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Genotypic concordance of *streptocccus sobrinus* ATCC® 33402™ in mother and child pairs detecting by molecular AP-PCR assays

Abstract

Background: *Streptococcus mutans* and *Streptococcus sobrinus* are major pathogens causing Early Childhood Caries. The experience of caries in children is associate with mother's caries experience and the acquisition and transmission of this strain in various populations have received extensive attention. **Objective:** the aim of this study was to use a simple and rapid molecular method named Polymerase Chain Reaction (AP-PCR) to verify the transmission and genotypic concordance of *Streptococcus sobrinus* in mother child (0-36months) pairs.

Materials and methods: A hundred one of mother-child pairs were evaluated. Plaque samples were taken in both mother and child with a cotton swab each one. Mothers completed a questionnaire regarding their sociodemographical behaviors and dental behaviors. Detection of *Streptocccus sobrinus* ATCC® 33402™ was performed by AP-PCR. Group mean values were compared using *Pearson* Chi-Square and Fisher's Exact Test. (IBM SPSS 22.0, Chicago, Illinois, USA).

Results: The results showed 16 mother-child pairs (15.8%) had *Streptocccus sobrinus* ATCC® 33402™ and 6 mother-child pairs of 16 (37.5%) had genotypic concordance. In addition there was no statistical difference of sociodemographical behaviors and dental behaviors associated with the genotypic concordance of *Streptococcus sobrinus* ATCC® 33402™ *(p>0.05)*.

Conclusion: AP-PCR is a useful molecular method for strain identification of *Streptocccus sobrinus* ATCC® 33402™ in dental plaque.

Keywords: early childhood caries, polymerase chain reaction, streptococcus sobrinus, dental plaque, genotypic concordance

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Sabina Mungi, Guido Perona
Department of Pediatric Dentistry, Scientific University of the South, Peru

Correspondence: Sabina Mungi, Department of Pediatric Dentistry, Scientific University of the South, Peru, Email saby1000@hotmail.com

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Introduction

The most prevalent species of *Streptococcus* in humankind are *Streptococcus mutans* and *Streptococcus sobrinus*, with *Streptococcus mutans* dominating in prevalence[1] Children harbouring both species have more tendency to a higher caries risk than children carrying only *Streptococcus sobrinus*.[2,3] Dental caries is a multifactorial disease in which *Streptococcus mutans* and *Streptoccocus sobrinus* play a major role.[3] Early acquisition of *Streptococcus mutans* and *Streptoccocus sobrinus* has been shown in many studies to be a major risk factor for early childhood caries and future caries experience.[4] Vertical transmission from mother to child has been suggested as the main pathway for *Streptococcus mutans* acquisition.

Several studies reported similar *Streptococcus sobrinus* genotypes between parents and child.[5] Recent studies have suggested that horizontal transmission occurs readily within families.[6–9] *Streptococcus mutans* and *Streptococcus sobrinus* has been found in animal models. In these studies, *Streptococcus mutans* has been associated with pit and fissure caries, while *Streptococcus sobrinus* with smooth surface caries; therefore, it is, important to identify both species in future investigations. Some epidemiological studies have suggested that the presence of *Streptococcus sobrinus* is closely related to severity of dental caries rather than *Streptococcus mutans* by itself.[5]

Some methods of Streptococcus identification from oral cavity include isolation with selective growth mediums such as trypticase yeast-extract cystine sucrose bacitracin (TYCSB) and mitis salivarius bacitracin (MSB). Nevertheless, bacterial culture is a time consuming procedure and the culture yeld depends on the chosen medium. A modern and molecular method is an Arbitrary Primer Polymerase chain reaction (AP-PCR) which is faster and has a higher sensitivity to identify different strains. Also it has been already used to discriminate genotypic diversity of *Streptococcus sobrinus*. AP-PCR technique has been widely used to discriminate genotypic diversity of *Streptococcus sobrinus*.[10]

Information regarding the genetic groups of *Streptococcus sobrinus* associated with dental decay in children is important in understanding and evaluating the trends of dental caries in cross-sectional studies.[9] The aim of this study was to use a simple and rapid molecular method named AP-PCR to verify the transmission and genotypic concordance of *Streptococcus sobrinus* in mother child (0-36 months) pairs.

