Mitochondrial 3-Hydroxy-3-Methylglutaryl-CoA Synthase Deficiency: Urinary Organic Acid Profiles and Expanded Spectrum of Mutations

Deleted: Characteristic

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Abstract

Mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase (HMCS2) deficiency results in episodes of hypoglycaemia and increases in fatty acid metabolites. Metabolite abnormalities described to date in HMCS2 deficiency are non-specific and overlap with other inborn errors of metabolism, making the biochemical diagnosis of HMCS2 deficiency difficult. Urinary organic acid profiles from periods of metabolic decompensation were studied in detail in HMCS2 deficient patients from 4 families. An additional six unrelated patients were identified from clinical presentation and/or the qualitative identification of abnormal organic acids. The diagnosis was confirmed by sequencing and deletion/duplication analysis of the *HMGCS2* gene. Seven related novel organic acids were identified in the urine profiles. Five of them (3,5-dihydroxyhexanoic 1,5 lactone, *trans*-5-hydroxyhex-2-enoate, 4-hydroxy-6-methyl-2-pyrone, 5-hydroxy-3-ketohexanoate, 3,5-dihydroxyhexanoate) were identified by comparison with synthesized or commercial authentic compounds. *trans*-3-hydroxyhex-4-enoate and 3-hydroxy-5-ketohexanoate were provisionally identified by their mass spectral characteristics. These metabolites were found in samples taken during periods of decompensation and normalized when patients recovered. When cut-offs of adipic>200 and 4-hydroxy-6-methyl-2-pyrone>20 µmol/mmol creatinine were applied, all eight samples taken from five HMCS2 deficient patients during episodes of decompensation were flagged with a positive predictive value of 80% (95% confidence interval: 35-100%). Some ketotic patients had increased 4-hydroxy-6-methyl-2-pyrone. Molecular studies identified a total of 12 novel mutations including a large deletion of *HMGCS2* exon 1 in two families, highlighting the need to perform quantitative gene analyses. There are now 26 known *HMGCS2* mutations, reviewed in the text. 4-Hydroxy-6-methyl-2-pyrone and related metabolites are markers for HMCS2 deficiency. Detection of these metabolites will streamline the biochemical diagnosis of this disorder.

Take home message: Urinary 4-hydroxy-6-methyl-2-pyrone is a biomarker for mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase deficiency.

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Abbreviations

4HMP	4-hydroxy-6-methyl-2-pyrone
HMCS2	mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase protein
MLPA	multiplex ligation-dependent probe amplification

Compliance with Ethics Guidelines

Conflict of interest: James Pitt, Heidi Peters, Avihu Boneh, Joy Yaplito-Lee, Stefanie Wieser, Katrin Hinderhofer, David Johnson and Johannes Zschocke declare that they have no conflict of interest.

Informed consent:

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

Contributions of the individual authors:

James Pitt conceived the study, wrote the manuscript, identified the metabolites and analysed metabolite data and acts as guarantor.

Heidi Peters managed patients and revised the manuscript.

Avihu Boneh managed patients and revised the manuscript.

Joy Yaplito-Lee managed patients and revised the manuscript

Stefanie Wieser performed mutation analysis and revised the manuscript.

Katrin Hinderhofer performed mutation analysis and revised the manuscript.

David Johnson synthesized and identified the metabolites and revised the manuscript.

Johannes Zschocke wrote the manuscript and performed mutation analysis.

Introduction

Mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase (HMCS2) catalyzes the first step of ketone biosynthesis from fatty acids and is critical for the provision of energy to the brain during periods of fasting. Human HMCS2 deficiency (OMIM 605991) was first described in 1997 (Thompson et al. 1997) and a small number of patients have subsequently been described with mutations identified in the *HMGCS2* gene that encodes HMCS2 (Aledo et al. 2006, Aledo et al. 2001, Bouchard et al. 2001, Ramos et al. 2013, Shafqat et al. 2010, Wolf et al. 2003). HMCS2 deficiency is characterized by episodes of severe hypoglycaemia and metabolic acidosis that occur during fasting and can progress rapidly to life-threatening coma. During these episodes, blood levels of free fatty acids are increased with inappropriately low levels of ketones (Fukao et al. 2014, Zschocke et al. 2002). Levels of urine dicarboxylic acids, which are secondary metabolites of incompletely oxidized fatty acids, are also increased. However, these biochemical features also occur in several inborn errors of fatty acid metabolism or secondary to other causes such as liver failure and they are therefore not specific for HMCS2 deficiency. Typically, no clinical nor biochemical abnormalities are apparent between clinical episodes. The lack of specific biochemical markers and the need to obtain samples during a metabolic crisis make the diagnosis of HMCS2 deficiency challenging. Consequently only a few individuals with HMCS2 deficiency have been reported.

