Higher incidence of emergence agitation in children with genetic intronic variant GABRγ2 rs2279020 after sevoflurane anesthesia

Zeinab Bayoumy Y.[a], Horea Ahmad F.[a], Heba Nabil Baz[b]

[a]Department of Anesthesia & Intensive Care, Faculty of Medicine, Al Azhar University, Cairo, [b]Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt

Correspondence to Nabil Heba Baz, MD, PhD, Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt
E-mail: hebaelbaz@yahoo.com

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Emergence agitation (EA) is very common in Preschool children who receive Sevoflurane anesthesia. The GABA receptors are the target effect site of Sevoflurane.

The aim of this study

Is to investigate if GABRγ2 Genetic variant (or Single nucleotide polymorphism SNP) rs211037 and an intronic nucleotide number rs2279020 polymorphism from A to G, is associated with EA after Sevoflurane anesthesia in Preschool children.

Patients and Methods

This study included 60 pre-school children, ASA I, of both sexes who underwent lower abdominal surgery, anesthetized by Sevoflurane and caudal blockade. At emergence, children were assessed for EA every 5 min, in PACU by Watcha scale1: (0: Asleep, 1: calm, 2: crying, can be consoled, 3: crying, cannot be consoled, or 4: agitated and thrashing). Children were divided into 2 groups. Gr.I: Non-agitated children; scored (0, 1 or 2) Gr.2: Agitated children; scored (3 or 4). GABA receptor gene polymorphisms rs211037 and intronic rs2279020 were genotyped by PCR-RFLP.

Results

GABRγ2 rs211037 stratified the cases into wild homozygous CC in (65%) and Heterozygous CT in (35%) of children, none of the cases was homozygous mutant (TT). The two groups showed insignificant EA between them. The intronic polymorphism rs2279020, however, was heterozygous AG in 73.3% of the cases; it was associated with EA in 54.50% of children versus 9 % in AA and 0.0% in GG Introns, ($P < 0.001$).

Conclusion

There was high incidence of EA in children with genetic intronic variant GABRγ2 (SNP) rs2279020 (A/G) after Sevoflurane anesthesia.

Keywords:
emergence agitation, GABRγ2, rs211037, rs2279020, sevoflurane

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Background

Emergence agitation is a dissociated state of consciousness in which the child is inconsolable, irritable, uncompromising, and/or uncooperative immediately after general anesthesia [1]. g-Amino butyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system. It regulates many physiological functions [2]. It acts on three types of GABA receptors: GABAA, GABAB, and GABAC receptors [3]. A genetic polymorphism is an allelic variant that exists stably in a population in a frequency that cannot be accounted for by new mutations. A number of laboratory techniques have been developed in recent years to analyze DNA rapidly with the advent of PCR. Restriction fragment length polymorphism is a method that uses specific restriction endonucleases to detect the differences in homologous DNA sequences [4]. The low blood/gas solubility of sevoflurane allows rapid elimination from the central nervous system, resulting in rapid recovery (emergence) from anesthesia [5]. However, the more rapid the emergence, the higher the incidence of agitation and excitement encountered [6].

Patients and methods

Patients

This case-control study was approved by the ethical committee of Alazhar University. Sixty preschool children aged 2–6 years, of ASA physical status I, of both sexes, undergoing lower abdominal operations were recruited into this study. History taking, physical examination, and laboratory investigations were carried out. Patients with a history of allergies, previous adverse anesthetic experiences, developmental delay,

psychological disorders, and children with congenital anomalies were excluded.

Methods

Anesthetic methods

Standard monitoring was applied throughout the study using Datex Omeda monitors (Louisville, Kentucky, USA): ECG, $SPaO_2$, heart rate, and mean arterial pressure were recorded. Anesthesia was induced by inhalation of sevoflurane 8%, followed by insertion of an intravenous cannula and laryngeal mask airway. Two milliliters of peripheral venous blood on EDTA tube was withdrawn for isolation of genomic DNA. Thereafter, caudal blockade was done using bupivacaine 0.25% (1 ml/kg). At the end of surgery, the laryngeal mask airway was removed and the children were transferred to the postanesthesia care unit and assessed every 5 min for emergence agitation (EA) by means of the Watcha scale [7]. Children with an agitation score of 3 or 4 were classified as agitated. The children were allocated into two groups according to the presence or absence of emergence agitation in the operation room (OR) or postanesthesia care unit:

Group 1 (nonagitated children): These children had scores of 0, 1, or 2.