Materials and methods

This cross-sectional study involved 101 mothers and child pairs all of them attending in Aurelio Diaz Ufano y Peral maternal hospital. An informed and voluntary written consent from parents was obtained prior to clinical examination and samples recollection according to

the ethical guidelines of the Helsinki Declaration (1975), and the protocol was approved by the research Ethical Committee of the faculty of Dentistry of Cientifica del Sur University with approval number 000265. All mothers who participated in this study had signed the written informed consent. The inclusion criteria were mothers with minimum ten teeth, mothers or family members who are caregivers for their children. The exclusion criteria: Mothers and/or children with systemic diseases (diabetes mellitus, cardiac abnormalities, renal abnormalities), mothers and/or children physically Committed (quadriplegia, hemiplegia, visual disturbances, hearing and language), mothers and/or mentally Committed children (schizophrenia, mental retardation, Down syndrome, autism) mothers who present bad habits (alcohol, tobacco, drugs). Detection of *Streptocccus sobrinus* ATCC® 33402™ was performed by AP-PCR. And a survey according to Caufield and Li that included information about sociodemographical behavior and dental behavior was used to collect data. The kappa statistic indicated that the intra-examiner and inter-examiner correlation for the variables was 0.9 according to Fleiss scale. A pilot study was conducted to determine the efficacy of molecular AP-PCR test concluding that the tool were adequate. Chi-Square Tests and Fisher's Exact Test for Data Analysis was used. Statistical Significant was calculated with a level confidence of 95% ($p < 0.05$)

Results

In this cross-sectional study the presence of *Streptocccus sobrinus* ATCC® 33402™ for mother and child pairs is presented in Table 1: 16 (15.8%) of mothers and 17 (16.8%) of children present *Streptocccus sobrinus* ATCC® 33402™. The statistical analysis for the genotypic concordance is presented in Table 2. DNA fingerprint patterns showed that 6 of 16 mother and child pairs whose presented *Streptocccus sobrinus* ATCC® 33402™ had genotypic concordance (37.5%). The Chi-square test and Fisher's exact test were used for comparison of the proportions among various category groups. A p-value of less than or equal to 0.05 was considered statistically significant. There was no statistical difference between sociodemographical behavior and dental behavior of the mother and child and genotypic concordance (Table 1–6).

Table 1 Presence of *Streptococcus sobrinus* ATCC® 33402™ on mother and child pairs

Variable	n	Streptococcus sobrinus ATCC® 33402TM			
		Abscent		Presence	
Mother	101	85	84.20%	16	(15,8%)
child	101	84	83.20%	17	-16.80%

Table 2 Genotypic concordance of *Streptococcus sobrinus* ATCC® 33402™ on mother and child pairs

Variable	n	Streptococcus sobrinus ATCC® 33402TM			
		Abscent		Presence	
Mother-child pair	16	10	-62.50%	6	-37.50%

Table 3 Association of child's sociodemographical behavior and the genotypic concordance of *Streptococcus sobrinus* ATCC® 33402™

			Genotypic concordance				
Variable	**Categories**	**n**	**Abscent**		**Presence**		**P**
Gender	Female	9	6	(66.7%)	3	(33.3%)	0.549**
	Male	7	4	(57.1%)	3	(42.9%)	
Age (months)	0-6 m	4	2	(50%)	2	(50%)	0.763*
	7-18 m	8	5	(62.5%)	3	(37.5%)	
	19-3 6m	4	3	(75%)	1	(25%)	
Weight	high	11	6	(75%)	5	(25%)	0.346*
	Adecuate	5	4	(95.8%)	1	(4.2%)	
Size	low	10	6	(94.2%)	4	(5.8%)	0.608*
	Adecuada	6	4	(93.8%)	2	(6.2%)	
Way of delivery	Eutosic	9	7	(77.8%)	2	(22.2%)	0.302**
	Distosic	7	3	(42.9%)	4	(57.1%)	
Breast-feeding	Exclusive	9	7	(77.8%)	2	(22.2%)	0.348*
	Artificial	2	1	(50%)	1	(50%)	
	Both	5	2	(40%)	3	(60%)	
Person in charge	Parents	12	6	(50%)	6	(50%)	0.202*
	Other Family	2	2	(100%)	0	(0%)	
	Caregiver	2	2	(100%)	0	(0%)	

