

Sudden Infant Death Syndrome and Sudden Intrauterine Unexplained Death: Correlation Between Hypoplasia of Raphé Nuclei and Serotonin Transporter Gene Promoter Polymorphism

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ABSTRACT: This study, besides to delineate the cytoarchitecture and the localization in the brainstem of the human raphé nuclei, aims to evaluate the correlation between neuropathological raphé defects and serotonin transporter gene (*5-HTT*) promoter region polymorphisms in a cohort of 28 SIDS victims, 12 sudden intrauterine unexplained deaths (SIUD), and 17 controls. Hypoplasia of one or more nuclei of both the rostral and caudal raphé groups was found in 57% of SIDS, in 67% of SIUD, and only in 12% of controls. Furthermore, a significant correlation among *5-HTT* Long (L) allele, hypoplasia of the raphé nuclei, and maternal smoking in pregnancy was observed in sudden fetal and infant deaths. The presence of the L allele represents a predisposing factor for sudden fetal and infant death in association with morphologic developmental defects of the raphé nuclei and prenatal smoke exposure. A further consideration of the authors is that SIUD should not be regarded as a separate entity from SIDS, given the potentially shared neuropathological and genetic bases. (*Pediatr Res* 66: 22–27, 2009)

Neuropathological studies have implicated the serotonin [5-HT (5-hydroxytryptamine)] system, morphologically located in the brainstem “raphé” complex, in the etiology of SIDS. Specifically, a decrease in serotonergic receptor binding in medullary regions containing raphé neurons was reported in small cohorts of SIDS cases (*versus* controls), in the United States and Japan (1–4). Panigrahy *et al.* (1) demonstrated decreased 5-HT receptor binding in medullary regions important for carbon dioxide chemoreception in SIDS cases *versus* unmatched controls. Similarly, Ozawa and Okado (2) reported reduced expression of 5-HT receptors in serotonin cell bodies of the raphé system among SIDS cases *versus* unmatched controls. Kinney *et al.* (3) confirmed altered 5-HT receptor binding in these medullary regions in Native American Indians, a group at increased risk for SIDS. Paterson *et al.* (4) additionally described an increase in number and density of serotonin cell bodies in the raphé, particularly in the raphé obscurus nucleus, in SIDS victims.

The above-described neuropathological studies served as the impetus for investigation of genes regulating the synthesis, storage, membrane uptake, and metabolism of 5-HT, as well as related receptors in SIDS cohorts (4–15). Key positive

findings include the promoter region and intron 2 of the serotonin transporter gene (*5-HTT*) Long (L) allele, the L/L genotype, and the haplotype including the L alleles of both polymorphisms, and low frequency but apparent genetic variants in the *FEV* gene (11) and the *PHOX2B* gene (12). These results likely have a role in serotonin network dysfunction, resulting in a failure of autonomic and respiratory responses to hypoxia and/or hypercapnia, potentially resulting in risk for sudden death.

Although the association of the promoter polymorphism L allele of the *5-HTT* with SIDS has been widely documented, correlation with brainstem neuropathology and identification of all nuclei of the raphé complex in histopathologic studies in SIDS is limited. Further, the medullary serotonergic network in fetal life has not yet been described. Therefore, we aim to 1) clearly delineate the histology of the human raphé system in a cohort of SIDS victims and sudden intrauterine unexplained death (SIUD) cases; 2) determine the *5-HTT* promoter region polymorphism in the same cohort of SIDS and SIUD; and 3) clarify the relationship of the *5-HTT* L allele to cytoarchitectural alterations of the raphé complex in both the SIDS and SIUDs. Because maternal smoking is consistently identified as a risk factor for SIDS (16,17), the possible influence of maternal smoking in the serotonergic development will also be examined.

MATERIALS AND METHODS

Study Subjects. The study included three groups of Caucasian infants: a SIDS cohort, a SIUD cohort, and a control group.

