INTRODUCTION

Review of Literature

Malocclusion is most often caused by a combination of genetic and environmental factors, though the most severe Class II Division 1, Class III, and Class II Division 2 (CII/D2) cases are predominantly hereditary.[1] Patients with Class II Division 1 have significantly smaller mandibles associated with a reduced mandibular length, causing the mandible to be more retruded than Class I patients.[2-3] The correlation between the patient and the immediate family is greater than the correlation between unrelated subjects paired at random.[2-3] Environmental factors may also influence Class II Division 1 malocclusions. A digit sucking habit can increase the proclination of the maxillary incisors producing a Class II Division 1 incisal relationship.[4] Patients that are lip incompetent may also have an increased maxillary incisal proclination due to a disproportionate balance of pressure on the dentition.[4] Class III malocclusions are polygenic in nature.[4-5] Of the previously mentioned types of malocclusions, Class III cases are most often determined to be hereditary. The "Hapsburg jaw" is perhaps the most notorious case of genetically related malocclusion; this form of Class III malocclusion has been passed through several generations.[6]

A CII/D2 phenotype is characterized by an Angle Class II molar relationship with retroclined maxillary central incisors, deep overbite, and obtuse interincisal angle and soft tissue components such as a high lip line and active mentalis muscle.[4, 7] This phenotype also has a maxilla that is orthognathic, a mandible that is relatively short and retrognathic and a hypodivergent facial pattern.[7] The occurrence of a CII/D2 phenotype

is comparatively rare in the Western white population, only 1.5% to 5%.[7] Similarly, the incidence is only slightly higher in white populations of European descent.[6]

There are significant genetic patterns in patients with CII/D2, even though the origin of CII/D2 has not been determined.[8] The cause of CII/D2 may be a combination of both environmental and genetic factors.[9]

Although both genetic and environmental factors affect the craniofacial phenotype, there is "evidence for a significant genetic influence" in dental variations.[4] The genetic influence varies dependent upon the trait being studied. Manfredi et al. compared thirty-nine cephalometric parameters of monozygotic and dizygotic twins and same sex pairs and found that there was strong evidence of heritability, particularly among monozygotic twins.[10] Both environmental and genetic factors must be given credence when determining methods of treatment and eventual outcomes.[4,10]

Similarly, in a comparative study between monozygotic and dizygotic twins the genetic component was the main factor in the development of the CII/D2 malocclusion.[1] Upon discovery of certain genetic markers, Markovic concluded that it will be possible to map genes and establish characteristics of their structures. The discovery of these markers would confirm the long held notion that "malocclusions associated with some congenital syndromes are predominantly caused by genetic factors."[1]

It is necessary to try to establish the cause of malocclusion as predominantly genetic or environmental, although both can and do play a part. If the source is determined to be primarily genetic, orthodontic treatment will not necessarily be capable

of resolving malocclusions.[11] However, the discovery of genetic markers might make it possible to resolve malocclusions in other manners in the future.

The retroclined upper incisors may present with non-physiologically high lip pressure. This may be due to a local soft tissue genetic factor in the development of a CII/D2.[12]

Although Class II Division I and Class III malocclusions do not appear to be related to congenital tooth anomalies when compared to the general population, CII/D2 malocclusions have a three-fold frequency of tooth anomalies compared with general populations.[14] Patients with CII/D2 are more likely to have developmental dental anomalies such as impaction of one or both upper canines, agenesis of and/or peg-shaped maxillary lateral incisors, agenesis of third molars, supernumerary teeth, and transpositioned teeth, than patients without a CII/D2 craniofacial type.[8, 13]

A preliminary study completed by Dr. Michael Morrison at Indiana University School of Dentistry (IUSD) in July 2008 analyzed "dental developmental anomalies in II/2 (CII/D2) probands and first-degree family members in order to possibly extend and define the II/2(CII/D2) phenotype and develop a discernible mode of inheritance."[15] Subjects with the CII/D2 phenotype had a higher frequency of congenital tooth anomalies than the general public. Furthermore, this study found that first degree relatives of CII/D2 probands had an increased incidence of permanent tooth agenesis, excluding third molars, as compared with the general population. [15] From the aforementioned studies, it can be concluded that those with the CII/D2 malocclusion have an increased incidence of congenital tooth anomalies, e.g. hypodontia, when compared to the general population.

Tooth agenesis is classified by the number of teeth involved and is generally the most pervasive developmental defect observed in permanent dentition. It can be broken down into three categories. Hypodontia is the most common form of tooth agenesis and is characterized by the absence of one to six teeth. Oligodontia excludes third molars and is typified by six or more undeveloped permanent teeth. Anodontia, though extremely rare, is a genetic disorder commonly defined as the absence of all teeth.[16] As the most commonly occurring form of tooth agenesis, hypodontia is often referred to as congenitally missing teeth. The breakdown of communication during the initiation phase of tooth development between the mesenchymal tissues and overlying epithelium results in hypodontia.[17] The literature suggests that mutations in genes can lead to hypodontia. For example, mutations in the PAX9 or MSX1 genes have been indicated as causative factors in hypodontia.[18-21, 23-31]

Between 2% and 10% of the general population have dental agenesis of teeth other than third molars.[18] The most common teeth missing are the maxillary lateral incisors and the mandibular second premolars, when excluding the third molars.[18] There is an ethnic difference in the prevalence of hypodontia.[22] The prevalence of hypodontia in the Japanese population is 9.2%, whereas the incidence in the Caucasian population is between 3% and 7.5%.[22] The most commonly missing teeth in the adult dentition are the third molars, occurring in approximately 20% of a Caucasian population.[19]

Several studies have suggested that dental agenesis is a genetic abnormality. It is transmitted as an autosomal dominant trait, an autosomal recessive or an X-linked inheritance pattern.[16, 20]

The PAX9 gene is located on chromosome 14.[21] PAX9 codes for a transcription factor that participates in interactions between epithelial and mesenchymal tissues during tooth development.[17] Numerous publications link mutations within the PAX9 gene to individuals with dental agenesis. [21, 23-25] Hypodontia was reported in the molar region in multiple studies.[21, 23-25] In the premolar region, hypodontia was observed by Klein et al. [26], Kula et al. [24], and Das et al.[21,] Kula et al.[24] and Das et al. [21] also noted hypodontia in the anterior region.

The MSX1 gene is located on chromosome 4 at 4q16.1.[27] This gene encodes for a transcription factor that is present in several tissues that deal with tooth formation, branchial arches, tooth buds, and salivary glands.[28] Mutations in the MSX1 gene are responsible for dental agenesis in the molar and premolar region.[29, 30]

Purpose of the Study and Objectives

The purpose of this study was to determine if there was an association between the CII/D2 malocclusion and genes associated with hypodontia namely: PAX9 or MSX1. An association analysis is performed to determine whether a genetic variant (polymorphism) of the PAX9 or MSX1 genes, has an increased probability of occurring with the CII/D2 malocclusion when compared to a non-CII/D2 population. If there is a significant increase of this genetic variant in this population it will increase our understanding of overall growth and development of the craniofacial complex. Increasing our understanding of the genetic influence on malocclusion will aid in our

diagnosis and treatment suggestions to patients and also help orthodontists provide better care.