The HMCS2 enzyme is poorly expressed in readily available tissues such as skin fibroblasts; thus enzymatic diagnosis requires an invasive liver biopsy. This is further complicated by the presence of the cytosolic form of the enzyme encoded by the *HMGCS1* gene. Diagnosis can be confirmed by DNA analysis but the mutations described to date have generally been private and pathogenicity is not always definitive. It would therefore be useful to have more specific biomarkers available to improve the diagnosis of HMCS2 deficiency and as a means of prioritizing patients for *HMGCS2* sequencing.

In this report we describe the identification of seven novel organic acids in urine that may serve as biomarkers for HMCS2 deficiency during periods of metabolic decompensation. Combining data from other families with HMCS2 deficiency, we report 12 novel disease-causing mutations including a deletion of

exon 1 which requires quantitative gene analyses for its detection, and provide an update on *HMGCS2* mutations identified so far.

Material and Methods

Subjects

Biochemical data were available from eight individuals with HMCS2 deficiency residing in Victoria, Australia (cohort A). Their clinical features are summarized in Suppl. Table 1. The proband in each family presented with hypoglycaemia and metabolic acidosis. Ammonium concentration was increased in one patient (A1-2) but was normal or only slightly increased in the other patients when measured. Lactate concentration was increased in one patient (A1-3) but was normal in the other patients when measured. Variable hepatomegaly was noted in several subjects during acute presentations. The parents in family A1 are of Lebanese ancestry and are distantly related. Four out of eight children in this family are affected. The mode of presentation was quite similar in all affected children. The first presentation was between 5 and 9 months of age and each child had one or more symptomatic episodes and multiple admissions to hospital for prophylactic management during intercurrent illnesses. Two siblings from this family had one or more episodes of rapid onset severe metabolic acidosis requiring haemofiltration during inter-current illnesses, despite preventive measures.

The parents in family A3 are first cousins of Egyptian ancestry. Patient A3-1 presented with significant hepatomegaly in addition to hypoglycaemia, which initially suggested the diagnosis of glycogen storage disease type1. However, subsequent investigation and resolution of the hepatomegaly excluded this diagnosis. A sibling (A3-2) with the same *HMGCS2* genotype was subsequently born but has had no episodes so far (current age: three years).

Excluding patient A3-2, the median age for the first episode of metabolic decompensation in cohort A was 8 months (range 5 – 37). Overall, these episodes and their severity tended to diminish with age and no patient had any episode after 5 years of age. The oldest patient (A1-1) is now 19 years of age. Newborn screening performed at 48-72 hours of age by tandem mass spectrometry was normal for patients A1-3, A1-4, A2-1, A3-1 and A4-1. In particular, acetyl carnitine and other

acyl carnitines were normal. Newborn screening acyl carnitine results were not available for the other patients.

Molecular data were available from these patients and six additional patients with HMCS2 deficiency who reside outside Victoria (cohort B). All presented in the first or second year of life with acute metabolic acidosis and hypoglycaemic hypoketotic coma. Full biochemical and clinical details were not available for cohort B and it was therefore not possible to compare their urine organic acid profiles with cohort A.

Biochemical analyses

4-hydroxy-6-methyl-2-pyrone was purchased from Sigma Aldrich. Other metabolites were synthesized as outlined in the Supplementary Material.

Urine organic acids were analysed by gas chromatography-mass spectrometry after ethyl acetate extraction and formation of methoxyamine/trimethylsilyl derivatives. Alternative derivatives (ethoxyamine/trimethylsilyl and butyl/trimethylsilyl) were prepared to assist with the identification of the new metabolites. Some of the novel organic acids are volatile so it was important to include precautions, such as adding ammonia during the drying stages, to minimize evaporative losses. Details of these methods are given in the Supplementary Material.

