Journal of Oral Science, Vol. 53, No. 4, 467-474, 2011

Original

Genetic polymorphism of Fcγ-receptors IIa, IIIa and IIIb in South Indian patients with generalized aggressive periodontitis

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(Received 2 April and accepted 24 September 2011)

Abstract: Recent evidence suggests that polymorphisms in Fcγ receptors are associated with different forms of periodontitis. However, the FcγR genotypes and their allele frequency differ among subjects from different ethnic backgrounds. The aim of the present study was to determine whether specific FcγRIIa, FcγRIIIa, and FcγRIIIb alleles and/or genotypes are associated with susceptibility to generalized aggressive periodontitits (GAgP) in a South Indian population. Buccal scrapings were obtained from 60 subjects with GAgP and 60 periodontally healthy individuals, and DNA was extracted from each of the samples. FcγRIIa and FcγRIIIa genotyping was performed by polymerase chain reaction (PCR) amplification of DNA with allele-specific primers followed by allele-specific restriction digestion of the products, whereas FcγRIIIb genotyping was done by allele-specific PCR. There was no significant difference in the distribution of the FcγRIIa H/R genotype between GAgP patients and healthy subjects, although significant over-representation of the R allele was noted in GAgP patients. With regard to FcγRIIIa F/V genetic polymorphism, the homozygous V/V genotype and V allele were significantly over-represented in the GAgP group, whereas the F/F genotype and F allele were over-represented in the controls. Furthermore, there was significant over-representation of the FcγRIIIb-NA2 allele and NA2/NA2 genotype in GAgP patients, and of the NA1/NA1 genotype and NA1 allele in the controls. These data suggest that the FcγRIIIa V/V genotype and/or V allele, as well as the FcγRIIIb NA2/NA2 and/or NA2 allele, along with the FcγRIIa- R allele, may be risk factors for GAgP in the population of South India. (J Oral Sci 53, 467-474, 2011)

Keywords: aggressive periodontitis; genetic polymorphism; South Indian; FcγR.

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Introduction

Periodontal disease is a multifactorial entity wherein bacteria, host and environmental factors interplay and manifest as disease. In this infectious-inflammatory process, subgingival anaerobes play an essential role in disease initiation, but inter-individual differences in immune responses to periodontal infection define the degree of host susceptibility. The medical literature contains an increasing number of reports linking genetic polymorphisms with inflammatory diseases (1-3). As periodontitis is also an inflammatory disease, research in this area has also contributed to our understanding of the process of inflammation (4-7). Genetic polymorphism has been proposed as a risk factor that influences susceptibility to periodontitis (8,9).

Aggressive periodontitis (AgP) generally affects systemically healthy young individuals and occurs as both localized (LAgP) and generalized (GAgP) forms. GAgP usually affects individuals younger than 30 years, although they may be older. It affects at least three permanent teeth other than first molars and incisors, and is mainly characterized by pronounced episodic destruction of attachment and alveolar bone loss usually in the

interproximal region (10). Several studies have indicated that the prevalence of GAgP is disproportionately higher in certain families (11-13). Such a dramatic familial aggregation of cases indicates that genetic factors such as polymorphism of cytokine genes and Fc receptors on leucocytes may play an important role in susceptibility to AgP.

In humans, three structurally distinct and closely linked families of Fcγ receptors are currently recognized, and are defined based on their molecular weights, ligand binding properties and certain monoclonal antibodies reactive with them. These families of receptors (FcγRs) represent different gene products of eight genes, and are present on a characteristic population of leucocytes. All of these genes have been mapped to the long arm of chromosome 1 (1q21 & 23-24). These receptors include FcγRIa, Ib, Ic (CD64); FcγRIIa, IIb, IIc (CD32) and FcγRIIIa and IIIb (CD16) (14). Among them, bi-allelic polymorphism has been identified for four FcγR subclasses: FcγRIIa, FcγRIIb, FcγRIIIa and FcγRIIIb, which have been identified as potential heritable risk factors for certain autoimmune and infectious diseases (15,16).

