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Genetic Perspective of Periodontal Diseases- Road less Travelled

Abstract

This paper reviews the effect of genetic variations on periodontal diseases. With increased understanding and discovery of new avenues to study the functional interrelationships between gene products with each other. It is becoming increasingly evident that many human diseases are influenced by heritable alterations in the structure or function of genes. A small number of outstanding achievements in the field of medical and molecular genetics during the last few decades have a quite remarkable impact not only on clinical genetics, but also on other areas of medicine including dentistry. Thousands of inherited human disorders have been catalogued to date, but the underlying genetic causes of less than 20 percent of those disorders have been discovered. The complex multifactorial etiologies of these conditions, together with methodological problems, have limited progress until recently. Present studies are clarifying previously unrecognized genetic and phenotypic heterogeneities and attempting to study the complex interactions between genes and environment by applying new statistical modeling approaches to twin and family data. In the present review we discuss some of the potential applications of human molecular genetics for the diagnosis and treatment of oral diseases. This discussion is presented in the context of the ongoing technological advances and conceptual changes that are occurring in the field of medical genetics. To realize the promise of this new molecular genetics, we must be prepared to foresee the possibilities and to incorporate these newly emergent technologies into the evolving discipline of dentistry.

Key Words

Oral Health; genetics; periodontal; disease

Sumit Sabharwal[1], Anant Raghav Sharma[2], Vedati Prathima[3], Vivek Kumar Rai[4], Mohit Miglani[5], Ambika Kaul[6]

[1]Post Graduate Student, Department of Conservative and Endodontics, Saraswati Dental College & Hospital, Lucknow, Uttar Pradesh, India
[2]Senior Lecturer, Department of Periodontics, Pacific Dental College, Udaipur, Rajasthan, India
[3]Senior Lecturer, Department of Public Health Dentistry, Army College of Dental Sciences, Secunderabad, Hyderabad, India
[4]Post Graduate Student, Department of Conservative and Endodontics, Saraswati Dental College & Hospital, Lucknow, Uttar Pradesh, India
[5]Post Graduate Student, Department of Conservative and Endodontics, Saraswati Dental College & Hospital, Lucknow, Uttar Pradesh, India
[6]Senior Lecturer, Department of Periodontics, Indira Gandhi Government Dental College

INTRODUCTION

Genetics is the branch of biology that deals with heredity, especially the mechanism of hereditary transmission and the variation of inherited characteristics among similar related organism.[1] Genetics had already proven its importance in art of knowing about the species from the basic cells of reproduction and also about the various features that a species adopt from his Ancestors.[2] The vast majority of diseases are caused by mutations or subtle changes in the DNA sequence of a gene. However, some human anomalies are caused by defects in chromosomes that can be visualized under a microscope, such as the extra copy of chromosome 21 that is seen in Down syndrome. The most common type of mutation in DNA is a single nucleotide polymorphism (SNP), of which most are silent and cause no visible, or phenotypic, consequences. However, at times, a nucleotide substitution can cause a change at a location that result in an altered protein product that does not function appropriately or is not even synthesized if it occurs in the gene's control elements.[3,4]

The three most common problems in dentistry today remain Dental Caries, Periodontal diseases and Malocclusion while there have always been anecdotal evidences of a genetic basis to each of these problems.[5,6] Out of the 3 most common

dental diseases, periodontal disease, are the most prevalent chronic inflammatory disease in humans, is an infection in the gums that results in destruction of tooth-supporting tissue and bone, ultimately causing tooth loss. Bacteria accumulate along the gum line initiating an immune response. The microbial causation of the inflammatory periodontal diseases is well established. Risks of many diseases including periodontal diseases are not borne equally by all individuals.[7] A variety of microbial, environmental ,behavioral and systemic factors are reported to influence risk for moderate to severe periodontitis.[8,9] It is also increasingly evident that genetic variance is a major determinant of the differential risk for many human diseases. However the contribution of an allelic variant to a diseases can vary from being determinants to having only a minor effect on the etiology. The contribution of an allelic variant to diseases has major implications for the diseases characteristics.[10]