Mutation analysis

All nine coding exons and adjacent intron regions of the *HMGCS2* gene were PCR-amplified and sequenced with the Sanger method and fluorescent dye-labeled terminators using standard methods. In patients with uninformative sequencing results, quantitative gene analyses were performed by multiplex ligation-dependent probe amplification (MLPA) using a new commercial kit (P068, MRC Holland, Amsterdam, The Netherlands). Mutations were confirmed in both parents where available. Functional consequences of mutations were assessed with the Alamut prediction software suite (Interactive Biosoftware, Rouen, France). The mutations are not listed as polymorphisms in various gene variant databases such as dbSNP (www. ncbi.nlm.nih.gov/projects/SNP/). All missense mutations were predicted to disrupt the structure of HMCS2 based on the experimental structure (Shafqat et al.

2010) and “probably damaging” with scores >0.995 using the Polyphen 2 prediction algorithm.

Results

Organic acid profiles

A representative urinary organic acid profile obtained from patient (A1-3) during a period of metabolic decompensation is shown in Fig. 1. The levels of dicarboxylic acids such as adipic and suberic acids were greatly increased, dominating the profile and reflecting increased fatty acid metabolism. A large number of other metabolites related to fatty acid metabolism, such as 3-hydroxydicarboxylic acids and 5-hydroxyhexanoic acid, were also prominent as previously reported (Aledo et al. 2006, Bouchard et al. 2001, Morris et al. 1998, Thompson et al. 1997, Zschocke et al. 2002). In common with previous reports, the levels of metabolites rapidly normalized with treatment and were normal when the subjects recovered. This was exemplified by the first episode in patient A1-3 whose organic acid profile completely normalized after 27 hours.

Seven unknown peaks were consistently present during periods of metabolic decompensation in affected members of family A1. They are labeled A to G in Fig. 1 and their GC-MS characteristics are given in Table 1 and Supplementary Fig. 1. These peaks were undetectable or present at much lower levels in control urine samples.

Peak D was identified as 4-hydroxy-6-methyl-2-pyrone (4HMP) by comparison with a commercial standard. Using GC-MS data obtained from alternative derivatives (butyl esters and ethoxyamine derivatives), the other peaks were tentatively identified as saturated and unsaturated hexanoic acids containing hydroxyl and/or keto groups. Peaks B, C, E and G were subsequently identified by synthesis of the authentic compounds with matching mass spectra and co-elution with patients' samples whereas peaks A and F were provisionally identified from their mass spectral characteristics and comparisons with peaks C and E, respectively (see Supplementary Material).

A qualitative assessment of the organic acids profiles from cohort A indicated that *trans*-3-hydroxyhex-4-enoic acid (peak A) and *cis*-5-hydroxyhex-2-enoic acid (peak C) were typically the most abundant peaks in the chromatograms during decompensation episodes (Fig. 1) and were present at much lower levels in control samples. However, significant levels of these two metabolites were also observed in

some patients with ketosis. Preliminary observations indicated that 4HMP appeared to be the most consistently increased metabolite and offered superior discrimination between HMCS2 deficient patients and ketotic patients.

In order to test the diagnostic specificity of 4HMP for HMGCS2 deficiency during routine organic acid screening, we retrospectively analysed archived data from all GC-MS urine organic acid profiles obtained in our laboratory over a four year period. Since it was not feasible to retrospectively analyse all archived profiles for the presence of 4HMP, a filtering process was used in which samples with significant dicarboxylic aciduria (arbitrarily defined as adipic>200 μmole/mmole of creatinine) were first identified. We also examined the relationship between urine 3-hydroxybutyric levels (an indicator of ketosis), adipic and 4HMP. Profiles obtained from a group of patients with fatty acid oxidation disorders (7 cases of MCAD deficiency, 3 of VLCAD deficiency, 2 of CACT deficiency, 1 of CPT2 deficiency and 1 of multiple acyl CoA dehydrogenase deficiency) were also included to further assess specificity. These profiles were obtained from samples collected during acute episodes, frequently accompanied by dicarboxylic aciduria.

Examination of a total of 7,369 urine organic acid profiles revealed 104 profiles with adipic>200 μmole/mmole of creatinine. Of these, eight profiles were from patients with disorders of fatty acid oxidation (Fig. 2). Eight samples were from five patients in the HMCS2 deficiency cohort collected during episodes of metabolic decompensation. Archived electronic data were not available for two members of family 1 but hard copy spectra and chromatograms showed that the abnormal metabolites were increased in these two patients during decompensation episodes. The data from these 104 urine profiles were then re-analysed to determine the levels of 4HMP and the results summarized in Fig. 2B. A cut-off of 20 μmole/mmole of creatinine for 4HMP distinguished all eight samples of HMCS2 deficient patients at decompensation from controls, patients with fatty acid oxidation defects and almost all of the patients with ketosis. The criteria of adipic acid>200 and 4HMP>20 μmole/mmole creatinine resulted in a positive predictive value of 80% for HMCS2 deficiency (95% confidence interval 35 – 100%).