Recent studies have demonstrated an association between polymorphism in Fcγ receptors and a particular form of periodontitis (17,18). For example, FcγRIIIb – NA2 allele and/or FcγRIIIb NA2/NA2 genotype and the composite genotypes FcγRIIIb NA2/NA2 plus FcγRIIa H/H131 may be associated with AgP, although a different situation exists in healthy controls (19). The genotype distribution also varies among different races, including African Americans (20), Caucasians (21) and Asians (22). These conflicting data are difficult to interpret due to variation in ethnic backgrounds of the study population, the reported allele frequencies, and the definitions of periodontal disease. Also, the association between FcγR polymorphism and periodontal health and disease may be race-related.

The relevance of distinct FcγR genotypes as risk determinants for susceptibility to various forms of periodontal diseases should be considered in different racial groups. To our knowledge, no previous study in the dental literature has determined polymorphism of different Fcγ receptors in CP or AgP patients from the Indian population. Hence, the aim of the present study was 1) to determine the association of polymorphism of the FcγRIIa, FcγRIIIa and FcγRIIIb genotypes with generalized aggressive periodontitis (GAgP) and periodontal health, and 2) to determine whether the presence of these genotypes can be considered a risk factor for susceptibility to generalized aggressive periodontitis (GAgP) in the population of South India.

Materials and Methods

Study population

Between December 2007 and July 2010, 120 Indian subjects including 60 patients with generalized aggressive periodontitis (30 males and 30 females; mean age 26.66 years) and 60 periodontally healthy controls (32 males and 28 females; mean age 32.23 years) were recruited from the outpatient Department of Periodontics, Bapuji Dental College & Hospital, Davangere, Karnataka, India for the study. Cases as well as controls were of South Indian origin. Signed informed consent was obtained from all participants. The protocol for the study was approved by the Review Committee for Human Subjects of Bapuji Dental College & Hospital. The GAgP group was defined according to the classification criteria established at the International Classification Workshop, 1999 (23). Subjects < 30 years of age, who had more than eight teeth with clinical attachment loss > 5 mm and probing depth > 6 mm and at least three affected permanent teeth other than first molars and incisors were diagnosed as having generalized aggressive periodontitis. Subjects with no evidence of attachment loss at more than one site, a probing depth of less than 3 mm, and no history of previous periodontal disease, were defined as periodontally healthy controls. None of the patients had a history or current signs of systemic disease affecting their periodontal status. Exclusion criteria included the presence of < 15 teeth, use of antibiotics in the last 6 months before entry into the study, pregnant and lactating mothers, and patients with a history of smoking.

Clinical assessments

The following clinical parameters were recorded in all the selected patients: a) Plaque index (24); b) Gingival index (25); c) Gingival bleeding index (26); d) Probing depth (PD) measured as the mean distance from the crest of the gingival margin to base of the pocket; e) Clinical attachment level (CAL) expressed as the mean distance from the cemento-enamel junction to the base of the pocket. The PD and CAL were assessed with the help of a Williams periodontal probe (Hu Friedy, Bangalore, India) at six locations around the tooth: mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual. The measurements were recorded to the nearest millimeter, and every observation close to 0.5 mm was rounded down to the lower whole number. Full-mouth intraoral periapical (IOPA) radiographs and orthopantomograms (OPGs) were also obtained to show evidence of bone loss. All clinical recordings and radiographic evaluations were performed by two certified examiners.

Isolation of genomic DNA

Buccal scrapings were obtained from all participants using a custom-made sterile wooden spatula, and each collected sample was immediately immersed in a sterile tube containing 1000 µl Krebs buffer solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM each KH_2PO_4 and $MgSO_4$, 4.2 mM $NaHCO_3$, 2.0 mM $CaCl_2$, 10 mM glucose). Krebs buffer maintains the homeostasis and the viability of cells so that the DNA is not lost during transit. The tubes were labeled according to a code number allotted to each individual patient and sealed tightly. Care was taken to keep the identity of the patients confidential; hence only the code numbers were used. Within 24 h of collection, the samples were sent to the laboratory, and stored at 4˚C before processing for genomic analysis. Genotyping was performed twice for each sample, and samples for which any discrepancy was found between the two results were subjected to a third genotyping; the third result was considered final. Only three such cases were encountered: one in a periodontally healthy control, and two in GAgP patients.

