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Assessment of Diversity and Fidelity of Transmission of *Streptococcus mutans* Genotypes in American Indian and Southeast Iowa Mother-Child Dyads

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Early childhood caries (ECC) is a common chronic infectious disease of childhood with a complex etiology and many contributing risk factors. Its prevalence is greater in certain racial and ethnic minority groups and populations with low socioeconomic status. Among the species of bacteria that contribute to the progression of dental caries, *Streptococcus mutans* (SM) has long been considered a primary etiological agent of the disease. We report here on the genotypic diversity, commonality, and fidelity of mother-child transmission of *S. mutans* in mother-child dyads in two high-risk populations.

Methods: Thirty-eight mother-child dyads from a Southeast Iowa population and 40 dyads from a Northern Plains American Indian Tribe were selected for a comparison of *S. mutans* genotype profiles within and between populations. *S. mutans* was isolated from whole mouth plaque samples collected from each subject. DNA was extracted and AP-PCR using OPA2 primer was performed. Amplified DNA was electrophoresed and images of the resulting patterns were compared via GelCompar ®IIv6.5 software.

Results: Thirty unique *S. mutans* genotypes were identified from the 1,638 *S. mutans* isolates analyzed. Fifteen genotypes (50%) were seen in both cohorts, while 11 were unique to the American Indian (AI) cohort and 4 were unique to the Southeast Iowa (SEI) population. Within the AI cohort, 61.5% (16/26) of the *S. mutans* genotypes identified were seen in ≥2 individuals and 14 of the 26 (53.8%) were seen in ≥2 dyads. In the SEI cohort, 78.9% (15/19) of the *S. mutans* genotypes identified were seen in ≥2 individuals and 13 of the 19 (68.4%) were present in ≥2 dyads. Fifty-seven percent of AI children and 23% of SEI children displayed fidelity of mother-child transmission of ≥1 *S. mutans* genotype.

Conclusion: In comparing the populations, we observed large variation in genotypic diversity and fidelity of mother-child transmission, while the amount of commonality seen in both cohorts was similarly high in both groups. This study furthers our understanding of the genotypic diversity of *S. mutans* in both of these populations and provides a basis for further comparison to other populations at greater risk for developing ECC.

Keywords: ***Streptococcus mutans*, early childhood caries, oral health disparities, genotyping, AP-PCR**

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INTRODUCTION

Dental caries is the most common chronic disease of childhood (1). Tooth decay present in children under the age of 6 years old is designated early childhood caries (ECC), or in its more acute form, severe early childhood caries (S-ECC). Children impacted by ECC/S-ECC are at greater risk for cavitation of permanent dentition and other health issues, such as nutritional deficiencies and below average growth (2–6). While ECC is present in all populations, it is a substantial public health challenge in certain racial and ethnic minority groups. Hispanic children have almost twice the amount of untreated decay as Caucasian children (20 and 11% respectively) (7). The highest rate of untreated decay, by far, is found in American Indian and Alaska Native (AI/AN) children at 43%, almost four times the amount reported in Caucasian children (7). There has been a national trend indicating a decrease in untreated decay in children over the past decade. While this trend has also been observed in minority populations, the disproportionate burden of disease in these populations remains (8). Although a full understanding of the underlying causes of the increased rates of ECC/S-ECC in minority and indigenous populations has not been reached, it has been posited that many risk factors contribute to the progression and severity of the disease. These risks include behavioral choices (e.g., sugar intake and oral health hygiene routine), oral microflora, sociodemographic characteristics (e.g., household income, parental education and crowded living conditions), limited access to care, environmental (e.g., fluoridated/non-fluoridated water source) and host factors (5, 7–10).

Mutans streptococci (MS) have been the focus of much caries research. Numerous studies support the belief that this group of bacteria play a pivotal role in the development of dental caries and that MS levels may be a potential indicator of ECC risk (11–17). Species within the mutans streptococci group, specifically *Streptococcus mutans*, have long been considered primary etiological agents of dental caries with time of colonization in children associated with severity of disease (18–21). Many studies have investigated genotypic diversity of *S. mutans* within various high-risk populations in an effort to understand the oral health disparities present in low-SES (socio-economic status) and racial/ethnic minority groups (22–25). While much work has been done looking at *S. mutans* genotypes identified in individual populations, there is a lack of comparisons between populations displaying high rates of ECC/S-ECC.

