Lack of association between PAX6/SOSTDC1/FAM20B gene polymorphisms and mesiodens

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Abstract

Background: The purpose of this study was to analyze the association between the genetic polymorphism of genes (PAX6, SOSTDC1 and FAM20B) and the susceptibility to mesiodens.

Methods: This study was carried out on 50 patients with mesiodens and 50 controls. The family history of each patient with mesiodens were recorded. Genomic DNA was extracted from saliva samples, and single nucleotide polymorphisms were detected in all exons and exon/intron boundaries of PAX6, SOSTDC1 and FAM20B using Sanger sequencing. The data were analyzed using Pearson chi-square test with theoretical frequency ≥ 5. For theoretical frequency less than 5 but at least 1 (≤ 20% cell), the data were analyzed by continuity correction. For the rest, Fisher’s Exact test was used. A P-value < 0.05 was considered statistically significant. The Odds ratio (OR) and confidence intervals (CI) were recorded.

Results: Three polymorphisms were detected in PAX6. Two polymorphisms were detected in SOSTDC1. Twenty-nine polymorphisms were detected in FAM20B. Although, the T allele of FAM20B (rs3766626) appears to be associated with mesiodens (P = 0.051), there were no significant differences of PAX6/SOSTDC1/FAM20B gene polymorphisms between the two groups. The T allele of FAM20B (rs3766626) was associated with susceptibility to two mesiodens (P < 0.001; OR = 8.333; CI = 2.516–27.600).

Conclusions: Lack of association between PAX6/SOSTDC1/FAM20B gene polymorphisms and mesiodens in the population studied was detected. Further studies with large samples on T allele of FAM20B (rs3766626) are needed.

Keywords: Mesiodens, PAX6, SOSTDC1, FAM20B, Genetic polymorphism

Background

Mesiodens is the most common supernumerary teeth located in the central position of the upper or lower jaw [1]. Mesiodens can be either erupted or impacted in alveolar bone placed and oriented vertically, horizontally or in an inverted manner [2, 3]. The prevalence of mesiodens in the population ranges from 0.09 to 2.2%, according to previous studies [4–6]. A series of clinical complications can be caused by mesiodens, including malposition or delayed eruption of the permanent incisors or the formation of a dentigerous cyst [7–9]. However, the etiology of mesiodens is still unclear.

An increasing number of studies indicate that genetic polymorphisms are associated with oral diseases. For instance, in Polish children, the prevalence of the AG genotype of the Enamelin (ENAM) gene (rs12640848) was higher in subjects with dental caries compared to that in controls [10]. The polymorphism of COX2-765G/C had significant influence on periodontitis risk [11]. The genetic polymorphism of axin 2 (AXIN2) and Gremlin-2 (GREM2, also called PRDC) were related with tooth agenesis [12, 13].