Statement of Hypotheses

Null Hypothesis: There is not a significant association of each SNP genotype with the presence of CII/D2 malocclusion when compared to a non-CII/D2 population.

Alternative Hypothesis: There is a significant association of each SNP genotype with the presence of CII/D2 malocclusion when compared to a non-CII/D2 population.

METHODS AND MATERIALS

Experimental Design

A total of 215 patients (probands) were enrolled in this study. 100 with CII/D2 malocclusions and 115 probands without CII/D2 and no hypodontia were obtained from Indiana University School of Dentistry (IUSD) Graduate Orthodontics Clinic. The sample number was arbitrarily chosen because there is no preliminary data or published data available in this area. The probands were contacted either by phone or when they presented for treatment in the IUSD Graduate Orthodontic Clinic by one investigator. An informed consent form approved by the IUSD Institutional Review Board (IRB) Committee was reviewed with the proband and/or legal guardian to verify that all questions and concerns had been addressed. Following a previous protocol for analyzing diagnostic clinical records of CII/D2 patients, each of the two hundred probands were analyzed in the following manner: [15]

- Clinical examination: Screening exam recording occlusion for molar, canine, incisor position and any clinically missing teeth. Patients were asked if the missing teeth were previously extracted. This exam was completed by a co-investigator or another orthodontist.
- Digital photographs of the proband's occlusion: Photographs were reviewed to determine occlusion and to verify clinically missing teeth. A series of extra-oral (3) and intra-oral (5) photographs were taken. The three extra-oral photographs included frontal view relaxed, frontal view with smile, and profile view relaxed. The five intra-oral photographs included maxillary occlusal view, mandibular

occlusal view, centric occlusion frontal view, and centric occlusion lateral view right and left.

- Study models: Pretreatment models of the subjects' occlusion were evaluated to verify the clinical exam. If a screening examination did not allow adequate visualization of molar occlusion, the assumption was that the intercuspation of the models was correct. We accepted the evaluation of the study models over the clinical examination and pictures. If the patient had no history of orthodontics, study models were obtained. Alginate impressions were made and poured with orthodontic plaster. A wax bite in centric occlusion was obtained. The plaster models were trimmed using the wax bite to verify centric occlusion.
- Panoramic and lateral cephalometric radiographs that were taken as pretreatment records were used. Instrumentarium Imaging Orthopantomograph OP100D and Orthoceph OC100D were used. Panoramic radiographs were used to verify missing teeth that are not apparent on photographs or models. Lateral cephalometric radiographs were used for measurements stated in the Cephalometric Analysis Section.

Using the information gathered, probands having CII/D2 malocclusion and probands without CII/D2 malocclusions or hypodontia were confirmed by one investigator. Any questions regarding acceptance or disqualification of a proband were directed to the principal investigator.

The probands were accepted as a CII/D2 if all of the following criteria were met:

- Class II molar relationship.
- Decreased axial inclination of permanent maxillary incisors, i.e. axial inclination of permanent maxillary incisors from Sella-Nasion -7º (SN -7) is less than 110 degrees.
- Flat mandibular plane: Mandibular Plane (MP) to SN -7 is less then 25 degrees.
- Deep bite: Over bite is greater than 50%.
- Late mixed to permanent dentition: Probands in late mixed dentition to full permanent dentition will be included in this study.

The proband was accepted as a non-CII/D2 with no hypodontia if all of the prior criteria were not met. Fifteen probands were eliminated from study due to hypodontia.

Cephalometric Analysis

One investigator was responsible for performing all measurements and radiographic interpretation. The following radiographic measurements were evaluated on the CII/D2 probands (Appendix 1): sella-nasion-A-point angle (SNA), sella-nasion-B-point angle(SNB), A-point-nasion-B-point angle(ANB), mandibular plane angle(MP-HP), and axial inclination of permanent maxillary incisors(1/ to HP). In order to evaluate reliability and minimize variability all measurements were performed on ten probands and repeated on a different day following primary analysis.

Salivary Sample

Saliva samples (4ml) of each CII/D2 and non-CII/D2 proband were collected for using Oragene-DNA (DNA Genotek Inc, Ottawa, Ontario, Canada). According to the manufacturer, the median yield of DNA per ~2 ml of saliva collected was approximately 100µg (with a range of >20µg to 300µg). The Oragene-DNA immediately stabilized the DNA for long term storage at -15°C, until DNA purification was completed. To minimize sample loss, each sample was divided into two tubes. One tube was used to isolate DNA while the other was kept frozen in case of contamination. Genomic DNA was isolated from the saliva/OrageneDNA mixture by ethanol precipitation as per the manufacturer's instructions and was resuspended in 10mM Tris-HCl, 1mM EDTA pH 8.0. All DNA concentrations were measured on the NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific., Wilmington, DE).

Laboratory protocol for manual purification of DNA from the previously frozen Oragene-DNA/ saliva samples was:

Step 1: Mix the Oragene-DNA/saliva sample in the Oragene-DNA vial by inversion and gentle shaking for a few seconds. This step was done at each collection of saliva prior to freezing. Once we collected the samples and were ready to purify, the samples were removed from the freezer and we continued with the purification process.

Step 2: Incubate the sample at 50°C in a water incubator for one hour.

Step 3: Transfer 500µL of the mixed Oragene-DNA/ saliva sample to a 1.5mL microcentrifuge tube.

Step 4: Into each 500µL Oragene-DNA/saliva specimen, add 20µL of Oragene-DNA Purifier to the microcentrifuge tube and mix by vortexing for a few seconds.

Step 5: Incubate on ice for ten minutes.

Step 6: Centrifuge at room temperature for five minutes at 13,000 rpm.

Step 7: Carefully transfer the clear supernatant with a pipet tip into a fresh microcentrifuge tube. Discard the pellet containing impurities.

Step 8: To 500µL of supernatant, add 500µL of room-temperature 95-100% ethanol. Mix gently by inversion ten times.

Step 9: Allow the sample to stand at room temperature for ten minutes to allow the DNA to fully precipitate.

Step 10: Place the tube in the microcentrifuge in a known orientation. Centrifuge at room temperature for two minutes at 13,000 rpm.

Step 11: Carefully remove the supernatant with a pipet tip and discard it. Take care to avoid disturbing the DNA pellet.

Step 12: Add 100µL of DNA buffer to dissolve the DNA pellet. Vortex for at least five seconds.

Step 13: (optional) Additional steps to ensure complete hydration of the DNA.

