DISTRIBUTION AND ALLELIC FREQUENCY OF THE ENAMELIN GENE VARIANT RS7671281 ASSOCIATED TO AMELOGENESIS IMPERFECTA AND HIGH-RISK CARIES IN PUERTO RICO

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ABSTRACT

Amelogenesis imperfecta (AI) is an inherited, rare dental disorder that affects only the formation of tooth enamel and shows both clinical and genetic heterogeneity. Similarly, the enamelin gene (ENAM) has a fundamental role in the mineralization and structural organization of enamel and any change in its sequence can affect the thickness of the enamel. The objective of this study is to be able to confirm the association between the genetic variant rs7671281 in the ENAM gene with AI and high-risk caries subjects in the Puerto Rican population. This nonsense variant has been associated with the local, autosomal dominant hypoplastic form (type IB) of AI. Through Real-time PCR, we determined the allelic frequency and geographic distribution of rs7671281 by genotyping 622 samples of healthy subjects from Puerto Rico. Due to its association with a reduction in enamel thickness, rs7671281 is also genotyped to check its role in the susceptibility to the risk of developing dental caries. The present study was carried out in the Puerto Rican population with phenotypes associated with amelogenesis imperfecta and subjects with a high to moderate prevalence of dental caries. In turn, subjects with a low to no prevalence of dental caries were considered as controls. Through the collection and analysis of saliva samples, the prevalence of the enamelin variant rs7671281 was confirmed in the Puerto Rican population. The island-wide distribution pattern is non-regionalized and influenced by coastal regions, but mostly predominated in the South and North area of the island. According to the 1000 Genomes Project, the allelic frequency of rs7671281 for the T allele was 0.885 (184) and for the C allele was 0.115 (24) based on a total of 208 samples. The present study shows a significant decrease in the allelic frequency of rs7671281 for the mutated allele C (0.0530) in comparison to the 1000 Genomes Project (0.115). Therefore, we concluded that the allelic frequencies of rs7671281 analyzed in this study are more accurate than the values given in the 1000 Genomes Project.

INTRODUCTION

- Amelogenesis imperfecta (AI) is any defect in the process of enamel formation.
- The ENAM gene plays a role in the mineralization structure of the enamel as one of the principal enamel matrix proteins.
- The enamelin gene variant rs7671281 has been correlated to:
  - the subtype IB and IC of AI, and
  - to a higher susceptibility of caries.
- Affected subjects meet one or more of the following phenotype groups: enamel hypoplasia, complete or partial yellowish discoloration of teeth; anterior open bite; sensitive, tender, and easily chipped teeth.
- Given the diverse ethnicity of Puerto Rico, there is a higher prevalence of wide-ranging genetic variants that are narrowed studied.
- This present study aims to determine the presence and distribution of the enamelin variant rs7671281 in the Puerto Rican population.

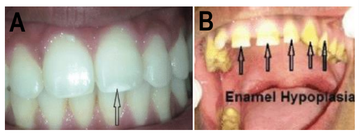


Figure 1. Enamel hypoplasia, one of the clinical feautures in amelogenesis imperfecta patients. (A) Mild case, as seen partial off-white discoloration in the central incisive tooth. (B) Severe case, as seen yellowish discoloration and pitted in general denture.

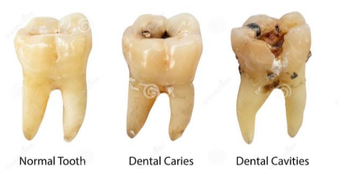
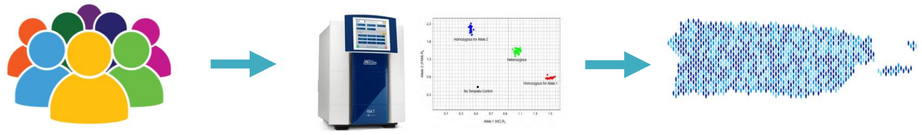


Figure 2. A clinical comparison of a normal tooth to dental caries and cavities. Shows a progresive decay of the tooth enamel, leaving the dentin exposed and eventually reaching the pulp, leading to tooth loss, gingivitis and periodontitis.