The results summarized in Fig. 2 also show the expected approximate correlation between adipic acid and 3-hydroxybutyric levels due to increased fatty

acid oxidation during ketosis. There was no obvious correlation between 4HMP and 3-hydroxybutyric levels. Surprisingly, we observed relatively high levels of 3-hydroxybutyric in some of the HMCS2 deficiency patients. For example, patient A1-3 had a 3-hydroxybutyric level comparable with ketotic patients (Fig. 2) and has a homozygous exon 1 deletion expected to completely abolish enzyme activity.

Mutations

All affected children in family A1 are homozygous for a novel, large deletion encompassing exon 1 of the *HMGCS2* gene, denoted c.1-?_104+?del. Although the exact break points of this deletion have not been determined, it is expected to completely destroy gene function and to cause complete lack of HMCS2. Patient A2-1 is compound-heterozygous for c.797T>C (p.L266S) in exon 4 and c.1220T>C (p.I407T) in exon 7. p.L266 lies in a conserved alpha-helical structure while p.I407 lies in a beta-sheet structure. Patients A3-1 and A3-2 are homozygous for a mutation c.1162G>A (p.G388R) in exon 6, predicted to disrupt the thiolase fold. Since our preliminary report (Pitt et al. 2009), this mutation has also been described in a Caucasian patient (Ramos et al. 2013). Patient A4-1 is compound heterozygous for c.506G>A (p.G169D) in exon 2 and c.1514G>A (p.R505Q) in exon 9. p.G169D is predicted to disrupt the thiolase fold while p.R505Q is predicted to disrupt the dimer interface.

The homozygous exon 1 deletion c.1-?_104+?del was also identified in a child of Mediterranean origin (B1-1) who presented at the age of one year with postoperative hypoglycaemic coma, seizures, and brain damage. One patient from England (B2-1) was found to be compound heterozygous for the missense mutation c.634G>A (p.G212R) in exon 3, previously detected in two independent German patients (Aledo et al. 2001), and the novel frameshift deletion c.431_432del (p.V144fs) in exon 2. One Australian patient (B3-1) is compound heterozygous for a novel nonsense mutation c.847C>T (p.Q283*) and a novel missense mutation c.695G>T (p.G232V), both in exon 4. Two novel missense mutations, c.553T>C (p.W185R) in exon 2 and c.1508A>G (p.Y503C) in exon 9, were detected in a patient from Northern Ireland (B4-1). One patient from an Arab country (B5-1) is homozygous for the novel missense mutation c.1078T>C (p.S360P) in exon 6. Finally, an Australian patient of mixed Chinese/Caucasian descent (B6-1) is compound heterozygous for the missense mutations c.502G>A (p.G168S) and

c.520T>C (p.F174L), both in exon 2. The p.G168S mutation is novel while the p.F174L mutation has previously been described in a patient of Chinese descent (Bouchard et al. 2001). All new and previously reported *HMGCS2* mutations are listed in Table 2.

Discussion

Mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase (HMCS2) is a critical enzyme in the hepatic synthesis of ketones from fatty acids. Patients with HMCS2 deficiency are therefore prone to episodic metabolic decompensation triggered by fasting or periods of catabolism that require fatty acid utilization for the provision of energy. Fatty acid oxidation is unimpaired but ketogenesis fails as a consequence of the HMCS2 deficiency and patients can become severely hypoglycaemic and encephalopathic. Urine organic acid profiles from episodes of metabolic decompensation were consistent within our cohort A of HMCS2 deficiency patients and were dominated by secondary products of fatty acid oxidation such as dicarboxylic acids (adipic, suberic and sebacic acids), 3-hydroxydicarboxylic and 5-hydroxyhexanoic acids, similar to previously published profiles. These metabolites are also frequently detected in patients with inborn errors of mitochondrial beta-oxidation (Duran 2003) and reflect the accumulation of fatty acid intermediates in enzyme steps upstream of the metabolic block. Subsequent metabolism by secondary pathways such as microsomal omega and omega-1 oxidation and peroxisomal beta-oxidation is proposed as a mechanism to produce many of the metabolites detected on the organic acid profiles (see Supplementary Fig. 2).