Step 14: Storage of the fully rehydrated DNA. Long term storage at -20°C was utilized.

Genetic Analysis

Purified DNA was analyzed at the University of Kentucky, Center for Oral Health Research in the Hereditary Genomics Laboratory. Single nucleotide polymorphisms (SNPs) for each gene of interest were selected based upon reported heterozygosity and linkage disequilibrium. The SNPs were chosen to best provide a clue if any genetic variation in or close by the gene was related to variance in the phenotype. Relying on the experience of the geneticist, Dr. James K. Hartsfield Jr., two SNPs for PAX9 and one SNP for MSX1 were identified that each exhibited heterozygosity (Minor Allele Frequency, MAF) as close to 0.5 as possible within a Caucasian population, that were not in linkage disequilibrium with each other. Based on these criteria, non-synonymous single nucleotide polymorphisms (nsSNP) were chosen whenever possible. For the PAX9 gene, the following SNPs were examined: rs1955734, rs8004560. For the MSX1 gene the following SNP was examined: rs3821949.

SNPs are DNA sequence variations that occur when a single nucleotide (Adenine, Thymine, Cytosine, Guanine) in the genome is altered. For example, a SNP may change to the DNA sequence A**A**GGCTAA to A**T**GGCTAA. This means this SNP would have two alleles (A or T). In populations alleles are assigned a minor allele frequency (MAF), which is the lesser of the two allele frequencies. MAFs and alleles may vary between populations. [32] Since every subject receives one set of DNA from each parent, each

proband will have two alleles which is referred to as a genotype. The proband's genotype may be homozygous for the major allele, homozygous for the minor allele or heterozygous (Appendix 2).

The MSX1 rs3821949 SNP is located upstream of the gene. The major allele is Adenine (A) and the minor allele is Guanine (G) with a MAF of 0.22. The PAX9 rs1955734 SNP is located in an intron. The major allele is a Cytosine (C), and the minor allele is a Thymine (T) with a MAF in the Caucasian population of 0.48. The PAX9 rs8004560 is located at an intron. The major allele is Adenine and the minor allele is Guanine with a MAF of 0.43. [33]

SNPs within the PAX9 and MSX1 genes were examined utilizing SimpleProbe® technology. [34] Briefly, 100 to 200 base pairs of DNA, centered on each SNP sequence to be analyzed, were amplified by Polymerase Chain Reaction (PCR) using appropriate forward and reverse primers with the LightCycler® 480 Genotyping Master Mix in the LightCycler® 480 Instrument (Roche Applied Science, Indianapolis, IN). This phase involved an enzyme activation step at 95°C for 10 minutes followed by 50 rounds of cycling (95°C/15s → 60°C/15s → 72°C/15s). Following amplification, each SNP allele was identified based on its strength of bonding with a single sequence-specific nucleotide probe coupled to the green fluorescent dye fluorescein. Single nucleotide mismatches between the probe and a SNP allele reduced the strength of bonding of that heteroduplex and melted more rapidly than exact matches. As the probe melted away from each SNP allele with increasing temperature (45°C to 95°C in 0.1°C increments), a specialized linker molecule present within the probe differentially quenched the fluorescent dye

resulting in unique melting temperatures (Tms) or melting curve profiles for each distinct SNP allele. SNP alleles were identified by the LightCycler® 480 software based on analysis of genotype-specific standards (wild type, mutant, and heterozygous) in a plot of -d (Fluorescence)/dT versus temperature. All probe and primer sets utilized in this study were designed with the LightCycler® 2.0 Probe Design Software (Roche).

Statistical Analysis

The primary hyposthesis was tested in the statistical analysis is the association of each SNP genotype with the presence of CII/D2 malocclusion.

Specified Level of Significance and Calculation of Statistical Power

The meaning of statistical significance is derived from considerations of probability. The rejection of a null hypothesis when it is actually true, is a type I error. The probability of committing a type I error, the specified significance level, is α. The specified significance level (α, typically called the p value) for this study will be 0.05. It should be noted that this implies that 1 out of 20 tests will likely say there is a significant difference when actually there is not. So, it is often recommended that the specified significance level be lowered if more than one independent analysis is going to be performed on the same data. For example, one simple method is to divide 0.05 by the number of tests (SNPs) that will be run. If this method were to be employed, then the

specific significance level for the analysis of three SNPs in a study would be 0.05/3 = 0.017.

The probability of not rejecting the null hypothesis when it is in fact false, of missing a difference that is real, a type II error, referred to as β. The power of a statistical test is defined as 1-β.[35] The greater the power of the test, the less likely it is to miss a difference that is real. Although intuitively α and β appear to be directly related, they actually are measures of different probabilities, although they both affect the sample size required.

Statistical power analysis utilizes the relationships amongst four statistical parameters: significance criterion (Type I error rate, α, or p value), power, effect size (sometimes placed on a scale from 0.1 [small], to 0.3 [medium], to 0.5 [large]), and sample size. When any three of these four parameters are fixed, the remaining one can be determined. [36-38] Using G*Power software (version 3.0.10) to perform an *a priori* power analysis at an α of 0.05, and 0.017, power of 0.80, and varying effect sizes, minimum total sample sizes were estimated (See Table I). [39]

Hardy-Weinberg test

As a screen for general genotyping errors and independence of alleles for each SNP, the Pearson χ^2 statistic was used to test for departure from the expected values in Hardy-Weinberg equilibrium.[40-42] A Hardy-Weinberg in equilibrium would indicate there is not a genetic bias in the sample.

Chi-Square test

A chi-square (χ^2) test was used to evaluate the association of the SNP genotypes in the two groups (affected and unaffected). [43]

RESULTS

Hardy-Weinberg test

In Table II, the expected and observed counts for the *MSX1* rs3821949 genotype are not significantly different. The chi-square test used to compare the expected and observed counts did not yield a statistically significant difference (χ^2_1= 0.676; p = 0.41), thus, the genotype frequencies appear to be in Hardy-Weinberg equilibrium, and it is reasonable to use them in the association analysis. Note that it is appropriate to use one degree of freedom in this test instead of two because it involves two alleles in three combinations. The heterozygosity for these data is 52/183 = 0.28.

In Table III, the expected and observed counts for the *PAX9* rs1955734 genotype are not significantly different. The chi-square test used to compare the expected and observed counts did not yield a statistically significant difference (χ^2_1= 1.048; p = 0.31), thus, the genotype frequencies appear to be in Hardy-Weinberg equilibrium, and it is reasonable to use them in the association analysis. The heterozygosity for these data is 77/174 = 0.44.

In Table IV, the expected and observed counts for the *PAX9* rs8004560 genotype are not significantly different. The chi-square test used to compare the expected and observed counts did not yield a statistically significant difference (χ^2_1= 0.251; p = 0.62), thus the genotype frequencies appear to be in Hardy-Weinberg equilibrium, and it is reasonable to use them in the association analysis. The heterozygosity for these data is 81/183 = 0.44.