Studies have shown that several genes can result in the formation of mesiodens. The Paired box gene 6 (PAX6) mutant in rats can result in the formation of a supernumerary upper incisor [14]. The inactivation of Family with sequence similarity member 20-B (FAM20B) in the
| ID       | Primer sequences                  | Amplicon size (bp) | Sequencing size (bp) |
|----------|-----------------------------------|--------------------|----------------------|
| PAX6     | F CAAACGGACCAATTGCACCA            | 432                | 432                  |
| PAX6     | R GGTTGGTGTGTGAGAGCAATTCTC        |                    |                      |
| PAX6     | F CAGAGTCTAGGGCCTTCCCTC           | 449                | 449                  |
| PAX6     | R TCGCTGGAAGTAGAAAGGTTTGG         |                    |                      |
| PAX6     | F TGACTGAGCCTCATAGTCAGATG         | 466                | 466                  |
| PAX6     | R TCCCCAATCTGTTCCTCCCTACAT        |                    |                      |
| PAX6     | F GAACCGGAGATTCTCTCTCTCTCA        | 364                | 364                  |
| PAX6     | R CAGTATCGAGAGAGGCAAGGC           |                    |                      |
| PAX6     | F AGGATGCATTGTGGTTGTCCTC          | 405                | 405                  |
| PAX6     | R TCCCAATGCTGGAATCTCGGAATCAA      |                    |                      |
| PAX6     | F AGAGCACAGACAGACTAAGAGACA        | 707                | 707                  |
| PAX6     | R GGTATATGCTGCAAATTCACCCCA        | 470                | 470                  |
| PAX6     | R CAATGTGGTCGATGTGTGCCCA          |                    |                      |
| PAX6     | F AAAGCTGACAGATTTCTCCGTGGGAA      | 398                | 398                  |
| PAX6     | R TCTTCTATGCAAAGGGGCGCTG          |                    |                      |
| PAX6     | F TGGTTGGAGATATGAGGAGT            | 334                | 334                  |
| PAX6     | R TGCGACGAGGAGATTTAGACAG          |                    |                      |
| PAX6     | F CCAGAGACAGACAGATTCTCGTAGTA      | 614                | 614                  |
| PAX6     | R GCAGACACAGCCAAATGAGG            |                    |                      |
| PAX6     | F AGCTCAGAGGGCCAAATTCCTAGAT       | 436                | 436                  |
| PAX6     | R AGGGACAAGGAAAGCAAGGAGT          |                    |                      |
| PAX6     | F1 CTCTTCTGTTGAGTGGGCTG           | 654                | –                    |
| PAX6     | R1 CACAGATCAACATCCATCCAGTCT       |                    |                      |
| PAX6     | F2 CCTATAAAATTGATTTACATGCT        | Only used for sequencing | 149               |
| SOSTDC1  | F ACAAGTGATGAAGTGACATATC          | 550                | 550                  |
| SOSTDC1  | R ACAAGTGATGAAGTGACATATC          |                    |                      |
| FAM20B   | F GTCCTGCTGCTTGGCTGCCTACCTAC      | 832                | 832                  |
| FAM20B   | R CTTTACAACCTCCATCCAGTCT          |                    |                      |
| FAM20B   | F ACTGCTGCCATCATTAGGGTC           | 949                | 949                  |
| FAM20B   | R CAGTGGGTACAGCTGCTGTCTCT         |                    |                      |
| FAM20B   | F AATCAGGCTTGCTTAATGGGTG          | 417                | 417                  |
| FAM20B   | R AGGCCAGAAATGGAATGACCTA          |                    |                      |
| FAM20B   | F TTAATTGGCTGCTGTTGGCCTTAG        | 825                | 825                  |
| FAM20B   | R CACATGGTTTACCATGCTGTA           |                    |                      |
| FAM20B   | F AGAGTGAGCTGGGTAGAAGGA           | 1130               | 1130                 |
| FAM20B   | R TAGCCAAAGGGAAAGCATGCTAG         |                    |                      |
| FAM20B   | F AAGTTCCACAGTATCTGCTAG           | 800                | 800                  |
| FAM20B   | R AAATGAAAATTCATCCATCCAGTCT       |                    |                      |
| FAM20B   | F CATGGTGAAGGCAACAACA             |                    |                      |
| FAM20B   | F GTCCTGCTGCTTGGCTGCCTACCTAC      | 832                | 832                  |
| FAM20B   | R CTTTACAACCTCCATCCAGTCT          |                    |                      |
| FAM20B   | F ACTGCTGCCATCATTAGGGTC           | 949                | 949                  |
| FAM20B   | R CAGTGGGTACAGCTGCTGTCTCT         |                    |                      |
| FAM20B   | F AATCAGGCTTGCTTAATGGGTG          | 417                | 417                  |
| FAM20B   | R AGGCCAGAAATGGAATGACCTA          |                    |                      |
| FAM20B   | F TTAATTGGCTGCTGTTGGCCTTAG        | 825                | 825                  |
| FAM20B   | R CACATGGTTTACCATGCTGTA           |                    |                      |
| FAM20B   | F AGAGTGAGCTGGGTAGAAGGA           | 1130               | 1130                 |
| FAM20B   | R TAGCCAAAGGGAAAGCATGCTAG         |                    |                      |
| FAM20B   | F AAGTTCCACAGTATCTGCTAG           | 800                | 800                  |
| FAM20B   | R AAATGAAAATTCATCCATCCAGTCT       |                    |                      |
| FAM20B   | F CATGGTGAAGGCAACAACA             |                    |                      |
dental epithelium in mice results in supernumerary maxillary and mandibular incisors [15]. The deletion of Sclerostin domain-containing 1 (SOSTDC1, also known as Wise, Ectodin, or USAG-1) in mice leads to the development of extra molar and incisors [16, 17]. However, research regarding the association between genetic polymorphism and mesiodens formation has been reported less often. Therefore, the purpose of the current study is to analyze the association between mesiodens formation and the genetic polymorphisms of genes related to this process, identifying the importance of genetic polymorphisms in mesiodens formation-related genes.