*Chi cuadrado of Pearson test**Fisher's exact test

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Table 4 Association of child's dental behavior and the genotypic concordance of *Streptococcus sobrinus* ATCC® 33402™

			Genotypic concordance				
Variable	Categories	N	Abscent		Presence		P
ECC	Abscent	13	9	(100%)	4	(0%)	0.241*
	Presence	3	1	(64.7%)	2	(35.3%)	
Teeth	None	2	0	(0%)	2	(100%)	0.410*
	Incisive (S/I)	10	7	(70%)	3	(30%)	
	Incisive and 1°molar (S/I)	3	2	(66.7%)	1	(33.3%)	
	All teeth	1	1	(100%)	0	(0%)	
Brushing	None	5	3	(60%)	2	(40%)	0.393*
	1-2 p/day	3	1	(25%)	2	(75%)	
	≥ 3 p/day	7	5	(71.4%)	2	(28.6%)	
	Interdiary	1	1	(100%)	0	(0%)	
Drug use	None	15	10	(66.7%)	5	(33.3%)	0.375*
	ATB	1	0	(0%)	1	(100%)	
Dental tools	None	4	3	(75%)	1	(25%)	0.450*
	Gauze	7	3	(42.9%)	4	(57.1%)	
	brushteeth without paste	2	2	(100%)	0	(0%)	
	Brushteeth + Paste	3	2	(66.7%)	1	(33.3%)	

*Chi cuadrado of Pearson test**Fisher's exact test. ECC = Early childhood caries

Table 5 Association of mother's sociodemographical behavior and the genotypic concordance of *Streptococcus sobrinus* ATCC® 33402™

			Genotypic concordance				
Variable	**Categories**	**n**	**Abscent**		**Presence**		**P**
Age	18-30 years	11	6	(54.5%)	5	(45.5%)	0.588**
	>30 years	5	4	(80%)	1	(20%)	
Number of births	Unipara	47	7	(63.6%)	4	(36.4%)	0.654**
	Multipara	54	3	(60%)	2	(40%)	
Time of gestation	Prematurity	2	2	(100%)	0	(0%)	0.375**
	Normal	14	8	(57.1%)	6	(42.9%)	
Educational level	Primary	4	2	(50%)	2	(50%)	0.109*
	Secundary	6	4	(66.7%)	2	(33.3%)	
	Technical	2	0	(0%)	2	(100%)	
	University	4	4	(100%)	0	(0%)	
monthly	< 750 PEN	8	5	(62.5%)	3	(37.5%)	0.696**
expenses	>750 PEN	8	0	(62.5%)	3	(37.5%)	

*Chi cuadrado of Pearson test**Fisher´s exact test

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Table 6 Association of mother´s dental behavior and the genotypic concordance of *Streptococcus sobrinus* ATCC® 33402™

			Genotypic concordance				
Variable	**Categorías**	**n**	**Abscent**		**Presence**		**p**
Caries	Presence	16	10	(62.5%)	6	(37.5%)	***
	Abscent	0	0	(0%)	0	(0%)	
OHI	Good	0	0	(0%)	0	(0.0%)	0.304*
	Regular	8	6	(75%)	2	(25%)	
	Bad	8	4	(50%)	4	(50%)	
Brushing	Never	0	0	(0%)	0	(0%)	0.625*
	1-2 v/day	9	6	(66.7%)	3	(33.3%)	
	≥3 v/day	7	4	(57.1%)	3	(42.9%)	
	Interdiary	0	0	(0%)	0	(0%)	
Dental tools	Not even	0	0	(0%)	0	(0%)	0.668*
	Toothbrush+paste	15	9	(60%)	6	(40%)	
	Tootbtush+paste+floss	1	1	(100%)	0	(0%)	
	Others	0	0	(0%)	0	(0%)	

