Cariogenicity of Candida albicans of distinct genotypes among 3-5-year-old Uygur children in Kashgar, China- a case-control study

Wanting Zhang¹, Yan Li¹, Jing Lin¹, Aynur Abduryim² and Jin Zhao¹*

Abstract

Background: In recent years, the opportunistic fungus C.albicans has been linked to ECC. It is important to investigate the relationship between the distribution of C.albicans and early childhood caries (ECC) in 3–5-year-old Uygur and Han children in Kashgar, and the role of C.albicans of distinct genotypes in caries.

Methods: Two hundred fifty-six Uygur and 141 Han children were enrolled in the study. The identified C.albicans isolates were genotyped based on 25S rDNA Polymerase Chain Reaction(PCR) amplification, and their acidogenicity, aciduricity, and adhesiveness were examined. Moreover, secreted aspartic protease (Sap) activity and SAP1–5 transcriptional levels were compared in the ECC and caries-free (CF) groups of Uygur children.

Results: C.albicans detection rate was significantly higher in Uygur children than in Han children (44.5% vs. 31.2%; χ²= 6.739, P = 0.009); the A genotype was dominant in Uygur and Han children with ECC, the C genotype was dominant in Uygur CF children(P = 0.022). C.albicans acidogenicity and growth were more pronounced in the Uygur ECC group than in CF group, especially at pH 4.0 and 4.5 (pH 4.0, P = 0.012; pH 4.5, P = 0.029); the average ratios of glass-wall adhesion and the Sap activity was higher in ECC group than in CF group(P < 0.01), and SAP2(P < 0.001) and SAP5(P = 0.001) were expressed more robustly in ECC group.

Conclusions: The strong acidogenicity and aciduricity, Sap activity, and high SAP2 and SAP5 expression might be closely associated with ECC. C.albicans potentially plays a key role in the progression of caries, which most readily affects genotype A carriers and could be attributed to person-to-person environmental variation.

Keywords: Early childhood caries, Candida albicans, Ethnicity, Cariogenicity, Genotype

Background

Early childhood caries (ECC) is the most common disease in children [1]. Numerous epidemiological studies have demonstrated that the prevalence of ECC is lower in developed countries than in developing countries with low socioeconomic status [2, 3]. Hence, children in underdeveloped regions might be at a higher risk of caries, and stringent measures must be employed to treat them [4]. ECC is recognized as caused by a combination of diverse microorganisms and multiple factors. The research on ECC-associated microorganisms has mainly focused on the bacteria Streptococcus mutans, Lactobacillus, and Actinomyces [5]. In recent years, the opportunistic fungus Calbicans has also been linked to ECC: it is detected in the saliva, plaque, and caries tissues, and the detection rate is markedly higher in children with ECC than in caries-free (CF) [6].

Dental plaque, a microecological environment, where the microbes survive closely attached to the tooth surface, slows the local diffusion of saliva, enabling a continuous partial activity of acids; once the cariogenic pH (5.4, 5.5) is reached, enamel demineralization occurs, with caries formation [7]. Calbicans ferments various sugars and produces acids; it can reduce the pH of a
sugary broth from 7.0 to 3.5, potentially leading to tooth demineralization [8]. A key *C. albicans* virulence factor is secreted aspartic protease (Sap; gene: SAP), it can promotes *C. albicans* adhesion to tooth surface [9]. The probability and intensity of the disease caused by *C. albicans* of distinct genotypes are different, which may correlate with the ethnic and regional disparities between different geographical areas, and *C. albicans* strains harboured by different groups of people harbour distinct genes [10]. Until now, only a few studies have examined *C. albicans* gene polymorphism in relation to the underlying mechanism of caries, and fewer still have investigated the influence of ethnic and geographic factors.

Xinjiang province is located in Northwestern China, with the Uygur people as the predominant ethnic minority group. The incidence of ECC among 3–5-year-olds in Kashgar city is 74.58% [11], which is markedly higher than the average level of China. This suggested that these children are particularly ECC-prone. The Uygur account for 91.92% of the total population in Kashgar; Kashgar’s history, geography, and local customs render the city a isolated area with a unique representation of the Chinese population. Hence, in the current study, we investigated *C. albicans* distribution, and the relationship between *C. albicans* and ECC in 3–5-year-old children in Kashgar. We also examined the role of *C. albicans* of distinct genotypes in the mechanism of caries development, providing a theoretical reference point for dental caries prevention.