Association Test

Using the three *MSX1* rs3821949 genotypes (GG, GA, AA), a chi-square test yielded (χ^2_1= 2.015; p = 0.16); indicating no significant association between CII/D2 status and the *MSX1* rs3821949 genotype (Table V). Note that it is appropriate to use one degree of freedom in this test instead of two because it involves two alleles in three combinations.

Using the three *PAX9* rs1955734 genotypes (CC, CT, TT), a chi-square test yielded (χ^2_1= 1.753; p = 0.19). This indicates no significant association between the CII/D2 status and the *PAX9* rs1955734 genotype (Table VI).

Using the three *PAX9* rs8004560 genotypes (GG, GA, AA), a chi-square test yielded (χ^2_1= 3.428; p = 0.06); indicating a lack of association between CII/D2 status and the *PAX9* rs8004560 genotype at a threshold p value of 0.05 (Table VII). However, the value of 0.06 could be considered to be in a gray area and insufficient to conclude that there is no significant association.

Since the p value of the preceding test was insufficient to conclude that there is no association, a statistical analysis was performed on the *PAX9* rs8004560 genotypes of the CII/D2 affected subjects reported to have hypodontia compared to the control subjects (unaffected) who did not have a CII/D2 malocclusion. Two analyses were performed, one with those subjects considered to be affected reported to have hypodontia of any permanent teeth, and another with the subjects reported to have hypodontia of any teeth other than the third molars.

Using the three *PAX9* rs8004560 genotypes (GG, GA, AA), a chi-square test yielded (χ^2_1= 1.577; p = 0.21). This indicates no association between CII/D2 with hypodontia of any permanent teeth and the *PAX9* rs8004560 genotype (Table VIII).

Using the three *PAX9* rs8004560 genotypes (GG, GA, AA), a chi-square test yielded (χ^2_1= 3.008; p = 0.08); indicating a lack of association between CII/D2 with hypodontia for any permanent teeth other than third molars, and the *PAX9* rs8004560 genotype at a threshold p value of 0.05 (Table IX). However, the value of 0.08 could be considered to be in a gray area and insufficient to conclude that there is no significant association.

Another analysis was performed on the *PAX9* rs8004560 genotypes of the CII/D2 affected subjects, comparing the genotype frequencies of the CIID2 subjects with hypodontia, and the CII/D2 subjects reported to not have hypodontia, to see if there was a significant association of the *PAX9* rs8004560 genotype with hypodontia. Two analyses were performed, one with those affected subjects reported to have hypodontia of any permanent teeth, and another with the affected subjects reported to have hypodontia of any teeth other than the third molars.

Using the three *PAX9* rs8004560 genotypes (GG, GA, AA), a chi-square test yielded (χ^2_1= 0.282; p = 0.60). This indicates no significant association between CII/D2 subjects with hypodontia of any permanent teeth the *PAX9* rs8004560 genotype (Table X).

Using the three *PAX9* rs8004560 genotypes (GG, GA, AA), a chi-square test yielded (χ^2_1= 0.535; p = 0.46). This indicates no significant association between CII/D2 subjects with hypodontia of any permanent teeth other than third molars and the *PAX9* rs8004560 genotype (Table XI).

Post hoc Calculation of Statistical Power Achieved

Using G*Power software (version 3.0.10) to perform a post hoc estimation of achieved power for each analysis at the calculated α, sample size and different effect sizes for each analysis, the following estimates of power achieved for each analysis were determined (Table XII). [38-39] If the effect size was small, 0.1, we did not have enough power to indicated something has no association. If the effect size is 0.2 or larger then there is a reasonable level of power. In Table XII, with an effect size of 0.2-0.3, all three SNPs had a reasonable level of power.

TABLES

TABLE I

A priori sample size estimates **(**Power = 0.80)

α = 0.05			α = 0.017			α = 0.010	
Effect Size	**Total Sample**		**Effect Size**	**Total Sample**		**Effect Size**	**Total Sample**
0.1	785		0.1	1,043		0.1	1,168
0.2	197		0.2	261		0.2	292
0.3	88		0.3	116		0.3	130
0.4	50		0.4	66		0.4	73
0.5	32		0.5	42		0.5	47

TABLE II

Expected and observed counts for the *MSX1* rs3821949 genotype[a]

Genotype	**Observed**	**Expected**
GG	123	121.3
GA	52	55.4
AA	8	6.3
Total	183	

[a]Each parent gives an allele to each subject, either a G(major) or A(minor). Each subject may be homozygous major (GG), heterozygous (GA) or homozygous minor (AA).

TABLE III

Expected and observed counts for the *PAX9* rs1955734 genotype[b]

Genotype	**Observed**	**Expected**
CC	66	62.8
CT	77	83.5
TT	31	27.8
Total	174	

[b]Each parent gives an allele to each subject, either a C(major) or T(minor). Each subject may be homozygous major (CC), heterozygous (CT) or homozygous minor (TT).

TABLE IV

Expected and observed counts for the *PAX9* rs8004560 genotype[c]

Genotype	**Observed**	**Expected**
GG	77	75.4
GA	81	84.1
AA	25	23.4
Total	183	

[c]Each parent gives an allele to each subject, either a G(major) or A(minor). Each subject may be homozygous major (GG), heterozygous (GA) or homozygous minor (AA).

TABLE V

Frequencies and column percentages for affection status by *MSX1* rs3821949 genotype: Unaffected are the non-CII/D2 subjects

	GG	**GA**	**AA**	**Total**
Affected	63 (67%)	25 (27%)	6 (6%)	94
Unaffected	60 (68%)	27 (30%)	2 (02%)	89
Total	123	52	8	183

TABLE VI

Frequencies and column percentages for affection status by *PAX9* rs1955734 genotype: Unaffected are the non-CII/D2 subjects

	CC	**CT**	**TT**	**Total**
Affected	35 (37%)	39 (41%)	20 (21%)	94
Unaffected	31 (39%)	38 (48%)	11 (14%)	80
Total	66	77	31	174

TABLE VII

Frequencies and column percentages for affection status by *PAX9* rs8004560 genotype: Unaffected are the non-CII/D2 subjects

	GG	**GA**	**AA**	**Total**
Affected	39 (42%)	38 (40%)	17 (18%)	94
Unaffected	38 (43%)	43 (48%)	8 (9%)	89
Total	77	81	25	183

TABLE VIII

Frequencies and column percentages for CII/D2 with hypodontia for any permanent teeth by the *PAX9* rs8004560 genotype: Unaffected is non-CII/D2 subjects

	GG	**GA**	**AA**	**Total**
Affected	14 (45%)	12 (39%)	5 (16%)	31
Unaffected	38 (43%)	43 (48%)	8 (9%)	89
Total	52	55	13	120