**Methods**

**Study participants**

One hundred patients (50 mesiodens group, 50 unrelated controls) were recruited in this study in Bengbu, China. The diagnosis of mesiodens was based on oral examination combined with periapical radiograph, panoramic radiograph, and/or cone-beam computed tomography. The characteristics including gender, crown direction, the number of mesiodens, and the eruption status of mesiodens were recorded. All patients had no abnormalities in their head, ears, eyes, nose, throat, thyroid, trunk, or extremities and were without cleft lip or palate, congenital absence of teeth or tooth malformation. The family history was recorded.

**Saliva collection and genomic DNA extraction**

A total of 2 mL of unstimulated saliva sample for each recruited participant was collected and stored using Oragene DNA Self-Collection kits (Lang Fu, Shanghai, China). Genomic DNA was extracted using the MagBeads Saliva & Swab DNA Extraction Kit (Regular & Pre-loading Version, Enriching Biotechnology LTD, Shanghai, China) according to the manufacturer’s protocol. The Genomic DNA samples were stored at −20 °C until further analysis.

**Sanger sequencing of selected mesiodens formation related genes**

We were particularly interested in PAX6, SOSTDC1, and FAM20B, which were reported to result in the formation of mesiodens. All exons and exon/intron boundaries of these three genes in 100 samples were amplified using a GC-rich PCR Kit (Sangon Biotech, Shanghai, China) combined with Champagne Taq DNA Polymerase (Vazyme, Nanjing, China). The PCR products were purified using a MagBeads Gel DNA Extraction Kit (Enriching Biotechnology LTD, Shanghai, China) according to the manufacturer’s instructions. The PCR reaction mixture (50 μL) included 3 μL of template, 5 μL of buffer, 4 μL of dNTP, 1 μL of each of the specific forward and reverse primers for these three genes, 0.25 μL rTaq enzyme, and RNase-free water. The PCR was performed with the

| ID       | Primer sequences              | Amplicon size (bp) | Sequencing size (bp) |
|----------|-------------------------------|--------------------|----------------------|
| FAM20B exon-8 F1 | CCATAATTTAACTATTTCCCAAGTCG          | 862                | 862                  |
| FAM20B exon-8 R1 | CCAATCCCAATTCCATCTATCC            | 795                | 795                  |
| FAM20B exon-8 F2 | TGGTGACGGGACAGATGTCGCC          | 793                | 793                  |
| FAM20B exon-8 R2 | AGATGAGTGGGCACATCAGG            | 735                | 735                  |
| FAM20B exon-8 F3 | TGACTTTGCACCTAAGTAAATCTGTG      | 666                | 666                  |
| FAM20B exon-8 R3 | CGTGGCCTCATGCTGTAATC            | 842                | 842                  |
| FAM20B exon-8 F4 | CTGGACCCATGATGGTTGATTTA          | 764                | 764                  |
| FAM20B exon-8 R4 | AAAGCAATCTCTGGAGAACAA           | 879                | 879                  |
| FAM20B exon-8 F5 | TTCCTTGATCTCAGTCTCCTC           | 765                | 765                  |
| FAM20B exon-8 R5 | ATTCATCATGACACTGTGGCAAA          | 879                | 879                  |
| FAM20B exon-8 F6 | CTACCTTGCTACACCCAGA             | 765                | 765                  |
| FAM20B exon-8 R6 | TGGTGAGGGCTGCTGTTGGA            | 514                | 514                  |
| FAM20B exon-8 F7 | TAGTGAAAGGCTGCAATGGTG            | 514                | 514                  |
| FAM20B exon-8 R7 | CTGGGAATTAATCTGAGCAAA           | 514                | 514                  |
following temperature procedures: denaturation at 94 °C for 5 min, 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C, with a 10-min extension step at 72 °C. The purified products were used for Sanger sequenced using the ABI Prism 3730 platform (Applied Biosystems™, USA). The primers used for amplification and sequencing are listed in Table 1. The primers of PAX6 were selected according to previous study [18]. The amplification sequences were detailed in Additional file 1 and Additional file 2.