**Methods**

**Study participants**

This study was approved by the First Affiliated Hospital of Xinjiang Medical Ethics Committee (ethical review number 20150214–162), and the local health administrative departments. We used the following sample size estimation formula [10, 12]:

\[
N = \left( \frac{Z_{1-\alpha/2} + Z_{1-\beta}}{\delta} \right)^2 \left[ p_1(1-p_1) + p_2(1-p_2) \right]/\delta^2
\]

\[
= \left( 1.96 + 1.28 \right)^2 \left[ 0.5 \ast (1-0.5) + 0.75 \ast (1-0.75) \right]/(0.75-0.5)^2
\]

= 73.5.

This yielded a minimum total sample size of 296.

After applying the stratified cluster-sampling method, 397 3–5-year-old children were enrolled in the study. The children were healthy; presented no systemic, hereditary, or mucous diseases; did not wear orthodontic appliances; and had not received any antibiotic drugs or injections within 30 d prior to sampling. Written consent was obtained from the parents (legal guardians) before sampling.

**Oral examination and specimen collection**

Two trained oral physicians checked 20 teeth of each child. The children were divided into ECC and CF groups. ECC is usually defined as the presence of one or more decayed (both noncavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child under the age of six (by AAPD). The inter-examiner reproducibility was 0.83, and the intra-examiner reproducibility was 0.81. The children rinsed their mouths before sampling; the dental plaques were collected by a sterilized caries excavator from the third cervical on the buccal side of the first maxilla and the mandibular deciduous molar, and one third lips of the upper anterior teeth [10]. The samples were placed in 1.5 mL sterile Sabouraud liquid medium (SDB) maintained 4°C, and returned to the laboratory within 2 h.

**Isolation and purification of *C. albicans***

Each sample (20 µL) was used to inoculate on CHROMagar (CAC) medium and cultured aerobically at 37°C, the colonies were evaluated after 24–72 h. Emerald green single colonies were picked and streaked on another CAC plate, the procedure was repeated three times.

**Identification of *C. albicans***

The morphology of cells from *C. albicans* colonies were examined under a microscope after Gram staining. For germ-tube experiments, microbial suspensions and bovine serum were mixed, placed in a moist dish, incubated at 37°C; samples were stained every 60 min, and examined cell morphology.

*C. albicans* was also identified by PCR. Fungal DNA was extracted using the Biospin DNA extraction kit. The following primers were used: ITS1 (5′-GGAAGTAAA AGTCTGAAACAAGG-3′) and ITS2 (5′-GCTGCGTTC TTCACTGATGC-3′). The PCR mixture contained 2× Easy Taq PCR supermix (10 µL), 10 µmol·L⁻¹ of forward and reverse primers (0.5 µL each), DNA template (2.0 µL), and ddH₂O (7.0 µL). The cycling conditions were as follows: 95°C hot-start, 5 min; followed by 40 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 30 s; and 72°C for 10 min. The PCR products were imaged using a UV gel scanner after agarose electrophoresis.

Following identification, 20 *C. albicans* strains were randomly selected from ECC and CF Uygur children, and from ECC Han children for subsequent genotyping.

**25S rDNA PCR-based *C. albicans* genotyping**

The 25S rDNA PCR amplification mixture contained 2× Easy Taq PCR supermix (15 µL), 10 µmol·L⁻¹ of forward and reverse primers (1.0 µL of each), DNA template (2.5 µL), and ddH₂O (10.5 µL); the following primers were used: CA-INT-L (5′-ATAAGGAACTGG GTTGGCGGCCAATAAGATTCGTA-3′) and CA-INT-R (5′-GCTGCGTTC TTCACTGATGC-3′). The cycling conditions were as followed: 94°C hot-start, 3 min;
30 cycles of denaturation at 94 °C for 1 min, annealing at 67 °C for 1 min, extension at 72 °C for 2.5 min; and final extension at 72 °C, 7 min. The different genotypes were determined based on the sizes of amplification product bands.