TABLE IX

Frequencies and column percentages for CII/D2 with hypodontia for any permanent teeth other than the third molars by *PAX9* rs8004560 genotype: Unaffected is non-CII/D2 subjects

	GG	**GA**	**AA**	**Total**
Affected	6 (35%)	7 (41%)	4 (24%)	17
Unaffected	38 (43%)	43 (48%)	8 (9%)	89
Total	44	50	12	106

TABLE X

Frequencies and column percentages of all CII/D2 subjects for affection status of having hypodontia of any permanent teeth: Unaffected is CII/D2 with no reported hypodontia

	GG	**GA**	**AA**	**Total**
Affected	14 (45%)	12 (39%)	5 (16%)	31
Unaffected	25 (40%)	26 (41%)	12 (19%)	63
Total	39	38	17	94

TABLE XI

Frequencies and column percentages of all CII/D2 subjects for affection status of having hypodontia of any permanent teeth other than third molars: Unaffected is CII/D2 with no reported hypodontia

	GG	**GA**	**AA**	**Total**
Affected	6 (35%)	7 (41%)	4 (24%)	17
Unaffected	33 (43%)	31 (40%)	13 (17%)	77
Total	39	38	17	94

TABLE XII

Post hoc calculation of statistical power achieved

Table V, $\alpha = 0.16$, n = 174 MSX1 rs3821949			Table VI, $\alpha = 0.19$, n = 183 PAX9 rs1955734			TableVII, $\alpha = 0.06$, n=183 PAX9 rs8004560	
Effect Size	**Power**		**Effect Size**	**Power**		**Effect Size**	**Power**
0.1	0.469		0.1	0.521		0.1	0.299
0.2	0.891		0.2	0.919		0.2	0.795
0.3	0.995		0.3	0.997		0.3	0.985
0.4	0.999		0.4	0.999		0.4	0.999
0.5	1.000		0.5	1.000		0.5	0.999

DISCUSSION

The average incisor inclination of the 100 probands was 98.4°. The mandibular plane angle was 17.2° when measured to Sella-Nasion minus 7°. The ANB angle measured at 4.6°, indicating Class II skeletal. These measurements were similar to the findings in other CII/D2 studies.[4, 7] Sixty-one of the 100 CII/D2 probands(61%) were female (Appendix 1). Basdra et al. also found a female predilection in his study of CII/D2.[13] Whereas Peck et al. found a male predilection, as did Maj and Lucchese.[8, 44]

Thirty-one of the 100 probands were missing at least one tooth. Twenty-two of the thirty-one probands(67%) with hypodontia were female. Thirteen of the seventeen (76%) probands missing at least one tooth, not a third molar, were females (Appendix 1). This supports previous findings that the CII/D2 population is more likely to have developmental dental anomalies, such as missing teeth, when compared to the general population.[8,13-15,22] This female predilection to dental agenesis was also noted by other researchers.[13, 45-46]

Of the three SNPs genotyped only the PAX9 rs8004560 had a borderline suggestion of being linked to the CII/D2 phenotype when all of the CII/D2 subjects were included (Table VII), and when the few CII/D2 subjects with hypodontia not involving the third molars were included (Table IX). The analysis of the CII/D2 subjects with hypodontia of any permanent teeth, including at least one third molar, did not indicate a reasonable association (Table VIII), suggesting that the hypodontia of teeth other than the third molars are a part of the CII/D2 spectrum, while hypodontia of one or more third molars may not be a specific part of the CII/D2 spectrum. The findings support what Basdra et al. reported that a tooth to tooth comparison reveals a strong association of

CII/D2 and dental agenesis. [13] Basdra et al. also stated, when excluding third molars, all other teeth were missing at least three times more often in CII/D2, when compared to a general population. [13] In this study, seventeen of ninety-four CII/D2 subjects (18%) had missing teeth, third molars excluded.

Further analysis of the CII/D2 subjects with hypodontia versus those CII/D2 subjects without hypodontia showed no association with the PAX9 rs8004560 SNP (Table X and IX), suggesting that the genotype differences for CII/D2 include the associated hypodontia. It is important to note that these comparisons of subgroups had very small numbers.

Future studies could investigate different SNPs located on the MSX1 and PAX9 genes to establish an association between the CII/D2 phenotype and two genes associated with hypodontia. When possible it is beneficial to identify non-synonymous SNPs that are not in linkage disequilibrium for each gene with a minor allele frequency as close to 0.5. A non-synonymous SNP changes the amino acid, which is more likely to change the protein structure and have an effect on phenotype. On the other hand, a synonymous SNP does not change the amino acid, but can effect gene expression.

The Axis Inhibition Protein 2 (AXIN2) gene may be associated with CII/D2 and could be explored. Dental agenesis associated with AXIN2 mutations incorporate a wide range of teeth, including the lateral incisors. [31, 47-49] The AXIN2 gene acts as a negative regulator of the Wnt signaling pathway, which is important in tooth development. [31]

CONCLUSIONS

- There is no significant association of CII/D2 malocclusion and the SNPs MSX1 rs3821949 and PAX9 rs1955734.
- There is a suggestion that there is an association of the SNP PAX9 rs8004560 and CII/D2 malocclusion.
- There is a suggestion that there is an association of SNP PAX9 rs8004560 and CII/D2 subjects with hypodontia of any tooth except third molars.

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APPENDIX 1

Lateral Cephalometric measurements of CII/D2 probands

Number	SNA	SNB	ANB	1/-HP	MP-HP
101	79	75	4	91	21
104	84	72	12	78	24
105					
107	82	74	8	100	23
110	84	78	6	94	25
113	80	75	5	96	19
114					
116	84	82	2	105	12
118					
121	79	75	4	97	15
124	80	75	5	101	15
126	85	80	5	103	11
130	83	76	7	90	20
132					
137	81	75	6	93	22
138					
143	85	80	5	92	10
144					
148	82	76	6	89	15
151	82	79	3	99	15
154	85	81	4	104	9
158	79	73	6	89	24
162	82	76	6	100	20
165	77	75	2	100	18
166					
168	80	74	6	92	20
175	82	75	7	82	25
179	80	75	5	87	15
181	80	78	2	107	19
183	85	78	7	105	17
185	82	79	3	106	20
187	82	74	8	95	18