In our laboratory, we have investigated *S. mutans* genotypic diversity via arbitrarily primed PCR (AP-PCR) in mother-child dyads within two distinct high-risk populations – one located in Southeast Iowa and the second a Northern Plains American Indian Tribe. Studies have been conducted and reported on concerning initial colonization of *S. mutans* in American Indian children, including some analyses of genotypic diversity and transmission seen during initial acquisition (birth through 16 months of age) (26). Previous investigations have also examined the genotypic diversity seen in 2- to 5-year old children in this same Southeast Iowa population (mean age = 3.6 years) and compared it to what is seen in 3-year-old children in the American Indian cohort (27). Our focus to date has been on children. Therefore, we have a paucity of data looking at genotypic diversity of MS within and across families. A more comprehensive analysis of the overall diversity, commonality, and fidelity of transmission of *S. mutans* genotypes found in both cohorts is needed. Herein, we compare all *S. mutans* genotypes identified in a subset of mother-child dyads from both populations.

MATERIALS AND METHODS

Descriptions of populations and details of recruitment, consent, sample collection, sample processing, *S. mutans* isolation and identification, DNA extraction, AP-PCR and dendrogram analysis of *S. mutans* isolates have all been described in previous laboratory publications (26–29). Brief descriptions of study procedures are presented here. More detailed descriptions can be found in the previously published manuscripts.

Study Population 1 (American Indian)

Details regarding this study population recruitment, sample collection, sample processing and genotyping have been published previously (26, 28, 29).

Recruitment

Multiple methods were utilized to recruit Northern Plains American Indian women who were pregnant or had recently given birth ($n = 239$) for a longitudinal birth cohort study. The proposed study was described to pregnant women during prenatal visits with Indian Health Service (IHS). It was advertised in local papers and on local radio stations. Recruitment was also done by local dental clinic staff. The onsite research team was directed by a senior dental hygienist in the tribe and all team members were American Indian.

Selection of Subjects for Analyses

Of the 239 dyads recruited for the AI study, a subset of 80 were randomly selected for microbiological analyses. Due to the fact that the AI study was longitudinal, *S. mutans* isolates ($n = 1,401$) from multiple time points were analyzed for all mother-child dyads. Selection of mother-child dyads for this study was partially a convenience sampling that included the subjects from the previously completed comparison of SEI and AI children. We also took into consideration what dyads had all available samples processed for genotyping. This resulted in the selection of 40 dyads (50% of the subset; 17% of all dyads recruited) for comparison in this study. For the AI cohort, isolates from multiple time points throughout the study were analyzed. For AI children with *S. mutans* detected within the study period ($n = 35$), the mother's *S. mutans* isolates were genotyped at the baseline visit (1 month of age ±30 days), the visit when mutans streptococci presence was established in the child (child age varied), and the final visit (36 months of age ±30 days). *S. mutans* was not detected in 3 of the mothers.

Sample Collection and Processing

Samples were collected from each mother and child at 8 time points from birth to 36 months of age – baseline, 4, 8, 12, 16, 22, 29, and 36 months (±30 days). Whole mouth plaque samples were collected by swabbing all tooth surfaces with a sterile cotton swab and placing swabs into tubes containing TSB-YE (Tryptic Soy Broth supplemented with 0.5% Yeast Extract) (Difco, Sparks, MD, USA) with 10% glycerol. Prior to tooth eruption in children, the oral mucosa and tongue were swabbed. Samples were refrigerated until shipment via FedEX overnight in temperature controlled Saf-T-Temp™ packaging (Saf-T-Pak, Hanover, MD, USA) to the microbiology labs in the Iowa Institute for Oral health Research at the College of Dentistry, University of Iowa.