**Acidogenicity and aciduricity test**

SDB media containing different glucose concentrations (0.01-0.2 mol·L⁻¹) and at different pH values (4.0-7.0) were prepared. Microbial suspensions were adjusted to OD₅₄₀= 1.0, inoculated into SDB [1:10 (v/v) ratio], and aerobically cultured at 37 °C for 48 h. Next, each suspension was centrifuged (15 min, 5000 rpm × g), and the supernatant was transferred to another tube. The pH of the supernatant (terminal pH) was measured and the pH change calculated [ΔpH = pH(initial)−pH(terminal)]. The collected *C. albicans* pellet was diluted in 4 mL of sterile saline, vortex-mixed, and shocked for 25 s; OD₅₄₀ was then measured, with the sterile saline as a blank. Each sample was tested three times and the results were averaged.

**Adhesiveness test**

Microbial suspensions (1 mL; OD₅₄₀= 1.0) were added to SDB (1 mL), the tubes tilted at 30°, and cultured aerobically at 37 °C, overnight. Next, the tube containing the suspension (tube no. 1) was gently rotated three times. The content was transferred to a clean tube (tube no. 2); 6 mL of PBS was placed in tube 1, which was again gently rotated three times. The tube contents were transferred to a third tube (tube no. 3) [13]. Tubes no. 2 and 3 were centrifuged (10,000 rpm, 10 min), and the supernatant removed; 6 mL of PBS was placed in tubes no. 1–3, and the contents mixed evenly. Next, OD₅₄₀ of tube no. 1–3 contents was measured. The microbial adhesion ratio was calculated as follows: adhesion ratio = OD₁/(OD₁ + OD₂ + OD₃) × 100%. Each sample was evaluated three times and the data were averaged.

**C. albicans sap activity**

For the YNB-BSA-agar method [14]; *C. albicans* suspensions were adjusted to 10⁶ CFU·mL⁻¹ and centrifuged (1500 rpm, 4 °C, 10 min); the supernatants were added to 0.1 mol·L⁻¹ of citrate buffer containing 0.2% BSA, and incubated aerobically at 37 °C for 30 min. The reaction was terminated by the addition of 10% (w/v) trichloroacetic acid; the samples were then centrifuged (1300 rpm, 4 °C, 30 min), and OD₂₅₀ of the supernatant was measured. Next, the microbial suspension was transferred, in triplicate, to 96-well plates (200 μL per well); 20 μL of MTT was added to each well, and the plates were incubated in the dark (37 °C, 4 h). Finally, the supernatant was collected, mixed with dimethyl sulphoxide (150 μL), and agitated gently for 10 min; OD₄₉₀ of each sample was then measured. The increase in absorbance corresponded to Sap activity (calculated as the OD₂₅₀/OD₄₉₀ ratio) [15].

**SAP1–5 gene expression**

Total RNA was extracted from *C. albicans* cells and then reverse-transcribed to cDNA using a cDNA synthesis kit. RT-PCR quantification of *SAPI–5* expression was performed in 20 μL reaction mixtures containing cDNA template (2 μL), 10 μmol·L⁻¹ of forward and reverse primers (0.5 μL each; Table 1), 5× SYBR Green I PCR buffer (10 μL), and ddH₂O (7.0 μL). The cycling conditions were as follows: initial denaturation at 95 °C for 30 s; followed by 40 cycles of denaturation at 95 °C for 5 s, elongation at 60 °C for 30 s. The results were then analysed.

**Statistical analysis**

Data were analysed using SPSS 21.0. For categorical data, χ² test was used (α = 0.05, two-sided). For continuous data, when normality and homogeneity were detected, t test or the nonparametric rank-sum test was used. Finally, factorial analysis was employed to evaluate data from the following experiments: acidogenicity and aciduricity (ΔpH, ΔOD), adhesion ratio, Sap activity, and *SAPI–5* expression in *C. albicans* isolates of different genotypes. The differences were considered statistically significant when *P*<0.05.