Group 2 (agitated children): These children had scores of 3 or 4.

Drugs

Sevoflurane was manufactured by Abbott Company (Chicago, Illinois, USA), and bupivacaine 0.5% was manufactured by Astra Zeneca, Södartälje, Sweden.

Laboratory methods

Extraction of genomic DNA from peripheral blood leukocytes was done using the column extraction technique by Life Biosciences, Thermo Fisher Scientific Inc. (Massachusetts, USA). For the GABRγ2–211037 C/T polymorphism, the following primers were used: forward primer 5′GAGTGCCAATTACAATTGCAAAA3′; reverse primer 5′AATCAGAAAGACTGTAGGTGAGG3′. For the intronic variant *GABRγ2* RS2279020, the following primers were used: forward primer 5′AGA AATTTACCAACTGGTCTAGCCGG 3′and reverse primer AAATCAAATATTGTGTCATGCTTAGT3′. Reagents used included PCR Master Mix 1X AmpliTaq Gold 360 Master Mix (Life Biosciences, Thermo Fisher Scientific Inc.), along with the adjoined nuclease-free water. The PCR conditions were as follows: 35 cycles at 94°C for 30 s, at 55°C for GABRγ2–211037 C/T for 30 s, and at 60°C for GABRγ2-nucleotide position rs2279020 in intron A/G for 30 s and at 72°C for 45 s, followed by final extension at 72°C for 7 min The resultant amplicon size for genotyping the GABRγ2–211037 C/T polymorphism was 122, whereas the amplicon size for genotyping the intronic rs2279020 GABRγ2 polymorphism was 288 bp. The former amplicon was cut by *Apo*I restriction enzyme into two fragments of 102 and 20 bp if the C allele existed, whereas the later was cut by *Nci*I into two fragments of 165 and 123 bp in the G allele.

Statistical methods

SPSS 16.0 ((SPSS Inc., Chicago, USA) for Windows was used for data analysis and Microsoft Power point and Excel (Microsoft, USA) for charts. Quantitative data were presented as mean ± SD. For comparison of two group means, the Student *t*-test was used, whereas for comparison of three group means one-way analysis of variance was used, followed by the post-hoc test. Nonparametric quantitative data were expressed as median (range), and the Kruskal–Wallis and Mann–Whitney tests were used for comparison of medians. Qualitative data were expressed as frequency and percentage. Association between qualitative data was determined using Fisher's exact test. *P*-values less than 0.05 were considered significant [8].

Results

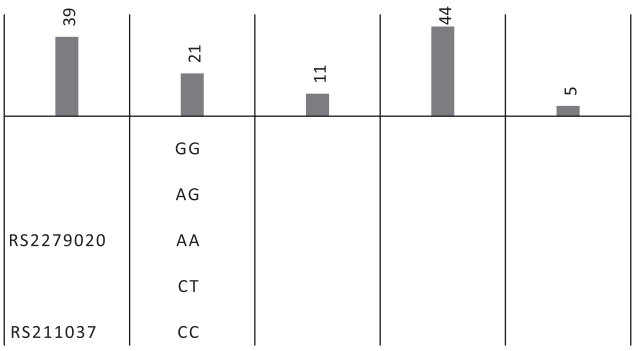
The demographic characteristics of the 60 patients are displayed in Table 1.

Patients were admitted for lower abdominal surgery. The most common type of surgery in the studied group was hernia surgery, accounting for 66.7% (Fig. 1).

Table 1 Demographic data and type of operation in the studied children

Variables	Mean ± SD	Range
Age (years)	3.33 ± 1.15	2–6
Weight (kg)	14.17 ± 2.01	11–21
Operation time (min)	59.15 ± 34.82	35–140
Sex [*n* (%)]		
Female	14 (23.3)	
Male	46 (76.7)	
Total	60 (100)	

Figure 1



The frequency of observed genotypes in both studied polymorphisms.