Swab samples were vortexed (3 min), followed by sonication (1 min) before sample dilution and spiral plating. Samples were plated on Mitis-Salivarius-Kanamycin-Bacitracin agar (MSKB) (Difco, Sparks, MD, USA) using an Autoplate® Spiral Plating System (Advanced Instruments, Inc., Norwood, MA, USA) for determination of *S. mutans* counts. Following incubation (37°C, 5%CO_2, 72–96 h), ten colonies displaying *S. mutans* colony morphology were selected from the MSKB plate. If fewer than 10 colonies were present, all available colonies were isolated. Identification was confirmed utilizing a previously described protocol that combines both morphological identification and PCR targeting the *gtfB* gene (28). Isolates were frozen in TSB-YE (10% glycerol) and stored at -80°C.

DNA Extraction

At the onset of the American Indian study, DNA was extracted using the Epicenter® MasterPure™ Gram Positive DNA Purification Kit (Epicenter, Madison, WI, USA) modified as described in a previous publication (26). However, the volume of samples being processed for isolation and identification of mutans streptococci (MS) necessitated the development of a more rapid, but equally effective, protocol for DNA extraction and *S. mutans* identification, as detailed in a previous manuscript (28). Quality of extracted DNA was verified using a Nanodrop® spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Study Population 2 (Southeast Iowa)

Details regarding this study population recruitment, sample collection, sample processing and genotyping have been published previously (27).

Recruitment

The cohort for the SEI population was recruited through two locations: the University of Iowa Muscatine Pediatric Dental Clinic and Muscatine Head Start. Children between 2 and 5 years of age (mean = 3.6 years) were recruited ($n = 190$). Nearly 45% of children recruited were Hispanic. The majority of the remaining children were Caucasian. Recruitment was completed in person by the study coordinator.

Selection of Subjects for Analyses

As with the AI population, selection of mother-child dyads for this study was a convenience sampling based on previous comparisons of the children. Thirty-eight dyads (20% of all dyads recruited) were selected for comparison here. The SEI study was a cross-sectional study, therefore *S. mutans* isolates ($n = 237$) were analyzed from one sample per subject. In addition to the child isolates previously analyzed, all *S. mutans* isolates from the mothers in the SEI cohort were analyzed. Eleven mothers in the SEI dyads did not have detectable levels of *S. mutans* at the time of sampling.

Sample Collection and Processing

Whole mouth plaque samples were collected on swabs that were placed into tubes containing reduced transport media (composition per liter: 0.045% KH_2PO_4, 0.045% K_2HPO_4, 0.09% NaCl, 0.09% $(NH_4)_2SO_4$, 0.018% $MgSO_4$, 0.038% EDTA, 0.04% Na_3CO_3, 0.02% dithiothreitol). Samples were placed on ice and transported to the microbiology labs.

Swab samples were processed and *S. mutans* isolated using the same method as in the American Indian study. Identification of isolates as *S. mutans* was confirmed by fermentation profile (mannitol, raffinose, salicin, and sorbitol) and arginine decarboxylase activity.

DNA Extraction

For the SEI dyads, the modified protocol for the Epicenter® MasterPure™ Gram Positive DNA Purification Kit (Epicenter, Madison, WI, USA) (26) was used for DNA extraction. DNA quality was verified by Nanodrop® spectrophotometry.

Evaluation of Genotypic Diversity

Genotypic diversity was examined in the same way for both populations. Arbitrarily Primed PCR (AP-PCR) using the OPA-2 primer was done on all *S. mutans* isolates. The positive control for both studies was *S. mutans* ATCC 25175. AP-PCR was set up and amplified products electrophoresed and imaged as previously described (26). Curve based cluster analysis using the Pearson correlation and Unweighted Pair Group Method using Arithmetic Averages (UPGMA) was used to generate dendrograms to assess genotypic diversity and strain relatedness (GelCompar ®IIv6.5, Applied Maths, Austin, TX, USA).

