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**PAPER**

**PATHOLOGY/BIOLOGY**

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# Significant Association of TH01 Allele 9.3 and SIDS

**ABSTRACT:** Sudden infant death syndrome (SIDS) constitutes a considerable percentage of infant death of unknown etiology. Individual catecholamine response variation is suspected to play a role in SIDS. TH01 is a tetrameric short tandem repeat marker in the tyrosine hydroxylase gene, which regulates gene expression and catecholamine production with allele 9.3 exerting a particularly strong effect on noradrenalin production. We investigated in an age-controlled study the TH01 allele frequencies in 127 cases of SIDS and 406 control cases to assess whether in SIDS cases a distinct TH01 allele distribution could be determined as has been reported by a previous study. We found that genotypes containing one or two 9.3 alleles were significantly more frequent in SIDS patients (58.2%) than in control subjects (48.4%, $p = 0.038$), whereas all other alleles were more frequent in the control subjects. Our findings support the notion that there exists a significant association between TH01 gene configuration and SIDS.

**KEYWORDS:** forensic sciences, sudden infant death syndrome, TH01 polymorphism, forensic genetics, catecholamine forensic pathology, tyrosine hydroxylase, genotyping

The sudden infant death syndrome (SIDS) is defined as "the sudden unexpected death of an infant <1 year of age with onset of the fatal episode apparently occurring during sleep, that remains unexplained after a thorough investigation, including performance of a complete autopsy and review of the circumstances of death and the clinical history" and subcategorized by revised definition (1). SIDS is still the major cause of death among infants between 1 month and 1 year of age in industrialized countries with an incidence of 0.529 per 1000 live births in 2003 (2). This disorder is influenced by situational and predisposing risk factors of which the situational, environmental risk factors, or triggers (exposure to smoke, sleeping in prone position, sleeping environment, overheating) are sufficiently well known (3–5). The predisposing, biological risk factors are diverse and less well understood including alterations in genes involved in metabolism, immune regulation, and catecholamine production, as well as conditions such as abnormal brainstem mechanisms in the control of respiration, chemosensitivity, autonomic regulation, and/or arousal (6,7).

It has been suggested that individual variations in reactive catecholamine production and in noradrenalinergic signaling in particular may play a role in SIDS. TH01 is a tetrameric short tandem repeat (STR) marker in the tyrosine hydroxylase (TH) gene, which regulates gene expression and catecholamine production (Fig. 1) with the TH01 allele 9.3, exerting a special impact on noradrenaline production. In a previous study, a strong association of longer TH01 alleles and SIDS cases ($n = 172$) was found when compared to a control group ($n = 390$) (8). Since these findings have not yet been replicated and many genetic findings in SIDS have not been confirmed by succeeding studies, the present study investigates whether these important findings can be confirmed and thereby strengthened in an independent robustly sized group of SIDS cases.

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## Materials and Methods

### *Patients and Diagnosis*

Our series consisted of 127 cases of SIDS and 406 age-matched control cases. No deceased controls were available. SIDS was diagnosed in all cases by a detailed postmortem investigation, consisting of an extensive autopsy and a case history review, excluding possible connections with previous vaccinations or infectious diseases, as well as toxicological, histological, and immunohistochemical analysis performed at the Institute for Forensic Medicine at the University Hospital in Bonn, Germany. All deceased infants were between 2 weeks and 1 year old. A doll scene re-enactment was not performed, and bed site and sleeping environment inspection was carried out based on police reports and photographs. Taken together, this classifies the cases of this study as "1B SIDS" according to a revised definition of Krous et al. (1). The control cases were healthy pediatric individuals of Caucasian origin. To prevent stratification, both SIDS patients and control subjects were chosen exclusively from the population of southern North Rhine-Westphalia.

### *DNA Extraction*

For SIDS cases, DNA was extracted from formalin-fixed and paraffin-embedded tissue using the All-tissue DNA Kit (Gen-Ial, Troisdorf, Germany). DNA from control cases was extracted from saliva swabs using a standard boiling lysis and chelation method (9).