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Of greater interest were seven novel metabolites observed in the HMCS2 deficiency patients during decompensation (Fig. 1). These metabolites were present at lower levels than the major secondary fatty acid oxidation products mentioned above yet they were consistent in the HMCS2 deficiency patients. Five of these were identified by synthesis of the authentic compounds and two were provisionally identified based on their mass spectral characteristics (Table 1). All contain six carbon atoms and their structures are consistent with a build-up of fatty acid metabolites upstream from the HMCS2 enzyme with subsequent secondary metabolism (Supplementary Fig. 2). The exact details of some of these metabolic inter-conversions are unclear (*e.g.* the formation of 4HMP) and require further investigation. We hypothesise that the *trans*-hex-2-enoate moiety, an intermediate in

fatty acid oxidation, could be a source for some of the metabolites after additional microsomal omega-1 and omega oxidation. This is analogous to the metabolism of the hexanoate moiety in MCAD deficiency to produce metabolites such adipate and 5-hydroxyhexanoate. Considering the metabolic pathway, there could be additional biomarkers for HMCS2 deficiency. Complementary analytical techniques, such as nuclear magnetic resonance, may be useful for further studies in this area.

Deleted: that are "closer" to the metabolic block, containing two and four carbon atoms. Indeed, a preliminary report described an increased level of crotonyl glycine in a HMCS2 deficient patient (Kouremenos et al. 2010). Crotonyl glycine is derived from crotonyl-CoA, a four carbon intermediate in the final part of the fatty acid oxidation pathway

While the pattern of metabolites appeared to be quite specific for HMCS2 deficiency, we occasionally observed some of these metabolites in subjects with severe ketosis. 4HMP was the most specific metabolite for HMCS2 deficiency in this regard and we focused on the diagnostic utility of this metabolite in a wider group of controls. A retrospective examination of routine urine organic acid profiles obtained over the period in which five of the patients presented showed that all eight decompensation samples from these five patients had levels of 4HMP>20 and adipic>200 µmol/mmole creatinine. Using these cut-offs, a positive predictive value of 80% was obtained in our study sample. Since our preliminary report of these findings (Pitt et al. 2009), two other laboratories have reported the value of these metabolites for the diagnosis of HMCS2 deficiency in abstract form (Carpenter et al. 2010, Carpenter et al. 2012, Hogg et al. 2012) and a third laboratory used them to diagnose an HMCS2 deficient patient (B6-1) (personal communication, Dr Shanti Balasubramaniam).

HMCS2 deficiency belongs to the group of "hypoketotic hypoglycaemias" (Aledo et al. 2006, Bouchard et al. 2001, Fukao et al. 2014, Morris et al. 1998) so the finding of moderately increased levels of ketones in urine or blood may result in this diagnosis being discounted. Importantly, increased 3-hydroxybutyrate concentration was observed in the urine of some of the patients during decompensation (Fig. 2), despite the fact that some of them had null mutations and were therefore expected to have negligible HMCS2 enzyme activity. It is plausible that other sources of ketones contribute a protective effect in HMCS2 deficiency (e.g. *via* leucine metabolism or reversal of ketone metabolism in extrahepatic tissues). Another possibility is that the 3-hydroxybutyrate is comprised of an increased proportion of the L-isomer, produced in the last steps of the fatty acid oxidation pathway, relative to the D-isomer which is observed in fasting ketosis. The measurement of parameters reflecting concentrations of circulating ketones relative to fatty acids (*e.g*. plasma D-3-

hydroxybutyric/free fatty acids or urine D-3-hydroxybutyric/adipic acid ratios) is more appropriate for determining if there is a functional block in ketogenesis that may indicate HMCS2 deficiency (Fukao et al. 2014, Zschocke et al. 2002).

The current report doubles the number of known disease-causing mutations to a total of 27 (Table 2). The impact of several of these on the structure of the HMCS2 protein has been described (Shafqat et al. 2010) and the functional consequences of some mutations have been assessed but there is no obvious correlation between mutation type and clinical or biochemical characteristics. Only five mutations have been observed in more than one family, including c.634G>A (p.G212R) in three families from Germany and England, and the exon 1 deletion c.1-?_104+?del in two families from Mediterranean countries. Our findings underline the importance of performing quantitative *HMGCS2* gene analysis by MLPA or a comparable method in individuals with suggested HMCS2 deficiency in whom standard sequence analysis yielded normal results since mutation analysis is the only realistic method that allows the diagnosis of HMCS2 deficiency when patients are not metabolically decompensated.