RESULTS

Genotypic Diversity and Fidelity of Transmission From Mother to Child

Diversity of *S. mutans* genotypes was evaluated by determining the number of unique *S. mutans* genotypes (GT) identified in individual subjects and within mother-child dyads (**Figure 1**). A total of 1,638 SM (AI = 1,401; SEI = 237) isolates were analyzed in this study. The number of *S. mutans* genotypes seen in individuals in the SEI population ranged from 0 to 2. When looking at mothers and children separately, the range was 0–2 in mothers and 1–2 in children. In the AI population, the range of *S. mutans* genotypes per individual was 0–6 (mothers: 0–5, children: 0–6). When looked at as mother-child dyads, the range

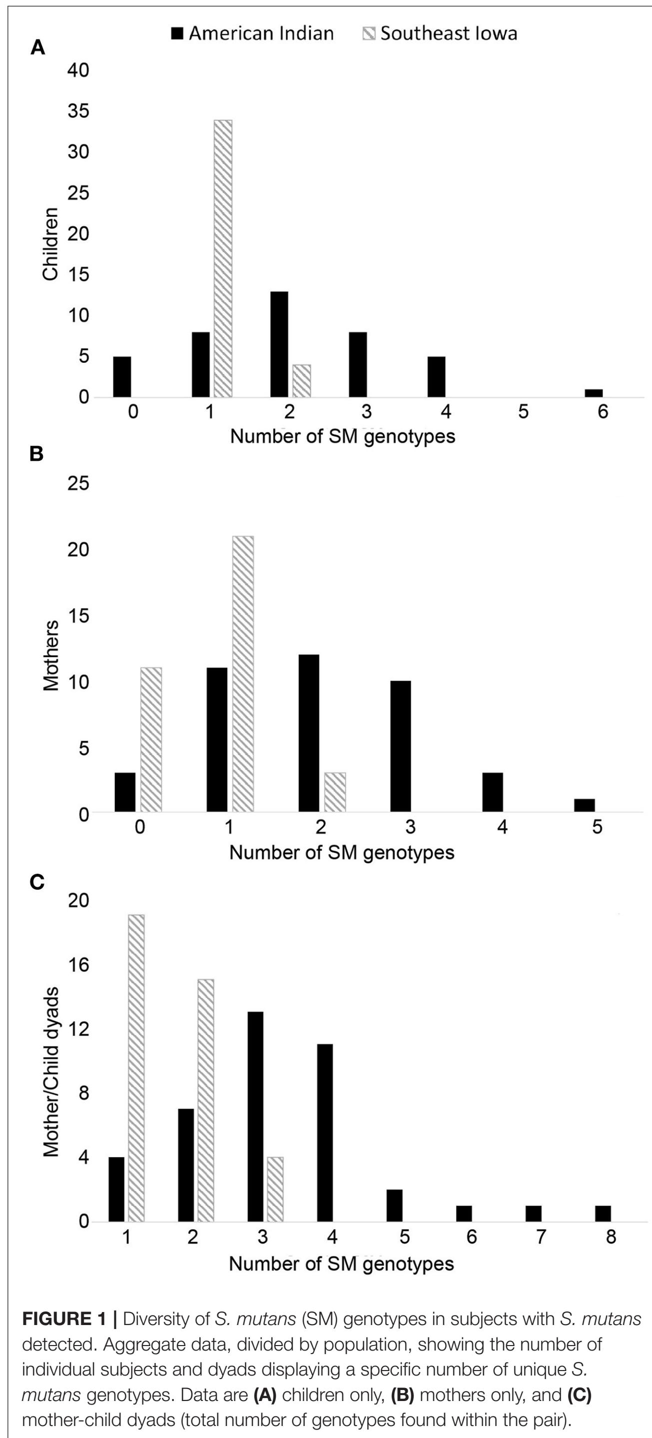

FIGURE 1 | Diversity of *S. mutans* (SM) genotypes in subjects with *S. mutans* detected. Aggregate data, divided by population, showing the number of individual subjects and dyads displaying a specific number of unique *S. mutans* genotypes. Data are **(A)** children only, **(B)** mothers only, and **(C)** mother-child dyads (total number of genotypes found within the pair).

of *S. mutans* genotypes per mother-child pair was 1–3 in the SEI population and 1–8 in the AI population.

Fidelity of transmission of *S. mutans* genotypes from mother to child was also evaluated. In the 38 mother-child pairs analyzed from the SEI cohort, 23% (8/35) of children shared a genotype with his/her mother and 77% (27/35) did not. Transmission status could not be determined (no *S. mutans* genotype profile available for mother) for the remaining 3 children in this cohort. The percent of children that shared at least 1 genotyped with his/her mother in the AI cohort was 57% (23/40). The remaining 43% (17/40) did not display fidelity of transmission of any *S. mutans* genotypes.

