Association Between *BRINP3* Genetic Variation and Aggressive Periodontitis Among Arab Descents.

by

Yazeed Mohammed Alhomrany

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This thesis was presented

by

Yazeed Mohammed Alhomrany

It was defended on

May 29, 2018

and approved by

Kelly L Williams, BS, DMD, Assistance Professor Director, Periodontics /Preventive dentistry department

Charles S Sfeir, DDS, PhD, Associate Professor, Periodontics and Preventive dentistry department

Thesis Director: Alexandre R Vieira, DDS, MS, PhD, Professor, Oral Biology department.

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Background: Aggressive periodontitis disease is a severe type of periodontal disease, which is characterized by enormous destruction of periodontal attachment and alveolar bone. A few loci have shown to be associated with aggressive periodontitis, which suggest a multifactorial genetic component. *BRINP3* locus shows correlation with aggressive periodontitis.

Aims: To replicate the initial findings of the relationship between aggressive periodontitis and *BRINP3* genetic variation (rs1342913) in group of Arab subjects.

Methods: 263 subjects were diagnosed with aggressive periodontitis. They are originally from Syria and Morocco. They are divided into: diseased (with aggressive periodontitis n= 200. Among them 49 from Syria and 151 from Morocco). and healthy (without aggressive periodontitis n= 63). Saliva was collected, then genomic DNA was extracted. One genetic marker was genotyped (*BRINP3* rs1342913). PCR was run with TaqMan chemistry. Chi-square with alpha of 0.05 was utilized to test over-representation of genotypes or alleles between two groups.

Result: The frequency AA genotype is higher than GG genotype. No significant difference between allele A and allele G.

Conclusion: The frequency *BRINP3* is higher in aggressive periodontitis compared with healthy cases in Arab population. Based on this result, *BRINP3* gene is associated with aggressive periodontists.

Key words: Aggressive periodontitis, *BRINP3,* Genotype, alleles, Dominant, Recessive**.**

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PREFACE

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I am very grateful for the opportunity to complete my master's degree here at the University of Pittsburgh.

1.0 INTRODUCTION

1.1 AGGRESSIVE PERIODONTITIS

Aggressive periodontitis is defined as a rapid destruction of periodontal attachment and the bone surrounding the tooth/teeth (Armitage et al., 1999), and it could be localized (localized to the first incisors or first molars) (Oettinger-Barak et al., 2013) or generalized (affecting at least three permanent teeth other than incisors or first molars) (Vaibhavi et al., 2015). Generalized periodontitis affects more males than females. The prevalence of aggressive generalized periodontitis is 0.13%, while the aggressive localized periodontitis is less than 1% (Vaibhavi et al., 2015). Children and young adults are less affected by this disease than older age groups (Susin et al., 2014). In the United States, it affects 47% of adults aged 30 years or older (Eke et al.,2015).

Genetic, socioeconomic, and environmental factors play a major role in the development of periodontal disease (Vieira et al., 2014). It is considered as one of the leading causes of tooth and alveolar bone loss.

There are several forms of periodontal disease ranging from its mildest form (gingivitis), to its severe one, which is called aggressive periodontal disease (APD). APD is characterized by loss of gingival attachment, bone tissue, and teeth loss. The progression of loss is much faster than other forms. Moreover, APD has been associated with the number of systemic conditions, including diabetes and cardiovascular diseases (Preshaw et al., 2012; Cullinan and Seymour, 2013), osteoporosis, and a low level of calcium and vitamin D (Genco et al., 2013).

1.2 PREVELANCE OF AGGRESSIVE PERIODONTITIS

The prevalence of APD is higher in undeveloped countries than the developed ones (Oppermann et al., 2007). APD has been reported to be more prevalent in Africans than Caucasians and Hispanics (Loe et al.,1991; Albandar et al., 2008; Falvia et al., 2010).

1.3 BACTERIA RELATED TO APD

The patient's periodontal status may not only be affected by the systemic condition but can also be a contributing factor to the development of more severe systemic complications. APD is associated with highly pathogenic bacteria, that are 65-75% of bacteria are gram- negative bacilli. In addition, there are a few spirochetes present. Aggressive periodontitis is characterized by rapid loss of attachment with association of pathogenic bacteria which impaired immune response. (Whiley et al., 2006).