| Table 1 | Primers for *SAPI–5* and GAPDH |
|---------|--------------------------------|
| Primer name | Primer sequence | Fragment length (bp) |
| SAPI1 | F TCAATCAATTACTTCTCCATTTCTAAACA | 161 |
| | R GCCAGTACATTTAACAGAGGATTCAAAATGACA | |
| SAPI2 | F TGAGTTGTTGGTGTGTTTTCGA | 108 |
| | R CCACGGCTTCATTGATTTTT | |
| SAPI3 | F CCTTCTCTAATAATTGATTGATGGAAC | 231 |
| | R TTGATTCCCTTTGGGACCGTAAACATTT | |
| SAPI4 | F CATTCATTCCTTTTAATACCAGACTAC | 156 |
| | R GGTCAAAACCCCTCTAGAATTTCCTT | |
| SAPI5 | F TGTTGTTGATAGCTGAGGACA | 107 |
| | R TTGTTCCCTACTAACAGAGAT | |
| GAPDH | F TTGACGGTCCATCCCCACAA | 103 |
| | R GGAAATAACCTTACACAGGCTT | |
Results

Distribution of *C. albicans* in the dental plaque of Han and Uygur children

Based on the proportion of local population distribution, 256 Uygur and 141 Han children were enrolled in the study. *C. albicans* detection rate was significantly higher in Uygur children than in Han (44.5% vs. 31.2%; $\chi^2 = 6.739$, $P = 0.009$), and in the ECC group than in the CF group of Uygur children (52.3% vs. 27.5%; $\chi^2 = 13.665$, $P < 0.001$) and in the ECC group than in the CF group of Han children (41.0% vs. 19.0%; $\chi^2 = 7.842$, $P = 0.005$). Among the Uygur children, the detection rate of *C. albicans* was significantly higher in male than in female children (51.2% vs. 37.8%; $\chi^2 = 4.630$, $P = 0.031$) (Table 2).

*C. albicans* identification

After 24 h culture on CAC plates, the *C. albicans* clinical isolates formed white or light green, flat, and round colonies; after 48 h, the colonies appeared emerald green, and had a smooth surface. Gram-positive *C. albicans* cells were round and scattered (Fig. 1, left). In the germ-tube test, the germinated spores of *C. albicans* clinical isolates were circular, with slender hyphae (Fig. 1, right). PCR analysis confirmed that all suspected clinical isolates were indeed *C. albicans*, and a clear 250-bp band was observed (Fig. 2).

*C. albicans* genotyping

The 25S rDNA PCR products were resolved on agarose gels: genotype A, 450-bp band; genotype B, 840-bp band; genotype C, 450- and 840-bp bands (Fig. 3). It revealed that genotype A was dominant in both Han and Uygur children with ECC, but the constituent ratio did not differ significantly. In Uygur CF children, genotype C was dominant, and the distribution between ECC and CF groups significantly differed. A, B, and C: $P = 0.2$, 0.407, and 0.022, respectively; the differences in A-, B-, and C-genotype constituent ratios were not statistically significant (Table 3).

The acidogenicity, aciduricity, and adhesiveness of *C. albicans*

The acidogenicity of *C. albicans* isolated from ECC and CF groups of Uygur children increased with increasing glucose concentration in the medium. When the concentration of glucose reached 0.1 mol·L⁻¹, both the acidogenicity and growth of *C. albicans* were more pronounced in ECC group than in CF; the differences were statistically significant (acidogenicity, $P = 0.004$; growth, $P = 0.012$). *C. albicans* isolates were still alive at pH 4.0 and 4.5; the acidogenicity and aciduricity of isolates from the ECC group were higher than the isolates from the CF group (pH 4.0, $P = 0.012$; pH 4.5, $P = 0.029$). Finally, the average glass-wall adhesion ratio was higher in the isolates from the ECC group (53.92%± 6.79%) than in isolates from the CF group (31.12%± 5.45%, $P = 0.02$).

*C. albicans* sap activity

As determined by the YNB-BSA-agar method, all strains showed Sap activity. The activity was significantly higher in ECC group isolates (0.160 ± 0.012) than in CF group (0.217 ± 0.031; $t = 7.713$, $P < 0.001$). Similarly, based on the results of the MTT assay, *C. albicans* Sap activity was

| Ethnicity | Caries | Gender | Total |
|-----------|--------|--------|-------|
|           | ECC (%) | CF (%) | P     | Male | Female | P     | Total | P     |
| Uygur     | 92/176 (52.3) | 22/80 (27.5) | < 0.001 | 66/129 (51.2) | 48/127 (37.8) | 0.031 | 114/256 (44.5) | 0.009 |
| Han       | 32/78 (41.0) | 12/63 (19.0) | 0.005 | 21/73 (28.8) | 23/68 (33.8) | 0.517 | 44/141 (31.2) |       |
| Total     | 124/254 (48.8) | 34/143 (23.8) | < 0.001 | 87/202 (43.1) | 71/195 (36.4) | 0.175 | 158/397 (39.8) |       |