Determination of FcγR genotypes

FcγRIIa-R-H131: PCR amplification for FcγRIIa was carried out according to the methodology described by Jiang et al. (27). Genomic DNA (100 ng) was added to a reaction mixture containing 200 µM dNTPs, 2.0 mM $MgCl_2$, 0.25 µM sense and antisense primers (Sigma Aldrich®, Bangalore, India) [5' GGA AAA TCC CAG AAA TTC TCG C 3' (forward), 5' CAA CAG CCT GAC TAC CTA TTA CGC GGG 3' (reverse)] and 1 unit of Taq Polymerase (Chromus Biotech®, Bangalore, India). The amplification protocol included 1 cycle of 95°C for 5 min followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s. The DNA obtained by PCR was digested using the restriction enzyme FnuDII (Fermentas®, Genetics Biotech Asia Pvt. Ltd., New Delhi, India) in accordance with the manufacturer's recommendations to obtain the allele distribution. The digested PCR product was resolved at 16 amp on 3% agarose gel, and then stained with ethidium bromide. The PCR product was digested with FnuDII, giving rise to 343-bp and 322-bp fragments for the H and R allele, respectively, followed by resolution on 3% agarose gel and observation under a UV transilluminator.

FcγRIIIa-158V-F: Amplification of FcγRIIIa was carried out by nested PCR and allele-specific restriction analysis assay according to a method described by Koene et al. (28). The DNA was amplified with sense and antisense primers (Sigma Aldrich®) 5' ATA TTT ACA GAA TGG CAC AGG 3' (forward) and 5' GAC TTG GTA CCC AGG TTG AA 3' (reverse). The amplification protocol consisted of 5 min at 95°C, and 35 cycles of 30 s at 95°C, 1 min at 50°C, and 1.5 min at 72°C. 0.5 µl of the first PCR product was used as a template for a nested PCR reaction with the primer pair: 5' ATC AGA TTC GAT CCT ACT TCT GCA GGG GGC AT 3' and 5' ACG TGC TGA GCT TGA GTG ATG GTG ATG TTC AC 3', using 30 cycles consisting of 30 s at 95°C, followed by 30 s at 62°C and 40 s at 72°C. The PCR product was digested with Nla III (Fermentas®, Genetics Biotech Asia Pvt. Ltd.) at 37°C followed by electrophoresis. The homozygous F/F158 fragments were not digested, whereas the heterozygous F/V158 fragments were partially digested yielding 3 bands of equal intensity (94 bp, 61 bp and 33 bp). On the other hand, the homozygous V/V158 fragments were maximally digested yielding 3 bands (a very low-intensity 94-bp band and two equal-intensity 61-bp and 33-bp bands).

FcγRIIIb-NA1-NA2: FcγRIIIb genotyping was performed by PCR employing allele-specific sense and anti-sense oligonucleotides, as described by van Schie et al. (29). Two different PCRs were carried out for detection of the FcγIIIb alleles: NA1 and NA2. For NA1 gene amplification, 5' CAG TGG TTT CAC AAT GTG AA 3' (forward) and 5' CAT GGA CTT CTA GCT GCA CCG 3' (reverse) (Sigma Aldrich®) were used. The PCR reaction conditions for amplifying this 142-bp product included 1 cycle of 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s. The NA2-specific sense primer '5 -CTC AAT GGT ACA GCG TGC TT-3' and antisense primer '5 -CTG TAC TCT CCA CTG TCG TT-3' (Sigma Aldrich®) were employed to amplify the 169-bp product. Otherwise, the composition of the reaction mixture was similar to that for NA1 PCR. This reaction was carried out using 1 cycle of 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 45 s. Five microliters of the NA1 PCR product and 5 µl of the NA2 PCR product mixed with ethidium bromide dye were loaded into the same well of the electrophoresis unit. The 142-bp and 169-bp products were resolved on 2% agarose gel run at 16 A.