Finally, the clinical observations in cohort A also support previous observations that HMCS2 deficiency is a disease of early childhood (Bouchard et al. 2001). None of the patients in cohort A had any episode of metabolic decompensation after the age of five years and all are able to tolerate normal lengths of fasting. In cohort A, three patients required intensive care, including haemofiltration in two. The use of haemofiltration has been described for other patients with HMCS2 deficiency (Sass et al. 2013). All patients continue to have normal growth and cognitive and motor development. Provided metabolic decompensations are quickly recognized and treated aggressively, the prognosis for HMCS2 deficiency is very good.

In conclusion, subjects with HMCS2 deficiency exhibit characteristic urine organic acid patterns during periods of metabolic decompensation and recognition of this pattern should facilitate the diagnosis of HMCS2 deficiency. Our results also indicate that sequencing of the coding regions and adjacent exon intron boundaries of the *HMGCS2* gene followed by MLPA testing for deletions will ensure the confirmation of the diagnosis at a molecular level.

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Table 1 GC-MS characteristics of urine organic acids in HMCS2 deficiency as methoxyamine/trimethylsilyl derivatives

Metabolite	Structure	Retention time (min)	M+	Major ions *m/z*
A	*trans*-3-hydroxyhex-4-enoic[3]	9.25	274	73,143,147,157,259
B	3,5-dihydroxyhexanoic 1,5 lactone[1]	9.58/9.63	202	73,101,145,187
C	*trans*-5-hydroxyhex-2-enoic	9.82	274	73,117,147,230,259
D	4-hydroxy-6-methyl-2-pyrone	11.55	198	73,170,183,198
E	5-hydroxy-3-ketohexanoic[2]	11.63	319	73,117,147,275,304
F	3-hydroxy-5-ketohexanoic[2,3]	12.02	319	73,147,233,304
G	3,5-dihydroxyhexanoic[1]	12.11/12.25	364	73,117,147,259,349

[1] Double peak due to two chiral centers

[2] methoxyamine derivative

[3] provisional identification

Table 2 Summary of novel and previously reported mutations identified in the *HMGCS2* gene (independent families)

Exon	Mutation	Protein effect	No. of families	Reference
1	c.1-?_104+?del	Deletion exon 1	2	**This Report**
2	c.160G>A	p.V54M	1	(Wolf et al. 2003)
2	c.177T>A	p.I56N	1	(Shafqat et al. 2010)
2	c.252T>G	p.Y84*	1	(Shafqat et al. 2010)
2	c.431_432del	p.V144fs	1	**This Report**
2	c.500A>G	p.Y167C	1	(Wolf et al. 2003)
2	c.502G>A	p.G168S	1	**This Report**
2	c.506G>A	p.G169D	1	**This Report**
2	c.520T>C	p.F174L	2	(Bouchard et al. 2001)
2	c.553T>C	p.W185R	1	**This Report**
3	c.563G>A	p.R188H	1	(Aledo et al. 2006)
3	c.634G>A	p.G212R	3	(Aledo et al. 2001)
4	c.695G>T	p.G232V	1	**This Report**
4	c.697A>G	p.T233A	1	(Shafqat et al. 2010)
4	c.727A>G	p.K243E	1	(Shafqat et al. 2010)
4	c.797T>C	p.L266S	1	**This Report**
4	c.847C>T	p.Q283*	1	**This Report**
5	c.920T>C	p.M307T	1	(Aledo et al. 2006)
6	c.1016+1G>A	IVS5+1g>a	1	(Zschocke et al. 2002)
6	c.1078T>C	p.S360P	1	**This Report**
6	c.1162G>A	p.G388R	2	(Pitt et al. 2009)
7	c.1220T>C	p.I407T	1	**This Report**
7	c.1270C>T	p.R424*	2	(Bouchard et al. 2001)
9	c.1499G>A	p.R500H	1	(Aledo et al. 2001)
9	c.1508A>G	p.Y503C	1	**This Report**
9	c.1514G>A	p.R505Q	1	**This Report**