THE HUMAN GENOME PROJECT AND PERIODONTITIS

It was once reasoned that the most complex organism on the face of the earth-the Human genome must have the greatest number of genes found in nature. Early estimates on the number of genes ranged from 80,000 to 150,000 genes (US Dept of Health and Human Sciences). However it is more apparent that the human genome contains only approximately 30,000-40,000 genes.[11] In periodontics as well as in medicine genetics of both humans and the pathogens and their interactions are of great importance. Presently the complete sequence of a putative periodontal pathogen, *P. gingivalis* has been sequenced in its entirety. Other organisms of dental and periodontal relevance with ongoing genomic sequencing include *Aa comitans, S. mutans, S. sanguis, T. denticola, Candida albicans and F. nucleatum.* Most common forms of periodontitis represent a lifelong account of interactions between our genome, our behavior and our environment. Humans share 99.9% of their genetic information, which is why all humans belong to the same species. The final 0.1% differs from one person to the next. This seemingly small variation may very well be involved in disease susceptibility and drug and treatment response in periodontitis. Periodontosis is an idiopathic degeneration of the periodontium that results in migration and loss of teeth. The disease begins in the regions of the incisors and first molars. Late in the disease, other areas of the dental arches may be involved. The gingivae are not initially inflamed, and there are no associated systemic abnormalities. Local irritants cannot account for the marked alveolar destruction, which leads to the tooth loss. Several heritable syndromes and periodontitis also may be associated with alveolar bone destruction. Periodontosis can be differentiated each of these on -the basis of negative laboratory tests, lack of associated anomalies, distinctive pattern of bone loss and timing of onset of gingival inflammation. A family in which periodontosis was present in three of six sibs and in which ichthyosis was segregating independently of periodontosis was reported.[12]

EVIDENCE FOR THE ROLE OF GENETICS IN PERIODONTITIS

1. Familial aggregation
2. Twin studies
3. Segregation studies.
4. Linkage studies
5. Polymorphism Studies

1) FAMILIAL AGGREGATION

There is literature reporting familial aggregation of periodontal diseases, but, due to different terminology, classification systems, and lack of standardized methods of clinical examination, it is difficult to compare reports directly. Although periodontal disease nosology has changed many times over the timeframe of these reports, most familial reports for periodontitis are for early-onset forms now called aggressive periodontitis.[13,14] Reports of the familial nature of chronic forms of periodontitis are less frequent, although German studies of the familial nature of chronic forms of periodonitis from the early 20th century have been reviewed by Hassell and Harris (1995). This aggregation within families strongly suggests a genetic predisposition. It must be borne in mind that familial patterns may reflect exposure to common environmental factors within these families. Thus it is important to consider the shared environmental and behavioral risk factors in any family. These would include education, socio-economic grouping, oral hygiene, possible transmission of bacteria, diseases such as diabetes, and environmental features such as passive smoking, sanitation, etc. Some of these factors, such as lifestyle and behavior and education, may be under genetic control and may influence the standard of oral hygiene. The complex interactions between genes and the environment must also be considered in the evaluation of familial risk for the periodontal

diseases.[15] In chronic periodontitis, the phenotype or disease characteristics do not present significantly until the third decade of life, whereas in the aggressive forms of periodontal disease, the presentation can occur in the first, second, third, and fourth decades. This variability in presentation of significant signs of disease makes diagnosis difficult, not only in declaring if a patient suffers from the disease but also in detecting patients who do not suffer from the disease, and differentiating between adult and aggressive forms of periodontitis. The problems associated with the clinical differentiation of periodontal disease are not uncommon in medical genetics, since similar problems arise in the study of other delayed-onset hereditary traits.[16] Attempts to correlate cellular, functional, and immune response variables with early-onset periodontitis phenotypes in families have been generally unproductive, except to indicate that a simple mode of transmission was not evident and that early-onset forms of periodontitis were likely to be etiologically complex and heterogeneous,[17,18] Although bacterial transmission between subjects has been suggested as a feasible explanation of why aggressive periodontitis may cluster within families, the observation of bacterial transmission within families is insufficient on its own to account for familial clustering.[19]. While the heterogeneity paradigm discussed by Potter (1989) is borne out in subsequent familial studies of what is now classified as aggressive periodontitis, the striking familial aggregation of the trait is consistent with a significant genetic etiology. Characterization of the genetic components of etiology requires more formal genetic analyses.[18]

