

Genetic Power of a Brazilian Three-Generation Family with Generalized Aggressive Periodontitis. II

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The genetic power of a Brazilian three-generation family with generalized aggressive periodontitis (GAgP) has been reported. The empirical logarithms of the odds (LOD) score thresholds for genetic linkage analysis of complex diseases proposed by Haines rely on confirmation from independent datasets. This study estimated the power of another large Brazilian family with GAgP for future linkage analysis. The three-generation family was seen at the Dental School of the Federal University of Bahia. Following the previously described methodology, full-mouth periodontal probing at 6 sites/tooth was performed in all 19 family members. Six out of 12 siblings were affected with GAgP. All affected family members were non-smokers and did not present diabetes or any other systemic condition or consanguinity. A parametric simulation ($\theta=0$) was performed on 100 replicates using the statistical software SLINK for linkage analysis. There was maximum expected LOD scores of 3.75 and 3.45 at penetrance rate $F=0.98$, and both studied phenocopy rates $P=0.0$ and $P=0.02$, respectively. The power of the study increased with the increase of the adopted penetrance rates in both studied phenocopy rates. The studied Brazilian three-generation family showed statistical power for future genetic linkage analysis of candidate genes to GAgP.

Key Words: family study, genetic linkage, aggressive periodontitis.

INTRODUCTION

The term complex disease implies that a single, causative, completely penetrant gene does not always produce the phenotype in question. Instead, a combination of effects from more than one gene or one or more genes with the environmental (nongenetic) factors may produce the phenotype. Complex phenotypes are causally heterogeneous. This usually means that over an extended population, the causes of a particular phenotype, trait, or disease include both low-frequency, high-penetrance "causative" alleles and common, low-penetrance "susceptibility" alleles interacting with environment factors. Most of the common disorders of children and adults are complex phenotypes. The common disorders of childhood include birth defects, mental retardation, short stature,

and cancer. Common disorders in adults include cancer, diabetes, cardiovascular disease, hypertension, stroke, psychoses and conditions once thought to be specific single entities such as Alzheimer's disease, macular degeneration, Parkinson's disease, and spina bifida. Even the phenotypic manifestation of conditions usually considered to be straightforward monogenic disorders may be the result of gene-environment interaction. Phenylketonuria (PKU; MIM 261600) phenotypic expression (i.e., mental retardation) depends not only on genotype but also on exposure to the amino acid phenylalanine in dietary protein (1).

Periodontitis is a multifactorial inflammatory disease that leads to the destruction of periodontal tissues in the presence of infection. Twin studies are a highly powerful method to estimate the influence of environmental and/or genetic factors on the expression

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of a phenotype. The findings of Michalowicz et al. (2) on 117 pairs of twins confirmed previous studies, which showed that 50% of the variance in the severity and extent of clinical parameters of periodontitis, but not gingivitis, can be attributed to genetic factors. In that investigation (2), both studied models of phenotypic variation strictly due to environmental factors were rejected. Aggressive periodontitis (AgP) comprises a group of rare, often severe and rapidly progressive forms of periodontitis. In spite of its rare occurrence, AgP has been the focus of several investigations that aimed at understanding its etiology and pathogenesis. There is a distinctive tendency for AgP to aggregate within families. Although, in general, AgP can occur at any age, it is frequently characterized by a clinical manifestation at early age, which indicates that the responsible etiologic agents are able to cause clinically detectable levels of the disease within a relatively short time (3). By means of segregation analysis, which provides information about the mode of inheritance of a genetic trait, 74 Brazilian families from the cities of Rio de Janeiro and Duque de Caxias in the Rio de Janeiro state comprising 475 individuals with at least one member presenting AgP were studied. The results showed an unequivocal genetic influence on the expression of AgP and suggested that a few loci, each with relatively small effects, contribute to AgP, with or without interaction with environmental factors in the studied model and population (4).

Family linkage analysis is a successful method for detecting causative or predisposition genes. It combines detailed clinical analyses of family members with genome-wide scans, using known genetic markers to locate disease loci. This method has been applied with success in mapping complex diseases such as Alzheimer's disease, breast cancer and cardiovascular diseases (5). One basic assumption of this method of analysis is that genes on the same chromosome segregate together at a rate related to the chromosomal distance between them, measured as recombination fraction (θ). The shorter the distance between the loci, the higher is the possibility that they will segregate together during meiotic recombination (combination of chromosome fragments from father and mother). In case two loci segregate together at a rate higher than 50%, they are, to some extent, genetically linked. Thus, the strength of linkage between two analyzed loci depends on the genetic proximity between them, and can be estimated by means of logarithms of the odds (LOD scores). In complex diseases the empirical LOD score thresholds for genetic

linkage proposed by Haines relies on confirmation from independent datasets (6).