### *Genotyping of TH01 Alleles*

The TH01 locus was amplified by polymerase chain reaction (PCR) using the primers listed in Table 1 and the following cycling conditions: initial denaturation at 95°C for 10 min, followed by 10 cycles of denaturation at 94°C for 1 min, annealing at 62°C for

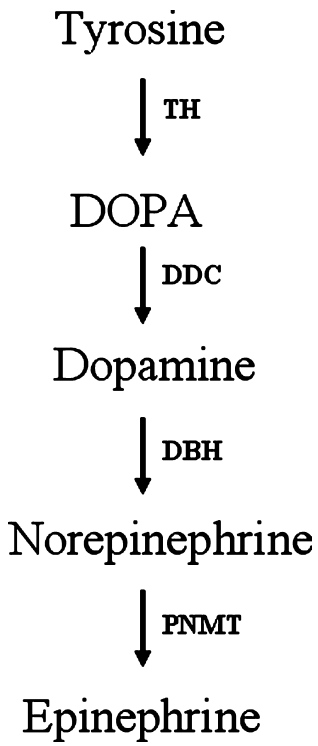


FIG. 1—*Simplified diagram of catecholamine synthesis and breakdown. TH, tyrosine hydroxylase; DOPA, 3,4-dihydroxyphenylalanine; DDC, DOPA decarboxylase; DBH, dopamine beta-hydroxylase; PNMT, phenylethanolamine N-methyltransferase.*

TABLE 1—*Primers for TH01 amplification—concentration and sequence.*

| Primer | Final Concentration | Sequence (5′ → 3′) | Reference |
|---|---|---|---|
| P1 | 1 μM | GTG GGC TGA AAA GCT CCC GAT TAT | (10) |
| P2 | 1 μM | GTG ATT CCC ATT GGC CTG TTC CTC | (10) |

1 min, elongation at 70°C for 2 min and 17 cycles of denaturation at 90°C for 1 min, annealing at 62°C for 1 min, elongation at 70°C for 2 min, and a final elongation at 72°C for 5 min. PCR was set up using standard conditions with 3 ng of DNA template and 1 U of genRES Plus DNA-Polymerase (serac, Bad Homburg, Germany) used per reaction.

PCR products were separated electrophoretically in a 6.9% PAA gel, and TH01 alleles were determined by comparison with a size standard.

### *Statistical Analysis*

Chi-squared tests and calculations of odds ratios and confidence intervals were performed using the program SPSS (SPSS Inc., Chicago, IL).

## Results

Table 2 lists TH01 allele frequencies in SIDS and control cases. The TH01 allele 9.3 is significantly more frequent in subjects affected by SIDS than in control cases (one-sided $p = 0.05$, odds ratio = 1.287). Conversely, all other alleles (non-9.3) are more frequent in control cases when compared to SIDS cases. Analysis of correlation of TH01 genotype frequencies and occurrence of SIDS shows that there is a significant association ($p = 0.038$, odds ratio = 1.467) of genotypes containing at least one 9.3 allele and the occurrence of SIDS (Table 3). Assessment of the influence of the infants' age of death did not point to differential frequencies of the 9.3 alleles in the respective age groups; however (Table 4), there was also no association between genotypes with no or at least one 9.3 allele and the respective age groups (Table 5).

TABLE 2—*Distribution of TH01 allele 9.3 in SIDS and control cases.*

| Allele | SIDS Cases (254 Alleles) Frequency [%] | Control Cases (812 Alleles) Frequency [%] |
|---|---|---|
| 5 | 0.0 | 0.12 |
| 6 | 20.87 | 24.26 |
| 7 | 17.72 | 18.47 |
| 8 | 11.81 | 11.95 |
| 9 | 15.35 | 15.64 |
| 9.3 | 34.25 | 28.82 |
| 10 | 0.0 | 0.74 |
| 9.3 | 34.25 | 28.82 |
| non-9.3 | 65.75 | 71.18 |
| $p(\chi^2) = 0.05$ OR = 1.287 | | |

9.3, 9.3 allele; non-9.3, all alleles other than 9.3; $p(\chi^2)$, one-sided $p$-value; OR, odds ratio.