2) TWIN STUDIES

Twin studies have been a valuable source of information about the genetic basis of simple as well as complex traits. To maximize the potential of twin studies, large, worldwide registers of data on twins and their relatives have been established. Twin studies have been used to obtain insights into the genetic epidemiology of complex traits and diseases and also to study the interaction of genotype with sex, age, and lifestyle factors. Twin studies of periodontitis have been limited in scope and generally of small numbers. However, studies of concordance for periodontitis and for clinical indices related to periodontal health and disease generally support a significant heritable component for periodontitis. Most twin studies have studied the more prevalent forms of chronic periodontitis.[20] These results confirm previous studies and indicate that approximately half of the variance in disease in the population is attributed to genetic variance. The basis for the heritability of periodontitis appears to be biological and not behavioral.

3) SEGREGATION ANALYSIS

While familial aggregation is consistent with a heritable component of aggressive periodontitis, and twin studies support a genetic component to chronic periodontitis, neither observation nor analysis is appropriate to identify the genetic model or specific gene loci that contribute to periodontal disease. Segregation analyses can evaluate the relative support for different models to identify that which most closely represents the clinical data observed. Segregation analyses can evaluate the relative support for different models to identify that which most closely represents the clinical data observed. here have been few rigorous segregation analyses of aggressive periodontitis, and many are actually studies of one or a few families and are realistically underpowered for definitive conclusions to be drawn. Early studies of aggressive forms of periodontitis were hampered by clinical diagnostic and classification issues (particularly in older individuals) and an overrepresentation of affected females .[21, 22] The female ascertainment bias would lead to false support for X-linked transmission. A detailed review of the topic is presented elsewhere and concludes that several reports suggesting X-linked transmission actually support autosomal-dominant transmission for aggressive forms of periodontitis in North American families when the ascertainment bias is corrected.[23] The most definitive segregation analysis in North American families was performed by Marazita and co-workers (1994), who studied more than 100 families, segregating aggressive forms of periodontitis, and found support for autosomal-dominant transmission. They concluded that autosomal-dominant inheritance with approximately 70% penetrance occurred for both Blacks and non-Blacks.[24]

4) LINKAGE STUDIES

To date, linkage studies have been performed on two families with localized aggressive periodontitis (LAgP). Boughman *et al*., (1986)[25] identified an autosomal-dominant form of LAgP in an extended family from Southern Maryland. In this family, type III dentinogenesis imperfecta (DGI-III) and a localized form of AgP were segregating as dominant traits. Since the gene for DGI-III had been

previously localized to chromosome 4, they performed a linkage analysis on this chromosome and demonstrated relatively close linkage with the suspected locus for AgP. Although the support for linkage for AgP to chromosome 4 in this Brandywine kindred from Maryland was the minimum required for statistical significance (LOD score = 3.0), this was an important study, because it supported autosomal-dominant inheritance of a single major gene locus, clearly indicating a major genetic component to the disease etiology. Hart *et al*., (1993)[26] evaluated support for linkage to this region of chromosome 4 in a different population of families (14 African-American and four Caucasian). Results of their linkage studies found evidence to exclude a gene of major effect from this region of chromosome 4 as being a major etiologic contributor in these families for any of the genetic models tested. They suggested that these findings supported genetic locus heterogeneity AgP. Thus, this Brandywine population appears to have a different form of periodontal disease, and a different gene is responsible for the disease in the Brandywine population than in the African-American and Caucasian families studied by Hart and co-workers. Results of linkage analyses to date have not identified a gene locus for AgP, but findings do support genetic heterogeneity, with at least one gene locus responsible for AgP located on chromosome 4.

