

Association between polymorphisms in the P2RY1 and SSTR2 genes and sudden infant death syndrome

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Abstract

Introduction Sudden infant death syndrome (SIDS) is a multifactorial syndrome and we believe that an inefficient respiratory response to certain homeostatic stressors, such as hypoxia and hypercapnia, is a key factor in the etiology of SIDS. Hence, we genotyped two single nucleotide polymorphisms (SNPs) in genes of importance for respiratory control: P2RY1 (adenosine diphosphate/adenosine triphosphate receptor) and SSTR2 (somatostatin receptor).

Methods Two SNPs, Rs1466113 (C>G dimorphism in SSTR2) and rs701265 (A>G dimorphism in P2RY1), were typed in 175 SIDS cases and 195 controls and 275 SIDS cases and 338 controls, respectively. Genotyping was performed using TaqMan technology.

Results The determined genotype frequencies were SSTR2: CC (14.4 %), CG (49.7 %), GG (35.9 %) in controls and CC (17.1 %), CG (49.1 %), and GG (33.8 %) in SIDS; P2RY1: AA (70.6 %), AG (28.7 %), GG (0.7 %) in SIDS and AA (68.3 %), AG (27.9 %), and GG (3.8 %) in the control group. For rs701265 in P2RY1, homozygous G carriers were significantly more frequent in the control group ($p=0.02$).

Conclusion We think that allele G provides a protective effect in events of ventilatory stress. Moreover, the significant lack of P2Y1 G allele homozygotes in the SIDS group shows that respiratory response plays an important role in the etiology of SIDS. Thus, we believe it is worthwhile to further investigate functional polymorphisms within genes that are involved in respiratory control in the future.

Keywords SIDS · Respiratory control · ADP/ATP receptor P2Y1 · Somatostatin receptor SSTR2

Introduction

The etiology of sudden infant death syndrome (SIDS), the leading cause of death in infants aged between 1 month and 1 year, still remains unclear [1]. According to the triple risk hypothesis, SIDS is a multifactorial syndrome [2] and the endogenous risk of SIDS might also be determined by genetic factors [3]. A widely spread theory is the so called *brainstem hypothesis* which indicates a relationship between autonomic nervous system dysfunction and SIDS [1, 4]. Abnormalities in the medullary serotonergic system seem to be one leading contributor to the brainstem hypothesis, supported, e.g., by the fact that SIDS was associated with a functional polymorphism (serotonin transporter-linked polymorphic region, 5HTTLPR) in the promoter region of the serotonin transporter gene SLC6A4 [5, 6]. As several other neurotransmitter systems also control the activity of the autonomic nervous system and might contribute to possible brainstem pathologies, our study focuses on the neurotransmitters somatostatin and adenosine triphosphate.

The tetradecapeptide somatostatin (SST) is one of the main inhibitory regulators of the endocrine system [7]. Its receptors (SSTRs) are widely distributed in the central nervous system [8]. During the first six postnatal months, the density of somatostatin receptors in respiratory nuclei of the human brainstem decreases. Furthermore, this remodeling is accompanied by a decrease in the frequency of apneas during the postnatal period [9]. Interestingly, a significantly higher density of SSTRs has been found in several respiratory nuclei (e.g., the medial and lateral parabrachial nuclei) in the brainstems of SIDS victims [10]. This imbalance might indicate a delay in maturation of this transmitter system, which may contribute to an inadequate ventilatory response to, e.g., hypoxia.

On the other hand, adenosine triphosphate (ATP) is an important mediator of peripheral and central chemosensory

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transduction. An increase in the level of carbon dioxide (hypercapnia) and protons triggers an immediate release of ATP from chemosensitive areas on the ventrolateral surface of the *medulla oblongata* [11]. As a result, an adaptive enhancement in breathing is generated in the preBötzing complex (preBötC) [11–13]. Our study concentrates specifically on the metabotropic adenosine diphosphate (ADP)/ATP receptor P2Y1 that is found in neuronal as well as glial compartments [14, 15] and mediates an increase in inspiratory frequency in the preBötC as a response to hypercapnia and hypoxia [13]. To further investigate the role of somatostatin and ATP neurotransmission in SIDS, we analyzed two single nucleotide polymorphisms (SNPs): Rs1466113, a G>C change in intron 1 of the SSTR2 gene, which is correlated with a high body mass index and increased food intake in Mediterranean adults [7]. Rs701265 is a common A>G polymorphism in the coding region (exon 1) of P2RY1 whose G allele has been associated with an increased platelet fibrinogen binding in response to ADP [16]. We concluded the G allele provides a more efficient receptor, which might offer a protective effect by increasing the ADP/ATP transmission during events of homeostatic (e.g., ventilatory) stress. Thus, we proposed either an association between the control group and the G allele or between the SIDS group and allele A, which we assumed to be less effective.