Genetic linkage of localized AgP (LAgP) to chromosome 1q25 in four multigenerational African American families has been published (7). Thus, extending the spectrum of investigations of the previously reported chromosomal regions 4, 6, 9, and 11 (8-10), which still require confirmation in other families and linkage studies. An essential step prior to performing linkage analyses is to estimate the power of the study design in order to determine whether the available pedigree information is sufficient to allow the detection of gene(s) underlying the trait(s) of interest and to optimize the cost of the study (11). In a previous report, we described the power in a Brazilian three-generation family to detect linkage to candidate gene(s) to generalized aggressive periodontitis (GAgP) (12). As one more large family seen at the Dental School of the Federal University of Bahia has been diagnosed with GAgP, and given the relevance of confirming genetic findings on different datasets in a multistage genetic mapping, the aim of the present study was to estimate the power of this new family for future genetic linkage analysis of candidate gene(s) to GAgP.

MATERIAL AND METHODS

In the course of the diagnostic process of GAgP in a 23-year-old woman (proband) at the Dental School of the Federal University of Bahia, Brazil, the entire family (19 family members in total) was periodontally examined and a pedigree was constructed. The family members ranged in age from 9 to 78 years and presented mixed ethnic features related to Portuguese Caucasian and Brazilian Indian ethnicities. In all 19 family members, a trained examiner performed a full-mouth periodontal probing at 6 sites/tooth with a manual probe (CP 10 William's periodontal probe; Hu-Friedy, Chicago, IL, USA). Six of the 12 siblings (second generation) were diagnosed on GAgP (13).

The clinical attachment loss in the mentioned family members was due to pocketing. In addition, routine full-mouth periapical radiographs revealed a generalized distribution of severe alveolar bone loss. In the affected siblings, the disease was classified as GAgP since at least 3 permanent teeth other than first molars and incisors were involved in the periodontal destruction. The only living grandmother (first generation) and both parents (second generation) were edentulous.

In direct interview, the mother and the grandmother reported pyorrhea as the cause of tooth loss, whereas the father informed that he had lost his teeth due to caries. Consequently, the phenotype of both mother and grandmother were considered positive, while it was negative for the father.

As previously described (12), given the age dependence of the expression of periodontitis concerning the time needed for the sequels to accumulate, family members under the age of 14 were classified as unknown phenotype in order to avoid misclassification of the phenotype in the study model (14). None of the family members had ever received any information about their periodontal status or had ever undergone periodontal treatment prior to the present study. They were not enrolled in any oral hygiene program by the time of this study either. All affected family members were non-smokers and tested negative for diabetes. There was no suspicion of any other systemic condition or consanguinity in this family. The pedigree structure and the phenotypic outcome (affected or not affected) of the studied family is presented graphically in Figure 1.

All subjects agreed to participate in the study by signing an informed consent form. The Brazilian Commission of Ethics in Research approved the study protocol (CONEP 12649).

As previously described (12), the statistical analysis involved the estimation of the probability for reaching a LOD score of $Z \geq 3$. An autosomal dominant inheritance mode was assumed. Three different penetrance values ($F=0.98, 0.75$ and 0.50) and 2 phenocopy rates ($P=0.0$ and 0.02) were tested. One hundred replicates of the studied pedigree were simulated using the software SLINK, which is a general simulation program for linkage analysis (15). For the simulations, a recombination fraction (θ)=0,

allele frequencies of 0.0001 for the mutated allele and of 0.25 for each of 4 equally frequent allele markers were assumed. The results were evaluated following the linkage LOD score criteria described by Haines (6) for complex diseases (polygenic or multifactorial).

RESULTS

Maximum expected LOD score frequencies, and expected average and maximum LOD scores for the given genetic parameters in the investigated Brazilian family are presented in Tables 1 and 2.