Statistics
The association between susceptibility to mesiodens and the genetic polymorphism of PAX6, SOSTDC1 and FAM20B were assessed using IBM SPSS 20.0 software (IBM, Armonk, NY, USA). The data were analyzed using Pearson chi-square test with theoretical frequency ≥ 5. For theoretical frequency less than 5 but at least 1 (≤20% cell), the data were analyzed by continuity correction. For the rest, Fisher’s Exact test was used. A P-value<0.05 was considered statistically significant. The relationships between the characteristics of mesiodens and the polymorphisms with P value less than 0.05 were further analyzed using the same method described previously.

Results
Basic characteristics of patients with mesiodens
Four of the 50 patients with mesiodens (8%) patients had a family history of mesiodens. The basic characteristics of mesiodens are listed in Table 2.

Associations between mesiodens formation and genetic polymorphisms
Considering the specific role of family history in mesiodens is still unknown, hence, careful family history is record in our study and excluded when we analyzed the association between mesiodens formation and gene polymorphisms. Removing patients with family history, three polymorphisms (rs750093295, rs667773 and rs3026393) were detected in PAX6. Two polymorphisms (rs6945425 and rs12699799) were detected in SOSTDC1. Twenty-nine polymorphisms (chr1:179025841, rs19319619, rs72707294, rs1024965514, rs745360443, rs778968805, rs2025584, rs140751029, rs9726948, rs16853612, rs9725887, rs9725888, rs4652352, rs147003645, rs72709441, rs4652353, rs4652354, rs56006430, rs3766625, rs3766626, rs775951319, rs16853619, rs2018786, rs16853621, rs18854154, rs530920451, rs9249, rs117216397, rs1220) were detected in FAM20B. Although, the T allele of FAM20B (rs3766626) appears to be associated with susceptibility to two mesiodens (P < 0.001; OR = 8.333; CI = 2.516–27.600).

Discussion
A total of 8% of patients have a family history of mesiodens, which may indicate that the occurrence of mesiodens is partially determined by genetics. The patients with mesiodens were mostly concentrated in the northern and southern regions of Bengbu. The occurrence of mesiodens might have regional distribution characteristics.

PAX6 is an important gene involved in a series of diseases including eye diseases, diabetes, autism spectrum disorder and mesiodens [14, 19–21]. Variants of PAX6 are correlated with eye diseases and the insulin response [22–24]. Lei HH et al. identified that variants of rs677773 and rs3026393, and showed that the GG

| Table 2  | Characteristic of patients with mesiodens, mean ± SD, or n (%) |
|---------|-------------------------------------------------------------|
| Numbers | 50                                                          |
| Age (years) | 11.8 ± 9.3                                                  |
| Gender | Females 16 (32.00) Males 34 (68.00)                           |
| Number of mesiodens per patient | 1 35 (70.00) 2 15 (30.00)                                  |
| Growth status | 1 erupted 13 (26.00) 1 impacted 22 (44.00) 1 erupted and 1 impacted 8 (16.00) 2 erupted 2 (4.00) 2 impacted 5 (10.00) |
| Crown direction | 1 vertical 13 (26.00) 1 horizontal 4 (8.00) 1 inverted 17 (34.00) 1 inverted and 1 vertical 6 (12.00) 1 horizontal and 1 vertical 4 (8.00) 2 vertical 4 (8.00) 1 horizontal and 1 inverted 1 (2.00) 2 inverted 1 (2.00) |
| Family history | 4 (8.00) |
| Located in maxilla | 49 (98.00) |
| Located in mandible | 1 (2.00) |
genotype of rs302693 was less prevalent in 20 patients with mesiodens than in 31 controls [18]. These results were further supported by our study. Polymorphisms in rs667773 and rs3026393 of PAX6 were detected in the current study, and the mesiodens group might have fewer genotypes of GG (rs3026393) than do the controls. Polymorphisms related to other diseases were not detected in this study; however, this may be because the patients with mesiodens did not have any other diseases.