Statistical analysis

Statistical analysis was carried out with the help of software, '*Primer of Biostatics*'. Results were represented as numbers or percentages. The mean value was calculated for all clinical parameters, and intergroup comparisons were made using one-way ANOVA. The distributions of FcγR genotypes (3×2 contigency table) and their allele frequencies in both groups were compared using chi-squared test. Odds ratios with 95% confidence interval

Table 1 Full mouth clinical parameters of GAgP and control groups

Clinical Parameters	GAgP (n = 60)	Control (n = 60)	P value*
Age (years)	26.667 ± 3.262	32.23 ± 2.804	> 0.05 NS
Gender % (Males/Females)	50 / 50	53.33 / 46.66	> 0.05 NS
Plaque index	1.106 ± 0.278	0.709 ± 0.290	< 0.001 HS
Gingival index	1.905 ± 0.292	0.078 ± 0.137	< 0.001 HS
Gingival bleeding index	85.741 ± 10.771	2.201 ± 6.065	< 0.001 HS
Mean probing depth	4.897 ± 1.329	1.441 ± 0.278	< 0.001 HS
Mean CAL	6.389 ± 1.288	0.000	< 0.001 HS

*One way ANOVA test, HS: Highly significant, NS: Not significant

Table 2 Single locus test for hardy weinberg equilibrium

Group	FcγR IIa				FcγR IIIa				FcγR IIIb			
	H allele	R allele	X^2	P value	V Allele	F allele	X^2	P value	NA1 allele	NA2 Allele	X^2	P value
GAgP (n = 60)	0.36	0.64	0.155	0.925	0.72	0.28	1.688	0.430	0.33	0.67	0.165	0.921
Healthy control (n = 60)	0.48	0.52	5.006	0.082	0.32	0.68	2.721	0.257	0.70	0.30	0.989	0.610

were determined to assess the strength of the relationship between allelic frequency and disease groups. Any difference at $P \leq 0.05$ was considered statistically significant.

Results

Clinical parameters

The clinical characteristics of the two groups are presented in Table 1. The subjects in both groups were age-matched and thus showed no significant difference in mean age. The two groups also had an almost equal distribution of males and females. The GAgP patients presented significantly higher mean PD and CAL values than the controls. The plaque index, gingival index and gingival bleeding index scores for the GAgP group were also significantly higher than in the control group.

Distribution of genotypes

The genotype distribution of FcγRIIa, FcγRIIIa and FcγRIIIb in the GAgP and healthy controls did not deviate from a Hardy-Weinberg equilibrium (P > 0.05) (Table 2). The distribution of FcγRIIa, FcγRIIIa and FcγRIIIb genotypes and their allelic frequencies are shown in Table 3. For FcγRIIa, R/R homozygosity was the more frequent genotype in both groups: GAgP 43.33%, and controls 36.66%. The inter-group difference in genotype distribution was not statistically significant (χ^2 = 5.65). Significant over-representation of the FcγRIIa R allele was found in the GAgP group relative to the control group [P = 0.05, odds ratio (OR) = 1.67, 95% confidence interval (CI) (0.99-2.80)]. With regard to the FcγRIIIa genotype distribution, F/F homozygosity was most frequent in the control group (53.33%), whereas V/V homozygosity was most frequently present in the GAgP group (56.66%). Comparison between the GAgP and control groups (χ^2 = 27.49) showed that the distribution of the genotypes was highly significant (P < 0.001). Intergroup comparison showed that the distribution of the F and V alleles between GAgP and controls was highly significant [P < 0.001, OR = 5.46, 95% CI (3.14-9.45)]. The distribution of the FcγRIIIb NA1/NA1 homozygous genotype was maximal in the control group (53.33%) whereas NA2/ NA2 was more frequently present in the GAgP group (46.66%). Comparison between the GAgP and control groups showed that the difference in genotype distribution for FcγRIIIb was highly significant (χ^2 = 25.87, P < 0.001). The differences in the allelic distribution of NA1 and NA2 between the GAgP and control groups were highly significant [P < 0.001, OR = 4.6, 95% CI (2.70-8.04)]

Discussion

In recent years, a great number of investigations have attempted to determine distinct FcγR genotypes as potential risk determinants for developing a particular form of periodontitis. For instance, the genotype FcγR IIIa V/V genotype has been associated with GAgP in North European Caucasians (30). Conversely, Kobayashi et al. found no correlation of FcγR IIIa F/V with GAgP in a