Figure captions

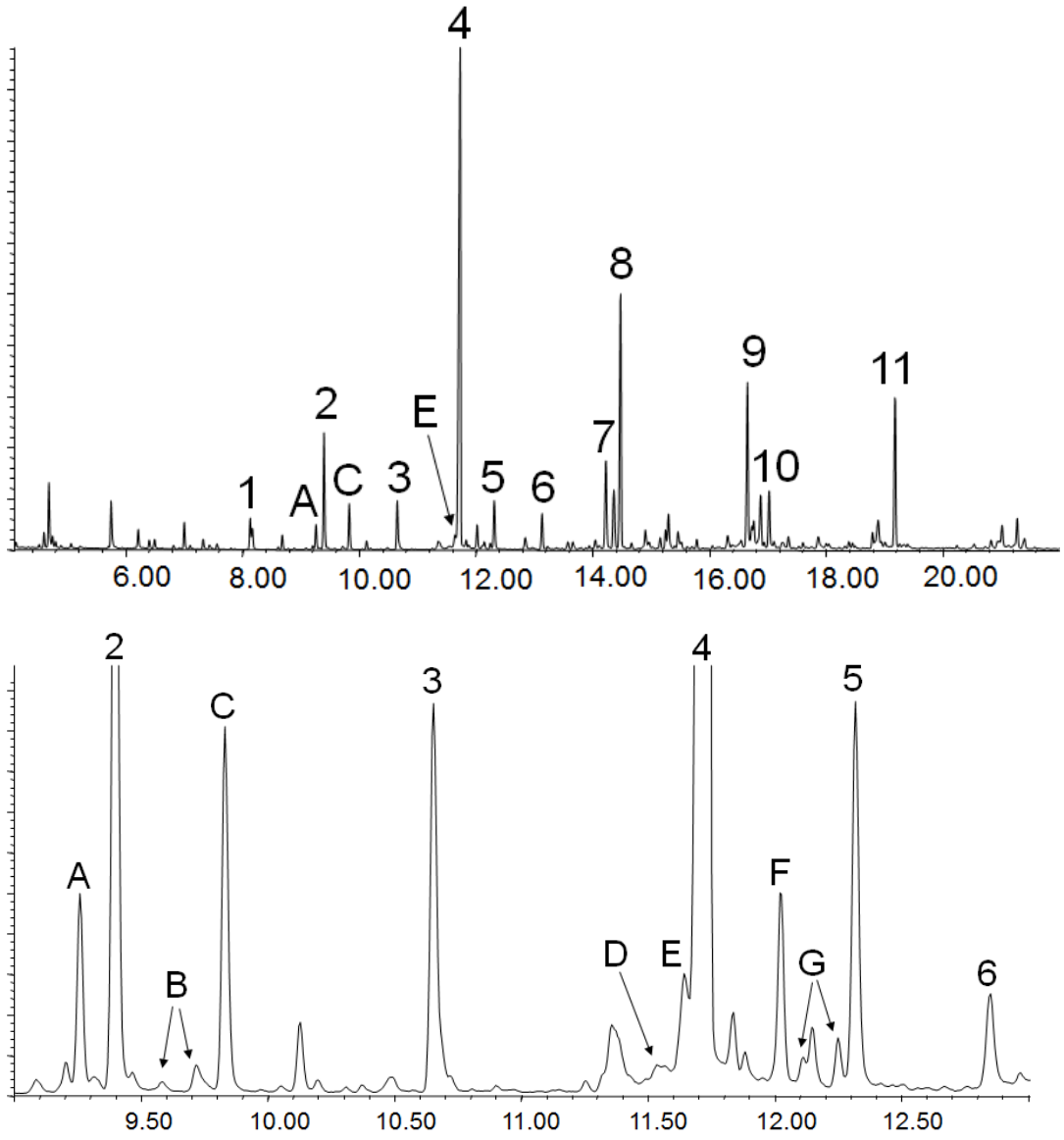
Fig. 1 Total ion chromatogram of urine organic acids from an HMCS2 deficient child during decompensation.

The lower panel shows an expansion between 9 and 13 mins. Novel organic acids (A to G) are identified in Table 1. Other metabolites: 1: urea, 2: 5-hydroxyhexanoic, 3: internal standard, 4: adipic, 5: hex-2-enedioic, 6: pimelic, 7: unsaturated suberic, 8: suberic, 9: unsaturated sebacic, 10: sebacic, 11: 3-hydroxysebacic.

Fig. 2 Urine levels (µmol/mmol of creatinine) of adipic acid (A) and 4HMP (B) relative to 3-hydroxybutyric (NB: log-log scales).

