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A common *UCP2* polymorphism predisposes to stress hyperglycaemia in severe sepsis

A Pyle¹, I M Ibbett¹, C Gordon², S M Keers¹, M Walker³, P F Chinnery¹, and S V Baudouin²

¹Mitochondrial Research Group, Institute of Ageing and Health, Newcastle University, Newcastle upon Tyne, UK

²Institute of Cellular Medicine, Newcastle University & Department of Anaesthesia, Leazes Wing, Royal Victoria Infirmary, Newcastle upon Tyne, UK

³Diabetes Research Group, Newcastle University, Newcastle upon Tyne, UK

Abstract

Background—Insulin resistance and hyperglycaemia are common in severe sepsis. Mitochondrial uncoupling protein 2 (UCP2) plays a role in insulin release and sensitivity.

Objectives—To determine if a common, functional polymorphism in the *UCP2* gene promoter region (the –866 G/A polymorphism) contributes to the risk of hyperglycaemia in severe sepsis.

Results—In the prospective group 120 non-diabetic patients who were carriers of the G allele had significantly higher maximum blood glucose recordings than non-carriers (mean (SD) AA 8.5 (2.2) mmol/l; GA 8.5 (2.4) mmol/l; GG 10.1 (3.1) mmol/l; $p = 0.0042$) and required significantly more insulin to maintain target blood glucose ($p = 0.0007$). In the retrospective study 103 non-diabetic patients showed a similar relationship between maximum glucose and UCP genotype (AA 6.8 (2.3) mmol/l; GA 7.8 (2.2) mmol/l; GG 9.2 (2.9) mmol/l; $p = 0.0078$).

Conclusions—A common, functional polymorphism in the promoter region of the *UCP2* gene is associated with hyperglycaemia and insulin resistance in severe sepsis. This has implications for our understanding of the genetic pathophysiology of sepsis and is of use in the stratification of patients for more intensive management.

The family of uncoupling proteins (UCPs) are evolutionary conserved, nuclear encoded transport proteins which are located within the inner mitochondrial membrane.¹ A common variant single nucleotide polymorphism (SNP) in the promoter region of UCP2 (–866 G/A) modifies UCP2 transcription, alters insulin release from pancreatic β cells, and is associated with type II diabetes and insulin resistance.¹² UCP2 also plays a role in the defence against reactive oxygen species and infection, but the underlying mechanism is not clear.¹

Correspondence to: Dr P F Chinnery, Mitochondrial Research Group, The Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, UK; p.f.chinnery@ncl.ac.uk.

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Hyperglycaemia is common in the critically ill. Observational studies and randomised controlled trials of insulin therapy suggest that hyperglycaemia is associated with a worse outcome in severely ill hospitalised patients.³ We hypothesised that the –866 G/A UCP2 SNP would be associated with the hyperglycaemia of severe sepsis. We examined this hypothesis in two independent studies before reporting our findings. In the first we prospectively compared UCP2 –866 G/A genotype frequencies with the maximum blood glucose recorded, total insulin given, and area under the curve (AUC) for all glucose recordings in the first 24 h of admission in 155 patients with severe sepsis. In the second study we replicated the association between maximum glucose and UCP genotype using archived DNA from a separate cohort of 120 critically ill patients with severe sepsis.

METHODS

Ethical approval was obtained from the Newcastle and North Tyneside Joint Ethics Committee. A prospective study was conducted from August 2005 to December 2006 in the adult critical care unit at the Royal Victoria Infirmary, Newcastle upon Tyne. Patients fulfilled the Joint Consensus severe sepsis definitions,⁴ and were excluded if <18 years old, pregnant, immunosuppressed, on high dose steroid therapy, or of non-Caucasian ancestry. Demographic data, the components of APACHE-II severity scoring system, and hospital outcomes were collected. Body mass index (BMI) was calculated from height and weight. Height was measured on admission. Body weight was recorded from patient notes where available. If no weight was recorded then admission weight was estimated (in 45%). Blood glucose was measured on admission and subsequently every 2 h for the first 24 h. Hyperglycaemia was managed according to a protocol based on the Surviving Sepsis guidelines.⁴ A blood glucose target of 6.0–8.3 mmol/l was set and a continuous insulin infusion commenced and adjusted according to protocol to maintain target blood sugars. The total amount of insulin given in the first 24 h was recorded. The total AUC for blood glucose during the first 24 h was calculated using Prism Graph Pad software (Prism, San Diego, California, USA).

The retrospective replication study was performed on archived samples from 120 patients with severe sepsis previously collected in the same unit between January 2004 and July 2005 with blood glucose controlled using an identical protocol, but only the maximum blood glucose in the first 24 h was recorded for this dataset. No BMI data were available for the retrospective group of patients.