TABLE 3—*Differential distribution of TH01 genotypes in SIDS and control cases.*

| | SIDS Cases | | Control Cases | | | |
|---|---|---|---|---|---|---|
| Genotype | *n* | [%] | *n* | [%] | OR | 95% CI |
| non-9.3/non-9.3 | 53 | 41.7 | 208 | 51.2 | 0.682 | 0.456–1.020 |
| non-9.3/9.3 | 61 | 48.0 | 162 | 39.9 | 1.392 | 0.932–2.078 |
| 9.3/9.3 | 13 | 10.2 | 36 | 8.9 | 1.172 | 0.601–2.286 |
| One or two 9.3 alleles | 74 | 58.2 | 198 | 48.8 | 1.467 | 0.980–2.194 |
| $p(\chi^2) = 0.038$ | | | | | | |

9.3, 9.3 allele; non-9.3, all alleles other than 9.3; $p(\chi^2)$, $p$-value; OR, odds ratio; 95% CI, 95% confidence interval.

TABLE 4—*Season-dependent distribution of TH01 alleles in SIDS cases.*

| Allele | Winter [%] | Spring, Summer, Fall [%] |
|---|---|---|
| 6 | 17.50 | 17.91 |
| 7 | 25.0 | 11.94 |
| 8 | 7.5 | 14.93 |
| 9 | 17.50 | 16.42 |
| 9.3 | 32.50 | 38.81 |
| 9.3 | 32.50 | 38.81 |
| non-9.3 | 67.50 | 61.19 |
| $p(\chi^2) = 0.399$ | | |

9.3, 9.3 allele; non-9.3, all alleles other than 9.3; $p(\chi^2)$, $p$-value.

## Discussion

Recently, associations between genetic markers in populations and disease are frequently reported. However, many of these associations have no evident biological relevance and rarely encourage hypothesizing about pathophysiology. Of late, Klintschar et al. (8) reported a strong association of TH01 polymorphism and

TABLE 5—*Age-dependent distribution of TH01 genotypes in SIDS cases.*

| Genotype | Winter [%] | Spring, Summer, Fall [%] |
|---|---|---|
| non-9.3 allele | 50.0 | 37.31 |
| one or two 9.3 alleles | 50.0 | 62.69 |
| $p(\chi^2) = 0.310$ | | |

$p(\chi^2)$: *p*-value.

occurrence of SIDS ($n = 172$) when compared to a control group. The polymorphism results in tandem repeats that modulate gene activity and thus catecholamine synthesis. Catecholamines play an essential role in regulating respiration and also response to stress. Therefore, this association of a polymorphism with SIDS can be biologically meaningful and should be thoroughly analyzed, backed up, and confirmed with independent evidence.

The present study hence comes up with a robust sample size and is in concordance with the study by Klintschar et al. (8) and thus for the first time confirms the important notion that there indeed exists a significant association between the allelic configuration of the TH01 locus and the occurrence of SIDS, although our data do not support a correlation between age of death and TH01 allelic configuration that was reported by Klintschar et al. This incongruence could be explained by an untypical age distribution in our series of SIDS cases, which does not show a higher frequency of infants of an age between 2 and 4 months.

A possible interpretation of these data hints at the potential existence of distinct genetically variant subgroups within the SIDS entity in which an impairment of the regulation of noradrenalinergic signaling, imparted by dysfunctional polymorphic gene variants, contributes to an adverse outcome, independent from the infants' age or season. Thus, a conceivable pathomechanism could include deficient regulation of respiration mediated by dysregulated catecholamine expression. To assess whether such a mechanism does exist and how strong its pathogenic influence may be, quantitative gene expression studies should be performed to directly correlate TH01 genotype and catecholamine expression.

## Conclusion

This study is the first to confirm the existence of a significant association of the TH01 allele 9.3 as well as the TH01 locus genotype configuration and the occurrence of SIDS. It therefore supports the necessity of further and functional analysis of the role of catecholamine expression in the pathomechanism of SIDS.

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