1.4 PREVIOUS RESERACHES

Several reports have shown a strong evidence of genetic predisposition to the development of APD (Carvalho et al., 2009). Studies were done on extended families, siblings and twins (reviewed by Kinane and Hart, 2003). These studies showed the genetic contribution to aggressive forms of periodontitis is apparent (Laine et al., 2000; Baker et al., 2002), due to perceived strong gene effect, it has been suggested that APD'S mode of inheritance is autosomal dominant. Earlier studies used segregation analysis technique (Van der Valden et al., 1993, Tinoco, 1998). More recently, a multifactorial mode of inheritance has been suggested (Carvalho et al., 2009). A few loci have been reported as associated with APD, including the *BRINP3* locus (Carvalho et al., 2010; Casado et al., 2015).

1.5 ACID AND INDUCIBLE NEURAL SPECIFIC 3 GENE (BRINP3)

Bone morphogenetic protein/retinoic acid inducible neural specific 3 gene (*BRINP3*) is located at 1q31.1 (figure.1). *BRINP3* is expressed in cortex adult at E14 and E18, frontal lobe and cerebellum (Yue et al., 2014).

There are syndromes or diseases that are etiologically diverse and included APD as phenotype, such as Papillon–Lefèvre syndrome, Chediak–Higashi, hypophosphatasia, congenital

and cyclic neutropenia, leukocyte adhesion deficiency type I and II (Khocht and Albandar, 2000).

1.6 HYPOTHESIS AND AIM

Our study aimed to replicate our initial findings of an association between *BRINP3* and APD in a group from a study done in Syria and Morocco. The hypothesis is that the same *BRINP3* genetic variants that were associated with APD in Brazilians is associated in a group of Arabs.

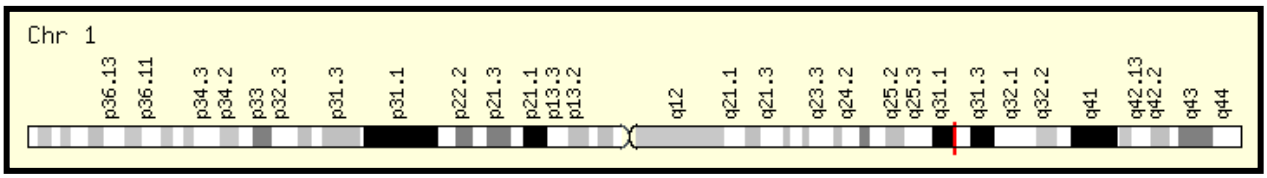

Figure 1 Location of *BRINP3* on chromosome 1 at q31.1.

2.0 MATERIALS AND METHODS

2.1 SUBJECTS

Two hundred and sixty-three individuals were included in this study. Among them, there are 200 subjects who are diagnosed with aggressive periodontitis, originally from Syria (n=49) and Morocco (n=151). Sixty-three individuals of Arab descent who are periodontally healthy were used as comparison. The inclusion criteria for APD was attachment loss of >3mm to 6mm with resorption of the bone or loss of the teeth. This protocol was approved by the University of Pittsburgh Institutional Review Board and all participants signed their informed consent form.

2.2 EXTRACTION OF GENOMIC DNA

Saliva samples were collected from all 263 participants with Oragene^{TM} DNA self- collection kit. The DNA was extracted, and the concentration and the purity of the DNA was determined by spectrophotometer. After that, samples were diluted with buffer to concentration of DNA of 2ng/µl. Polymerase chain reaction mix was made based on the following formula: Master Mix – 1.5µl, 40X SNP – 0.037 µl, Water - 0.462 µl, the total will be 2 µl per well, allowing at least 4 extra wells for a negative control and general loss. Adding 2µl of the reaction mix to each well. Then, adding 1µl of DNA to each well by the use a multi-channel pipettor. For the negative

controls, 1µl of water was added instead of DNA with 2µl of reaction mix. PCR was run with TaqMan chemistry in the thermocycler once at 95°C for 10 minutes, and then 40 cycles on 92°C for 15 seconds, and 60°C for 1 minute.