Patients and methods

In the initial study, the SIDS group was composed of German Caucasian infants who had died unexpectedly in their first year of life, and whose death remained without a definite cause of death despite thorough postmortem

examination ($n=175$; 106 male and 69 female, with an average age of 142 days). The control group consisted of children and adults, whose samples were obtained from either routine paternity testing performed by the laboratory or during autopsy ($n=195$; 110 male and 85 female). This initial study showed a higher frequency of the G allele of the ATP receptor P2Y1 (rs701265) in the control group (data not shown). Thus, a total number of 275 SIDS (168 male and 107 female, with an average age of 133 days) and 338 control cases (187 male and 151 female) were included in the final data analysis of this SNP. This study has been approved by the local ethics committee.

Genomic DNA was extracted from either thymus tissue, blood, or saliva samples using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genotyping of the samples was performed on a 7500 Real-Time PCR System (Life Technologies, Darmstadt, Germany) with a predesigned TaqMan SNP genotyping assay (rs1466113, SSTR2, Assay ID: C__2167504_10, Life Technologies) and a Custom TaqMan SNP genotyping assay (rs701265, P2Y1) with primers and fluorescently labeled probes adapted from Sibbing et al. [17]: primer F: 5'-CTCTCCTCTGAGGAGAAAATCGAT-3'; primer R: 5'-AAGGGATGTAAGACACAGCAAAAAC-3'; and probes: 5'-FAM-TACCTGGTATCATTG-3' and 5'-VIC-TACCTGGTGATCATTG-3' (with letters in italics indicating the SNP position). Amplification parameters for both assays were: 95 °C for 10 min followed by 40-cycles of 95 °C for 15 s and 62 °C for 1 min and a final hold of 60 °C for 1 min. To identify any differences in genotype and allele frequencies between SIDS samples and controls, exact tests for deviation from Hardy–Weinberg equilibrium and a Fisher's exact test was performed.

Fig. 1 Allele and genotype frequencies for SNP rs1466113 (G>C change in SSTR2) in SIDS and controls

