed using the natural log. In all cases SNPs were modelled as an additive effect with the major allele representing the referent group. Additional analytes captured on the newborn screen were tested for association (using Kruskal-Wallis tests) with SNPs that met Bonferroni correction, to determine what other

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metabolites were co-regulated with each SNP. These analytes are products of β-oxidation and are likely correlated with one another. In order to detect potentially novel associations in newborns we expanded our analysis to the wider range of metabolites.

These additional metabolites include amino acids: ALA (alanine), ARG (arginine), CIT (citrulline), GLU (glutamate), LEU (leucine), MET (methionine), PHE (phenylalanine), TYR (tyrosine) andVAL (valine); medium-chain acylcarnitines: C6 (hexanoylcarnitine), C6-DC (methylglutarylcarnitine), C8 (octanoylcarnitine), C8:1 (octenoylcarnitine), C10 (decanoylcarnitine), C10:1 (decenoylcarnitine), C12 (dodecanoylcarnitine) and C12:1 (dodecenoylcarnitine); and long-chain acylcarnitines: C14 (tetradecanoylcarnitine), C14:1 (tetradecenoylcarnitine), 14:2 (tetradecadienoylcarnitine), C16 (palmitoylcarnitine), C16:1 (palmitoleylcarnitine), C16:1-OH (3-hydroxypalmitoleylcarnitine), C18 (stearoylcarnitine), C18:1 (oleoylcarnitine) and C18:2 (linoleoylcarnitine).

Metabolite ratios have been shown to be informative for newborn screening (Gieger *et al*, 2008) and several ratios are routinely reported. Metabolomic studies have also shown that when a gene co-regulates several metabolites, increased power to detect these effects can be gained by examining the ratios(Gieger *et al*, 2008). We therefore, examined 5 short-chain acylcarnitine ratios (C3/C2, C4/C2, C4/C3, C5/C2 and C5/C3) with SNPs significant after Bonferroni correction.

RESULTS

A total of 58 markers in 11 genes were considered for association with 9 short-chain acylcarnitine metabolites (Table 1).Our study population consisted of 743 total infants; 402 were born at 40 weeks gestation and 341 were born preterm (<37 weeks gestation) (Table 2). After correction for multiple testing ($P<1\times10^{-4}$), 13 SNPs in 4 genes were associated with one or more metabolite levels in the full population.

Six SNPs were significantly associated with C4; all are located in the *ACADS* gene (rs2066938, rs3916, rs3794215, rs555404, rs558314, rs1799958) (Table 3). Independently, these six SNPs explained8% to 25% of the variance in neonatal C4 (Table 3). In a multivariate model containing all 6 SNPs only rs2066938 remained significantly associated ($p<0.05$) with C4. All six SNPs in *ACADS* (rs2066938, rs3916, rs3794215, rs555404, rs558314, rs1799958) were also significantly ($p<1\times10^{-4}$) associated with C4/C2 and C4/C3 ratios (Supplemental Table 1). The same association patterns were observed for both preterm and term infants when analyzed separately (Supplemental Table 2).

Two SNPs in *SLC22A4*(rs1050152 and rs11950562), two SNPs in *SLC22A5*(rs11746555 and rs1762208) and two SNPs in *MCCC1* (rs4859156 and rs12486983) were associated with C5-OH levels (Table 4). Independently, these SNPs explained 3% to 7% of the variation in neonatal C5-OH. In a multivariate model containing all six SNPs only rs4859156 remained significantly associated with C5-OH levels ($p<0.05$). This is due to the strength of the association between *MCCC1* and C5-OH as *MCCC1* encodes for the enzyme that converts 3-methylcrontonyl-CoA to 3-methylglutaconyl-CoA; rare defects in this gene lead to Methylcrontonylglycinuria (OMIM: 210200) for which C5-OH is the biomarker.The A

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allele of rs11950562 in *SLC22A4*, which is associated with higher levels of C5-OH, was also associated with higher levels of C16 (Supplemental Table 1). The same association patterns were observed in both preterm and term infants for *SLC22A4* and *SLC22A5* SNPs; however, both SNPs in *MCCC1* were only significant in term but not preterm infants (Supplemental Table 2).The direction of the effect was the same in term and preterm infants; however, the significance of the effect was only marginal in preterm infants (p=0.12 for both SNPs), likely due to reduced power in the preterm group.