Continued

188	81	80	1	109	6
189	86	80	6	89	23
191	83	80	3	105	18
192	81	74	7	89	18
193	88	80	8	105	15
196	89	83	6	92	15
198	81	76	5	99	19
200	81	77	4	101	19
203	83	77	6	106	20
204	81	79	2	103	21
205	80	76	4	99	13
206	82	81	1	102	20
208	85	82	3	104	15
209	86	83	3	97	21
210	82	75	7	92	18
211	83	78	5	99	23
212	85	82	3	107	18
213	83	82	1	101	17
214	83	77	6	105	19
215	86	81	5	93	13
217	82	80	2	107	16
218	84	80	4	98	15
219	82	78	4	104	18
220	84	80	4	95	18
222	85	81	4	103	13
223	91	82	9	101	19
225	86	82	4	104	12
226	81	74	6	95	18
227	82	79	3	103	19
229	86	80	6	97	16
230	83	77	6	107	18
231	81	76	5	94	13
232	89	85	4	109	15
234	81	75	6	94	24
235	82	76	6	100	18
236	86	81	5	99	11
237	78	72	6	99	25
238	80	76	4	103	23

Continued

239	82	79	3	110	19
240	75	71	4	77	20
241	87	80	7	102	23
242	81	78	3	102	17
243	81	80	1	95	5
245	82	77	5	102	13
246	78	75	3	102	21
247	80	76	4	102	17
248	82	76	6	105	21
249	78	72	6	105	22
250	89	84	5	97	18
251	84	80	4	92	22
252	84	80	4	107	15
253	82	79	3	101	5
255	75	71	4	107	25
256	79	76	3	98	24
257	78	72	6	105	24
259	89	84	5	107	11
260	86	79	7	101	24
261	84	80	4	99	10
262	83	81	2	109	12
263	80	79	1	102	14
264	80	75	5	99	15
265	85	79	6	99	18
266	90	85	5	110	3
267	86	80	6	103	17
269	85	78	7	96	21
273	82	80	2	110	15
276	84	80	4	105	14
285	82	78	4	97	15
AVERAGE	81.7	77.13	4.64	98.4	17.17

APPENDIX 2

CIID2 Genotypes

Caucasian

	Number	M/F	Ethnicity	Age	DNA	Ceph	Hypodontia	*MSX1* rs3821949	*PAX9* rs1955734	*PAX9* rs8004560
1	101	F	Caucasian	19	X	X	20	GG	CC	GG
2	104	F	Caucasian	21	X	X	1,17,32	GG	CT	GA
3	105	F	Caucasian	57	X		1,16,17,32	GG	CC	GG
4	107	M	Caucasian	15	X	X		AA	CC	GG
5	110	F	Caucasian	59	X	X		GG	CC	GG
6	113	F	Caucasian	10	X	X		GA	CT	GG
7	114	F	Caucasian	40	X			GG	CC	GG
8	116	F	Caucasian	19	X	X	13,16,17,20,2	GG	CT	GA
9	118	F	Caucasian	49	X		4,13,16,29	GG	CC	GG
10	121	M	Caucasian	18	X	X	16,17,32	AA	CT	GA
11	126	F	Caucasian	15	X	X	1	GA	CC	GG
12	130	F	Caucasian	16	X	X		AA	CT	GA
13	132	F	Caucasian	43	X			GA	CT	GA
14	137	F	Caucasian	25	X	X		GG	CT	GA
15	138	M	Caucasian	47	X	X		GG	CC	GG
16	143	M	Caucasian	29	X	X		GG	TT	GA
17	144	F	Caucasian	52	X			GG	TT	AA
18	148	F	Caucasian	31	X	X		GG	CT	GA
19	151	F	Caucasian	12	X	X		GA	TT	GA
20	154	F	Caucasian	16	X	X		GG	CC	GG
21	158	F	Caucasian	14	X	X		GG	CC	GG
22	162	F	Caucasian	14	X	X		GG	CT	GA
23	165	F	Caucasian	41	X	X		GG	CC	GG
24	166	F	Caucasian	17	X		7	GG	CC	GG
25	168	F	Caucasian	11	X	X		GG	CT	GA
26	175	M	Caucasian	11	X	X		GG	TT	AA
27	179	F	Caucasian	19	X	X		GG	TT	AA
28	181	F	Caucasian	12	X	X		GG	CT	GA
29	183	F	Caucasian	12	X	X		GA	CC	GG
30	185	M	Caucasian	14	X	X	17, 32	GG	CC	GG
31	187	F	Caucasian	13	X	X		GG	TT	AA
32	188	F	Caucasian	13	X	X		GA	CC	GG
33	189	M	Caucasian	14	X	X	16	GG	CC	GG
34	191	F	Caucasian	10	X	X		GG	CT	GG
35	192	F	Caucasian	12	X	X	17,32	GG	CC	GG
36	196	F	Caucasian	39	X	X	13, 20, 29	GA	CT	GA
37	198	F	Caucasian	12	X	X		GG	CC	GG
38	200	F	Caucasian	14	X	X		GG	TT	AA
39	204	M	Caucasian	12	X	X	17,32	GA	CT	GA
40	205	F	Caucasian	14	X	X		AA	CT	GA

Continued

41	206	M	Caucasian	19	X	X		GA	CT	GA
42	208	F	Caucasian	14	X	X		GA	CC	GG
43	209	M	Caucasian	16	X	X		GG	CT	GA
44	210	M	Caucasian	14	X	X		GG	CC	GG
45	211	F	Caucasian	14	X	X		GG	CT	GA
46	212	M	Caucasian	19	X	X	1,16,17	GG	CT	GA
47	213	F	Caucasian	19	X	X	29,32	GA	TT	AA
48	214	F	Caucasian	13	X	X	4	GG	CT	GA
49	215	F	Caucasian	15	X	X	1,16,17,32	GA	CT	GA
50	217	M	Caucasian	15	X	X		GG	CT	GA
51	218	M	Caucasian	13	X	X		GA	CT	GA
52	219	M	Caucasian	17	X	X		GA	CT	GA
53	220	F	Caucasian	15	X	X		GG	CT	GA
53	222	M	Caucasian	15	X	X		GG	CT	GA
54	223	F	Caucasian	14	X	X		GA	TT	AA
55	225	M	Caucasian	16	X	X		GA	TT	GA
56	226	F	Caucasian	15	X	X	17	GG	CC	GG
57	227	F	Caucasian	15	X	X		GG	TT	AA
58	229	F	Caucasian	16	X	X	16	GG	TT	AA
59	230	F	Caucasian	13	X	X		GG	CC	GG
60	231	M	Caucasian	20	X	X		GG	TT	AA
61	232	M	Caucasian	11	X	X		GG	CT	GA
62	234	F	Caucasian	17	X	X	10,16	GG	CC	GG
63	235	F	Caucasian	11	X	X		GA	TT	AA
64	236	F	Caucasian	13	X	X	17,32	GA	CC	GG
65	238	F	Caucasian	13	X	X	7,10	GG	CT	GA
66	239	M	Caucasian	16	X	X	17,20,29,32	GG	TT	AA
67	240	F	Caucasian	11	X	X		GG	CC	GG
68	241	F	Caucasian	10	X	X		GG	TT	AA
69	242	M	Caucasian	16	X	X		GG	CC	GG
70	243	M	Caucasian	13	X	X		GG	CT	GG
71	245	M	Caucasian	11	X	X		GA	CT	GA
72	246	M	Caucasian	19	X	X		AA	CC	GG
73	247	M	Caucasian	17	X	X		GG	CT	GG
74	248	F	Caucasian	13	X	X		GG	CT	GA
75	249	M	Caucasian	14	X	X		GG	CC	GG
76	250	F	Caucasian	22	X	X	17,20,29,32	GG	CC	GG
77	251	F	Caucasian	17	X	X	7,10,20,23	GG	TT	AA
78	252	F	Caucasian	14	X	X	17,32	GA	CC	GG
79	255	M	Caucasian	15	X	X	29	GG	CT	GA