Table 3 Distribution of FcγR genotypes and alleles in study subjects

		GAgP $n = 60$ (%)	Controls $n = 60$ (%)
FcγR IIa			
Genotype	R/R 131	26 (43.33)	22 (36.66)
	R/H 131	25 (41.66)	18 (30)
	H/H 131	9 (15)	20 (33.33)
Allelic frequency	R131	77**	62
	H131	43	58
FcγR IIIa			
Genotype	158V/V	34 (56.66)[#]	10 (16.66)
	158V/F	18 (30)	18 (30)
	158F/F	8 (13.33)	32 (53.33)
Allelic frequency	158V	86[##]	38
	158F	34	82
FcγR IIIb			
Genotype	NA1/NA1	8 (13.33)	32 (53.33)
	NA1/NA2	24 (40)	20 (33.33)
	NA2/NA2	28 (46.66)[£]	8 (13.33)
Allelic frequency	NA1	40	84
	NA2	80[££]	36

**: $P = 0.05$, S $X^2 = 3.846$, OR = 1.67, 95% CI (0.99-2.80)
[#]: $P < 0.001$ HS, $X^2 = 27.491$; [##]: $P < 0.001$ HS, $X^2 = 38.443$, OR = 5.45, 95% CI (3.14-9.48); [£]: $P < 0.001$ HS, $X^2 = 25.87$; [££]: $P < 0.001$ HS, $X^2 = 30.85$, OR = 4.66, 95% CI (2.70-8.04)
S = significant, HS = highly significant, OR = odds ratio, CI = confidence interval

Japanese population (31). However, the conflicting data derived from these studies may be due to the fact that populations with different ethnic backgrounds seem to show varying frequencies of FcγR genotypes. Therefore, the relevance of distinct FcγR genotypes as risk determinants for susceptibility to various forms of periodontal disease should be considered in different racial groups. The present study was designed to determine whether there is any relationship between the genotype distribution of polymorphic FcγRIIa, FcγRIIIa and FcγRIIIb and susceptibility to generalized aggressive periodontitis in a South Indian population. The results showed that the FcγRIIIa V/V and FcγRIIIb NA2/NA2 genotypes, as well as the FcγRIIa-R, FcγRIIIa-V and FcγRIIIb NA2 alleles, may represent risk markers for susceptibility to generalized aggressive periodontitis.

Polymorphisms of Fcγ receptors may possibly explain individual susceptibility to gram-negative bacteria present in subgingival plaque. Periodontitis lesions contain large numbers of lymphocytes and plasma cells (32). Localization of $CD19^+$B lymphocytes bearing FcγRII has been observed in gingival tissue of periodontitis patients (33). Gene polymorphism in the FcγRIIa 131 H/R gene has been associated with periodontitis. Sanders et al. (34) observed that neutrophils with the R/R genotype are less efficient at phagocytosis of IgG2-mediated opsonized bacteria than H/H-bearing neutrophils, thus associating R/R with disease. Wilson and Kalmer (35) noted that FcγR IIa genotypes profoundly influence the IgG2-mediated phagocytosis and killing of *Aggregatibacter actinomycetemcomitans* by neutrophils and suggested that FcγRIIa alleles constitute a risk factor for localized early-onset periodontitis. In our study, no association of FcγR IIa genotype with GAgP was found, similar to observations made in African American (20) and Taiwanese populations (36). Furthermore, when the distribution of the R allele was assessed, it was significantly over-represented in GAgP group with an odds ratio of 1.6, which is in line with a study of a Taiwanese population (36). Although the present study was designed to keep confounding factors to a minimum, some unknown factors may have affected the result. Therefore, the association of the R allele with GAgP might have been a false positive finding. Further studies may be required to assess the role of the R allele in causation of GAgP in this South Indian population.

FcγR IIIa shows a single-nucleotide polymorphism that corresponds to a change of phenylalanine (F) to valine (V) at position 158 of Ig-like domain 2. Although V/V homozygous natural killer (NK) cells and macrophages are more effective than F/F NK cells and macrophages, the V/V genotype is associated with periodontal disease. This can be explained by the destructive role of hyperactive NK cells with the higher-affinity FcγRIIIa genotype in response to bacterial antigen (37). In our study, the V/V genotype was found in a higher proportion of GAgP patients, similar to previous findings obtained in North European Caucasian patients with periodontitis (30). We also observed a very high association of the V allele with GAgP patients, with an odds ratio of 5.46. Similar observations were made by Meisel et al. (38), who associated the V allele with the severity of alveolar bone destruction.