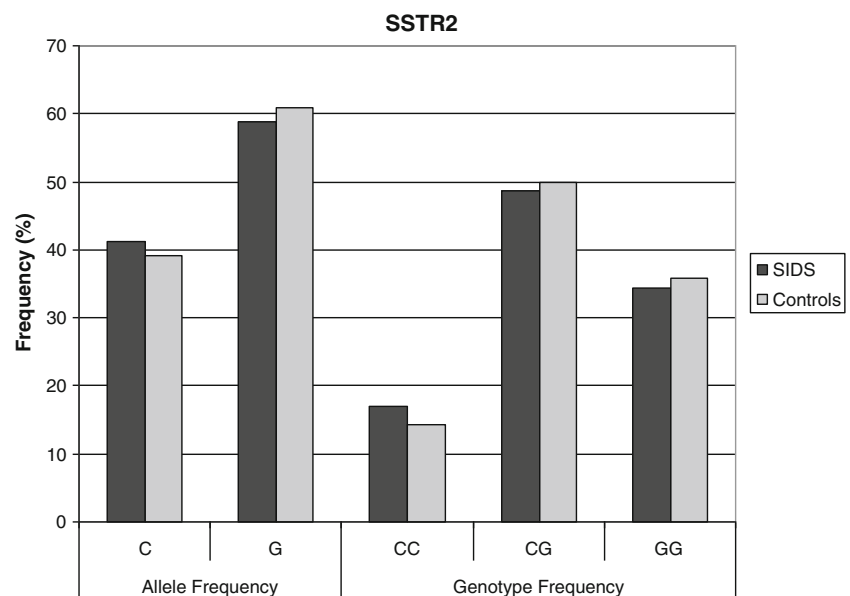
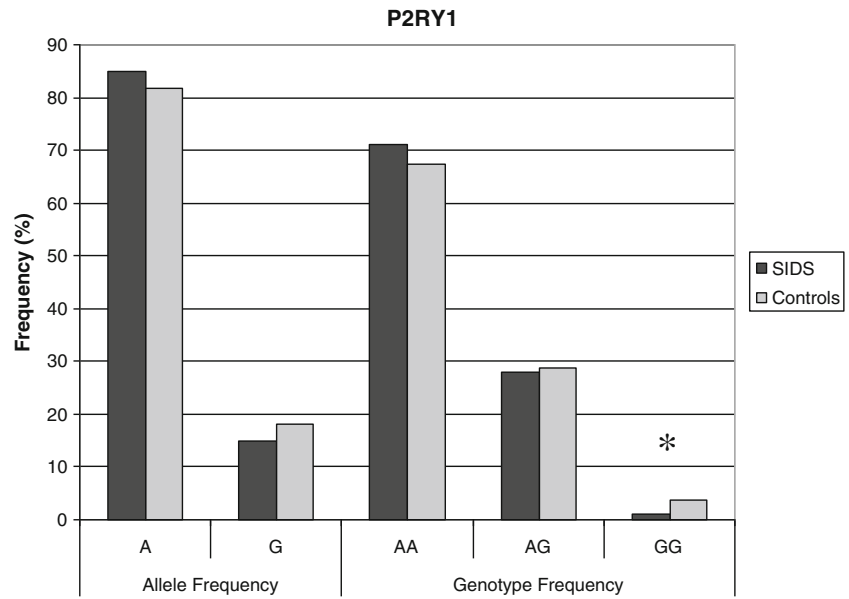


Fig. 2 Allele and genotype frequencies for SNP rs701265 (A>G change in P2RY1) in SIDS and controls. The significant difference in GG genotype frequency is marked by an *asterisk* ($p=0.02$)



Results

The results of the statistical tests are shown in Fig. 1 and 2 and Table 1. For the SNP rs1466113 in the SSTR2 gene, genotype frequencies of both groups comply with Hardy–Weinberg equilibrium (Hardy–Weinberg exact test; SIDS, $p=0.89$; controls, $p=0.55$). Genotype frequency of the SNP rs701265 in the P2RY1 gene shows Hardy–Weinberg compliance for the control group (Hardy–Weinberg exact test; $p=0.38$); however, it does not for the SIDS group (Hardy–Weinberg exact test; $p=0.04$). For the SSTR2 polymorphism, the genotype distribution is CC (17.1 %), CG (49.1 %), and GG (33.8 %) in the SIDS group and CC (14.4 %), CG (49.7 %), and GG (35.9 %) in the control group. In the two groups, allele G and C show a comparable frequency (allele C, 41.7 %; allele G, 58.3 % in SIDS; allele C, 39.2 %; and allele G, 60.8 % in controls) and a significant association with SIDS was not found for this

polymorphism regarding the allele distribution (Fisher’s exact test; $p=0.5$). Regarding the allelic distribution of the P2Y1 polymorphism, a slightly higher frequency of allele A (84.9 %) was observed in SIDS cases compared to controls (82.2 %). This difference, however, could not be proven significant (Fisher’s exact test; $p=0.21$). The heterozygous AG genotype was quite similar in both groups (28.7 and 27.9 %, respectively). The AA genotype was more frequent in SIDS cases (70.6 %) than in controls (68.3 %), and the GG genotype had a significantly higher frequency in the control group compared to the SIDS group (3.8 and 0.7 %, respectively; Fisher’s exact test; $p=0.02$).

Furthermore, we looked for associations for allele and genotype frequencies according to gender and age at death (0–45, 46–150, and 151–365 days). The former significant result of the GG genotype could be confirmed in males (Fisher’s exact test; $p=0.006$), however not so in females (Fisher’s exact test; $p=0.54$). Regarding the age at death, our data showed that children who died at an age between 46 and 150 days had significantly less often the G allele and the GG genotype compared to both controls (data not shown) and SIDS cases that died between 151 and 365 days (see Table 1).