ECC early childhood caries, CF caries-free

Table 2 The distribution of *Calbicans* among Uygur and Han children
significantly higher in ECC group isolates (1.876 ± 0.373) than in CF group (1.166 ± 0.348; \( t = 6.226, P < 0.001 \)).

SAP1–5 gene expression
The SAP1–3 and SAP5 genes were expressed at higher levels in ECC group than in CF, but the difference in expression was only statistically significant for SAP2 (\( P < 0.001 \)) and SAP5 (\( P = 0.001 \)) expression; conversely, SAP4 expression was lower in ECC group than in CF, but the difference was not significant (\( P = 0.114 \)).

Cariogenicity of C. albicans of different genotypes: Factorial analysis
By using 3× 2× 2 factorial analysis, at pH 4.0 and 4.5 the aciduricity did not differ between the different genotypes, but was higher in the ECC group than in the CF group isolates.

Acidogenicity C. albicans of different genotypes from ECC and CF groups in the presence of 0.1 mol·L\(^{-1}\) of glucose was compared using 3× 2 factorial analysis; it significantly differed between the groups (group comparison, \( P = 0.020 \); genotype comparison, \( P = 0.019 \)); ECC group: A > C > B; CF group: B > A > C (Table 4).

C. albicans adhesion rate and Sap activity were examined using 3× 2 factorial analysis, which revealed that these did not show genotype-dependent differences. Finally, factorial analysis of gene expression in ECC and CF group isolates revealed that only the expression of SAP2 was significantly different in C. albicans of different genotypes in the ECC and CF groups (group comparison, \( P = 0.001 \); genotype comparison, \( P = 0.020 \)); SAP2 expression: ECC group, A > B > C; CF group, B > A > C (Table 5, Fig. 4).

Discussion
The recent research into the aetiology of ECC has been focused on the relationship between C. albicans and ECC [16]. Klinke et al. verified that C. albicans is associated with an increased prevalence of caries [17]. In the current study, the C. albicans detection rate in the ECC group (48.8%) was more than double of that in the CF group (23.8%), which again demonstrated the close association of C. albicans with ECC.

The C. albicans detection rate was significantly higher in Uygur children than in Han (\( P = 0.009 \)), in contrast to our previous study of Urumqi [18]. This discrepancy might be due to regional differences: compared with Urumqi, Kashgar is a more isolated city, with a higher proportion of Uygur people and unique customs. Hence, C. albicans distribution is likely affected not only by ethnic differences, but also by geographic differences.

We found that in the presence of 0.1 mol·L\(^{-1}\) of glucose, the acid produced by C. albicans caused the broth pH to drop from 7.00 to 4.06, which is lower than the enamel demineralization pH threshold (5.5) [19]; further, C. albicans acidogenicity and growth were both more pronounced in ECC group isolates than in CF group. This suggests that C. albicans isolates were strongly cariogenic in the presence of 0.1 mol·L\(^{-1}\) of glucose. Furthermore, the aciduricity and growth capacity of both ECC and CF group C. albicans isolates decreased as the initial pH was lowered; nevertheless, at pH 4.0, C. albicans continued to produce acid, in agreement with the observations of Klinke et al. [18] This is in contrast with clinical isolates of S. mutans, whose growth is inhibited, and acidogenicity and aciduricity are abolished at pH below 5.0 [20], indicating that in an acidic environment, the acidogenicity and aciduricity of C. albicans are more pronounced than in S. mutans.
Calbicans is able to colonize the oral cavity because of its strong adhesion ability. The activity of Calbicans Saps has been shown to be associated with Calbicans invasiveness, and cell adhesion is proportional to the amount of Calbicans produced Saps \([21]\). Recently, considerable progress has been made in the Calbicans Sap research. Taylor et al. reported that Sap5 is expressed early in infection; Sap4 is expressed in the final infection stage, while the expression of Sap5 is reduced \([22]\). The difference in Sap expression is linked to the infection environment \([23]\), and this might due to the adaptation of Sap to the specific host environment, promoting an overlap in the activities of distinct Sap proteins produced in diverse environments and their roles. The results of the current study differ from those of Li et al. \([24]\), who reported significantly higher or lower expression of SAP1 and SAP4 respectively in ECC group isolates than in CF group. This discrepancy might be associated with the differences between the selected populations, geographical differences, or ethnic differences, but further investigation is required to clarify this issue. In the future, DNA-chip technology and microarray analysis may allow the elucidation of the relationship between the expression of individual SAP genes and the biological activity or virulence of Calbicans, facilitating the development of new infection prevention strategies based on targeting of specific SAP genes.