SIDS and SIUD victims. The SIDS cases included 28 infants, 11 females, and 17 males, aged from 1 to 6 postnatal months (median age: 3.4 mo). The SIUD cases included 12 unexplained fetal deaths, 6 females and 6 males, aged 24–40 gestational weeks (median age: 38 wk). All cases were diagnosed after application of the 2006 guidelines provided by Italian law n.31 “Regulations for Diagnostic Post Mortem Investigation in Victims of SIDS and Unexpected Fetal Death.” This law imposes that all the infants suspected of SIDS, deceased suddenly within the first year of age, and all fetuses deceased after the 25th week of gestation without any apparent cause must undergo in depth anatomo-pathologic examination, particularly of the autonomic nervous system. For every case, a complete clinical history was collected. Additionally, mothers were asked to complete a questionnaire on their smoking habit,

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Abbreviations: 5-HT, serotonin (5-hydroxytryptamine); 5-HTT, serotonin transporter gene; L, long (allele); S, short (allele); SIUD, sudden intrauterine unexplained death

detailing the number of cigarettes smoked before, during, and after pregnancy. Eighteen of the 40 SIDS/SIUD mothers (45%) were active smokers before and during the pregnancy, taking more than three cigarettes/d. The remaining mothers (55%) admitted no history of cigarette smoking before or during the pregnancy. In addition, smoking habits of fathers and/or other persons living in the infant environment were investigated, but with poor achievement of information.

Controls. This group included 17 suddenly deceased subjects (11 infants—2 females, 9 males; aged from 2 to 8 postnatal months, median age: 3 mo) and 6 fetuses (2 females, 4 males; aged 25–40 gestational weeks, median age: 36 wk) in whom a complete autopsy and clinical history analysis established a precise cause of death. Specific diagnoses among the infant deaths were congenital heart disease ($n = 4$), severe bronchopneumonia ($n = 3$), myocarditis ($n = 1$), pulmonary dysplasia ($n = 2$), and mucopolysaccharidosis type I ($n = 1$). Specific diagnoses among the fetal deaths included chorioamnionitis ($n = 3$) and congenital heart disease ($n = 3$). Five of the 17 control mothers (29%) reported smoking of more than three cigarettes every day already before the pregnancy. The remaining mothers (71%) denied smoking.

Parents of all SIDS/SIUD cases and all controls provided written informed consent to both autopsy and genetic study, with the Milan University L. Rossi Research Center institutional review board approval.

Autopsy protocol. The autopsy protocol included published specific guidelines (18,19), as well as in depth anatomic-pathologic examination of the central autonomic nervous system and molecular analysis of the 5-HTT gene. The four samples obtained from each brainstem are shown schematically in Figure 1.

Specimen description. Fresh specimen marked in green was first collected near the obex and conserved in RNA-later reagent (AMBION, Inc, Austin, TX) for the genetic studies. Successively, the three samples marked in red in the figure were cut for the histologic examination.

The first “red” specimen, ponto-mesencephalic, includes the upper third of the pons and the adjacent portion of mesencephalon. The second red specimen extends from the rostral limit of the medulla oblongata to the adjacent caudal pons. The third red specimen takes as reference point the obex and extends 2–3 mm above it and below it. Red marked samples were embedded in paraffin and serially cut at intervals of 60 μm . For each level, twelve 5- μm sections were obtained, two of which were routinely stained using alternately hematoxylin-eosin and Klüver-Barrera stains and two undergone to serotonergic immunohistochemistry. The remaining sections were saved and stained as deemed necessary for further investigations.

Brainstem in depth histologic examination. Care was taken in examination of the main nuclei, including the following: parafacial nucleus, locus coeruleus and parabrachial/Kölliker-Fuse complex in pons/mesencephalon, and the hypoglossus, dorsal motor vagal, tractus solitarius, ambiguus, pre-Bötzing, inferior olivary, and arcuate nuclei in medulla oblongata.