TABLE 1 | Genotypes displaying fidelity of transmission from mother to child in ≥1 mother-child dyad.

	Percent of dyads with trasmission	
Genotype	**American Indian (AI)**	**Southeast Iowa (SEI)**
1	27.5%	5.7%
6	10%	0%
8	15%	2.8%
11	10%	2.8%
12	0%	2.8%
14	0%	2.8%
15	0%	2.8%
18	25%	0%
19	12.5%	0%
20	2.5%	0%
21	2.5%	0%
27	2.5%	0%
29	0%	2.8%

Thirteen of the *S. mutans* genotypes identified showed fidelity of transmission in at least 1 mother-child dyad (**Table 1**). Additional genotypes were found in both mothers and children, but not within both subjects of a mother-child dyad. Genotypes 1 and 8 were the genotypes most commonly acquired by children from their mothers.

Commonality of Genotypes

A total of 30 unique *S. mutans* genotypes were identified within the study population (**Figure 2**). Of these 30 genotypes, 26 were seen in the AI population and 19 in the SEI cohort. Analysis of commonality, the sharing of genotypes by at least 2 people (30), within and between the two populations, showed that half (15/30) of the *S. mutans* genotypes identified were present in both populations. Eleven *S. mutans* genotypes were unique to the AI cohort and 4 were unique to the SEI cohort. Within the AI cohort, 61.5% (16/26) of the *S. mutans* genotypes identified were seen in ≥2 individuals and 14 of the 26 (53.8%) were seen in ≥2 dyads. In the SEI cohort, 78.9% (15/19) of the *S. mutans* genotypes identified were seen in ≥2 individuals and 13 of the 19 (68.4%) were present in ≥2 dyads. The three most common *S. mutans* genotypes in this study (GT1, GT8, and GT11) were seen in both populations and were identified in 35.82, 23.13, and 16.42% of all individuals, respectively (**Table 2**).

DISCUSSION

While each cohort within this study can be classified as a Midwest population, they are both geographically and culturally distinct from each other. The populations were selected for study because, demographically, they were at risk for higher incidence of early