Mesiodens is the most common type among supernumerary teeth, and the development of supernumerary teeth is closely associated with bone morphogenetic protein (BMP) and Wnt signaling pathways [25]. BMP is

**Table 3** The gene polymorphisms in patients with mesiodens and controls

| Marker | Gene polymorphism | Mesiodens | Controls | P value |
|--------|-------------------|-----------|----------|---------|
| rs2025584 (FAM20B) | AA/AG/GG | 4/23/16 | 10/24/15 | 0.326 |
|        | A/G              | 31/55    | 44/54    | 0.223  |
| rs140751029 (FAM20B) | CC/CT | 40/4 | 43/4 | 1.000 |
|        | C/T              | 84/4     | 90/4     | 1.000  |
| rs9726948 (FAM20B) | GG/GT | 41/2 | 41/6 | 0.270 |
|        | G/T              | 84/2     | 88/6     | 0.282  |
| rs16853612 (FAM20B) | AA/AG/GG | 23/12/8 | 28/16/3 | 0.206 |
|        | A/G              | 58/28    | 72/22    | 0.171  |
| rs9725887 (FAM20B) | CC/CT/TT | 15/22/6 | 14/19/15 | 0.146 |
|        | C/T              | 52/34    | 47/49    | 0.120  |
| rs9725888 (FAM20B) | CT/TT | 2/41 | 6/42 | 0.273 |
|        | C/T              | 2/84     | 6/90     | 0.284  |
| rs4652352 (FAM20B) | AA/A/C/CC | 5/15/23 | 6/12/30 | 0.584 |
|        | A/C              | 25/61    | 24/72    | 0.537  |
| rs147003645 (FAM20B) | GG/AG | 43/0 | 47/1 | 1.000 |
|        | G/A              | 86/0     | 95/1     | 1.000  |
| rs7209441 (FAM20B) | CC/TT/CT | 22/6/15 | 28/3/17 | 0.477 |
|        | C/T              | 59/27    | 73/23    | 0.262  |
| rs4652353 (FAM20B) | GG/GT/TT | 6/14/23 | 6/12/30 | 0.668 |
|        | G/T              | 26/60    | 24/72    | 0.430  |
| rs4652354 (FAM20B) | CC/CT/TT | 19/22/1 | 18/23/7 | 0.146 |
|        | C/T              | 60/24    | 59/37    | 0.159  |
| rs56006430 (FAM20B) | CC/CG/GG | 7/15/21 | 3/17/29 | 0.269 |
|        | C/G              | 29/57    | 23/75    | 0.123  |
| rs3766626 (FAM20B) | GG/GT/TT | 16/21/4 | 14/19/14 | 0.067 |
|        | G/T              | 53/29    | 47/47    | 0.051  |
| rs3766625 (FAM20B) | AA/AG/GC/CC | 6/15/20/0 | 2/17/27/1 | 0.277 |
|        | A/G/C            | 27/55/0  | 21/71/2  | 0.131  |
| rs16853619 (FAM20B) | AA/AG/GG | 2/39 | 6/41 | 0.276 |
|        | A/G              | 2/80     | 6/88     | 0.287  |
| rs7207294 (FAM20B) | GG/GC/CC | 8/18/22 | 3/18/27 | 0.249 |
|        | G/C              | 34/62    | 24/72    | 0.116  |
| rs2018786 (FAM20B) | AA/AG/GG | 7/14/23 | 1/18/30 | 0.061 |
|        | A/G              | 28/60    | 20/78    | 0.076  |
| rs16853621 (FAM20B) | AA/AG/GG | 36/4/1 | 40/8/0 | 0.361 |
|        | A/G              | 76/6     | 88/8     | 0.802  |
| rs188554154 (FAM20B) | GG/GT/TT | 43/0 | 47/1 | 1.000 |
|        | G/T              | 86/0     | 95/1     | 1.000  |
| rs530090451 (FAM20B) | AA/AG | 43/1 | 47/1 | 1.000 |
|        | A/G              | 87/1     | 95/1     | 1.000  |
| rs775951319 (FAM20B) | CC | 41     | 47     | –     |
| rs778068805 (FAM20B) | CC/CT | 46/0 | 46/1 | 1.000 |
|        | C/T              | 92/0     | 93/1     | 1.000  |