In addition, a careful study was performed on the raphé complex, with attention to the serotonergic neurons grouped in clusters around the midline of the brainstem, along its rostro-caudal extension, according to the classification of Hornung (20).

Serotonergic immunohistochemistry. An immunohistochemical study by using a MAb to 5-HT was undertaken for mapping the 5-HT-containing neurons and fibers and to provide validation of the anatomical organization of

the raphé system. Tissue sections, after deparaffinizing and rehydrating, were immersed in EDTA 0.05M pH8, boiled three times for 5 min in a pressure cooker, and washed with TBS buffer. Each section was placed on the Dako cytomation automated immunostainer and incubated with the specific MAb at room temperature for 45 min, washed with TBS pH 7.6, and incubated in biotinylated goat anti-mouse anti-rabbit immunoglobulins (Dako REAL, cod.K5005; Dako cytomation, Glostrup, Denmark) at room temperature for 30 min. After incubation with the secondary antibody and a new wash with TBS pH 7.6, sections were incubated with streptavidin conjugated to alkaline phosphatase at room temperature for 30 min. A red chromogen solution was prepared as indicated by Dako REAL datasheet and used as enzyme substrate. Finally, each section was counterstained in Mayer’s Hematoxylin solution and coverslipped.

Serotonin transporter genotyping methods. DNA was isolated by the specific specimens conserved in RNA-later reagent, using Invisorb Spin Tissue Mini Kit (INVITEK, GmbH; Germany) and following the kit procedure.

The 5-HTT polymorphism was genotyped using specific primers according to Heils *et al.* (21) (forward: 5-GGCGTTGCCGCTCTGAATGC-3; reverse: 5-GAGGGACTGAGCTGGACAACCAC-3).

PCR was carried out in a final volume of 50 μL consisting of ~50 ng genomic DNA. Amplification was performed using 20 μL of genomic DNA, 1 \times PCR Gold buffer (Applied Biosystems, Foster City, CA), 2 mM MgCl₂, 0.4 mM dNTPs, 50 pmol each of the required primers, and 2U AmpliTaq Gold (Applied Biosystems). Temperature cycling was performed using an Applied Biosystems 2720 Thermal Cycler with the following new protocol, which was performed in our laboratory: 10 min at 95°C, followed by 40 cycles of 95°C for 1 min, and 61°C for 10 min, with a final extension for 7 min at 72°C. PCR amplification products were electrophoresed through a 1.5% agarose gel and visualized by UV-light in presence of ethidium bromide.

Statistical analysis. The statistical significance of direct comparison among polymorphisms of the serotonin transporter, morphologic alterations of the raphé nuclei, and smoking in SIDS, SIUD, and controls was evaluated with a JMP software (SAS Institute, Cary, NC). Contingency table and χ^2 tests were performed. Significance was assumed when $p < 0.05$.

RESULTS

Morphologic analysis of the raphé serotonergic system.

The anatomic-pathologic examination of the raphé system allowed for identification of the normal topography and cytoarchitecture of the different raphé nuclei. We identified two main clusters of nuclei located in the midbrain/rostral pons (the superior raphé group) and in the caudal pons/rostral medulla oblongata (the inferior raphé group), respectively, separated by a gap in the middle pons. The normal topography of the two raphé groups is shown in Figure 2 that repropose the Figure 1 with addition of the schematic brainstem sections where the raphé nuclei can be identified.