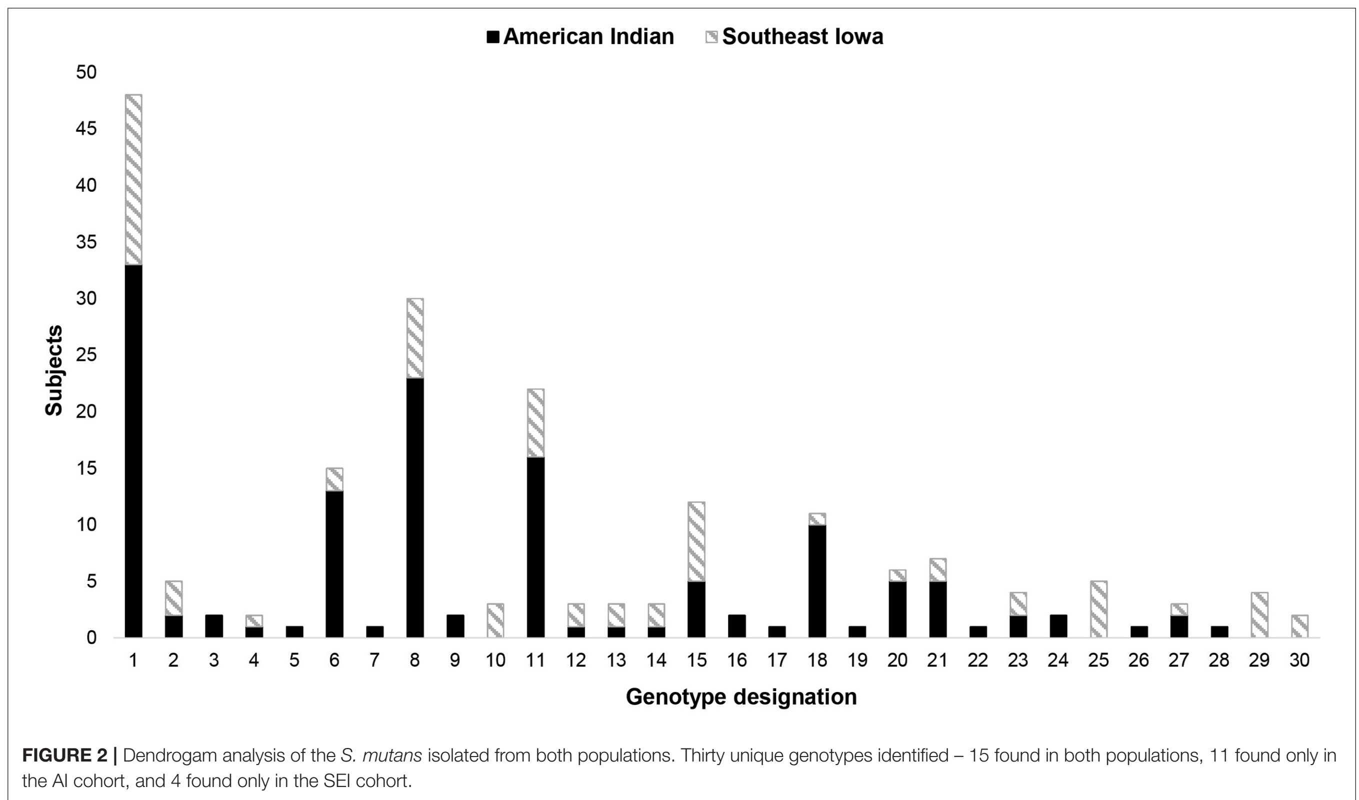

FIGURE 2 | Dendrogam analysis of the *S. mutans* isolated from both populations. Thirty unique genotypes identified – 15 found in both populations, 11 found only in the AI cohort, and 4 found only in the SEI cohort.

childhood caries and, especially in the AI cohort, severe early childhood caries. The children from both populations displayed a higher incidence of caries than that of children in the same age group within the general U.S. population (9).

Genotypic diversity was the most challenging comparison to make in this study. The AI mothers and children were part of a longitudinal birth cohort study, so there were multiple samples to isolate *S. mutans* from for most subjects. The SEI study was cross-sectional, so *S. mutans* was only isolated from samples collected at one time point. This resulted in significantly more *S. mutans* isolates to analyze from the AI population. There were a total of 1,638 *S. mutans* isolates analyzed in this study, of which, 1,401 were from AI subjects and 237 were from SEI subjects.

Many studies have reported on genotypic diversity in both longitudinal and cross-sectional studies of various populations. Previous investigations have shown in cross-sectional studies, a range of 1–5 *S. mutans* genotypes per subject, with some reporting as few as 1–2 genotypes per subject (23, 24, 31–33). In a 7-year longitudinal birth cohort study, Lindquist et al. identified 2–7 *S. mutans* genotypes in children and 1–5 in mothers over the course of the study (34). While some longitudinal studies report slightly higher *S. mutans* genotypic diversity than cross-sectional studies, most subjects within the longitudinal studies displayed $\leq$3 unique genotypes per subject at a single time point (30, 34, 35). Similarly, the mothers and children in the AI population reported on here displayed a greater amount of genotypic diversity than subjects in the SEI population, but none displayed more than 3 unique genotypes at any single time point. The majority of studies discussed here utilized AP-PCR for the genotyping method, but rep-PCR (repetitive element sequence-based PCR) and RFLP (restriction fragment length polymorphism) analysis were also used. Variation in the method of genotyping could have an impact on results. Ages of participants also varied in these studies. Of the cross-sectional studies, two worked with pre-school aged children (3–5 years), two with kindergarten aged children (5–6 years), and one worked with adults (18–28 years). For the longitudinal studies, two worked with birth cohorts and one with children who were kindergarten aged (5–6 years) at the start of the study. While we recognize that it is challenging to compare the results from these two studies, overall, our data presented here were consistent to previously-published cross-sectional and longitudinal investigations and represent a first step toward comparing *S. mutans* diversity in multiple populations.