**Table 4** The distribution of AA genotype of FAM20B (rs2018786) according to eruption status

| genotype | 1 erupted | 1 impacted | 1 erupted | 1 impacted | 2 erupted | 2 impacted |
|----------|-----------|------------|-----------|------------|-----------|------------|
| AA       | 1         | 3          | 0         | 0          | 3         | 2          |
| others   | 8         | 17         | 8         | 2          | 2         | 2          |

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required for SHH expression during early tooth development and postnatal root development [26]. However, SOSTDC1 is an inhibitor of BMP, and the deletion of SOSTDC1 in mice induces the formation of mesiodens [15]. Wnt, another signaling pathway, can be inhibited by SOSTDC1, located in the upstream of Sonic hedgehog (Shh), and induces the expression of Shh, followed by the induction of high SOSTDC1 expression. Insufficient SOSTDC1 enhances WNT signaling, which increases proliferation and continuous development of vestigial tooth buds and results in the formation of supernumerary teeth [27, 28]. In our study, two polymorphisms (rs6945425 and rs12699799) were detected in SOSTDC1, but none of them were found related to susceptibility to mesiodens.

FAM20B is a member of Family with sequence similarity 20 (Fam20) proteins containing FAM20A, FAM20B, and FAM20C in the human genome [29]. FAM20A knockout mice have biomineralization defects, and mutations in FAM20A have been found to be associated with amelogenesis imperfecta subsequently [30–32]. FAM20B null mice showed mesiodens [15]; however, the relationship between variants of FAM20B and mesiodens has not yet been reported. Our results suggest for the first time that individuals with T allele of FAM20B (rs3766626) appear to have a low risk of mesiodens, which was located in the 3′ untranslated region (3′ UTR) of corresponding gene. Although it isn’t translated into protein, previous and recent studies showed that variant in 3′ UTR region could impact the expression of mRNA [33, 34].

The current study provides information on the association between genetic polymorphisms and the occurrence of mesiodens; however, there are some limitations. The sample size (mainly the control size) and the number of genes analyzed in this study were limitations. The mechanism by which these polymorphism affect mesiodens is unknown. Further studies including more samples, more genes, and the mechanism of these polymorphism on mesiodens are needed.

Conclusions
There were no significant differences of PAX6/SOSTDC1/ FAM20B gene polymorphisms between the two groups. Further studies with large samples on T allele of FAM20B (rs3766626) are needed.

Table 5 The distribution of AA genotype of FAM20B (rs2018786) according to crown direction

| genotype | 1 vertical | 1 horizontal | 1 inverted | 1 inverted + 1 vertical | 1 horizontal + 1 vertical | 2 vertical | 2 horizontal + 1 inverted | 2 inverted |
|----------|------------|--------------|------------|-------------------------|---------------------------|------------|---------------------------|-----------|
| AA       | 2          | 0            | 2          | 0                       | 2                         | 0          | 1                         | 0         |
| others   | 8          | 3            | 14         | 5                       | 2                         | 4          | 0                         | 1         |

Additional files

Additional file 1: The amplification sequences of FAM20B. (DOCX 22 kb)
Additional file 2: The amplification sequences of SOSTDC1. (DOCX 13 kb)

Abbreviations
AXIN2: Axin 2; BMP: Bone morphogenetic protein; ENAM: Enamelin; Fam20: Family with sequence similarity member 20; Grem2: Gremlin-2; PAX6: Paired box gene 6; SOSTDC1: Sclerostin domain-containing 1; UTR: Untranslated region

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Not applicable

Authors’ contributions
KZ and SSL conceived and designed the experiments; JCL and JCX contributed to the data acquisition; SKL, YFC, RXZ and RTP analyzed the data; SSL wrote the manuscript; KZ revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The data and materials of the present study were available from the corresponding author.

Ethics approval and consent to participate
This study was approved by the Ethics Committee of [2017] KY010 by the First Affiliated Hospital of Bengbu Medical College. Informed consents were written before recruitment. Written informed consent for participation under 16 years old in the study was obtained from their parent or guardian.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflict of interest.

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