Rostral serotonergic raphé group. The rostral serotonergic raphé group, confined to the mesencephalon and rostral pons, includes three nuclei: the caudal linear raphé nucleus, the dorsal raphé nucleus, and the median raphé nucleus. In histologic sections of caudal mesencephalon, the caudal linear nucleus, located in the tegmental area ventrally to the superior cerebellar decussation, along the midline toward the ventral surface, has a limited number of neurons that are lengthened and arranged in parallel to the midline. The dorsal raphé nucleus is visible around the ventral periaqueductal gray, dorsally to the trochlear nucleus and to medial longitudinal fasciculus. Neurons of the dorsal raphé nucleus, with similar morphology to the caudal linear nucleus neurons, are found in the midline and extending laterally. The median raphé nucleus is identifiable in sections of rostral pons, as a median/paramedian cluster of neurons below the superior cerebellar decussation, ventral to the medial longitudinal fasciculus.

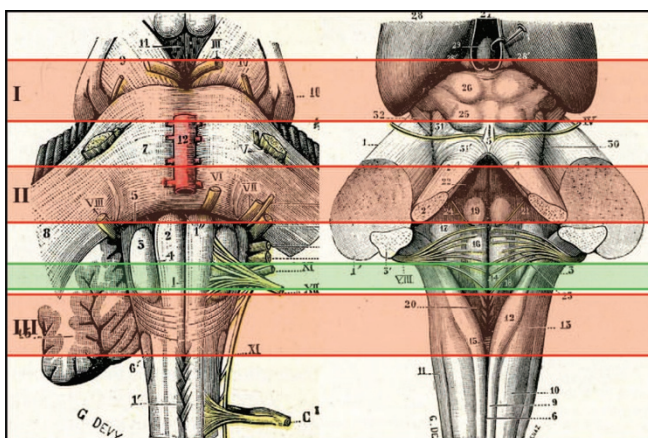


Figure 1. Schematic representation of the brainstem sampling: ventral (left) and dorsal (right) surface. Figure from Testut L 1923 [Human Anatomy, Book V: Central nervous system.] Unione Tipografico-Editrice Torinese, Turin, Italy.

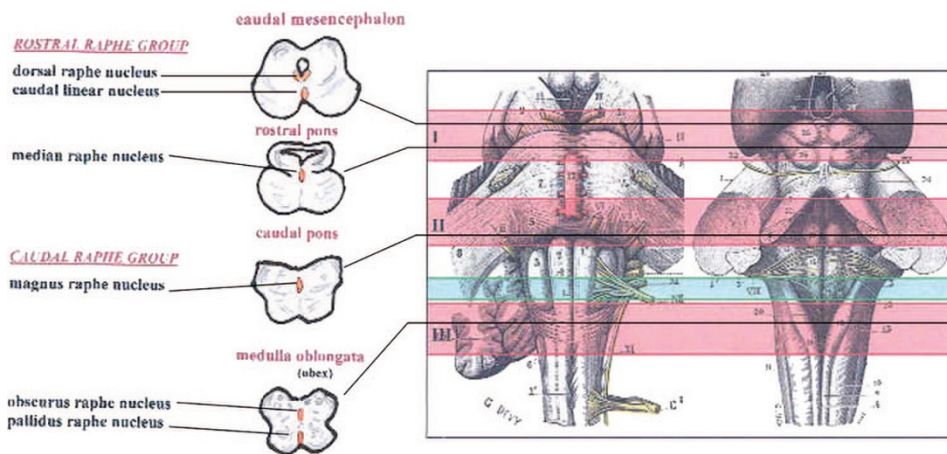


Figure 2. Schematic representation of the localization of the raphe nuclei in the brainstem histologic sections. Figure from Testut L 1923 [Human Anatomy, Book V: Central nervous system.] Unione Tipografico-Editrice Torinese, Turin, Italy.