FcγRIIIb is found specifically on neutrophils, and shows NA1/NA2 polymorphism that determines the function of IgG1- and IgG3-mediated neutrophils. NA2 has been found to confer lower efficiency at neutrophil phagocytosis of IgG1-opsonized bacteria (39). This polymorphism has previously been associated with susceptibility to aggressive periodontitis (31). Other investigators have also associated the FcγRIIIb-NA2/NA2 genotype with severity (17) and recurrence (40) of chronic periodontitis. On comparison between the GAgP and control groups, we observed a highly significant distribution of NA2/NA2 in the GAgP group and NA1/NA1 in the control group. Therefore, the NA1 allele might be associated with periodontal health, whereas the NA2 allele may be a risk factor for GAgP. These

findings are in agreement with previous studies (20,31) of the association of this genotype with GAgP. A recent meta-analysis has also suggested an association between FcγRIIIb- NA1/NA2 polymorphism and aggressive periodontitis (41).

The distributions of FcγR genotypes/alleles in Indian healthy controls in our present study, and in a study by Morgan et al. of Fcγ receptor haplotypes in rheumatoid arthritis (42), were quite different. This difference was probably attributable to the ethnic types chosen for the two studies. The study by Morgan et al. involved a North Indian cohort, whereas the samples used in our study were obtained from a South Indian population. "Ancestral South Indian" is the term used to describe the population residing in the southern region of India, and this population is believed to be genetically unique and unrelated to any other human population of the world (43). This would explain the difference in genotype distribution between the two studies.

Generalized aggressive periodontitis shows a short disease onset and rapid tissue destruction. This may be due either to more aggressive microorganisms associated with the disease, or the immune response of the individual to microbial challenge. It is believed that both hypo- and hyperactive immune responses can lead to periodontopathology. The results of our study associate the less efficient R and NA2 alleles and the hyperactive V allele with susceptibility to aggressive periodontitis. This can be explained by the fact that the immune response is the result of interplay among many genes and their products. Furthermore, no single gene can explain the activity of immune cells, although it may partly account for the immune effect.

The distribution of the FcγR genotype varies widely in different ethnic groups. The reason for the difference in the results of these previous studies might lie in differences of ethnic background, the criteria used for placement of subjects in different groups, or a small sample size that does not accurately reflect the larger population as a whole. Since the prevalences of alleles vary in different populations, the results of a study on one population cannot be applied to other populations with different ethnic backgrounds. Identification of genetic factors contributing to disease in different populations could aid in the development of more specific therapeutic strategies for different phenotypes of periodontitis.

In the present study, subjects were placed in various groups to remove confounders as far as possible. Previous studies have revealed that smoking affects the IgG subclass concentrations in patients with early-onset periodontitis (44,45). Also, smoking has previously been shown to be associated with increased disease severity in patients with generalized aggressive periodontitis (46,47). For this reason, patients who smoked were excluded from this study. Similarly, patients with systemic disease that may mimic the aggressive course of the disease were also not included. The subjects in both groups showed no significant difference in mean age or male/female ratio. Defining a subject population according to the type of periodontal disease may also have contributed to significant differences in the results of genetic studies. The patients in the present study were selected on the basis of classification criteria set by in 1999 at the World Workshop on Periodontics (23).

In summary, our present results indicate that the FcγR IIIa V/V genotype and/or V allele, as well as the FcγR IIIb NA2/NA2 genotype and/or NA2 allele appear to be associated with susceptibility to GAgP in a South Indian population. The R131 allele of FcγRIIa occurred more frequently in the GAgP group than in the controls, and was also suggested to be a risk factor for GAgP in this South Indian population. To confirm the utility of these risk factors for prediction of GAgP, studies of distinct populations should be carried out. This identification/determination of specific genetic risk factors responsible for GAgP will aid in the development of more satisfactory classification systems, diagnosis and therapeutic strategies.

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