Genotyping is an effective and classical molecular biology approach for studying the relationship between species, clarifying the association between different strains. The genotype of Calbicans continually changes after host infection to enable fungal adaptation to new environments \([25]\). In this regard, the transmission and colonization of oral microorganisms is not only limited by the source and route of infection, but is also affected by the host’s genetic background, lifestyle, and oral microbial environment \([26]\). The constitution of genotype A in the current study is the same as reported by Wu et al. \([10]\), and da Silva-Rocha et al. \([27]\), implying that the Calbicans of genotype A might cause more caries. Genotype C of Calbicans was dominant in Uygur CF children, and the difference between Uygur ECC and CF group isolates was statistically significant. This results differ from those of Qiu et al \([28]\), possibly on account of location differences, and the unique eating habits and lifestyles of the subjects. Calbicans exhibits extremely high gene polymorphism in hosts from different ethnic groups and in hosts with different degree of caries \([10], [18]\) this suggests that more attention should be devoted to genotype variation and host microecological balance when addressing such topics as Calbicans pathogenicity, prevention of dysbacteriosis, and drug selection. In the current study, the constituent Calbicans genotype was different, probably because of differences in pathogenicity, or selective growth of Calbicans in different host environments. The following conclusion may hence be drawn pertaining to the differences in genotype constitution: the mouth microenvironment differs among different groups of people, and thus the colonization capacity of different genotypes of Calbicans is also different.

In conclusion, dental caries develops when the dynamic equilibrium of the oral microflora is destroyed. The results might be correlate with the differences in population genetics and ethnicity, geographical distribution, and eating habits. Further investigation is required to verify that. To fully understand the ecological processes of caries formation, future molecular biology research is essential: sophisticated methods, such as metabolomics and metagenomics, are needed for the reconstruction of microbial metabolism networks and the dynamic stability of physiological mechanisms within a dental biofilm.

### Table 3 Gene polymorphism of Calbicans isolates from Uygur and Han children with ECC or CF

| Ethnicity     | A-genotype (%) | B-genotype (%) | C-genotype (%) | P   |
|---------------|----------------|----------------|----------------|-----|
| Han ECC       | 13 (65)        | 4 (20)         | 3 (15)         | 0.827 |
| Uygur ECC     | 11 (55)        | 5 (25)         | 4 (20)         |     |
| Uygur CF      | 6 (30)         | 2 (10)         | 12 (60)        |     |
| P(Uygur ECC/CF) | 0.2            | 0.407          | 0.022          | 0.035|

*ECC early childhood caries, CF caries-free*  
Calbicans gene polymorphism in Uygur and Han children with ECC: \(n = 40\); theoretical frequency \(1 \leq T \leq 5\); Fisher’s exact test, \(P = 0.827\)

### Table 4 Acidogenicity of A-, B-, and C-genotype Calbicans isolates from the ECC and CF groups in the presence of 0.1 mol·L\(^{-1}\) glucose

| Genotype | ECC         | CF           | P   |
|----------|-------------|--------------|-----|
| A        | 3.092 ± 0.230 | 2.772 ± 0.125 | 0.019 |
| B        | 2.656 ± 0.308 | 2.825 ± 0.078 |     |
| C        | 2.870 ± 0.424 | 2.617 ± 0.193 |     |
| P        | 0.020        |              |     |

*ECC early childhood caries, CF caries-free*  
ECC group: A > C > B; CF group: B > A > C