Caudal serotonergic raphe group. The caudal serotonergic raphe group, extending from caudal pons to caudal portion of the medulla oblongata includes three nuclei: the magnus raphe nucleus, the obscurus raphe nucleus, and the pallidus raphe nucleus. In histologic sections of caudal pons, at the level of the parafacial complex and the trapezoid body, the magnus raphe nucleus includes a large group of neurons, adjacent to the midline and above the medial lemniscus. The obscurus raphe nucleus is identified in the dorsal half of the medulla oblongata, adjacent to the magnus raphe nucleus, composed of medium-sized neurons arranged in a main cluster along the midline, ventral to the fourth ventricle, with two small lateral subnuclei. The pallidus raphe nucleus is also found in the dorsal half of the medulla, with a small number of neurons in the ventral midline between the pyramids, overlying medial lemniscus. The identification of the different raphe serotonergic nuclei has been validated by the distribution of the cell bodies and axonal arborization 5-HT-immunopositive. Figure 3 shows as example the dorsal raphe nucleus in the mesencephalon, consisting of neurons bordering the ventricle. In Figure 3B, the neurons of this nucleus appear 5-HT-immunolabeled.

Brainstem raphe nuclei pathologic results. Table 1 summarizes the neuropathological findings of rostral and caudal serotonergic raphe groups. A subset of SIDS victims (57%) and a subset of SIUD victims (67%) demonstrated hypodevelopment/hypoplasia (*i.e.*, no or few neurons) of one or more nuclei of the raphe system of both the caudal and the rostral

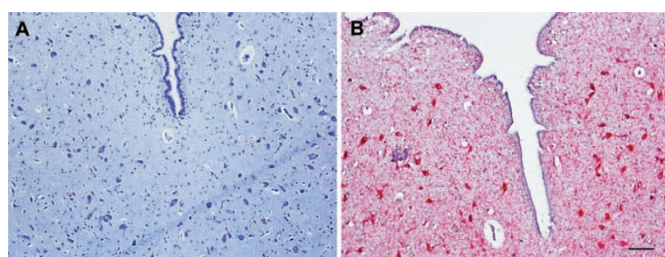


Figure 3. Dorsal raphe nucleus. A, Photomicrograph of its periventricular neurons. Klüver-Barrera stain. B, Photomicrograph of its immunolabeled periventricular neurons. 5-HT-immunohistochemistry. Scale bar: 50 μ m.

Table 1. Distribution of the raphe system hypoplasia observed on the whole in 16 of 28 SIDS (57%), 8 of 12 SIUD victims (67%), and 4 of 17 controls (24%)

Number of cases with hypoplasia (%)	Rostral raphe group			Caudal raphe group		
	CliR	DoR	MeR	MaR	ObR	PaR
SIDS						
8 (29%)	+	-	+	-	+	-
3 (11%)	+	-	-	-	+	-
1 (4%)	+	-	-	-	-	-
2 (7%)	-	+	-	+	-	-
1 (4%)	-	-	+	-	-	+
1 (4%)	-	-	-	-	+	-
Infant controls (n = 11)						
3 (27%)	-	-	-	-	+	-
SIUD						
5 (42%)	+	-	+	-	+	-
2 (17%)	+	-	-	-	+	-
1 (8%)	-	-	-	-	+	-
Fetal controls (n = 6)						
1 (17%)	-	-	-	-	+	-

groups. In 27% of the infant controls and 17% of the fetal controls, hypoplasia of the only obscurus raphe nucleus was observed.

Figure 4B shows, as example, the hypoplasia of the obscurus raphe nucleus (in Fig. 4A the normal morphology).

Brainstem pathologic results overall. Table 2 summarizes the histologic neuropathological findings of the brainstem nuclei. A subset of SIDS cases had hypoplasia of the medullary arcuate nucleus or the pre-Bötzing nucleus, or different raphe nuclei. A subset of SIUD cases had hypoplasia of the parafacial nucleus or different raphe nuclei (as specified later). A small subset of controls, both fetal and infant deaths, had hypoplasia of the arcuate nucleus and of the raphe obscurus nucleus. In all, a significantly greater proportion of neurologic alterations were observed in SIDS-SIUD cases compared with controls ($p < 0.01$).