2.3 SINGLE NUCLEOTIDE POLYMORPHISM (SNP)

The variant (*BRINP3* rs1342913) that was previously associated with APD in Brazilians (Carvalho et al., 2010) was genotyped.

2.4 STATISTICAL ANALYSIS

To test the presence of over-representation of genotypes or alleles in individuals affected by APD in comparison to unaffected individuals, chi-square with alpha of 0.05 was utilized.

3.0 RESULTS AND DISCUSSIONS

The results of association between *BRINP3* and aggressive periodontitis are summarized in table1. The AA genotype of *BRINP3* rs1342913 was over-represented in aggressive periodontitis cases. The frequency of the GG genotype was significantly higher in patients with aggressive periodontitis compared to unaffected individuals (p=0.02). For recessive alleles, A- and GG alleles is higher in aggressive periodontitis disease cases (p=0.059). Moreover, for dominant alleles G- and AA are higher in APD cases than in the unaffected individuals.

Genotype	Cases	Controls
AG	69	35
AA	75	20
GG	33	5
Allele		
A	219	75
G	135	45
Dominant		
AA	75	20
G-	102	40
Recessive		
A-	144	55
GG	33	5

Table 1 Genotyping distribution (AA, GG, AG), p-value=0.02. Frequency of alleles (A and G), p-value= 1.0. Recessive and dominant models p-values= 0.059 and 0.22 respectively. For the recessive model, the OR = 2.5 (95% CI 0.94-6.79) was found.

Aggressive periodontitis is one of the most common types of periodontal diseases combined with destruction of bone, according to American Academy of Periodontology. There are several factors that increase the risk of aggressive periodontitis disease such as systemic disease, environmental or genetic linkage (Kinane et al., 2000; Meng et al., 2000). There was one study done in Rio de Janeiro, Brazil. They found an association between *FAM5C*, which is another name for *BRINP3*, and aggressive periodontal diseases. The test the tissues and they found high expression of *FAM5C*.

The original study from our laboratory looked at linkage (family-based design) and association between *BRINP3* and aggressive periodontitis by using two different markers (Carvalho et al., 2010). In this replication study, we are focusing on a different population group, with different ethnic group (Arab).

The individuals who are diagnosed with aggressive periodontitis have shown over-representation of two copies of the G allele of *BRINP3* rs 1342913, similar to the previous result. *BRINP3* gene is expressed in mitochondria and could cause proliferation and migration of the cells (Shorts-Cary L et al., 2007; Casado et al, 2015).

Figure 2 shows *BRINP3*‘s location in regard to other genes in the region. There are some diseases associated with *BRINP3*, such as transitional cell carcinoma and bladder cancer.

This gene encodes a mitochondrial protein which contains a BH3 domain and acts as a pro-apoptotic factor by interacting with anti-apoptotic proteins, including the E1B 19 kDa protein and BCL2.

The sequence of variant site in homo sapiens *BRINP3* (rs1342913) we found associated with aggressive periodontitis is CCTAAAATCAAAAAACTGGAGAAAA**[A/G]**ATGACGTGACACTGATCTCCAAGCA, which is located on an intron 3 (Berkowicz et al., 2016).

To test for association, we analyzed the genotypes and alleles (table 1).
The frequency of allele G was found to be higher in affected in comparison to unaffected individuals. The allele A represents the dominant (wild type), while allele G is the less common one (represents the recessive allele (figure.3). The expression level of mRNA *BRINP3* was found to be higher in gingival tissue from aggressive periodontitis cases compared to healthy tissue from control cases (Carvalho et al., 2010).

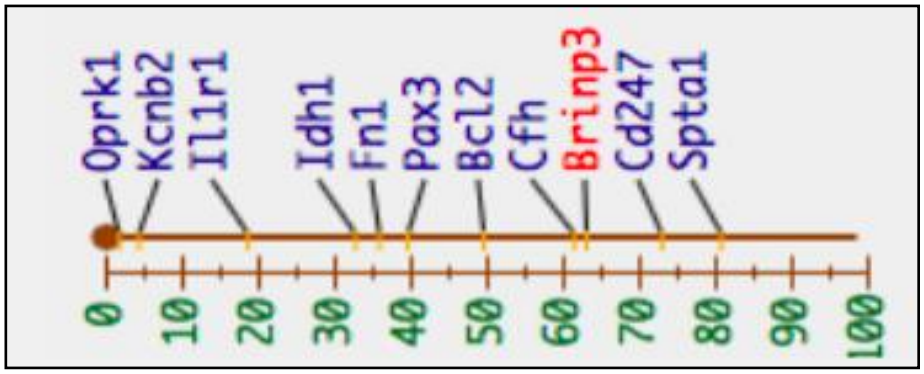

Figure 2 Location of *BRINP3* on genetic map of chromosome 1.