OBJECTIVES

- Confirm the prevalence of the enamelin gene variant rs7671281 in Puerto Rico
- Determine the distribution and the allelic frequency of rs7671281 in Puerto Rico.
- Differentiate the significant data of the allelic frequency of rs7671281 between the 1000 Genomes Project and the present study.

METHODOLOGY



622 Samples
30 Municipalities

DNA repository
J. C. Martínez-Cruzado Lab

Genotyping by RT-PCR
Allelic Discrimination

TaqMan Assay for SNP rs7671281
Allele Frequencies

Distribution and Frequency
in Puerto Rico

Distribution Maps
Map Viewer

RESULTS

Region	Allelic Frequency (%)
North	5.73%
South	**7.98%**
Center	1.06%
West	4.73%
East	5.99%
Puerto Rico	**5.30%**
1000 Genome Proyect	**11.53%**

Table 1. Porcentage-based allelic frequency of the enamelin gene variant rs7671281 regionally and globally in Puerto Rico; analogous data comparison of the 1000 Genome Proyect is included.

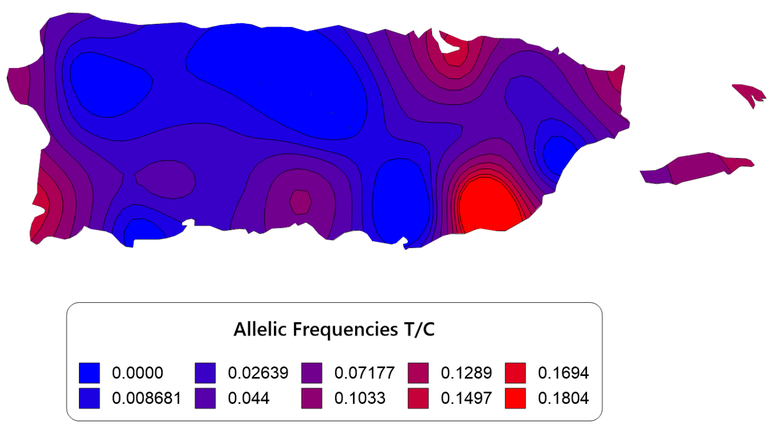


Figure 3. Frequency-based distribution for the mutated allele T of the enamelin gene variant rs7671281 in the Puerto Rico population.

PRELIMINARY CONCLUSIONS

- The enamelin variant rs7671281 is confirmed to have a prevalence in the Puerto Rican Population.
- The island-wide distribution pattern is non-regionalized and has a coastal behavior, but mostly predominated in the South and North area of the island.
- The present study shows a significant decrease in the allelic frequency of the enamelin variant rs7671281 for the mutated allele C (0.0530) in comparison to the 1000 Genomes Project (0.115).
- Therefore, we concluded that the allelic frequencies of rs7671281 analyzed in this study are more accurate than the values given in the 1000 Genomes Project.

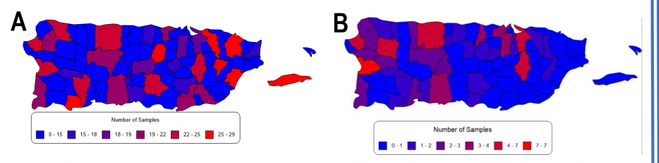


Figure 4. Municipality sample distribution for our study and Puerto Ricans in the 1000 Genomes Project. Our study sample distribution by municipality is shown in A (n= 622) and in B municipality distribution of Puerto Rican volunteers for the 1000 Genome Project until October 10, 2009 (n=77).

FUTURE PLANS

- Continue collecting and genotyping saliva samples from eligible subjects in order to verify the allelic frequency of the enamelin variant rs7671281.
- Determine the association between the variant rs7671281 with symptoms associated with amelogenesis imperfecta and candidates with a high prevalence of caries.
- Assess the role of the enamelin variant rs7671281 in the susceptibility to the risk of developing dental caries in the Puerto Rican population.
- Additionally, the next recruitment phase include subjects with the following clinical features and conditions:
  - Enamel hypoplasia (less developed enamel; easily chipped)
  - Complete or partial yellowish discoloration of teeth
  - Anterior open bite
  - Crown restorations
  - Smaller crown size
  - Dental sensitivity
  - Gastroesophageal reflux
  - High to moderate risk of caries
  - Low to none prevalence of caries (control)

ACKNOWLEDGEMENTS

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