5. POLYMORPHISM STUDIES IN PERIODONTITIS

A) Gene Polymorphisms Of Host Response Elements And Periodontitis[27]

Our current understanding is that periodontal disease (gingivitis and periodontitis) is initiated by the microbes within the plaque which accumulates in the gingival crevice region. Gingivitis will progress in many individuals to periodontitis, but this progression is governed by the subject's host response. The host response is determined to some extent by previous experience (acquired immunity) but is predominantly influenced by the person's genetic make-up. Individuals respond to different antigens in ways predicted by their genes. A good example of this is in the case of the atopy diseases (viz. eczema, hay fever, asthma). Sufferers of hay fever have specific IgE antibody responses to antigens, such as those in pollen, which initiate a mass release of inflammatory mediators from mast cells in the respiratory system. This excessive inflammation is seen as hay fever. Hay fever, asthma, and eczema are genetically related conditions that are grouped within families and show how responses of the immune system can be affected by genetics. Differences in host response between subjects are not solely confined to differences in immune response, as is the case in the above example, but may also be manifested through differences in the inflammatory response (e.g., complement C1 deficiency that produces angio-edema) or in basic innate immune aspects (an example is the dysfunction of sweat glands which predisposes to infection in cystic fibrosis patients)

b) Immunological Polymorphisms And Periodontal Disease

Variations in IgG2 levels influence the immune response to periodontal pathogens (Tew *et al*., 1996), and IgG2 antibodies are considered to be most effective in dealing with microbial carbohydrate moieties. A segregation analysis of IgG2 levels in AgP families has suggested an autosomal-co-dominant mode of inheritance.[24] Class II MHC molecules are part of the process of recognition of bacterial antigens and could therefore feasibly influence susceptibility to AgP.[28] The MHC or HLA genes determine our response to particular antigens and may thus influence our response to periodontal pathogens and, thus, the host response to periodontitis. Molecular biological techniques are now available to investigate, in detail, genetic polymorphisms, such as those demonstrated by the HLA gene cluster. A Japanese study of AgP patients has found a significant association for these patients with an atypical BamHI restriction site in the HLA.DQB gene. Another group has investigated HLA.DR polymorphisms in patients with GAgP and found a significant association between several DRB1 alleles and the disease. These alleles have previously been associated with rheumatoid arthritis.[29]

c) Il-1 Gene Polymorphisms In Periodontal Disease

The IL-1 gene polymorphisms associated with periodontitis provide a useful example for arguing the strengths and limitations of gene polymorphism in disease association studies in the periodontal diseases. In 1997, Kornman *et al*.,[30] found an association between polymorphisms in the genes encoding for IL-1a (-889) and IL-1ß (+3953) and an increased severity of periodontitis. This initial study has since spawned numerous publications and has been the most influential in creating interest in gene

polymorphisms and periodontal disease. The specific genotype of the polymorphic IL-1 gene cluster (periodontitis susceptibility trait, PST) was associated with severity of periodontitis in only non-smokers, and distinguished individuals with severe periodontitis from those with mild disease (odds ratio 18.9 for ages 40–60 years, but wide confidence intervals of 1.04 to 343.05). The interleukin-1 polymorphism in aggressive periodontitis Hodge *et al*., (2001)[31] examined IL-1A and IL-1B genetic polymorphisms in unrelated European white Caucasian patients with generalized early-onset periodontitis and found no significant differences between patients and controls for any of the composite genotypes described by Kornman *et al*., (1997).[30] No significant differences were found between patients and controls regardless of whether smoking was included as a covariate. It was concluded that there was a lack of association between the IL-1 polymorphisms and aggressive periodontitis, which questions the utility of these candidate genes as markers of susceptibility.

Summary Of The Findings On The Il-1 Composite Genotype In Periodontitis

It appears that this IL-1 composite genotype has equivocal ability in detecting susceptibility to periodontitis and may be limited in its utility to only specific populations at best. It would appear, from the mixed reports on this composite genotype, that:[32]

i. It is unlikely to be relevant in aggressive periodontitis;
ii. It is, at best, in linkage disequilibrium with the gene contributing susceptibility to chronic periodontitis.
iii. It confers risk independent of that attributable to smoking;
iv. The polymorphism is at best one of several involved in the genetic risk to chronic periodontitis, which is likely to be a disease in which multiple genes may confer risk.
v. The polymorphism is a useful marker in only defined populations, is relatively absent in some (Armitage et al., 2000), and is too prevalent in others to be a genetic marker with utility;
vi. Demonstration of the functional significance of this gene polymorphism has yet to be confirmed; and
vii. Clinical utilization of these composite polymorphisms for risk assessment and prognostic determination is currently premature.