Genomic DNA was extracted from peripheral blood from the sepsis patients and 263 healthy Caucasian controls from the same geographic region. DNA samples were amplified using previously published gene specific primers.⁵ The –866 genotype was determined by sequencing using Big Dye Terminator v3.1 chemistries on an ABI 3100 genetic analyser (Applied Biosystems, California, USA). Continuous variables were compared using unpaired t testing if normally distributed. Total insulin dosage in the first 24 hours was logarithmically transformed (Bartlett's test for unequal variance $p = 0.0048$ before, and $p = 0.1397$ after transformation). Genotype frequencies were compared using the χ^2 test. Maximum glucose values were grouped by genotype, analysed by analysis of variance

(ANOVA) and then tested for trend against the dose of the G allele (GraphPad Instat software).

RESULTS

Genotyping was successful in 262 out of 263 healthy volunteers. Genotype frequencies were AA 32 (12.2%), GA 135 (51.5%), and GG 95 (36.3%), which were in Hardy–Weinberg equilibrium ($p = 0.1294$).

There were three genotyping failures in the 155 patients in the prospective study. Hyperglycaemia was common in patients with severe sepsis and 63% had a recorded glucose of ≥ 8.3 mmol/l during their first 24 h. Genotype frequencies for the whole cohort were AA 16/152 (10.5%), GA 72/152 (47.4%), and GG 64/152 (42.1%), which were in Hardy–Weinberg equilibrium ($p = 0.5169$). Hospital mortality was 40.1%.

Patients with a pre-admission diagnosis of diabetes were analysed separately ($n = 32$). Mean (SD) maximum blood glucose in the first 24 h was significantly associated with UCP2 genotype in non-diabetic patients (AA 8.5 (2.2) mmol/l, GA 8.5 (2.4) mmol/l, GG 10.1 (3.1) mmol/l; $p = 0.0042$) (fig 1A). There was no significant association between genotype and illness severity, age, gender or source of sepsis (table 1). There was a significant relationship between the dose of G allele and the total 24 h insulin requirement (fig 2, $p = 0.0007$). Patients with one or more copies of the G allele required higher doses of insulin to maintain target glucose than those with the A/A genotype. Glucose control in the first 24 h, as assessed by AUC calculations, was similar in all three genotype groups and in those patients with previously diagnosed diabetes ($p = 0.2119$) (fig 3).

Mean (SD) BMI did not differ significantly between patients grouped by UCP2 genotype (A/A 26.2 (3.9) kg/m²; G/A 25.0 (6.2) kg/m²; G/G 26.0 (6.0) kg/m²; $p = 0.5846$) or between non-diabetic patients grouped by genotype (table 1). We also performed a linear regression analysis using maximum glucose as the dependent variable and BMI, age, genotype and APACHE II illness severity as the independent variables. Only GG and illness severity were independent predictors (GG $p = 0.046$; APACHE II $p = 0.039$) of maximum glucose.

The genotype frequency in the 120 patient retrospective cohort was A/A 12/120 (10%), G/A 75/120 (62.5%), and G/G 33/120 (27.5%). Again, the patients with a previous history of diabetes were analysed separately ($n = 17$). Genotype frequency did not differ significantly from either the control population or the prospective sepsis cohort ($p = 0.1111$). Maximum blood glucose in the first 24 h in the retrospective cohort was associated with the number of G alleles (AA 6.8 (2.3) mmol/l; GA 7.8 (2.2) mmol/l; GG 9.2 (2.9) mmol/l; $p = 0.0078$) (fig 1B). Patients were also well matched for age, gender, severity of illness and source of sepsis (table 2). Hospital mortality was 45.8%.

DISCUSSION

Here we show that maximum blood glucose, recorded during the first 24 h of critical care admission for severe sepsis, was associated with a common functional UCP2 polymorphism in two independent cohorts. Patients with the G allele had significantly higher blood glucose

recordings, and -866G homozygotes had a higher blood glucose than heterozygotes, consistent with a gene dosage effect, providing further support for our conclusions. Independent confirmation of our observations was also obtained by analysis of the total amounts of insulin given to the patients in the first 24 h. Our unit used a continuous intravenous insulin protocol to maintain blood glucose between 6.0–8.3 mmol/l. In effect this is a form of euglycaemic–hyperinsulinaemic clamp and the total dose of insulin is therefore a measure of insulin resistance. Total amount of insulin given was significantly related to the dose of the UCP2 G allele with carriers of the G allele receiving higher doses of insulin despite similar glycaemic control between groups.

