Monoamine Oxidase A Gene Polymorphism and the Pathogenesis of Sudden Infant Death Syndrome

Cornelius Courts, PhD*, Melanie Grabmüller, Dipl.-Biol.*, and Burkhard Madea, MD

Objectives To test the hypothesis that there is a significant association between functionally relevant allelic variants of the monoamine oxidase A (MAO-A) polymorphism and sudden infant death syndrome (SIDS).

Study design In a case-control study of 142 cases of SIDS and 280 sex-matched control cases, the distribution of allelic and genotype variants of a promoter polymorphism of the MAO-A gene was examined using polymerase chain reaction locus amplification and fluorescence based fragment length analysis.

Results There was a significantly differential distribution of allelic and genotype variants between females with SIDS and controls. Moreover, there was a significant association between SIDS in females and allelic and genotype variants, each related to a higher transcriptional activity at the MAO-A locus.

Conclusions Our results suggest a role of MAO-A in female SIDS pathogenesis exerted by functionally relevant allelic and genotype variants of the MAO-A polymorphism. However, with the complex and inconsistent evidence available to date, the impact of the MAO-A promoter polymorphism on SIDS etiology remains unclear. *(J Pediatr 2013;163:89-93)*.

Sudden infant death syndrome (SIDS)[1] is the major cause of death among infants between 1 month and 1 year of age in developed countries.[2-4] SIDS is generally regarded as a multifactorial disease and its occurrence is probably dependent on the coalescence of developmental, environmental, and predisposing (ie, genetic risk factors ['triple risk hypothesis']).[5,6] The latter include alterations in genes involved in catecholamine metabolism, and the control of serotonergic and noradrenergic regulation of autonomic respiration and/or arousal.[7-9]

Monoamine oxidase A (MAO-A) is involved both in serotonin and catecholamine metabolism and signaling. Its gene is located on the X-chromosome. Scant evidence of X-chromosomal genetic factors in SIDS has been produced[10] and there are few studies analyzing a possible relationship between SIDS and specific allelic variants of the length polymorphism in the promoter region of the MAO-A gene. This polymorphism appears to be associated to various disorders, and MAO-A was shown to be activated in cases of SIDS.[11] A complete deficiency of MAO-A is evidently compatible with life[12] but leads to behavioral alterations in mice[13,14] and humans.[15] Several of the 5 known variants of this MAO-A length polymorphism are apparently related to a measurable up- or down-regulation of transcription of the MAO-A gene,[16,17] thereby indicating an underlying functional context. Although there are no animal models for MAO-A overexpression, we hypothesize that MAO-A overexpression in the brain stem would lead to decreased serotonergic signaling, which could in turn favor SIDS in a subgroup of infants. This concept is supported by indirect evidence from animal models, where serotonergic signaling was impaired by mutated transcription factors,[18] autoinhibition by overexpression of serotonin 1A autoreceptors,[19] and a serotonin transporter knockout causing a SIDS-like phenotype.[20]

However, the evidence produced to date for associations of one or another variant of the MAO-A polymorphism with SIDS occurrence is conflicting. Nonnis-Marzano et al and Filonzi et al reported a significant relationship between allelic variants '4' and '3.5' with cases of SIDS (n = 20) and control cases.[21,22] In a 2010 reply to their article, Klintschar and Heimbold challenged these findings.[23] Filonzi et al thereupon revised their data and confirmed their original findings.[24] In the most recent study, Klintschar et al confirmed their preliminary data from 2010 by examining a considerably larger series of cases (n = 156) and presented evidence for a significant accumulation of the allelic variants '2' and '3' in males with SIDS.[25]

In any case, functional variants of MAO-A may be genetic determinants of significant individual differences in oxidizing capacity for critical MAO-A substrates. Hence, the aim of our study was to test the hypothesis that an association between functionally relevant allelic variants of the MAO-A polymorphism and SIDS can be established in an independent and similar sized set of cases.

Methods

Our retrospective study consisted of 142 formalin fixed paraffin embedded samples of German Caucasian infants with SIDS (90 males, 52 females) and 280 sex matched control cases (171 males, 109 females). In all cases, SIDS was diagnosed according to the current

MAO-A	Monoamine oxidase A
PCR	Polymerase chain reaction
SIDS	Sudden infant death syndrome

From the Institute of Legal Medicine, University of Bonn, Bonn, Germany

*Contributed equally.

The authors declare no conflicts of interest.

 http://dx.doi.org/10.1016/j.jpeds.2012.12.072

"San Diego" definition[1] and based upon a detailed and comprehensive post mortem investigation at the Institute of Legal Medicine, University Hospital Bonn, Germany. Males (45.6%) and females (61.5%) with SIDS, respectively, were in the risk age interval between 46 and 154 postnatal days. The controls were German Caucasian and consisted of biopsy material taken from the psoas major muscle during autopsy (153 males, 100 females, taken from cases where potentially confounding alterations of autonomic and central nervous system could be excluded) and voluntarily donated saliva and blood samples (21 males, 9 females). The study design was approved by the local ethics committee.