Profiles with adipic>200 µmol/mmol of creatinine (dashed line, panel A) included all eight samples from five HMCS2 deficient patients in cohort A during acute episodes. These samples also had 4HMP > 20 µmol/mmol of creatinine (dashed line, panel B). FAOD is a group of patients with fatty acid oxidation disorders.

Fig 1


4
8
9
11
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7
1
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3
5
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10
6.00
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A
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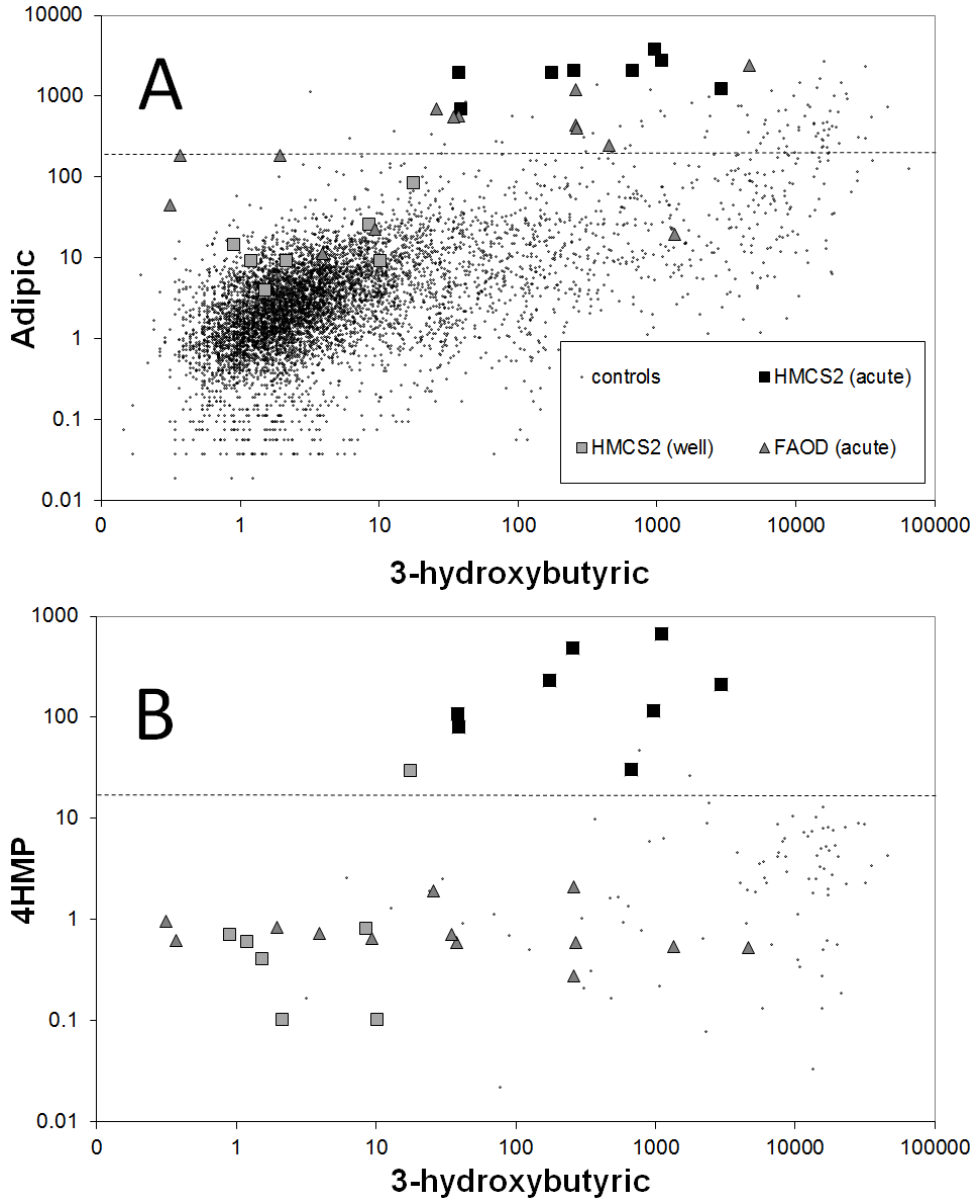
12.50

Fig 2


A
Adipic
3-hydroxybutyric
controls
HMCS2 (acute)
HMCS2 (well)
FAOD (acute)
B
4HMP
3-hydroxybutyric

Author/s:
Pitt, JJ; Peters, H; Boneh, A; Yaplito-Lee, J; Wieser, S; Hinderhofer, K; Johnson, D; Zschocke, J

Title:
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