The frequency of a LOD score of 3 (evidence

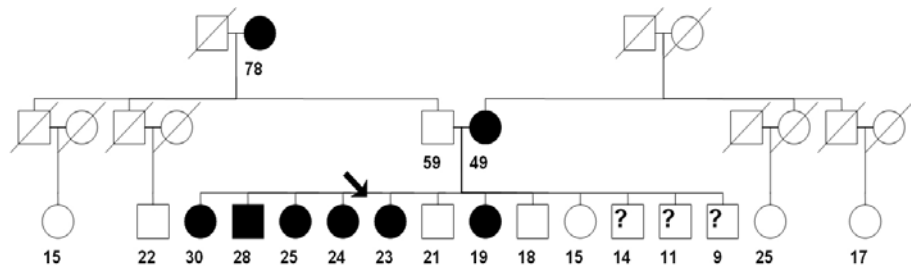


Figure 1. Pedigree structure of the studied Brazilian three-generation family. Numbers below the symbols indicate the age of the respective family member; arrow designates the proband; open squares: unaffected males; open circles: unaffected females; filled circles: affected females; crossed-out symbols: deceased or not available family members; ?=unknown phenotype.

Table 1. Frequency (%) of maximum ELOD scores at phenocopy rates $P=0$ and 0.02 , and penetrance rates $F=0.50, 0.75$, and 0.98 .

ELOD Score	P=0			P=0.02		
	F=0.50	F=0.75	F=0.98	F=0.50	F=0.75	F=0.98
1	31	59	71	46	46	76
2	0	29	63	4	20	57
3	0	0	52	0	0	46

Table 2. Average and maximum ELOD scores at phenocopy rates $P=0$ and 0.02 , and penetrance rates $F=0.50, 0.75$, and 0.98 .

F	P=0		P=0.02	
	Average	Max	Average	Max
0.50	0.65	1.95	0.88	2.2
0.75	1.27	2.81	1.15	2.78
0.98	2.22	3.75*	2.23	3.45*

*ELOD score above 3.

for genetic linkage) was 52% and 46% at penetrance rate $F=0.98$ and phenocopy rates $P=0.0$ and 0.02 , respectively. Maximum ELOD scores of 3.75 and 3.45 were found at penetrance rate $F=0.98$ and phenocopy rates $P=0.0$ and 0.02 , respectively.

DISCUSSION

Epidemiological surveys have shown a wide variation in prevalence of AgP among different populations. The prevalence of AgP ranged from 1% to 15% among Caucasians, Hispanics and African-Americans. A greater prevalence in African-Americans compared with Caucasians has been reported (16) with some studies showing up to 51.5% of affected individuals. These differences are probably due to differences in the employed epidemiologic methodologies and definition of the disease. However, all available investigations indicate that AgP is detectable in all age and ethnic groups (3).

In the absence of an etiologic classification, aggressive forms of periodontal disease have been defined based on the following primary features: (i) except for the presence of periodontitis, patients are otherwise healthy; (ii) patients present rapid attachment loss and bone destruction; (iii) familial aggregation (13). The diagnosis of any type of AgP requires the absence of systemic diseases that may severely impair host defenses and lead to premature exfoliation of teeth. In such instances the appropriate clinical diagnosis will be periodontal manifestation of systemic diseases. The second primary feature of rate of disease progression can only be determined in longitudinal studies by means of comparison between at least 2 examinations. As an alternative, inference based on the severity of the condition with respect to the patient's age has been used to address the progression rate of the disease during first examination (17). Family reports are considered an important diagnostic tool and are accepted as diagnostic criterion by the 1999 International Classification Workshop Consensus. They may help constructing individual's pedigree and estimation of the familial aggregation rate.

The family analyzed in the current study presented all 3 primary features of AgP. A sib-ship aggregation of 50% was found in the third generation. Out of 12 siblings, 6 presented clinical features of GAgP. Both parents and the only living grandmother were edentulous. In both mother and grandmother, pyorrhea was the

cause of tooth loss, whereas the father reported caries as cause of his edentulisms. In the study model, hence, the phenotype was considered positive for the mother and the father (second generation). Consequently, the high familial aggregation of 50% is held up even when considering both the second and third generation. The family aggregation rate of 50% of GAgP in this study family was therefore equal to the 50% aggregation rate in our previously reported three generation Brazilian family. Nevertheless, the main occurrence of members affected with GAgP in the current family was in the third generation whereas in the previously reported three generation Brazilian family it was in the second generation (12). As in the previously described family, the members of this study family also presented a high incidence of caries.