Continued

80	256	M	Caucasian	13	X	X	1,5,16,17,29,32	GA	CT	GA
81	259	F	Caucasian	27	X	X		GG	CT	GA
82	260	F	Caucasian	18	X	X	20,29	GG	TT	AA
83	261	M	Caucasian	14	X	X	20	GG	CC	GG
84	262	F	Caucasian	10	X	X		GA	CT	GA
85	263	F	Caucasian	17	X	X	10	GA	CT	GA
86	264	M	Caucasian	13	X	X		GG	CC	GG
87	265	F	Caucasian	13	X	X		GA	CT	GA
88	266	M	Caucasian	21	X	X		AA	TT	AA
89	267	F	Caucasian	12	X	X		GG	CC	GG
90	269	F	Caucasian	15	X	X		GG	TT	AA
91	273	F	Caucasian	14	X	X		GG	CC	GG
92	276	M	Caucasian	15	X	X		GA	CC	GG
93	285	M	Caucasian	17	X	X		GG	CT	GA

CII/D2 'Other Ethnicities

1	124	F	Asian	24	X	X		GA	CC	GG
2	193	F	Hispanic	25	X	X		GG	CT	GA
3	201	F	African Am		Withdrew from study					
4	203	M	Hispanic	14	X	X		GA	TT	AA
5	237	F	Asian	25	X	X		GG	CC	GG
6	253	F	African Am	38	X	X	7,17,32	GA	CT	GA
7	257	F	African Am	19	X	X		AA	TT	GA

		COUNT	COUNT	COUNT	
Total # Females		**GG=**	**CC=**	**GG=**	
61		**63**	**35**	**39**	
Total # Males		**GA=**	**CT=**	**GA=**	
33		**25**	**39**	**38**	
	Average Age	**AA=**	**TT=**	**AA=**	
	All	**6**	**20**	**17**	
	18.40	**Total=**	**Total=**	**Total=**	
		94	**94**	**94**	
		Female #'s	**Female #'s**	**Female #'s**	
		GG=	**CC=**	**GG=**	
		42	**24**	**26**	
		GA=	**CT=**	**GA=**	
		17	**23**	**22**	
		AA=	**TT=**	**AA=**	
		2	**14**	**13**	**2**
		Total=	**Total=**	**Total=**	**Total=**
		61	**61**	**61**	**2**
		Male #'s	**Male #'s**	**Male #'s**	**Male #'s**
		GG=	**CC=**	**GG=**	**GG=**
		21	**11**	**13**	**1**
		GA=	**CT=**	**GA=**	**GT=**
		8	**16**	**16**	**3**
		AA=	**TT=**	**AA=**	**TT=**
		4	**6**	**4**	**2**
		Total=	**Total=**	**Total=**	**Total=**
		33	**33**	**33**	**6**
		Hypodontia count	**Hypodontia count**	**Hypodontia count**	**Hypodontia count**
		GG=	**CC=**	**GG=**	**GG=**
		21,13	**14,6**	**14,6**	**1,0**
		GA=	**CT=**	**GA=**	**GT=**
		9,4	**12,7**	**12,7**	**3,2**
		AA=	**TT=**	**AA=**	**TT=**
		1,0	**5,4**	**5,4**	**2,0**
		Total=	**Total=**	**Total=**	**Total=**
		31,17	**31,17**	**31,17**	**6,2**

First number = subjects with hypodontia of any teeth

Second number = subjects with hypodontia of any teeth teeth excluding third molar

Non CIID2 Genotypes

	All Caucasian Subjects					*MSX1*	*PAX9*	*PAX9*
	Number	**M/F**	**Race**	**Age**	**DNA**	**rs3821949**	**rs1955734**	**rs8004560**
1	102	F	Caucasian	54	X	GG	CC	GG
2	103	F	Caucasian	30	X	GG	CC	GG
3	106	F	Caucasian	19	X	GG	CC	GG
4	109	F	Caucasian		X	GG	CT	GA
5	115	M	Caucasian	41	X	GA	CT	GG
6	120	F	Caucasian	16	X	GA	CT	GA
7	123	F	Caucasian	53	X	GA	CT	GA
8	128	F	Caucasian	19	X	GA	CC	GG
9	129	F	Caucasian	17	X	GG	CC	GG
10	133	M	Caucasian	47	X	GA	CC	GG
11	135	M	Caucasian	19	X	GA	CT	GA
12	139	F	Caucasian	46	X	GG	CT	GA
13	140	F	Caucasian	19	X	GG	CC	GG
14	141	M	Caucasian	18	X	GG	CC	GG
15	142	F	Caucasian	24	X	GG	CC	GG
16	145	M	Caucasian	54	X	GG	TT	GA
17	146	M	Caucasian	27	X	GG	TT	AA
18	149	F	Caucasian	12	X	GG	CT	GA
19	150	M	Caucasian	10	X	GG	CT	GA
20	152	F	Caucasian	41	X	GA	TT	AA
21	155	M	Caucasian	45	X	GG	CC	GG
22	160	M	Caucasian	11	X	GG	TT	AA
23	161	M	Caucasian	9	X	GG	CT	GA
24	163	F	Caucasian	45	X	GG	TT	AA
25	164	F	Caucasian	16	X	GG	CT	GA
26	167	F	Caucasian	16	X	GG	CC	GG
27	169	F	Caucasian	38	X	GG	TT	AA
28	170	F	Caucasian	12	X	GG	CT	GA
29	171	M	Caucasian	41	X	GG	CT	GA
30	172	M	Caucasian	54	X	GA	CT	GA
31	173	F	Caucasian	29	X	GG	CT	GA
32	174	M	Caucasian	33	X	GG	CT	GA
33	176	M	Caucasian	33	X	GG	CT	GA
34	177	F	Caucasian	26	X	GA	CT	GA
35	178	M	Caucasian	29	X	GG	CT	GA
36	180	M	Caucasian	18	X	GG	CC	GG
37	182	M	Caucasian	12	X	GA	CC	GG
38	184	M	Caucasian	29	X	GG	CT	GA
39	186	F	Caucasian	15	X	GA	TT	AA
40	190	M	Caucasian	12	X	AA	CT	GA