It is possible that we are underestimating genotypic diversity in the SEI population and additional genotypes may have been identified with sampling at additional time points. However, the high rate of commonality observed in this population and the fact that the results were consistent with other studies that have found lower rates of genotypic diversity within some populations (24, 32, 33), suggests that our data were a good assessment of overall genotypic diversity of *S. mutans* in this population.

Reported rates of vertical *S. mutans* transmission vary greatly between studied populations. The reasons for the differences in vertical transmission are not known. Rates as low as 21%

TABLE 2 | Distribution of *S. mutans* (SM) genotypes in subjects with detectable levels of SM present.

Genotype	Percent of subjects			Percent of total SM isolates	Number of mother-child dyads		
	AI	SEI	Total		AI	SEI	Total
Genotypes found in both populations							
1	45.83	24.19	35.82	25.89	22	13	35
2	2.78	4.84	3.73	1.22	2	3	5
4	1.39	1.61	1.49	0.49	1	1	2
6	18.06	3.23	11.19	7.51	9	2	11
8	31.94	12.90	23.13	14.53	17	7	24
11	18.06	9.68	16.42	10.20	12	5	17
12	1.39	3.23	2.24	0.67	1	1	2
13	1.39	3.23	2.24	0.85	1	2	3
14	1.39	3.23	2.24	1.10	1	1	2
15	6.94	11.30	8.96	4.27	5	6	11
18	13.89	1.61	8.21	4.64	8	1	9
20	6.94	1.61	4.48	2.14	4	1	5
21	6.94	3.23	5.22	4.15	4	2	6
23	2.78	6.23	2.99	0.79	2	2	4
27	2.78	1.61	2.24	0.43	1	1	2
Genotypes unique to American Indian population							
3	2.78		1.49	0.85	2		2
5	1.39		0.75	0.67	1		1
7	1.39		0.75	0.55	1		1
9	2.78		1.49	0.55	1		1
16	2.78		1.49	0.12	2		2
17	1.39		0.75	0.61	1		1
19	29.17		15.67	12.94	16		16
22	1.39		0.75	0.06	1		1
24	2.78		1.49	1.71	2		2
26	1.39		0.75	0.12	1		1
28	1.39		0.75	0.61	1		1
Genotypes unique to Southeast Iowa population							
10		4.84	2.24	0.24		3	3
25		8.06	3.73	1.16		5	5
29		6.45	2.99	0.79		3	3
30		3.23	1.49	0.12		2	2

and as high as 81% have been reported (35–38). We found that the transmission rates in both of our populations varied considerably. Fifty-seven percent (23/40) of AI mother-child dyads displayed fidelity of transmission of at least one *S. mutans* genotype. In the SEI dyads, only 24% (10/42) of children shared ≥1 genotype with their mother. The percentage of dyads in the AI and SEI cohorts that did not display fidelity of transmission (42% and 69% respectively) included: 1) dyads where both subjects had *S. mutans*, but did not share a genotype and 2) dyads where either mother or child exhibited no detectable *S. mutans*. In the AI cohort, there were five dyads where the child had no detectable *S. mutans* and three dyads where the mother had no detectable *S. mutans*. In the SEI cohort, there were eleven dyads where there was no detectable *S. mutans* in the mother's sample. There are many variables that may affect rates of vertical and horizontal transmission, including, but not limited to, dietary habits (e.g., prolonged bottle feeding, sugar intake, sharing food), behavioral and oral health practices (e.g., poor oral hygiene routines, lack of regular dental care), level of *S. mutans* colonization in the oral cavities of caregivers and crowded living conditions (39, 40).