As a group, aggressive forms of periodontitis are characterized by severe destruction of periodontal attachment apparatus at an early age. This early manifestation of clinically detectable lesions is generally interpreted as being the expression of highly virulent causative agents and/or high levels of susceptibility of the individual. Currently aggressive forms of periodontitis are considered to be multifactorial diseases developing as a result of complex interactions between specific host genes and the environment. There is evidence that besides genetic influences and individuals host susceptibility to infections, environmental factors may affect the clinical expression of AgP. In a large study, cigarette smoking was shown to be a risk factor for patients with GAgP. Smokers with GAgP had more affected teeth and greater mean levels of attachment loss than patients with GAgP who did not smoke. Environmental exposure to cigarette smoking, therefore, seems to add significant risk of more severe and prevalent disease to this group of already highly susceptibility (3,18). On the other hand, both Brazilian three-generation families with GAgP, the presently studied and the previously reported (12), showed a high prevalence and severity of GAgP in the absence of smoking. All studied family members were non-smokers. These data corroborate the inheritance model described by de Carvalho et al. (4), which showed an unequivocal genetic influence on the expression of AgP and suggested that a few loci, each with relatively small effects, contribute to AgP, with or without interaction with environmental factors in the 74 studied Brazilian families.

At the studied phenocopy rates $P=0$ and 0.02 , the power estimation calculated maximum ELOD

scores of 3.75 and 3.45 at penetrance rate $F=0.98$ (Table 2). The observed values of maximum ELOD in the currently studied Brazilian family are slightly superior to our previously studied three-generation family (max ELOD 3.56). This fact may reflect the greater rate of affected *versus* non-affected and unknown members in the currently studied family 8/23 compared to 6/19 in the previously reported family. It is estimated that in an autosomal dominant disease when linkage phase (one chromosome of each parent) is known and there is no recombination between the disease and marker locus, affected individuals contribute 0.30 to the LOD score. The contribution of unaffected individuals is dependent on the penetrance of the disease. As penetrance decreases, so does the contribution of an unaffected individual to the LOD score, because the ability to accurately score these unaffected individuals (who may or may not be gene carriers) as recombinants or non-recombinants is reduced (11).

In both Brazilian three-generation families, the frequency of maximum ELOD score of 3 was only seen in the highest studied penetrance rate of $F=0.98$, being respectively of 52% and 46% in the presently studied family and 36% and 27% in the previously reported family, in $P=0.0$ and $P=0.02$, respectively. Both average and mean ELOD scores increased gradually with the increase of the studied penetrance rates ($F=0.50$, $F=0.75$, $F=0.98$) in both studied phenocopy rates ($P=0.0$, $P=0.02$) in both studied Brazilian families. Interestingly, the same tendency of increase in the power of the study with the increase of the adopted penetrance rate was observed in the power study of a Brazilian three-generation family affected with generalized chronic periodontitis (max ELOD 2.37, $F=0.98$, $P=0.0$) (19). The ELOD scores of the presently studied family are comparable to previous results of studies on AgP in a single large Scottish family (max ELOD: 3.80) (20) and in four multigenerational African American families (max ELOD: 3.75) (11). Therefore, it may be concluded that the studied Brazilian three-generation family is suitable for future genetic linkage analysis of candidate genes of GAgP.

RESUMO

O poder genético em uma família brasileira de três gerações com periodontite agressiva generalizada (PAgG) foi reportado. Os valores dos escores logarítmicos (LOD) empíricos para análise genética de ligação de doenças complexas propostos por Haines se baseiam na confirmação em conjuntos de dados independentes. O objetivo deste estudo foi de estimar o poder de uma nova grande

família com PAgG para futura análise de ligação. A família de três gerações foi vista na Faculdade de Odontologia da Universidade Federal da Bahia. De acordo com metodologia previamente descrita, sondagem periodontal em 6 sítios/dente foi realizada em todos 19 membros da família. Seis de 12 irmãos apresentaram PAgG. Todos os membros afetados da família eram não fumantes, não apresentaram diabetes ou qualquer condição sistêmica ou consanguinidade. Uma simulação paramétrica ($\theta=0$) foi realizada em 100 réplicas usando software estatístico SLINK para análise de ligação. Houve escore LOD esperado máximo de 3,75 e 3,45 no valor de penetrância $F=0,98$ em ambas razões de fenocópia estudadas $P=0,0$ e $P=0,02$, respectivamente. O poder do estudo aumentou com o aumento do grau de penetrância adotado em ambas razões fenotípicas estudadas. A família brasileira de três gerações estudada mostrou poder estatístico para futura análise de ligação genética de genes candidatos para PAgG.

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