SNP rs270606 in *SLC22A4*, which was not associated with C5-OH, was significantly associated with C2, C3 and C4 after correction for multiple testing (Table 5). This SNP explained 3% of the variance for each metabolite. The T allele of rs270606 which is associated with higher levels of C2, C3 and C4 is also associated with higher levels of C14 and C16:1 (Supplemental Table1).The association between rs270606 and C4 was strongest in term infants and non-significant in preterm infants; while the associations with C2 and C3 were strongest in preterm but not term infants (Supplemental Table 2).

DISCUSSION

Recently, several genome-wide association studies have sought to identify genetic polymorphisms associated with the adult human metabolome (Gieger *et al*, 2008; Illig *et al*, 2010; Suhre *et al*, 2011). These studies have identified several variants that explain a substantial proportion of the variability within various metabolites, many of which have been implicated as biomarkers for chronic diseases, such as obesity, type 2 diabetes and metabolic syndrome (Adams *et al*, 2009; Bene *et al*, 2013; Suhre *et al*, 2011). One of the strongest associations identified in these studies was between the *ACADS* gene and adult levels of short-chain acylcarnitines and acylcarnitine ratios, namely C4 and C4/C3 (Gieger *et al*, 2008; Illig *et al*, 2010; Suhre *et al*, 2011). While novel, this was unsurprising as C3 and C4 are by-products of *ACADS*. Interestingly, a large portion of the variation in these traits (~25% for C4/C3 ratio) could be explained by a single polymorphism (Gieger *et al*, 2008; Illig *et al*, 2010; Suhre *et al*, 2011). Our current findings in neonates are consistent with what is observed in adults; the G allele of rs2066938 is associated with higher C4 levels and this SNP explains 25% of the variation in neonatal C4 levels.

This finding has significance for neonatal research, specifically newborn screening of short chain acylcarnitine deficiency (SCAD) and has important implications for adult chronic conditions which are composite conditions that involve many intermediate phenotypes including those involved in β oxidation, including the short-chain acylcarnitines. SCAD (OMIM#201470) is an autosomal recessive condition detected by elevated levels of C4 through newborn screening. SCAD is difficult to diagnosis due to a highly variable phenotype, and the more mild manifestations of this disorder are likely underdiagnosed (Gregersen *et al*, 2001; Nagan *et al*, 2003). Several coding mutations in *ACADS* have been implicated in SCAD, including rs1800556 and rs1799958 (Corydon *et al*, 2001; Gregersen *et al*, 2001; Nagan *et al*, 2003). One study found that the A allele of rs1799958 is associated with elevated C4 in infants who received a normal newborn screen and that the frequency of this variant was 25% in Caucasians (Nagan *et al*, 2003). We report nearly identical results with a minor allele frequency of 26% in our population which is largely Caucasian.

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The high frequency of the rs1799958 polymorphism in the general population not only strengthens the hypothesis that this mutation confers disease susceptibility only in conjunction with other genetic and non-genetic factors, but it also has important implications regarding false positive screens (Corydon *et al*, 2001; Nagan *et al*, 2003). Further research should determine the frequency of these *ACADS* variants in infants who screen positive for elevated C4 but are not diagnosed with SCAD. It is of note that the association of rs1799958 with elevated C4 disappeared in our population in multivariate models that included rs2066938. These two variants are highlycorrelated in our population ($r^2 = 0.89$) (Figure 1a); therefore rs2066938, located in the 3′ untranslated region of *ACADS* and to our knowledge has not been examined in conjunction with SCAD, should be considered as a variant of potential significance in SCAD and other disorders associated with elevated C4.

In addition to the associations with SNPs in *ACADS* and C4, we also identified polymorphisms in *SLCA22A4*, *SLCA22A5* and *MCCC1* that were associated with several short-chain (C2, C3, C4, C5-OH) and long-chain acylcarnitines (C14, C16 and C16:1). Multiple studies have found higher levels of short-chain acylcarnitines, specifically C2, C3 and C4 in individuals with type 2 diabetes or metabolic syndrome; however, results are inconsistent (Adams *et al*, 2009; Bene *et al*, 2013; Gall *et al*, 2010). The prevailing hypothesis is that insulin resistance can be conferred through accumulation of the by-products of incomplete fatty acid oxidation such as acylcarnitines (Schooneman *et al*, 2013). We posit that the common polymorphisms that predispose individuals from birth to higher (or lower) levels of metabolites, such as the shortchain acylcarnitines, may also increase their susceptibility to later life chronic conditions. This is supported by findings that the minor C allele of rs2014355, which is in complete linkage disequilibrium (r2 = 1.0) with the G allele of rs2066938 (Figure 1b), is associated with decreased insulin release (Hornbak *et al*, 2011). It is hypothesized that this effect is partly due to impaired β-oxidation of fatty acids, which is strengthened by our finding that the G allele of rs2066938 is associated with elevated neonatal C4 concentrations and elevated C4 concentrations are also observed in diabetic adults (Bene *et al*, 2013; Gall *et al*, 2010). We also find that the same alleles in SLC22A4 (rs1050152 and rs11950562) and SLC22A5 (rs11746555 and rs17622208) associated with lower C5-OH are also associated with an increased risk for Crohn’s disease. Individuals with Crohn’s disease have been shown to have lower levels of short-chain acylcarnitines compared to controls (Bene *et al*, 2007).