Many studies in fact support the idea that, while maternal transmission is an important contributing factor to the establishment of *S. mutans* within a child's oral microflora, it may not be the primary source of *S. mutans* colonization in children at risk for ECC and S-ECC. In one Alabama population, 41% of mother-child pairs showed some level of maternal transmission; however, acquisition of *S. mutans* from non-maternal sources was observed in 74% of the pairs (41). Zhan et al. reported that 90% of children in their study harbored genotypes of non-maternal origin and the overall rate of maternal transmission was only 21% (36). We report here similar findings. Low percentages of children in either population exhibited SM genotypes that all matched those in their mothers (AI: 20%, SEI: 24%). A majority of children had ≥1 SM genotype that was different from those isolated from their mothers (AI: 77.1%, SEI: 69%), suggesting acquisition from other sources. No children in the SEI cohort had both maternal and non-maternal sources of *S. mutans* colonization, while 69% of AI children displayed both vertical and apparent horizontal transmission of *S. mutans*. Determining the source of SM colonization in young children is complex as there appear to be multiple variables that influence directly or indirectly transmission and acquisition events. Assessing the impact of a complex set of variables that may interact in ways that are unknown in transmission and acquisition of MS will require additional investigations.

The high rate of commonality (30) of *S. mutans* genotypes observed between families in both of these cohorts (53.8% AI, 68.4% SEI) provided additional support to the idea that both vertical and horizontal transmission events played a key role in establishing the genotypic profile of *S. mutans* in individuals within both of these populations. As with transmission, there has been a high degree of variance seen across populations in the amount of sharing of SM genotypes observed between individuals. Many studies that include data on sharing of genotypes between unrelated study subjects report little to no horizontal transmission observed (33–35, 42). In contrast, Cheon et al. found that 92.5% of the children in their study shared at least 1 genotype with another child and that the most prevalent genotype was identified in 36% of the children (30). In our AI cohort, 100% of the individuals that had *S. mutans* colonization (n=72) shared ≥1 genotype with at least one individual from another mother-child dyad within that cohort, while 93.1% (67/72) shared ≥1 genotype with ≥1 individual in the SEI population. There were 28 AI subjects (38.9%) that harbored ≥1 genotype that was unique to that population. In our SEI cohort, 90.9% (60/66) of subjects with *S. mutans* colonization (n=66) shared ≥1 genotype with at

least one individual from another mother-child dyad within that cohort, while 84.8% (56/66) shared ≥1 genotype with ≥1 individual in the AI population. There were 14 individuals from the SEI cohort (21.2%) that harbored a genotype unique to that population.

CONCLUSIONS

We acknowledge that there are limitations and challenges to comparing these two populations due to differences in study design. However, as stated in the discussion, we do believe that the data obtained is a fair representation of the genotypic diversity of each population. Since the focus of this manuscript is a comparison of findings within populations, not side-by-side comparisons of individual mother child dyads, we feel it reasonable to make the following observations when looking at the data from both populations. Large variation between these high caries-risk populations was observed in genotypic diversity and fidelity of transmission from mother to child, whereas the amount of commonality and source of *S. mutans* colonization (maternal/non-maternal) numbers were more similar. The importance of these varied SM genotype profiles and acquisition patterns – vertical vs. horizontal – on colonization in these caries high-risk children is unknown. It is conceivable that specific SM genotypes are pivotal in caries development, or specific profiles of SM genotypes create conditions in the oral cavities of these children that, combined with other variables such as diet, behavior, crowded living conditions, etc. could set the stage for the development of early childhood and severe early childhood caries. This and future studies comparing multiple populations at increased risk of early childhood caries will contribute to gaining a better understanding of how these variables interact and will provide valuable information for development of new preventive and therapeutic treatment approaches to improve this area of health disparity.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available and would need approval from the American Indian Tribe. Requests to access the datasets should be directed to david-drake@uiowa.edu.

ETHICS STATEMENT

Consent was obtained for all subjects who participated in both studies. For both populations, approval was obtained from the University of Iowa Institutional Review Board (IRB). Additionally, for the American Indian cohort, approval was required and obtained from both the Tribal Research Review Board and Aberdeen Area IRB.

AUTHOR CONTRIBUTIONS

AV worked on processing samples, AP-PCR and dendrogram analyses of *S. mutans* isolates, data collection, and manuscript writing. DL worked on processing samples, data collection, and AP-PCR. JW was the Primary Investigator for the Southeast Iowa study and was involved in all aspects of that study. DD was the Primary Investigator for the American Indian study and was involved in overseeing all aspects of the study, including manuscript revision. All authors contributed to the article and approved the submitted version.

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