Genetic analysis of the serotonin transporter gene. Table 3 summarizes the 5-HTT analysis results. Examination of the polymorphic region of the 5-HTT gene demonstrated the prevalence of the L allele in both SIDS and SIUD. The short (S) allele was more prevalent among controls. Specifically, the

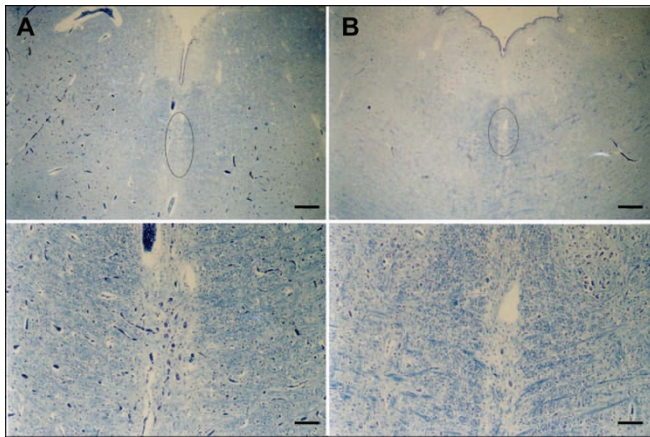


Figure 4. Obscure raphé nucleus. A, Normal structure. Below, higher magnification (Scale bar: 50 μ m). B, Hypoplasia. Below, higher magnification (Scale bar: 50 μ m). Klüver-Barrera stain.

Table 2. Overall neuropathological brainstem findings in 28 SIDS, 12 SIUD, and 17 controls

Study group	Number of cases (%)			
	Arcuate nucleus hypoplasia	Pre-Bötzing nucleus hypoplasia	Parafacial nucleus hypoplasia	Raphé nuclei hypoplasia
SIDS (n = 28)	9 (32%)	2 (7%)	—	16 (57%)
Infant controls (n = 11)	2 (18%)	—	—	3 (27%)
SIUD (n = 12)	5 (42%)	—	6 (50%)	8 (67%)
Fetal controls (n = 6)	2 (33%)	—	—	1 (17%)

ClR, caudal linear raphé nucleus; DoR, dorsal raphé nucleus; MaR, magnus raphé nucleus; MeR, median raphé nucleus; ObR, obscure raphé nucleus; PaR, pallidus raphé nucleus.

Table 3. Frequencies of 5-HTT promoter polymorphism allele and genotype frequencies in SIDS and SIUD cases and controls

	5-HTT promoter region polymorphisms		
	SIDS (n = 28)	SIUD (n = 12)	Controls (n = 17)
Allele frequencies	% of cases	% of cases	% of controls
S	38	45	65
L	62	55	35
Genotype frequencies	Number of cases (%)	Number of cases (%)	Number of cases (%)
S/S	4 (14)	2 (20)	7 (41)
S/L	13 (47)	6 (50)	8 (47)
L/L	11 (39)	4 (30)	2 (12)

Table 4. Distribution of the 5-HTT promoter polymorphism genotypes among SIDS and SIUD cases and controls by raphé nuclei neuropathology

5-HTT polymorphisms	Raphé nuclei hypoplasia			Raphé nuclei normal structure		
	SIDS (n = 16)	SIUD (n = 8)	Controls (n = 4)	SIDS (n = 12)	SIUD (n = 4)	Controls (n = 13)
S/S genotype	1	1	1	3	1	6
S/L genotype	4	3	1	9	3	7
L/L genotype	11	4	2	—	—	—

L/L homozygote and S/L heterozygotes genotypes were detected in 39 and 47% of SIDS cases, respectively. Similarly, the L/L and S/L genotypes were detected in 30 and 50% of SIUD cases, respectively. The L/L and S/L genotype frequencies in controls were 12 and 47%, respectively. The S/S genotype was detected in 14% of SIDS, in 20% of SIUD, and in 41% of control cases, respectively. Therefore, the frequency of the L allele was 62% in SIDS, 55% in SIUD, and 35% in controls.