There were no statistically significant differences in age (in years), sex, weight (in kg), and operation time (in min) between the patients of the two-genotype group GABRγ2 genetic SNP 211073 C/T ($P > 0.005$) (Table 2). No correlation was found between sex and degree of agitation.

There were no statistically significant differences in age, weight, and operation time among patients in the three-genotype group GABRγ2 genetic SNP rs2279020 intron A/G, as shown by the *t*-test ($P>0.05$) (Table 3).

There was no significant difference in the distribution of two-genotype GABR γ2 genetic SNP 211073 intron C/T in children with emergence agitation group (Table 4 and Fig. 2).

There was a highly significant difference in the distribution of GABR γ2 genetic SNP rs2279020 intron A/G in children with emergence agitation, as seen by the χ^2-test (Fig. 3 and Table 5).

Discussion

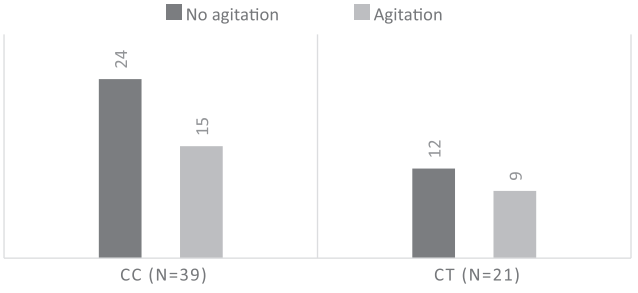
EA is very common in children who receive sevoflurane anesthesia; its incidence can range from 10 to 80%. It is a more frequent side effect in preschool children than in older children [9]. There are several factors that contribute to EA: preoperative anxiety, rapid emergence, intrinsic characteristics of the anesthetic drug, postoperative pain, surgery type, and age [10]. In this study, we tried to decrease preoperative anxiety with toys and balloons, and by keeping the children with parents until preinduction of anesthesia. Moreover, caudal blockade was done to provide intraoperative and postoperative analgesia, to alleviate anxiety and pain as risk factors for EA. The results showed that there were nonsignificant differences between the two genotype groups C/C and C/T in GABRγ2 genetic SNP 211073 intron C/T as regards age, sex, weight, operation time, and incidence of emergence agitation [42.9% among CT patients and 38.5% among CC patients were agitated ($P > 0.05$)]. Also, there were nonsignificant differences between the three genotypes (A/A, A/G, and G/G), in GABRγ2 genetic nucleotide position rs2279020 intron A/G, as regards age sex, weight, and operation time. These results are in agreement with those of Park *et al.* [11], who agreed upon the nonsignificant differences between the three genotype groups as regards emergence agitation, age, sex, weight, and operation time.

Among the patients with GABRγ2 SNPs rs2279020, the AG genotype was found in 73.3% of children. It was associated with highly significant differences in EA: 54.50% of children with the AG genotype versus 9% with AA and 0.0% with GG introns ($P < 0.001$). These results are similar to previously described work by Locatelli *et al.* [12], who reported that sevoflurane and desflurane anesthesia were associated with similar incidences of EA in children undergoing subumbilical surgery and receiving effective regional anesthesia. On the other hand Bajwa and Kalra [13] found an incidence of EA with sevoflurane of 40%. Also, it has been previously described that the incidence of EA in children undergoing sevoflurane anesthesia for bilateral myringotomy and insertion of ear tubes is 58% [14], which is comparable to our results.

Table 2 Comparison of the mean values of age, sex, weight, and operation time between the two genotype groups GABR γ2 genetic nucleotide position 211073 intron C/T

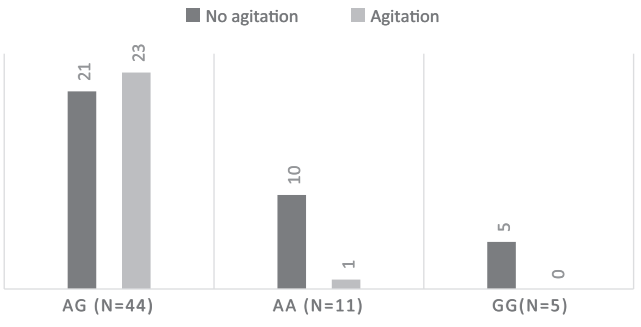
Demographic data	CC	CT	χ^2-Test	
	N = 39	*N* = 21	χ^2	*P*-value
Age				
Mean ± SD	3.24 ± 1.13	3.5 ± 1.19	−0.823	0.414
Range (years)	2–6	2–6		
Sex [*n* (%)]				
Female	11 (28.20)	3 (14.30)	1.478	0.224
Male	28 (71.80)	18 (85.70)		
Weight				
Mean ± SD	14.3 ± 1.93	14.43 ± 2.18	−0.738	0.464
Range (kg)	12–21	11–21		
Operation				
Mean ± SD	57.03 ± 33.4	63.1 ± 37.8	0.641	0.524
Range (min)	35–140	35–140		