### Table 5 SAP2 expression in A-, B-, and C-genotype C. albicans: differences between the ECC and CF group isolates

| Genotype | ECC         | CF           | P   |
|----------|-------------|--------------|-----|
| A        | 3.644± 1.415 | 0.910 ± 0.897 | 0.020 |
| B        | 2.617± 1.481 | 1.995 ± 0.332 |     |
| C        | 1.783± 1.373 | 0.435 ± 0.652 |     |
| P        | 0.001        |              |     |

*ECC early childhood caries, CF caries-free*  
SAP2 expression: ECC group, A > B > C; CF group, B > A > C
Conclusions
The strong acidogenicity and aciduricity, Sap activity, and high SAP2 and SAP5 expression might be closely associated with ECC. C.albicans potentially plays a key role in the progression of caries, which most readily affects genotype A carriers and could be attributed to person-to-person environmental variation.

Abbreviations
BSA: Bovine Serum Albumin; CAC: Chromagar Candida; ECC: Early childhood caries; MTT: Methy thiazolyl tetra zolium; PCR: Polymerase Chain Reaction; Sap: Secreted aspartyl proteinase; SDA: Sabourand Dextrose Agar; YNB: Yeast Nitrogen Base

Acknowledgements
Not applicable.

Funding
This study was funded by grant funding from the National Natural Science Foundation of China (grant no. 81760194) and the College of Xinjiang Medical University of China. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

Availability of data and materials
The data were upload as additional supporting files.

Authors’ contributions
WTZ contributed to Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization and Writing- original draft; YL contributed to Data curation, Formal analysis, Investigation and Methodology. JL contributed to Investigation, Software and Validation. AA contributed to Conceptualization, Data curation, Formal analysis and Software. JZ contributed to Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Visualization and Writing-review & editing. All authors gave final approval and agree to be accountable for all aspects of the work.

Ethics approval and consent to participate
This study was approved by the First Affiliated Hospital of Xinjiang Medical Ethics Committee (ethical review number 20150214–162), and the local heath administrative departments. The parents of all study participants provided written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
1Department of Endodontics, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang 830054, China. 2Xinjiang Kashgar First people’s Hospital, Xinjiang 844000, China.

Received: 24 July 2018 Accepted: 8 November 2018
Published online: 05 December 2018

