Eur J Pediatr (2002) 161: 337–339
DOI 10.1007/s00431-002-0940-x

ORIGINAL PAPER

**Ali Fatemi · Chike Item · Sylvia Stöckler-Ipsiroglu
Osman Ipsiroglu · Wolfgang Sperl · Wolfgang Patsch
Wolfgang Strobl**

Sudden infant death: no evidence for linkage to common polymorphisms in the uncoupling protein-1 and the β3-adrenergic receptor genes

Received: 8 November 2001 / Revised: 18 January and 30 January 2002 / Accepted: 31 January 2002 / Published online: 16 April 2002

Abstract Thermal stress has been postulated to play a major role in the aetiology of sudden infant death (SID). The human uncoupling protein-1 (UCP-1), expressed in brown adipose tissue dissipates the transmitochondrial proton gradient as heat and plays a central role in energy homeostasis and thermogenesis. A common Bcl I polymorphism in the promoter region of the *UCP-1* gene is associated with reduced UCP-1 adipose tissue mRNA and obesity. In addition, a common sequence variation in the β3-adrenergic receptor gene (β3-AR), Trp64Arg, has been linked to a decreased resting metabolic rate. To determine whether the UCP-1 Bcl I polymorphism and/or the Trp64Arg variant of β3-AR are associated with the occurrence of SID, we determined the allele frequencies of these polymorphisms in 53 Austrian SID victims and 54 controls by nested PCR and restriction digestion using DNA extracted from Guthrie cards. We found that the allele frequencies of both polymorphisms did not differ between the SID and control groups (0.65/0.35 versus 0.72/0.28 for UCP-1 Bcl I, and 0.89/0.11 versus 0.93/0.07 for β3-AR Trp64Arg in SID victims versus controls, respectively). *Conclusion:* Our data do not support a major association between the occurrence of sudden infant death and two common functional polymorphisms in the human uncoupling protein-1 and β3-adrenergic receptor genes.

A. Fatemi · W. Strobl (✉)
Department of Medical Chemistry,
University of Vienna, Waehringerstrasse 10,
1090 Vienna, Austria
E-mail: wolfgang.strobl@univie.ac.at
Tel.: +43-1-4277-60822

A. Fatemi · C. Item · S. Stöckler-Ipsiroglu · O. Ipsiroglu
Department of Paediatrics, University of Vienna,
Vienna, Austria

W. Sperl
Children's Hospital, LKA Salzburg, Austria

W. Patsch
Department of Laboratory Medicine,
LKA Salzburg, Austria

Keywords Polymorphism · Sudden infant death · Uncoupling protein

Abbreviations *β3-AR* β3-adrenergic receptor · *BAT* brown adipose tissue · *SID* sudden infant death · *UCP* uncoupling protein

Introduction

Evidence from epidemiological and metabolic studies strongly suggests that thermal stress is a major risk factor for sudden infant death (SID) [6, 10, 12, 13,14]. Regulation of thermogenesis of the newborn is achieved by non-shivering metabolic activity in the brown adipose tissue (BAT). When infants face cold stress, norepinephrine levels increase and act via β3-adrenergic receptors (β3-AR) in the BAT to stimulate lipolysis [6,8]. The released free fatty acids activate the uncoupling proteins (UCP) which are components of the inner mitochondrial membrane [8]. Human UCP-1, expressed only in BAT, promotes the dissipation of the mitochondrial proton gradient through the membrane and short-circuits ATP-synthase, which results in energy dissipation as heat [8].

Two conflicting hypotheses associating the UCPs with the aetiology of SID have been proposed. Lean and Jennings [11] found normal UCP content but active uncoupled thermogenesis in two cases of SID with an increased core temperature post-mortem. They hypothesised that BAT thermogenesis occurring inappropriately in a warm well insulated infant could be a cause of some cases of SID. Conversely, Douglas [3] suggested that a decreased level of UCP in BAT may cause SID due to a lowered thermogenic response to acute cold exposure. This hypothesis is based on the finding of a reduced UCP protein concentration found in axillary BAT of SID victims [12].

A Bcl I polymorphism located at –3826 bp relative to the transcription start site in the *UCP-1* gene promoter region [2] imparts a reduced UCP-1 mRNA expression

in abdominal adipose tissue [4]. Both, this UCP-1 Bcl I polymorphism and the Trp64Arg variant in exon 1 of the *β3-AR* gene [18] have been associated with indices of obesity [7,15]. The simultaneous occurrence of both polymorphisms is linked to a reduced basal metabolic rate [16]. Therefore these polymorphisms appeared particularly suitable for studying a possible relation of the *UCP-1* and *β3-AR* genes with the occurrence of SID.

The aim of our study was to determine the allele frequencies and genotype distributions of the UCP-1 Bcl-I polymorphism and the Trp64Arg variant of the *β3-AR* gene in Austrian SID victims and in a control group.