Figure 2



Agitation versus SNP 211073 intron C/T genotype.

Figure 3



Agitation versus rs2279020 genotype.

Table 3 Comparison of the mean values of age, sex, weight, and operation time between the three genotype groups GABR γ2 genetic nucleotide position rs2279020 intron A/G

Demographic data	AG	AA	GG	χ^2-Test	
	N = 44	*N* = 11	*N* = 5	χ^2	*P*-value
Age					
Mean ± SD	3.6 ± 1.20	3.73 ± 1.03	3.1 ± 0.89	0.832	0.44
Range	2–6	2–5	2–4		
Sex [*n* (%)]					
Female	8 (18.2)	4 (36.4)	2 (40)	2.473	0.29
Male	36 (81.8)	7 (63.6)	3 (60.0)		
Weight					
Mean ± SD	14.23 ± 2.19	14.09 ± 1.45	13.8 ± 1.6	0.108	0.898
Range	11–21	12–16	12–16		
Operation time					
Mean ± SD	61.23 ± 36.5	50.45 ± 26.5	60 ± 39.2	0.414	0.663
Range	35–140	40–130	40–130		

Table 4 Distribution of two-genotype groups GABR γ2 genetic nucleotide position 211073 intron C/T according to the presence of emergence agitation

Agitation	CC (*n* = 39) [*n* (%)]	CT (*n* = 21) [*n* (%)]	χ^2-Test	
			χ^2	*P*-value
No agitation	24 (61.50)	12 (57.10)	0.110	0.740
Agitation	15 (38.50)	9 (42.90)		
Total	39 (100.00)	21 (00.00)		

Table 5 Distribution of children in three-genotype groups GABR γ2 genetic nucleotide position rs2279020 intron A/G according to the presence of emergence agitation

Agitation	AG (*n* = 44) [*n* (%)]	AA (*n* = 11) [*n* (%)]	GG (*n* = 5) [*n* (%)]	χ^2-Test	
				χ^2	*P*-value
No agitation	21 (47.70)	10 (90.90)	5 (100.0)	10.47	0.005
Agitation	23 (54.50)	1 (9.10)	0 (0.00)		
Total	44 (100.00)	11 (100.00)	5 (100.0)		

As regard to the genetic polymorphism, our study showed an incidence of 73.3% for GABRγ2 SNPs rs2279020 AG, followed by 18.3% for AA and 8.3% for GG. As regards GABRγ2 SNP 211037 C/T, the intron CC was found in 65% of patients, followed by 35% for CT. These results partially disagree with those of Eroglu *et al.* [15] because their study on preschool children found the most common genotype in *GABRγ2*-SNP211037 C/T to be CT (44.7%), followed by CC (30.7%), and the most common genotype in *GABRγ2*-nucleotide position rs2279020 in intron A/G to be AG (45.6%), followed by GG (43.0%) and AA (11.4%). In addition, they reported significantly different distribution in GABRγ2-SNP rs2279020 in intron A/G compared with the Japanese population and in GABRγ2-SNP211037 C/T compared with the Chinese population. These differences can be explained by different distributions of genetic polymorphisms in different races.

Conclusion

There was a high incidence of EA in children with genetic polymorphism in *GABRγ2* (SNP) rs2279020 in intron A/G after sevoflurane anesthesia. The distribution of $GABA_A\gamma2$ genetic polymorphisms is different across races.

Recommendations

We recommend the following:

(a) Premedication to decrease anxiety at the time of separation from parents; and
(b) Adoption of genotyping to screen preschool children.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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