Our study is limited in the ability to directly connect genetic associations with neonatal levels of metabolites to laterlife chronic conditions. There is emerging evidence that acylcarnitine and amino acid levels captured at birth through newborn screening can be useful for prediction of complex conditions such as type 1 diabetes (la Marca *et al*, 2013); however, to our knowledge no study exists examining the association between levels captured at birth and laterlife development of chronic disease. We were also limited to examination of only the metabolite markers captured by the newborn screen. As metabolomic technology advances it may be possible to capture a much wider array of analytes using dried blood spots. While our study was well powered to detect strong SNP effects with analyte concentrations, we were less powered to detect moderate effect sizes or effects when stratified by prematurity. Additionally, we did not have any information on

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race or ethnicity; however, in 2005, the year these samples were collected the Iowa census reported that 94.5% of the population was Caucasian. Therefore, race/ethnicity is unlikely to dramatically affect our results. Expanding these studies to more diverse populations will be particularly important for determining the generalizability of our findings.

Newborn screening is a critical public health initiative with mandatory screening of every infant born in the United States. This important public health program may have substantial reach beyond the detection of rare inborn errors of metabolism. Capitalizing on the potential of metabolic profiles to predict risks for later life chronic conditions will be of paramount importance for personalized medicine and future research into the etiology of chronic conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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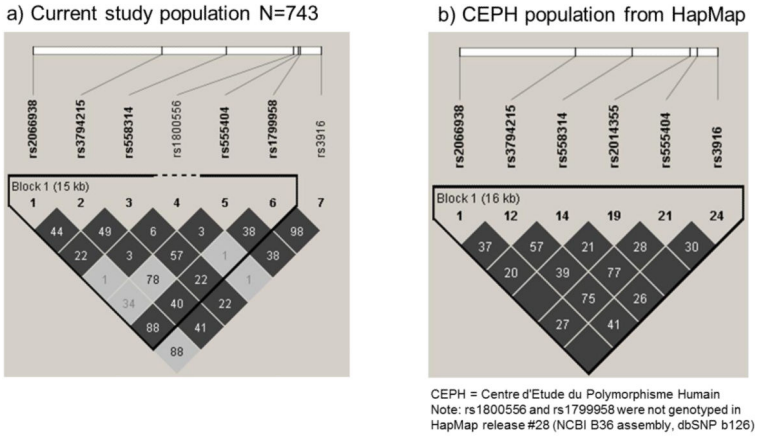

Figure 1.

Haplotype structure in the current study population (a) and CEPH population (b). Figures were generated using Haploview, version 4.2.[34] CEPH genotyping data was obtained from the International HapMap Genome Browser release #28 using NCBI B36 and dbSNP b126.

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Table 1

Candidate SNPs and genes analyzed for association with neonatal metabolite levels