Brainstem raphé nuclei results matched to serotonin transporter gene promoter region polymorphism results: Overall and with smoking exposure. Table 4 summarizes the raphé and polymorphism results overall. The L allele and hypoplasia of the raphé nuclei were highly correlated among SIDS cases and SIUD cases. Specifically, 62% of SIDS cases and 70% of SIUD cases with L allele (L/L or L/S genotypes) showed hypoplasia of variable raphé nuclei ($p < 0.01$). Conversely, raphé hypoplasia with the S/S genotype was observed in only 1 SIDS and 1 SIUD cases. Table 5 summarizes the raphé and polymorphism results related to maternal smoking. A significant correlation among maternal smoking, hypoplasia of the raphé nuclei, SIDS, and SIUD was observed ($p < 0.01$). Notably, 100% of sudden death victims with a smoking mother demonstrated hypoplasia of raphé nuclei and the L allele (L/L or S/L genotype).

DISCUSSION

This study, in addition to delineating the cytoarchitecture of the raphé nuclei in the human brainstem, is the first to establish a link between neuropathological raphé defects and serotonin transporter promoter region polymorphisms in SIDS and also in SIUD, never investigated in regard to the serotonin network. Yet, studies describing the topography and the histopathology of the human raphé nuclei, where the serotonin producing and containing neurons are located, are few in number (2,22–24). The anatomical organization of the 5-HT neurons and their projections in the brain and spinal cord, in fact, has been intensely studied only in experimental animals (25–28). A first value of this study was therefore the morphologic identification in the human brainstem of all nuclei of the raphé complex, *i.e.*, the caudal linear raphé nucleus, the dorsal raphé nucleus and the median raphé nucleus forming the rostral group, and the raphé magnus nucleus, the raphé obscurus nucleus, and the raphé pallidus nucleus forming the caudal group.

We observed hypoplasia of one or more nuclei of both the rostral and caudal raphé groups in 57% of SIDS and 67% of SIUD and only in 24% of control group. Our results are in contrast with the observation of Paterson *et al.* (4). These

Table 5. Distribution of the neurodevelopmental defects of the raphé system and 5-HTT polymorphisms among SIDS and SIUD cases and controls in relation to maternal smoking

	Smoking mothers SIDS + SIUD controls		Nonsmoking mothers SIDS + SIUD controls	
	(n = 18)	(n = 5)	(n = 22)	(n = 12)
Raphé nuclei hypoplasia and L/L or S/L genotype	18	2	4	1
Raphé nuclei hypoplasia and S/S genotype	—	1	2	—
Raphé nuclei normal structure and L/L or S/L genotype	—	2	12	5
Raphé nuclei normal structure and S/S genotype	—	—	4	6

authors reported in fact significantly higher number and density of serotonin neurons in the obscure raphé nucleus in SIDS cases compared with controls.

A notable finding of this study was the increased frequency of the 5-HTT L allele not only in SIDS cases, in agreement with the previously published data (7–9,14,15), but also in SIUD, never considered in relation to serotonin network dysfunction. An additional value was the significant correlation emphasized between L allele and hypoplasia of the raphé nuclei in sudden fetal and infant deaths. In fact, a high percentage of SIDS and SIUD victims with L/L or L/S genotype showed hypoplasia of one or more nuclei of both superior and inferior raphé groups, suggesting the L allele involvement in the raphé system hypodevelopment. It is known, in fact, that the serotonin system plays a trophic role during the neuronal development in fetal brain (29,30). Furthermore, L/L genotype increases serotonin transporter activity, in turn reducing serotonin concentrations in the synapses particularly of the serotonin-producing neurons of the raphé nuclei (7,8,21). Hence, the 5-HTT L allele could have a key role during early development of the raphé complex, giving rise to hypoplasia of its nuclei.