Other genetic factors which have influence on the periodontal disease includes:

TUMOR NECROSIS FACTORα (TNFα)

The TNFα cytokine is crucial to both the immune and inflammatory responses. For example, TNF up-regulates host defenses and has other effects on tissue physiology, including bone resorption.[33] Over-expression of TNFα in the periodontium may be harmful to the host. Normally, TNFα and other pro-inflammatory agents are regulated by IL-10, suggesting that some deficiency in this regulation mechanism may be linked with disease.

INTERLEUKIN-10 GENES

A study of the distribution of genes related to interleukin-10 (IL-10) found no association between the genes for this cytokine and aggressive periodontitis compared with healthy controls. There is little to suggest that variations at the IL10.R locus are relevant to the genetic transmission of GAgP, whereas in RA a significant trend toward IL10.R2 has been shown (Eskdale *et al*., 1998). It seems that the genotype represented by this allele plays little if any role in GAgP. IL10.G showed the greatest variation between GAgP and control populations, which seems mainly due to a 12% decrease in IL10.G9 occurrence vs. that of other alleles in the GAgP population.[34]

F_C-GAMMA RECEPTOR

The Fc-gamma receptor (FcγR) is the receptor present on phagocytes' which binds immunoglobulin G (IgG) and is thus crucial in the opsono-phagocytosis of bacteria. Polymorphisms that influence the binding affinity between the Fcγ receptors and IgG of different subclasses are considered important in susceptibility to periodontal disease.[35]

Genetic Screening For Periodontitis Risk

The current practical clinical utility of genetic knowledge in periodontics is limited. However, performing clinical periodontal assessments of siblings of AgP probands is one of the most useful actions we can perform to ensure the early diagnosis of this disease. By careful clinical diagnostic procedures, we may detect susceptible patients early and instigate therapy which may prevent the more significant disease aspects from occurring. In the pursuit of better genetic diagnostic tests for chronic and aggressive periodontitis, we must plan our research using plausible biological arguments and carefully avoid bias and misinterpretation of genetic associations with diseases.

CONCLUSION

One can conclude that, despite major advances in the awareness of genetic risk factors for periodontal disease, we are still some way from determining the genetic basis of both aggressive and chronic periodontitis. A strong plea in the discovery of better genetic diagnostic tests for chronic and aggressive periodontitis is that we plan our research using plausible biological arguments and carefully avoid bias and misinterpretation of genetic associations with disease.