Gene	Single Nucleotide Polymorphisms	Biologic Rationale
ACAD8 acyl-CoA dehydrogenase family, member 8	rs1130172, rs1551183, rs473041	Rare variants associated with elevated C4 on newborn screen (Pedersen *et al*, 2006)
ACADS acyl-CoA dehydrogenase, short-chain	rs2066938, rs3794215, rs558314, rs695950*, rs1800556, rs1800556, rs555404, rs1799958, rs3916, rs9204*	C3 and C4 are substrates of ACADS and SNPs in ACADs associated with C3 and C4 in adults(Nagan *et al*, 2003; Suhre *et al*, 2011)
IVD isovaleryl-CoA dehydrogenase	rs10518693, rs8033938, rs12440317**, rs2289330*, rs875767**	IVD critical for mitochondrial fatty acid β oxidation and associated with C5 in adults (Suhre *et al*, 2011)
LACTB lactamase, beta	rs2652789, rs2652822, rs8468**	Associated with C4DC in adults (Suhre *et al*, 2011)
MCCC1 3-methylcrotonyl-CoA carboxylase 1	rs12486983, rs13075596, rs6443842, rs11928508*,rs9823766, rs4859156, rs937652**	MCCC1 encodes α-subunit of MCCC which when defective results in MCG*** detected by elevated C5-OH (Jung *et al*, 2012)
MCCC2 3-methylcrotonyl-CoA carboxylase 2	rs7449316, rs277994, rs1553314*, rs1391180, rs277976, rs7443786	MCCC2encodes β-subunit of MCCC which when defective results in MCG*** detected by elevated C5-OH (Jung *et al*, 2012)
PPARG peroxisome proliferator-activated receptor-gamma	rs2972164, rs12495364, rs17793951, rs1247191, rs4135247, rs2921190, rs4135275, rs709156*, rs7645903, rs13099828, rs1797912, rs7626560, rs3856806	Short-chain fatty acids activate and bind to *PPARG*(Alex *et al*, 2013)
SLC16A9 solute carrier family 16, member 9	rs7094971	SLC16A9 shown to transport free carnitine and associated with C0 levels in adults (Suhre *et al*, 2011)
SLC22A1 solute carrier family 22, member 1	rs3798174, rs594709, rs3798173, rs3798172, rs1871388**, rs6937722, rs3798167, rs619598, rs622342, rs9295125, rs622591	Associated with C4 levels in adults (Suhre *et al*, 2011)
SLC22A4 solute carrier family 22, member 4	rs3792881**, rs270606, rs11950562, rs272889, rs273909*, rs1050152, rs13184379	SLC22A4 transports C5 and associated with C5 levels in adults (Suhre *et al*, 2011)
SLC22A5 solute carrier family 22, member 5	rs274570*, rs17622208, rs274555, rs17689550, rs11746555, rs274552, rs1045020	Mutations cause primary carnitine deficiency (Shibbani *et al*, 2013)

* denotes SNPs excluded due to poor genotyping efficiency (<95%).

** denotes SNPs excluded due to deviations from HWE ($p<0.01$).

MCG: methylcrotonylglycinuria (OMIM: 210200)

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Table 2

Demographic characteristics of study subjects

	Combined (N=743)	Term (N=402)	Preterm (N=341)
C0 level (μmol/L)	19.6 ± 7.2	18.7 ± 6.1	20.7 ± 8.1
C2 level (μmol/L)	25.5 ±8.7	25.9 ± 8.2	25.0 ± 9.2
C3 level (μmol/L)	2.3 ± 0.89	2.1 ± 0.7	2.5 ± 1.0
C3-DC level (μmol/L)	0.14 ± 0.06	0.14 ± 0.06	0.13 ± 0.07
C4 level (μmol/L)	0.28 ± 0.14	0.26 ± 0.12	0.32 ± 0.15
C4-DC level (μmol/L)	0.11 ± 0.04	0.12 ± 0.04	0.09 ± 0.03
C5 level (μmol/L)	0.13 ± 0.06	0.10 ± 0.04	0.16 ± 0.08
C5-OH level(μmol/L)	0.13 ± 0.05	0.12 ± 0.05	0.13 ± 0.05
C5-DC level (μmol/L)	0.06 ± 0.03	0.06 ± 0.02	0.07 ± 0.03
Male Gender	393 (52.9%)	204 (50.7%)	189 (55.4%)
Gestational Age (wks)	37.4 ± 3.2	40 ± 0	34.3 ± 2.3
Birth weight (g)	2984.2 ± 739.1	3451.9 ± 413.0	2432.8 ± 653.6
Age at screening (h)	37.1 ± 11.9	36.3 ± 10.6	38.1 ± 13.1
Total parenteral nutrition	40 (5.4%)	0 (0.0%)	40 (11.7%)