The decrease in neuronal number and consequently the depletion of 5-HT receptors in hypoplastic raphé nuclei would be of importance to explain the mechanism of the sudden infant death. We know that, usually, the serotonergic neurons are involved in breathing mechanism by modulating the ventilatory responses to hematic oscillation of PO_2 , PCO_2 , and pH to maintain it within physiologic levels (31,32). We may speculate that the hypoplasia of one or more raphé nuclei, such as we observed in a wide subset of SIDS victims, may cause a deficit in the serotonergic complex, preventing eupneic breathing, leading to death. Since the correlation of L allele with raphé nuclei hypoplasia was found in our study also in high percentage of SIUD, it is reasonable to speculate whether breathing alterations can lead to death during intrauterine life. We know that periodic fetal breathing regulates the release of tracheal fluid in the lung, and consequently alveolar expansion, to favor lung development (33,34). Nevertheless, defective intrauterine respiratory movements would not be sufficient to justify fetal loss. One possibility that we strongly support is that the serotonergic raphé system in fetal life participates not only in breathing but, more extensively, in the modulation of all the vital functions (35). Consequently, its developmental alterations can produce severe dysregulation of the ANS homeostatic control, triggering the death mechanism. Therefore, this study provides strong evidence that genetic

variation in 5-HTT contributes to SIDS and SIUD susceptibility through a mechanism involving morphologic developmental defects of the raphé nuclei. Thus, the genotypic presence of the 5-HTT L allele represents a predisposing factor for sudden death when it is in association with morphologic developmental defects of the raphé nuclei.

Finally, the role of cigarette smoking as an important causal risk factor for fetal and infant death must be stressed. In fact, in addition to the above-described findings, our results indicate a significant association among hypoplasia of the raphé nuclei, L/L or S/L genotypes, and maternal smoking in pregnancy. The effects of nicotine prenatal exposure on serotonin transporter expression have been examined till now only by Muneoka *et al.* (36,37) in rat brain. These authors showed significant differences in 5-HT turnover in the midbrain and pons-medulla between rats treated with nicotine injections compared with controls. Therefore, nicotine in pregnancy can produce direct effect on the expression of 5-HTTs, and consequently in the development of the raphé nuclei. Our previous studies already supported the hypothesis of a close relation between maternal cigarette smoking during pregnancy and developmental abnormalities of different structures of the human brain (38–40). Now we can extend this association to the serotonergic system. Thus, based on the careful analysis of this research, we suggest that a combination of the 5-HTT L allele, hypoplasia of one or more serotonergic nuclei, and maternal smoking in pregnancy, may result in a failure in mediating vital functions, triggering a death mechanism in both intrauterine and postnatal life.

This study has three apparent limitations. First is the relatively small number of cases of SIDS and particularly of SIUD and controls. This is in part due to the difficulty to apply our guidelines for molecular analysis of the 5-HTT gene that require the collection during the victim autopsy of fresh specimens in specific RNA-later reagent. Second is the default of specific examination by HPLC of the cotinine in hair of victims to validate the maternal self-reported smoking habit (though provided by our protocol, it is rarely applied). Third is the limited data related to smoking habit of fathers and/or other persons living in contact with the victims. Even recognizing these limitation, the data presented in this manuscript are compelling if only for a pilot dataset.

We conclude that SIDS and SIUD should be regarded as a unified pathology, given the common neuropathological and genetic substrates. A wide subset of these deaths is marked by 5-HTT L/L or S/L genotypes and hypoplasia of the raphé nuclei, and frequently related to cigarette smoking exposure in

pregnancy. By expanding the network of clinicians, pathologists, and genetic scientists working together and by combined efforts in a collaborative multicenter international study to identify the pathophysiology and genetics of SIUD and SIDS, the discovery of fetuses and infants at risk for sudden death can ultimately be determined and specific risk reduction strategies can be realized.

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