References
1. Benjamin RM. Oral health: the silent epidemic[J]. Public Health Rep. 2010; 125(2):158–9.
2. Nobile CG, Fortunato L, Bianco A, Pileggi C, Pavia M. Pattern and severity of early childhood caries in southern Italya preschool-based cross-sectional study.[J]. BMC Public Health. 2014;14(1):1–12.
3. Doğan D, Dülgergil CT, Mutluay AT, Yıldırım I, Hamidi MM, Coşkun H. Prevalence of caries among preschool-aged children in a central Anatolian population.[J]. J Nat Sci Biol Med. 2013;4(2):325–9.
4. Melo Nunes AM, da Silva AA, Coelho Alves CM, Hugo FN, Costa Ribeiro CC. Factors underlying the polarization of early childhood caries within a high-risk population.[J]. BMC Public Health. 2014;14(2):988–92.
5. Marchant S, Brailford SR, Twomey AC, Roberts GJ, Beighton D. The predominant microflora of nursing caries lesions. Caries Res. 2001;35(6):397–406.
6. Yoon SX, Zhang Q, Lu LY, Yang R, Liu Y, Zou J. Genotypic distribution of Candida albicans in dental biofilm of Chinese children associated with severe early childhood caries. Arch Oral Biol. 2017;57(8):1048–53.
7. Fan MW, Zhou XD. Endodontics-4th. ed.Beijing: PEOPLE’S MEDICAL PUBLISHING HOUSE.2012.
8. Hintao J, Teanpaisan R, Chongsuvivatwong V, Dahlen G. The microbiological profiles of saliva, supragingival and subgingival plaque and dental caries in adults with and without type 2 diabetes mellitus. Oral Microbiol Immunol. 2007;22(3):75–81.
9. Gao S, Zhao W, Qiu RM, Lin Y. Relationship between secreted aspartyl proteinase of Candida albicans and severe early childhood caries. Chin J Stomatol. 2010;3(5):20–3.
10. Wu N, Lin J, Wu L, Zhao J. Distribution of Candida albicans in the oral cavity of children aged 3-5 years of Uygur and Han nationality and their genotype in caries-active groups. Genet Mol Res. 2015;14(1):748–57.
11. A yinuer A, Zhao J, Ma L, Lian BJ. An epidemiological study of Uyghurs and Han infant caries in Kashgar,China. Health Statistics. 2014;31(3):234–7.
12. Wu XS. Clinical study sample size estimation. 2nd ed ed. Beijing: PEOPLE’S MEDICAL PUBLISHING HOUSE; 2008. p. 145–208.
13. Lian BJ, Zhao J, Liu ZH, Nurbiye M. Comparative study of Uyghur children with different caries sensitive clinical isolates of Streptococcus Mutans biofilm State’s extracellular polysaccharide synthesis capacity. J Oral Sci Res. 2012, 28(4):325–30.
14. Barros LM, Bortolli MF, Alves AC, Klein MI, Gonçalves RB, Höfling JF. Genetic diversity and exoenzyme activities of Candida albicans and Candida dubliniensis isolated from the oral cavity of Brazilian periodontal patients. Arch Oral Biol. 2008;53(12):1172–8.
15. Mendes A, Mores AI, Canvalho AP, Rosa RT, Samarayake LP, Rosa EA. Candida albicans biofilms produce more secreted aspartyl protease than the planktonic cells. Biol Pharm Bull. 2007;30(9):1813–5.
16. Maria B, Irafltonz MR, Diego C, Marcelo O, Osvaldo C. Mechanisms of interaction between Candida albicans and Streptococcus mutans: an experimental and mathematical modelling study. Acta Odontol Scand. 2013; 71(3):38–46.
17. Klinke T, Guggenheim B, Klimm W, Thurnheer T. Dental Caries in Rats Associated with Candida albicans. Caries Res. 2011;45(1):100–6.
18. Wu N, Liu F, Zhao J. Distribution of Oral Candida Albicans and its correlation with early child caries in Uygur and Chinese children. J Oral Sci Res. 2013; 29(4):338–41.
19. Fan MW. New Development of Stomatol Res. 2015.Xinjiang Medical University.
20. Liu ZH. Study on the ability of acidogeneity, aciduricity and genetic diversity within aciduric virulence of Streptococcus mutans from caries-active and caries-free individuals in Urumqi Uygur children[dissertation][Xinjiang China]2015.Xinjiang Medical University.
21. Sun HY, Zou X, Zou JC. Current research status on the virulence of common pathogenic Candida. Chinese Journal of Microecology. 2013;25(5):604–8.
22. Taylor BN, Staib P, Binder A, Biesemeier A, Sehnal M, Rillinghoff M, Morschhuser J, Schrøppel K. Profile of Candida albicans-secreted aspartic proteinase elicited during vaginal infection. Infect Immun. 2005;73(3):1828–35.
23. Ge YP, Hu Y. Secreted aspartic proteinase genes differential expression and influencing factors. Int J Oral Science. 2010;3(6):732–5.
24. Li WQ, Yu DS, Gao S, Lin JC, Chen ZY, Zhao W. Role of Candida albicans secreted aspartyl proteinases(saps) in severe early childhood caries. Int J Mol Sci. 2014;15(16):10766–79.
25. Paula S, Leonor G, Alexandra C, Cintia A, Acacio GR, Cidalia PV, Antonio A, Célia P. New microsatellite multiplex PCR for Candida albicans strain typing reveals microevolutionary changes. J Clin Microbiol. 2005;43(8):3869–76.
26. Chen KW, Chen YC, Lo HJ, Frank CO, Wang TH, Lin CY, Li SY. Multilocus sequence typing for analyses of Clonality of Candida albicans strains in Taiwan. J Clin Microbiol. 2006;44(6):2172–8.
27. Silva-Rocha WP, Lemos VL, Svidzinski TI, Milan EP, Chaves GM. Candida species distribution, genotyping and virulence factors of Candida albicans isolated from the oral cavity of kidney transplant recipients of two geographic regions of Brazil. BMC Oral Health. 2014;14(1):1420–5.
28. Qiu RM, Li WQ, Lin Y, Yu DS, Zhao W. Genotypic diversity and cariogenicity of Candida albicans from children with early childhood caries and caries-free children. BMC Oral Health. 2015;15(1):144.