Continued

41	194	M	Caucasian	18	X	GG	CT	GA
42	195	F	Caucasian	14	X	GA	TT	AA
43	197	M	Caucasian	15	X	GG	CT	GA
44	199	M	Caucasian	15	X	GA	CC	GG
45	207	M	Caucasian	18	X	GG	CT	GA
46	216	F	Caucasian	16	X	GA	CC	GG
47	224	F	Caucasian	14	X	GG	TT	GA
48	228	M	Caucasian	29	X	GG	CC	GG
49	244	F	Caucasian	12	X	GA	CC	GG
50	254	M	Caucasian	15	X	GG	CT	GA
51	258	M	Caucasian	31	X	GA	CC	GG
52	268	M	Caucasian	14	X	GG	CT	GA
53	270	M	Caucasian	16	X	GG	CT	GA
54	271	F	Caucasian	14	X	GG	CT	GA
55	272	F	Caucasian	17	X	GG	CC	GG
56	274	F	Caucasian	11	X	GA	CT	GA
57	275	F	Caucasian	15	X	GA	CC	GG
58	277	F	Caucasian	7	X	GG	CT	GA
59	278	F	Caucasian	18	X	GG	CC	GG
60	280	F	Caucasian	18	X	GG	CC	GG
61	281	M	Caucasian	16	X	GA	CC	GG
62	282	F	Caucasian	14	X	GA	CC	GG
63	283	F	Caucasian	24	X	GA	CT	GA
64	287	F	Caucasian	30	X	GG	CT	GA
65	289	F	Caucasian	17	X	GG	TT	AA
66	290	F	Caucasian	16	X	AA	CC	GG
67	292	M	Caucasian	16	X	GG	CT	GA
68	293	F	Caucasian	24	X	GG	CC	GG
69	294	M	Caucasian	16	X	GG	CT	GA
70	296	F	Caucasian	17	X	GG	CC	GG
71	297	M	Caucasian	26	X	GG	CT	GA
72	299	F	Caucasian	17	X	GG	CC	GG
73	300	F	Caucasian	13	X	GG	CT	GA
74	301	M	Caucasian	32	X	GA	CT	GA
75	302	M	Caucasian	29	X	GA	CC	GG
76	303	F	Caucasian	18	X	GG	CC	GG
77	305	M	Caucasian	15	X	GA	TT	GA
78	306	F	Caucasian	25	X	GG	CC	GG
79	308	F	Caucasian	17	X	GG	CT	GA
80	309	F	Caucasian	12	X	GG	CT	GA
81	310	M	Caucasian	11	X	GG		GG
82	311	F	Caucasian	13	X	GG		GG

Continued

83	312	M	Caucasian	25	X	GG	GA
84	313	F	Caucasian	29	X	GA	GG
85	314	M	Caucasian	26	X	GA	GG
86	316	M	Caucasian	17	X	GA	GG
87	317	F	Caucasian	30	X	GG	GG
88	318	F	Caucasian	13	X	GG	GA
89	319	M	Caucasian	21	X	GG	GA

Non CII/D2'Other Ethnicities

1	233	F	African Am	12	X	GA	TT	GA
2	279	M	African Am	21	X	GG	CT	GA
3	284	M	Asian	37	X	GG	CC	GG
4	286	F	African Am	18	X	GA	TT	AA
5	288	F	African Am	23	X	AA	CT	GA
6	291	F	African Am	39	X	GA	TT	AA
7	295	M	African Am	14	X	GA	TT	AA
8	298	F	African Am	15	X	GA	CT	GG
9	304	M	Hispanic	16	X	GA	TT	AA
10	307	M	Asian	27	X	GG	CT	GA
11	315	F	Hispanic	27	X	GA		AA

Total		**COUNT**	**COUNT**	**COUNT**
Female #'s		**GG=**	**CC=**	**GG=**
49		**60**	**31**	**38**
Male #'s		**GA=**	**CT=**	**GA=**
40		**27**	**38**	**43**
	Average	**AA=**	**TT=**	**AA=**
TOTAL=	**Age All =**	**2**	**11**	**8**
89	**22.67**	**Total=**	**Total=**	**Total=**
		89	**80**	**89**

Female #'s	**Female #'s**	**Female #'s**
GG=	**CC=**	**GG=**
34	**21**	**24**
GA=	**CT=**	**GA=**
14	**17**	**19**
AA=	**TT=**	**AA=**
1	**7**	**6**
Total=	**Total=**	**Total=**
49	**45**	**49**
1	**1**	**2**
Male #'s	**Male #'s**	**Male #'s**
GG=	**CC=**	**GG=**
26	**10**	**14**
GA=	**CT=**	**GA=**
13	**21**	**24**
AA=	**TT=**	**AA=**
1	**4**	**2**
Total=	**Total=**	**Total=**
40	**35**	**40**

ABSTRACT

Association Analysis for Class II Division 2 and Two Genes Associated with Hypodontia (PAX9, MSX1)

Purpose of the Study: Determine if there is an association of the CII/D2 malocclusion and genes linked to hypodontia, namely PAX9 and MSX1.

Methods and Materials: One hundred probands with CII/D2 and one hundred non-CII/D2 with no hypodontia were enrolled in this study. Clinical exam, photographs, models, radiographs, and saliva were gathered. DNA was isolated from the saliva and sent for genetic analysis. Single Nucleotide Polymorphisms (SNPs) from the PAX9 and MSX1 genes were analyzed using the LightCycler® 480 to verify the presence of each with the CII/D2 malocclusion. A Hardy-Weinberg test was used to screen for genotyping errors, then a chi-square test was used to evaluate the association of the SNP genotypes. A p-value of 0.05 was considered significant.

Results: The Hardy-Weinberg test showed no significant differences between observed and expected counts thus we used them for association analysis. Chi-square analysis indicated no significant association between CII/D2 and the MSX1 rs3821949 and the PAX9 1955734 genotypes. Although a p-value of 0.06 for the PAX9 rs8004560 suggested association, it was considered a grey area and insufficient to conclude that there was significant association. Since the SNP PAX9 rs8004560 was insufficient, additional statistical analysis was also performed on the PAX9 rs8004560 genotype of the CII/D2 affected subjects reported to have hypodontia of any tooth including third molars and excluding third molars. A chi-square test yielded a p-value of 0.08 on the analysis of CII/D2 with hypodontia for any permanent tooth except third molars, which suggested association, but insufficient to conclude a significant association. All other analyses indicated a lack of association of the PAX9 rs8004560 SNP.

Conclusions: There is no significant association of CII/D2 and the SNPs MSX1 rs3821949 and PAX9 rs1955734. There is a suggestion that there is an association of the SNP PAX9 rs8004560 and CII/D2. There is a suggestion that there is an association of SNP PAX9 rs8004560 and CII/D2 subjects with hypodontia of any tooth except third molars.