EFERENCES

1. Oxford dictionary. Oxford university press. Available at: www.oxforddictionaries.com/definition/genetics?view=uk. Accessed on 18.01.2014.
2. Pemberton TJ, Patel PI, Gee J, Pragna I. Gene discovery for dental anomalis. J Am Dent Assoc. 2006;137:743-752.
3. SarkarXA. Human Genetics.5th Ed.Dominant Publishers; 2001
4. Gardner S. Principle of Genetics. 8th Ed. Wiley publisher; 1971.
5. Sandford RN. "Clinical Genetics" Davidson's Principles and Practice of Medicine. 2002 Churchill Livingstone.
6. Townsend GC, Aldred MJ, Bartold PM. Genetic aspects of dental disorders. Aust Dent J. 1998;43(4):269-86.
7. Johnson NW, Griffiths GS, Wilton JMA. Detection of high risk groups and individuals for periodontal diseases. Evidence for the existence of high risk groups and individuals and approaches to their detection. J Clin Periodontol. 1988;15:276-82.
8. Page RC. The role of inflammatory mediators in the pathogenesis of periodontal disease. J Periodontal Res. 1991;26:230-42.
9. Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Periodontal disease and cardiovascular disease. J Periodontol. 1996;67: 1123-37.
10. Collins FS, McKusick VA. Implications of the Human Genome Project for Medical Science. J Am Med Ass. 2001;285:540-4.
11. Genco RJ, Mashimo PA, Krygier G, Ellison SA. Antibody-mediated effects on the periodontium. J Perio. 1974;45:330-7.
12. Grenby TH, Owen D. A gnotobiotic study to distinguish between heredity and the oral microflora as transmitters of dental caries activity in laboratory rats. Caries Res. 1980;14:434-440.
13. Korkhaus G. Über die erbliche Disposition zur paradentose. Dtsch Zahnärztl Z. 1952;7:441-448.
14. Cohen DW, Goldman HM. Clinical observations on the modification of human oral tissue metabolism by local intraoral factors (abstract). Ann NY Acad Sci. 1960;85: 68.
15. Harris SA, Ingram R. Molecular systematics of the genus Senecio LI. Hybridization in a British polyploid complex. Heredity. 1992;69:1-10.
16. Boughman JA, Beaty TH, Yang P, Goodman SB, Wooten RK, Suzuki JB. Problems of genetic model testing in early onset periodontitis. J Periodontol. 1988;59:332-337.
17. Astemborski JA, Boughman JA, Myrick PO, Goodman SB, Wooten RK, Agarwal S, *et al*. Clinical and laboratory characterization of early onset periodontitis. J Periodontol. 1989;60:557-563.
18. Potter RH. Etiology of periodontitis: the heterogeneity paradigm. J Periodontol. 1989;60:593-597.
19. Boughman JA, Astemborski JA, Suzuki JB. Phenotypic assessment of early onset periodontitis in sibships. J Clin Periodontol. 1992;19:233-239.
20. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. Nat Rev Genet. 2002;3:872-882.
21. Saxen L, Nevanlinna HR. Autosomal recessive inheritance of juvenile periodontitis: test of a hypothesis. Clin Genet. 1984;25:332-335.
22. Hart TC, Schenkein HA, Marazita ML, Brooks CN, Gunsolley JG, Diehl SR. No female predominance in juvenile periodontitis after correction for ascertainment bias. J Periodontol. 1991;62:745-749.
23. Hart TC, Marazita ML, Schenkein HA, Diehl SR. Re-interpretation of the evidence for X-linked dominant inheritance of juvenile periodontitis. J Periodontol. 1992;63:169-173.
24. Marazita ML, Burmeister JA, Gunsolley JC, Koertge TE, Lake K, Schenkein HA. Evidence for autosomal dominant inheritance and race-specific heterogeneity in early-onset periodontitis. J Periodontol. 1994;65:623-630.
25. Boughman JA, Halloran SL, Roulston D, Schwartz S, Suzuki JB, Weitkamp LF, *et al*.

An autosomal-dominant form of juvenile periodontitis: its localization to chromosome 4 and linkage to dentinogenesis imperfecta and Gc. J Craniofac Genet Dev Biol. 1986;6:341-350.

26. Hart TC, Marazita ML, McCanna KM, Schenkein HA, Diehl SR. Reevaluation of the chromosome 4q candidate region for early onset periodontitis. Hum Genet. 1993;91:416-422.
27. Kahn MC. The Merck Veterinary Manual. 10th Edition. Merck and Co. Publisher; 2006
28. Shapira L, Smidt A, Van Dyke TE, Barak V, Soskolne AW, Brautbar C, *et al*. Sequential manifestation of different forms of early-onset periodontitis. A case report. J Periodontol. 1994;65:631-635.
29. Takashiba S, Noji S, Nishimura F, Ohyama H, Kurihara H, Nomura Y, *et al*. Unique intronic variations of HLA-DQ beta gene in early-onset periodontitis. J Periodontol. 1994;65:379-386.
30. Kornman KS, Crane A, Wang HY, di Giovine FS, Newman MG, Pirk FW, *et al*. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol. 1997;24:72-77.
31. Hodge PJ, Riggio MP, Kinane DF. Failure to detect an association with IL1 genotypes in European Caucasians with generalised early onset periodontitis. J Clin Periodontol. 2001;28:430-436.
32. Lundstrom A. Tooth Size and Occlusion in Twins. 2nd Edition. Stockholm; 1948.
33. Mundy GR. Cytokines and growth factors in the regulation of bone remodeling. J Bone Mineral Res. 1993;8:S505-S510.
34. Kinane DF, Hodge PJ, Eskdale J, Ellis R, Gallagher G. Analysis of genetic polymorphisms at the interleukin-10 (IL-10) and tumour necrosis factor (TNF) loci in early-onset periodontitis. J Periodontal Res. 1999;34:1-8.
35. Kobayashi T, Westerdaal NA, Miyazaki A, van der Pol WL, Suzuki JB, Yoshie H, *et al*. Relevance of immunoglobulin G Fc receptor polymorphism to recurrence of adult periodontitis in Japanese patients. Infect Immun. 1997;65:3556-3560.