Subjects and methods

Subjects

Dried blood samples of 60 SID victims who died in the years 1988–1998 in three federal states of Austria (Vienna, Salzburg and Tyrol) and of 60 controls (born on the same day) were obtained from the National Neonatal Screening Laboratory at the Department of Paediatrics, University of Vienna. The diagnosis of SID was based on autopsies in all cases. The clinical characteristics of the majority of the SID patients have been reported previously [10]. The study was approved by the ethics review board of the Vienna General Hospital.

DNA extraction

DNA was extracted from the dried blood samples using Chelex 100 chelating resin (Promega, Mannheim, Germany) [17] and further purified by ultrafiltration (Microcon-50, Amicon).

Nested PCR and restriction digestion

A 310 bp fragment of the *UCP-1* promoter region containing the Bcl I site was amplified by nested PCR using the primers 5'-CTT GGG TAG TGA CAA AGT AT-3', 5'-CCA AAG GGT CAG ATT TCT AC-3' in the first round, and 5'-AGT GAT ATC TGT CAT TTG CAC-3', 5'-AGG TCA GTA TGA GCA AGG GC-3' in the second round. PCR amplification was successful in 53 of 60 SID cases and in 54 of 60 controls. The amplification product was digested with Bcl I resulting in a 130 bp and a 180 bp fragment for the wild type allele, and a 310 bp fragment for the mutant allele. A 210 bp fragment of exon 1 of the *β3-AR* gene was amplified using the primers 5'-GCT CTC ATG CCT TGC TGT C-3', 5'- AGG AGT CCC ATC ACC AGG TC-3' in the first round, and 5'-CGC CCA ATA CCG CCA ACA C-3', 5'-CCA CCA GGA GTC CCA TCA CC-3' in the second round. PCR amplification was successful in 51 of 60 SID cases and in 50 of 60 controls. The product was digested with BstN I resulting in fragments of 100 bp, 60 bp, 30 bp, 12 bp, and 7 bp for the wild type allele, and 160 bp, 30 bp, 12 bp, and 7 bp for the mutant allele. The fragments were separated on agarose gels. In 46 SID victims and in 44 controls, genotypes could be determined at both the *UCP-1* and the *β3-AR* locus.

Statistical analysis

The allele frequencies and the frequencies of genotype combinations in the SID versus control groups were compared by chi-square tests calculated using the SPSS-PC program package; P values < 0.05 were considered as significant.

Results

The allele frequencies of the UCP-1 Bcl I polymorphism and the Trp64Arg variant of the β3-AR did not differ significantly between the SID and the control group (Table 1) and were similar to those in earlier studies involving adult populations [4, 7, 16,18]. The observed genotype distributions were in accordance with the Hardy-Weinberg equilibrium as judged by chi-square goodness of fit tests (data not shown). As synergistic effects of the two polymorphisms have been described in adults [16], the six genotype combinations observed in the SID group and the controls were analysed for an association with the occurrence of SID by chi-square tests (Table 2). No significant associations were detected.

Discussion

Several explanations may be offered for our findings, which do not confirm two earlier studies suggesting a link between UCP and SID based on decreased UCP protein content [3,12] or altered uncoupling activity [11] of BAT in SID victims. First, both studies were post-mortem investigations conducted in very small numbers of subjects [11,12]. In contrast we investigated 53 autopsy confirmed SID cases that occurred in a specified geographic region over a period of 10 years and appropriate random controls. The allele frequencies in SID victims and controls in our study were very similar to the distributions in adult populations studied in Austria [4] and other countries [7, 16,18] confirming random selection of cases and controls. Second, the association of the *UCP-1* Bcl I and the *β3-AR* Trp64Arg polymorphisms with altered UCP-1 mRNA expression and altered energy metabolism has been documented in adults only and may not extend to infants. Moreover, several genes encoding proteins closely related to UCP-1 have been discovered recently [1,5]. Although UCP-1 is thought to be the major uncoupling protein mediating adaptive thermogenesis in BAT, it cannot be ruled out that UCP-2 or UCP-3 contribute to the pathogenesis of SID. Third, SID aetiology is likely to be quite heterogeneous.

Table 1 Allele frequencies of the *UCP-1* Bcl I polymorphism and the *β3-AR* Trp64Arg variant in SID victims and controls

Allele frequencies	*UCP-1* BcI I		*β3-AR* Trp64Arg	
	SID ($n=53$)[a]	Controls ($n=54$)	SID ($n=51$)[b]	Controls ($n=50$)
Wildtype	0.65	0.72	0.89	0.93
Variant	0.35	0.28	0.11	0.07

[a]Not significant, chi-square = 1.25, df = 1, $P=0.26$
[b]Not significant, chi-square = 1.25, df = 1, $P=0.26$

Table 2 Distribution of genotype combinations of the *UCP-1* Bcl I and the *β3-AR* Trp64Arg polymorphisms in 46 SID victims and 44 controls (*W* wildtype, *V* variant)