The hyperglycaemia of critical illness is caused by insulin resistance of peripheral tissues.³ Pancreatic β cell function is well maintained as demonstrated by high circulating insulin concentrations. The cellular basis of insulin resistance remains uncertain. However, recent work suggests that increased oxidative stress is a common feature of insulin resistance at the cellular level.⁶ There is good evidence that UCP2 plays an important role in the cellular protection against oxidative stress, probably by increasing mitochondrial transmembrane proton leak and reducing membrane potential.¹ An increase in cellular oxidative stress invariably occurs in severe sepsis and would provide the drive for the insulin resistance that occurs.⁷ The -866 G/A UCP2 polymorphism is located in a multifunctional cis regulatory site with functional consequences. A number of studies show that the common A allele variant increases UCP2 transcription in both human tissue and cell lines.²⁵⁸ Increased UCP2 transcription is explained by the finding that transcription factors preferentially bind to and more effectively transactivate the A compared to the G allele in human cell lines.²

These observations explain our current results. We found that insulin resistance in sepsis was independently associated with increase illness severity, as measured by the APACHE II scoring system. Sepsis causes an intense proinflammatory response which includes raised circulating concentrations of reactive oxygen species and depletion of antioxidants. We found that the UCP2 A allele protected against stress hyperglycaemia and insulin resistance in sepsis. This would be consistent with the cellular studies showing increased UCP2 transcription in the variant compared to the wild type allele. The increased UCP2 would then provide increased cellular antioxidative defence and therefore reduce insulin resistance.

In summary, we have found a significant association between a common UCP2 G/A promoter polymorphism, maximum glucose concentrations and insulin resistance in patients with severe sepsis. This association suggests that UCP2 may be involved in the development of insulin resistance in the critically ill.

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Key points

- ▶ Uncoupling proteins (UCPs) are a family of evolutionary conserved, nuclear encoded transport proteins located within the inner mitochondrial membrane. We investigated the role of UCP2 in insulin release and sensitivity.
- ▶ The common functional –866 G/A polymorphism, present in the *UCP2* gene promoter region, was studied in a cohort of patients with severe sepsis.
- ▶ 120 non-diabetic patients carrying the G allele had significantly higher maximum blood glucose recordings than non-carriers and required significantly more insulin to maintain target blood glucose.
- ▶ A similar relationship between UCP genotype and maximum glucose was observed in a retrospective study of 103 non-diabetic patients.
- ▶ The functional –866 G/A polymorphism in the *UCP2* gene is associated with hyperglycaemia and insulin resistance in severe sepsis.

Figure 1.

(A) Maximum blood glucose in the first 24 h in the prospective cohort study of 152 patients with severe sepsis. Group means are shown by horizontal bars. The diabetic patients are presented separately and the non-diabetic patients analysed by genotype. Carriers of the G allele had significantly higher blood glucose recordings (analysis for trend $p = 0.0042$). (B) Maximum blood glucose in the first 24 h in the retrospective analysis of 120 patients with severe sepsis. Group means are shown by horizontal bars. The diabetic patients are presented separately and the non-diabetic patients analysed by genotype. Carriers of the G allele had significantly higher blood glucose recordings (analysis for trend $p = 0.0078$).

Figure 2.

The total dose of insulin given to each patient in the prospective study in the first 24 h. Insulin by infusion was given to maintain a target blood glucose of 6.0–8.3 mmol/l. Diabetic patients are presented separately and the non-diabetic patients analysed by genotype. Geometric mean insulin dose and 95% confidence intervals are presented. There was a highly significant trend for greater insulin requirement with the dose of G allele ($p = 0.0007$).

Figure 3.

Mean blood glucose (error bar shows standard deviation) area under the curve (AUC) calculated for the first 24 h for the prospective cohort of patients. The mean AUCs are not significantly different comparing diabetic patients and non-diabetics grouped by UCP2 genotype ($p = 0.2119$; ANOVA).

Table 1

A comparison of age, body mass index (BMI), APACHE II score, gender and source of sepsis in the 120 non-diabetic patients in the prospective study grouped by UCP2 genotype

	AA (11)	GA (56)	GG (53)	p Value
Age* (years)	58 (21)	61 (18)	60 (19)	0.8801
BMI* (kg/m ²)	25.6 (4.1)	23.6 (4.6)	25.3 (6.1)	0.2162
APACHE II*	24 (7)	23 (7)	22 (6)	0.7607
Male/female	5/6	35/21	28/25	0.4367
Chest	4	20	20	0.9761
Abdomen	3	15	17	0.8229
Primary blood/stream	1	2	6	0.3010

* Standard deviations are shown in brackets.

The groups were well matched for these variables.

Table 2

Comparison of age, APACHE II score, gender and source of sepsis in the 103 non-diabetic patients in the retrospective analysis grouped by UCP2 genotype

	AA (11)	GA (65)	GG (27)	p Value
Age* (years)	64 (14)	65 (14)	58 (16)	0.06
APACHE II*	26 (6)	28 (7)	28 (8)	0.6603
Male/female	8/3	33/32	18/9	0.2049
Chest	6	30	12	0.8459
Abdomen	2	21	9	0.6175
Primary blood/stream	1	4	0	0.3601

* Standard deviations are shown in brackets.

The groups were well matched for these variables.