For cases of SIDS, DNA was extracted from formalin-fixed paraffin-embedded tissue using the QIAamp DNA formalin-fixed paraffin-embedded Tissue Kit (Qiagen, Hilden, Germany). For control cases, the QIAamp DNA Investigator Kit (Qiagen) was used to extract DNA from saliva swabs and the Qiagen DNA Micro Kit (Qiagen) was used with dried blood specimens. All kits were utilized according to the manufacturer's prescription, respectively. DNA extraction was successful for all samples.

The MAO-A promoter region containing the polymorphism was amplified by polymerase chain reaction (PCR) using the primers listed in Table I and the following cycling conditions: initial denaturation at 95°C for 10 minutes, followed by 34 cycles of denaturation at 95°C for 1 minute, annealing at 62°C for 1 minute, elongation at 72°C for 1.5 minutes, and a final elongation at 72°C for 5 minutes. PCR was set up using standard conditions with 1 ng of DNA template and 1 U Taq-Polymerase (New England Biolabs Inc, Ipswich, Massachusetts) used per reaction.

PCR products were then separated electrophoretically using a 310 Genetic Analyzer (Applied Biosystems, Darmstadt, Germany), and allelic variants were identified by comparison with a size standard using the Gene Mapper software (Applied Biosystems).

Statistical Analyses

To account for small sample sizes in the expected value slots of the contingency tables, a Monte Carlo operation with 10 000 simulations was performed (threshold of significance was set at 99%) to avoid grouping of categories.[26] Fisher exact tests, Monte-Carlo simulations, calculations of OR, and possible departure from Hardy–Weinberg equilibrium were performed using the SPSS software v. 19 (SPSS Inc, Chicago, Illinois).

Table I. Primers for MAO-A locus amplification

Primer	Final conc. [μM]	Sequence (5′→3′)	Reference
MAO-A-f	0,5	FAM-ACAGCCTGACCGTGGAGAAG	[16]
MAO-A-r	0,5	AACGGACGCTCCATTCGGA	

FAM, a fluorescein amidite (FAM)-fluorophor was added to this primer's 5′-end; *Final conc.*, final concentration.

Results

Distribution of Allelic Variants of the MAO-A Polymorphism

A significantly differential distribution of allelic variants was observed for all cases (Table II). To examine whether differential distribution was sex-specific, the analysis was repeated separately for each sex; a significantly differential distribution of allelic variants was found in female but not in male cases of SIDS versus controls (Table II). We also examined the distribution of allelic variants grouped according to their expression status: 'high' allelic variants '3', '5', and '4' and 'low' variants '2' and '3' were significantly associated with female cases of SIDS and control cases, respectively (Table II). Also, there was no relevant association between allelic variants or combinations thereof with all male or female cases of SIDS in the risk age interval between 46 and 154 postnatal days (data not shown).

Distribution of Genotype Variants of the MAO-A Polymorphism

Owing to hemizygosity for the MAO-A locus in male cases, genotype distribution was only analyzed for female cases. A significantly different distribution of genotype variants could be established between female cases of SIDS and control cases with the genotypes containing allele 3 accumulating in female control cases and the genotypes and 4/4 being relatively more frequent in cases of SIDS (Table III).

To further differentiate the 3 most common genotypes (3/3, 3/4, and 4/4) present in 94.2% and 95.4% of females with SIDS and controls, respectively, we dubbed them according to their hypothetical expression state: genotypes 3/3 and 4/4 being homozygous for allelic variants associated with low ('3') and high ('4') transcriptional activity, respectively, were termed 'low' and 'high', accordingly. Hence, the heterozygous genotype 3/4 was termed 'intermediate'. We then examined whether there was a differential distribution of expression states by genotype

Table II. Distribution of MAO-A promoter allelic variants in cases with SIDS and controls

	All		Males		Females	
Allele	SIDS n (%)	Control n (%)	SIDS n (%)	Control n (%)	SIDS n (%)	Control n (%)
2	7 (3.6)	0 (0.0)	3 (3.3)	0 (0.0)	4 (3.8)	0 (0.0)
3	59 (30.4)	152 (39.1)	31 (34.4)	58 (33.9)	28 (26.9)	94 (43.1)
3,5	2 (1.0)	5 (1.3)	1 (1.1)	1 (0.6)	1 (1.0)	4 (1.8)
4	126 (64.9)	231 (59.4)	55 (61.1)	112 (65.5)	71 (68.3)	119 (54.6)
5	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Total	194 (100)	389 (100)	90 (100)	171 (100)	104 (100)	218 (100)
	p_a .001*		p_a .089*		**p_a .002***	
	p_{exp} .239*		p_{exp} .586*		**$p_{exp\text{-}a}$ .038***	
	OR_{exp} 1.244		OR_{exp} .845		$OR_{exp\text{-}a}$ 1.706	
	CI 1.001-1.593		CI 0.358-1.673		CI 1.477-2.082	

p_a, *P* value for differential distribution of allelic variants between SIDS and control cases; p_{exp}/OR_{exp}, *P* value/OR for association between pooled 'low' alleles (2 and 3) and SIDS.
*Derived from Fisher exact test based on 10 000 Monte-Carlo simulations; significant *P* values are bolded.