Genotype combinations		
UCP-1 Bcl I/*β3-AR* Trp64Arg	SID[a]	Control
WW/WW	19	21
WW/WV	2	2
WV/WW	13	13
WV/WV	7	5
VV/WW	4	3
VV/WV	1	0
Sum	46	44

[a]Not significant, chi-square = 3.8, df = 5, $P = 0.57$

A possible association of *UCP-1* and *β3-AR* polymorphisms with SID in a subgroup of SID victims may have been missed due to this heterogeneity. Finally, whereas there is strong evidence for a contribution of thermal stress to the pathogenesis of SID, the underlying mechanisms remain unclear. Thermal stress may precipitate SID e.g. via disturbances of ventilatory control [6,9], rather than via abnormal thermoregulation involving the UCPs and the β3-AR. Thus, our findings are not inconsistent with current SID preventive rules calling for an avoidance of overheating.

Acknowledgements The authors gratefully acknowledge the assistance of M. Trnka and H. Rozboril. This work was supported by the "Anton Dreher-Gedächtnisschenkung für medizinische Forschung" Grant #331–1999.

References

1. Boss O, Samec S, Paoloni-Giacobino A, Rossier C, Dulloo A, Seydoux J, Muzzin P, Giacobino JP (1997) Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. FEBS Lett 408: 39–42
2. Cassard-Doulcier AM, Bouillaud F, Chagnon M, Gelly C, Dionne FT, Oppert JM, Bouchard C, Chagnon Y, Ricquier D (1996) The Bcl I polymorphism of the human uncoupling protein gene is due to a point mutation in the 5'-flanking region. Int J Obes Relat Metab Disord 20: 278–279
3. Douglas RJ (1992) Could a lowered level of uncoupling protein in brown adipose tissue mitochondria play a role in SIDS etiology? Med Hypotheses 37: 100–102
4. Esterbauer H, Oberkofler H, Liu YM, Breban D, Hell E, Krempler F, Patsch W (1998) Uncoupling protein-1 mRNA expression in obese human subjects: the role of sequence variations at the uncoupling protein-1 gene locus. J Lipid Res 39: 834–844
5. Esterbauer H, Schneitler C, Oberkofler H, Ebenbichler C, Paulweber B, Sandhofer F, Ladurner G, Hell E, Strosberg AD, Patsch JR, Krempler F, Patsch W (2001) A common polymorphism in the promoter of ucp2 is associated with decreased risk of obesity in middle-aged humans. Nat Genet 28: 178–183
6. Fleming PJ, Azaz Y, Wigfield R (1992) Development of thermoregulation in infancy: possible implications for SIDS. J Clin Pathol 45[Suppl]: 17–19
7. Fumeron F, Durack-Bown I, Betoulle D, Cassard-Doulcier AM, Tuzet S, Bouillaud F, Melchior JC, Ricquier D, Apfelbaum M (1996) Polymorphisms of uncoupling protein (UCP) and beta 3 adrenoreceptor genes in obese people submitted to a low calorie diet. Int J Obes Relat Metab Disord 20: 1051–1054
8. Garlid KD, Jaburek M, Jezek P, Varecha M (2000) How do uncoupling proteins uncouple? Biochem Biophys Acta 1459: 383–389
9. Guntheroth WG, Spiers PS (2001) Thermal stress in sudden infant death: is there an ambiguity with the re-breathing hypothesis? Pediatrics 107: 693–698
10. Kohlendorfer U, Kiechl S, Sperl W (1998) Sudden infant death syndrome: risk factor profiles for distinct subgroups. Am J Epidemiol 147: 960–968
11. Lean ME, Jennings G (1989) Brown adipose tissue activity in pyrexial cases of cot death. J Clin Pathol 42: 1153–1156
12. Lean ME, James WP, Jennings G, Trayhurn P (1986) Brown adipose tissue uncoupling protein content in human infants, children and adults. Clin Sci 71: 291–297
13. Ponsonby AL, Dwyer T, Jones ME (1992) Sudden infant death syndrome: seasonality and a biphasic model of pathogenesis. J Epidemiol Community Health 46: 33–37
14. Stanton AN (1984) Sudden infant death. Overheating and cot death. Lancet 2: 1199–201
15. Strazzullo P, Iacone R, Siani A, Cappuccio FP, Russo O, Barba G, Barbato A, D'Elia L, Trevisan M, Farinaro E (2001) Relationship of the Trp64Arg polymorphism of the beta3-adrenoceptor gene to central adiposity and high blood pressure: interaction with age. Cross-sectional and longitudinal findings of the Olivetti Prospective Heart Study. J Hypertens 19: 399–406
16. Valve R, Heikkinen S, Rissanen A, Laakso M, Uusitupa M (1998) Synergistic effect of polymorphisms in uncoupling protein 1 and beta3-adrenergic receptor genes on basal metabolic rate in obese Finns. Diabetologia 41: 357–361
17. Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10: 506–513
18. Widen E, Lehto M, Kanninen T, Walston J, Shuldiner AR, Groop LC (1995) Association of a polymorphism in the beta 3-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. N Engl J Med 333: 348–351