4.0 CONCLUSION

Based on our results, *BRINP3* is associated with aggressive periodontitis diseases in an Arab population. In order to avoid inheritance of aggressive periodontitis, consanguineous marriage must be discouraged. We recommend further studies related to genetic counseling that consider specific cultural beliefs.

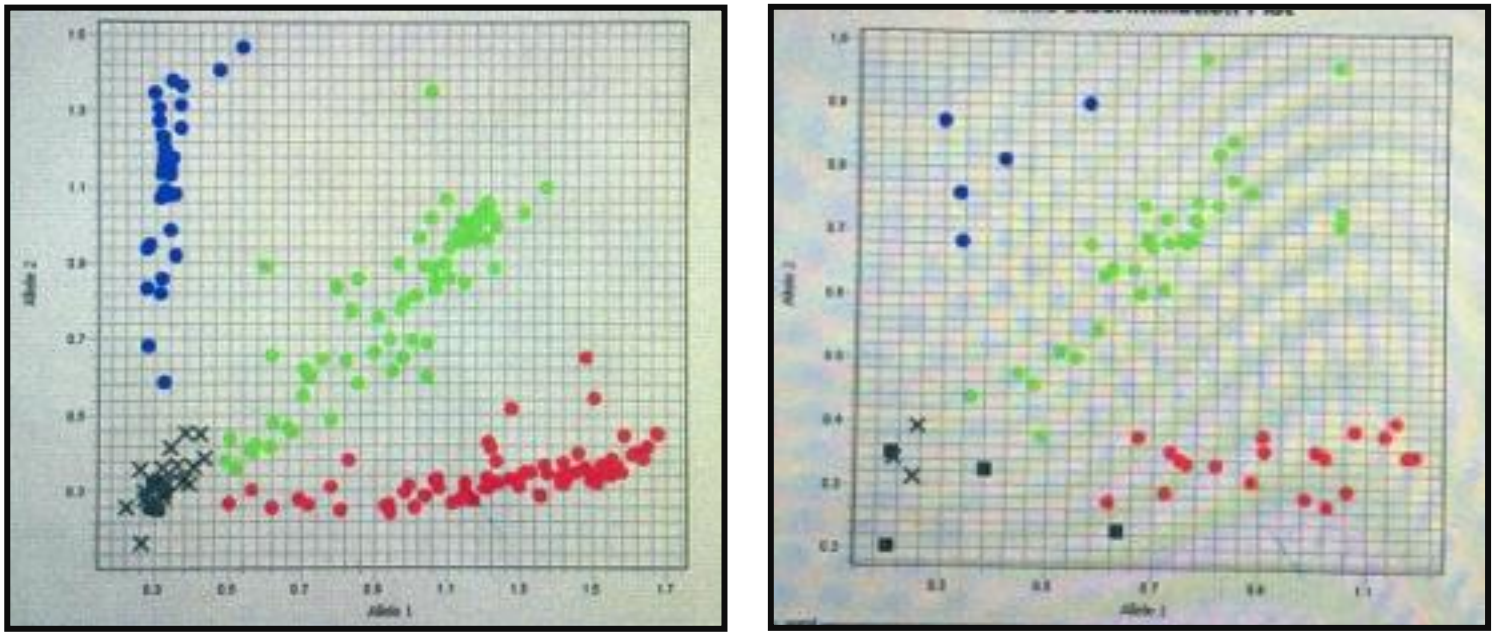

figure 3 Allelic discrimination plot (aggressive periodontitis disease on the left panel, while control cases on the right panel). frequency of allele a higher than frequency of allele g.

•: Homozygous allele 1 /allele 1 (A). • : Homozygous Allele 2/ Allele 2.

• Heterozygous Allele 1 / Allele 2. X: undetermined.

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