Table 1 Total counts of alleles and genotypes for P2Y1 in SIDS cases according to age at death. p values were calculated using chi-squared test and refer to the G alleles and GG genotypes, respectively

	0–45 Days	46–150 Days	151–365 Days
A	80	228	132
G	14	28	32
p value		n.s.	0.012
AA	34	100	50
AG	12	28	32
GG	1	0	0
p value		n.s.	0.007

n.s. not significant

Discussion

There is evidence that polymorphisms of relevance for various neurotransmitter systems associated with respiratory regulation are involved in the etiology of SIDS [18–22]. Our study focuses on both somatostatin and adenosine triphosphate, which play a notable role in respiratory control [11, 12] and we proposed an involvement of single nucleotide polymorphisms within the somatostatin receptor

2 gene SSTR2 and the ADP/ATP receptor gene P2RY1 in the pathogenesis of SIDS.

A plausible interpretation why there is a lack of association of SSTR2 and SIDS could be that although somatostatin does play a certain role in ventilation regulation, it may not be one of the crucial factors, at least not in respect to the etiology of SIDS. Moreover, there could be the possibility that the receptor, and this particular SNP rs1466113, is not one of the key players, as other parts of the pathway may be, such as an ineffective transporter or an enzyme that is important for synthesis or degradation of somatostatin.

The G allele of rs701265 in the P2RY1 gene, however, provides a more efficient receptor [16]. Therefore, it might contribute to a protective effect by increasing the ADP/ATP transmission during events of ventilatory stress. In fact, we could show that homozygous P2Y1 G allele carriers were significantly more frequent in the control group. Moreover, we were able to confirm this result by testing male SIDS cases as well as children who died between 46 and 150 days against the control group. The logical conclusion (allele A being more frequent in the SIDS group) could be shown, a statistical significance, however, could not be reached ($p=0.21$). P2Y1 receptors are highly expressed in the preBötC and therefore, P2Y1 seems to be a key mediator of preBötC frequency increase [13]. The facts that the ATP response can be mimicked by the selective P2Y1 receptor agonist MRS2365 [23] and inhibited by the P2Y1 receptor antagonist MRS2179 [13] further support this hypothesis. Since P2Y1 appears to be such an important player in respiratory regulation, it seems likely that other ATP receptors (e.g., the ionotropic P2X or other P2Y receptors) are also capable of mediating ATP responses in respiratory neurons and are therefore able to rescue a possible impairment of P2Y1 due to the A allele. This might be the reason, why allele A is not significantly more frequent in the SIDS group. Instead, it is likely that the G allele, by leading to an increasing ADP/ATP transmission, can be seen as a protective factor, which helps to successfully handle risky events of ventilatory stress.

Infants that died between days 46 and 150 of life, during the peak prevalence of SIDS, completely lacked the GG genotype and showed a significant decrease of allele G when compared to either SIDS cases that died after day 150 or to controls (Table 1). A similar finding was already reported for THO1 [19]. In our opinion, this finding further supports the heterogeneity of the diagnosis “SIDS” and that those infants that die during the peak prevalence are more likely to suffer from respiratory dysregulation, while those that die before or afterwards might show an accumulation of alternative etiologies (e.g., cardiac or metabolic disease).

Conclusion

In conclusion, we were not able to demonstrate a significant association of rs1466113 (C>G dimorphism in SSTR2) with SIDS. We could, however, show that homozygous carriers of the rs701265-derived allele G in P2Y1 are significantly more frequent in the control group and assume the G allele to be a protective factor. Nevertheless, these results have to be corroborated in further case–control studies. SIDS is a multifactorial syndrome and, in accordance to the triple risk hypothesis, a genetic component in the pathogenesis of the sudden infant death syndrome cannot be denied. We believe the genetic contribution that leads to an impaired arousal reaction or inadequate ventilatory response to either hypercapnia or hypoxia can be found within the medulla oblongata (brainstem hypothesis). Thus, and because of our findings, we find it worthwhile to further investigate functional polymorphisms within genes that are involved in respiratory control generated in medullary nuclei.

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Conflict of interest The authors declare no conflict of interest.

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