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Table 3

Association between neonatal C4 levels and SNPs in the *ACADS* gene

SNP	Mean (SD)	KW *P* value	[1]β (95% CI)	[2]r^2
rs2066938		1.50×10^{-29}		0.25
AA	0.25 ± 0.10		Referent (MAF=0.74)	
AG	0.28 ± 0.11		014 (0.08, 0.19)	
GG	0.54 ± 0.22		0.77 (0.67, 0.86)	
rs3916		5.10×10^{-27}		0.24
CC	0.54 ± 0.23		0.76 (0.66, 0.86)	
CG	0.28 ± 0.11		0.13 (0.08, 0.18)	
GG	0.25 ± 0.10		Referent (MAF=0.74)	
rs3794215		7.90×10^{-18}		0.13
GG	0.38 ± 0.20		−0.31 (−0.39, −0.24)	
GA	0.26 ± 0.10		−0.39 (−0.46, −0.31)	
AA	0.25 ± 0.11		Referent (MAF=0.56)	
rs555404		4.20×10^{-18}		0.13
CC	0.37 ± 0.20		0.38 (0.30, 0.45)	
CT	0.26 ± 0.10		0.07 (0.01, 0.14)	
TT	0.25 ± 0.11		Referent (MAF=0.53)	
rs558314		1.60×10^{-11}		0.08
CC	0.23 ± 0.09		−0.30 (−0.39, −0.22)	
CG	0.27 ± 0.11		−0.17 (−0.24, −0.11)	
GG	0.33 ± 0.17		Referent (MAF=0.61)	
rs1799958		1.10×10^{-28}		0.25
AA	0.56 ± 0.23		0.79 (0.69, 0.89)	
AG	0.28 ± 0.11		0.13 (0.07, 0.18)	
GG	0.25 ± 0.10		Referent (MAF=0.74)	

KW = Kruskal-Wallis non-parametric p-value

MAF = major allele frequency

[1] β coefficients presented are from the univariatemodel for the individual SNP and analyte level.

[2] r^2 values are for the total univariate model including only the association between the SNP and analyte level.

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Table 4

SNP associations with neonatal C5OH levels

Gene	SNP	Mean (SD)	KW *P* value	β (95% CI)	r^2
SLC22A4	rs1050152		8.9×10^{-10}		0.06
	CC	0.14 ± 0.05		Referent (MAF=0.63)	
	CT	0.12 ± 0.05		−0.10 (−0.15, −0.06)	
	TT	0.11 ± 0.03		−0.21 (−0.28, −0.15)	
SLC22A4	rs11950562		6.60×10^{-11}		
	AA	0.14 ± 0.05		Referent (MAF=0.57)	0.06
	AC	0.13 ± 0.05		−0.09 (−0.14, −0.04)	
	CC	0.11 ± 0.03		−0.22 (−0.28, −0.16)	
SLC22A5	rs11746555		9.70×10^{-10}		0.06
	AA	0.11 ± 0.03		−0.21 (−0.28, −0.15)	
	AG	0.12 ± 0.04		−0.10 (−0.15, −0.06)	
	GG	0.14 ± 0.05		Referent (MAF=0.65)	
SLC22A5	rs17622208		7.70×10^{-12}		0.07
	AA	0.11 ± 0.03		−0.23 (−0.30, −0.17)	
	AG	0.12 ± 0.05		−0.10 (−0.15, −0.06)	
	GG	0.14 ± 0.05		Referent (MAF=0.58)	
MCCC1	rs4859156		4.10×10^{-09}		0.05
	GG	0.12 ± 0.05		Referent (MAF=0.62)	
	GT	0.13 ± 0.04		0.08 (0.03, 0.13)	
	TT	0.14 ± 0.05		0.20 (0.14, 0.27)	
MCCC1	rs12486983		5.70×10^{-06}		0.03
	CC	0.12 ± 0.05		Referent (MAF=0.70)	
	CT	0.13 ± 0.05		0.10 (0.05, 0.14)	
	TT	0.14 ± 0.05		0.16 (0.08, 0.24)	

KW = Kruskal-Wallis non-parametric p-value

MAF = major allele frequency

[1] β coefficients presented are from the univariate model for the individual SNP and analyte level.

[2] r^2 values are for the total univariate model including only the association between the SNP and analyte level.

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Table 5

Associations between multiple neonatal short-chain acylcarnitine levels and rs270606 in *SLC22A4*.

Carnitine	SNP	Mean (SD)	KW *P* value	β (95% CI)	r^2
C2	rs270606		1.20×10^{-05}		0.03
	CC	24.1 ± 8.14		Referent (MAF=0.71)	
	CT	26.6 ± 8.87		0.10 (0.05, 0.15)	
	TT	28.2 ± 9.65		0.16 (0.07, 0.24)	
C3	rs270606		3.70×10^{-06}		0.03
	CC	2.14 ± 0.85		Referent (MAF=0.71)	
	CT	2.36 ± 0.89		0.09 (0.04, 0.15)	
	TT	2.62 ± 0.89		0.21 (0.12, 0.31)	
C4	rs270606		3.50×10^{-05}		0.03
	CC	0.27 ± 0.13		Referent (MAF=0.71)	
	CT	0.29 ± 0.14		0.07 (0.01, 0.13)	
	TT	0.35 ± 0.17		0.24 (0.14, 0.35)	

[1] β coefficients presented are from the univariate model for the individual SNP and analyte level.

[2] r^2 values are for the total univariate model including only the association between the SNP and analyte level.