*Chi cuadrado of Pearson test ** Fisher´s exact test***Constant

Discussion

Results from the present study suggest that mothers are one of the source of *Streptocccus sobrinus* ATCC® 33402™. Genotypes of *Streptocccus sobrinus* ATCC® 33402™ isolated from children were homologous to those isolated from the mother in 16 (15.8%) of the mother-child pairs. The findings are further enhanced by a cross-sectional study. The initial expectation was that the concordance between genotypes of mothers and their infants would be lower than the observed considering the fact of previously published studies.[11–17] Perhaps most of the infants acquired *Streptococcus sobrinus* from their mothers, but we were unable to detect all genotypes present in mothers or in the infants. Although saliva constitutes the vehicle of transfer from mother to child, there is not knowledge whether *Streptococccus sobrinus* found in saliva represent the total repertoire of *Streptococcus sobrinus* on the tooth surfaces.[18] Also the stability of *Streptococcus sobrinus* genome is not clear. Previously Caufield.[38,39] reported the stability of DNA fingerprints over a three-year period in one mother-infant pair. The chromosomal DNA fingerprint of *Streptococcus mutans* strain originally isolated 25 years ago by Krasse.[40] showed a pattern identical to that of a recent isolate from this same subject, so in some individuals, *Streptococcus mutans* appears to be stable. Hence, the lack of total homology with mother's genotypes could be due, in part, to the instability of restriction patterns or a form of genetic drift as a function of time. The possible biological relevance between concordance and disease outcome may not be illustrated by the fact that there was no significant association with sociodemographical and dental behavior. However, there was no way of assessing either the virulence or the transmissibility of individual genotypes. Evidence from an earlier study of Hagan et al. 1989, now expanded to include ten families, shows that all ten of the *Streptococcus mutans* colonized children who were adopted prior to tooth emergence were caries-free. Since their natural mothers were not a likely source of *Streptococcus mutans*, because *Streptoccocus mutans* does not colonize the oral cavity until after teeth emerge, it is tempting to speculate that a child who acquires *Streptococcus mutans* from a source other than the mother may be less prone to caries. Further studies are needed, however, to confirm this association.

Among the seven families in whom *Streptococcus mutans* was obtained from the father, the mother, and the infant, DNA fingerprints failed to show commonality of genotypes between fathers and infants or fathers and spouses. Similar findings were reported by Rogers.,[34] Davey & Rogers.,[36] Caufield et al.,[37] and Kulkarni et al.[22] The obvious explanation is that mothers enjoy the most contact with their infants and, therefore, constitute the major source of *Streptococcus mutans*. If, however, as we hypothesize, passively acquired immunoglobulins derived from the mother play a role in determining which strains of indigenous bacteria can colonize the infant, then fathers' strains may be excluded. The mechanism may be that intimacy between a mother and her spouse results in the appearance of an immunological recognition of the father's indigenous bacteria as non-self, and this immunological awareness is transferred to the child through the placenta or breast milk. Caufield et al.[25–29] showed that the conservation of specific strains of *Streptococcus mutans*, not only among mother-child pairs

but also within racial cohorts, underscores the notion that mother-to-child transfer of indigenous bacteria such as Streptococcus may be a multi- generational theme Li et al.[1,4,18–20] hypothesize that mothers transfer to their infants not only maternal immunoglobulins via the placenta and colostrum, but also a complementary set of indigenous bacteria capable of co-existing with directed immunity factors. How passively acquired immunoglobulins from the mother influence the infant's "selection" of which bacteria are allowed to persist and those which are eliminated remains to be solved.

In summary, dental caries is an infectious and transmissible disease. Detailed knowledge regarding the acquisition and transmission of infectious agents facilitates a more comprehensive approach toward prevention. *Streptococcus sobrinus* are important organisms in the initiation and pathogenesis of dental caries. Recent evidence demonstrates that these bacteria are found in the mouths of pre-dentate infants and are acquired via vertical and horizontal transmission from human reservoirs.[41] This information should facilitate the focusing of clinical interventions that prevent or delay infant infection, thereby reducing the prevalence of dental caries.

Conclusion

1. There was *Streptococcus sobrinus ATCC® 33402™* genotypic concordance from mother to child from 0 to 36 months.
2. There is no significant association between mother's sociodemographical behavior and the genotypic concordance of *Streptococcus sobrinus ATCC® 33402™*.
3. There is no significant association between children's sociodemographical behavior and the genotypic concordance of *Streptococcus sobrinus ATCC® 33402™*.
4. There is no significant association between mother's dental behavior and the genotypic concordance of *Streptococcus sobrinus ATCC® 33402™*.
5. There is no significant association between children's dental behavior and the genotypic concordance of *Streptococcus sobrinus ATCC® 33402™*.

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Conflict of interest

None.

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