GENETICS OF SHORT ROOT ANOMALIES

by

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DEDICATION

To my mother **Wendy Thian**, father **Victor Choong**, older brother **Clement Choong**, and my younger brother **Clarence Choong**. I would be nowhere without your love and support from day one, and am so proud to be your daughter and sister.

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ABSTRACT

GENETICS OF SHORT ROOT ANOMALIES

Purpose: The purpose of this study is to define clinical features and identify specific gene mutations that are associated with short root anomalies (SRA) among five affected families. This study aims to determine the potential association between clinical features and genetic mutations in order to provide scientific evidence for oral health providers to enhance diagnosis and management of patients with short root anomalies.

Methods: Participants were recruited through the University of Pittsburgh and the University of Michigan. Study explanation, pedigree construction, subject enrollment, clinical examinations and collection of blood or non-stimulated saliva samples were completed under the proper consenting procedure as approved by Institutional Review Board. Each sample was coded and a small aliquot was used for genomic DNA isolation. Samples from parents and proband of each family were selected for whole-exome sequencing. Sequencing results were analyzed according to establish algorism. DNA samples from all other family members were used for confirmation analyses. Prioritized DNA sequence variations and their segregation with short root anomalies within each family were assessed. Phenotypic comparisons of the affected subjects within the five study families were performed to determine whether these families can be considered as having similar if not identical clinical presentation of SRA. Phenotypic features analyzed include root length, root width, taurodontism, missing teeth, whether cases were localized or generalized, and other pertinent dental anomalies.

Results: Genomic analyses identified 22 genes with rare and potentially damaging variants in more than one affected individual of the five study families. Four out of five probands have variants in *THAP11* gene, three out of five probands have variants in *PODXL*, *NIPA1* and *VEZF1* and two out of five probands have variants in additional 18 genes. The role of these gene variants in tooth and root development is not immediately clear. There were no variants involving the same gene present in all five or four families. Given WES data and literature evidence, there were no logical variants or candidate genes that can be targeted for segregation analysis. Phenotypic features documented in the affected study participants include short roots of various types of teeth, wider than normal root widths, taurodontism in both maxillary and mandibular arches, microdontia, ectopic eruption, and pulpal obliteration. Localized and generalized SRA cases showed differences in phenotype features.

Conclusion: Phenotypic features of SRA vary from patient to patient. Correlating genotypes and phenotypes of those with short root anomalies may facilitate clinical diagnosis. By determining the genetic etiology of SRA, we may better understand the disease mechanism and be able to make sound decisions on whether applying forces and manipulating the teeth might lead to continued changes in root length and structure. Exploring the molecular mechanisms of SRA allows an understanding of whether the condition is largely a developmental anomaly or a progressive, long-term process. This foundational knowledge is relevant to many facets of dentistry where root to crown ratio must be carefully considered in treatment plan development.

Chapter I

Introduction

1. Study Aim

The purpose of this study is to define clinical features and identify specific gene mutations that are associated with short root anomalies (SRA) among five affected families. This study aims to determine the potential association between clinical features and genetic mutations in order to provide scientific evidence for oral health providers to enhance diagnosis and management of patients with short root anomalies.

2. Definition and Diagnosis

Short root anomalies were first described in 1972 by Lind, who observed that the roots of maxillary central incisors were, in few instances, so short that they must be anomalous. The roots were described to have a characteristic “plump” and “onion shape”, and were found to always affect the central incisors bilaterally. While the abnormally short and plump roots of the maxillary central incisors were observed in some patients, the teeth and surrounding tissues appeared normal both clinically and radiographically. In the same study, the “relative root length” was described as a diagnostic reference and continues to be used as a reference in subsequent studies (Lind, 1972). The relative root length is described as the ratio between root length (R) and crown length (C), calculated by the equation: r-m/i-m= R/C. The apex marker represents (r) while the midpoint of the incisal edge represents (i). (m) represents the midpoint line between x and y, which mark the outer contours on either side of where the root and crown meet. In this pioneer study, the mean relative root length of 1.6 was noted in unaffected individuals while a mean relative root length of 1.1 was noted in affected individuals. Subsequent studies support the average relative root length of the maxillary central incisor to be 1.6, with short root teeth R/C presenting as less than or equal to 1.1

(Lind, 1972; Edwards et al.,1990; Jakobsson et al.,1973)**.** While Lind's method has been used extensively in studies assessing relative root length, recent studies have also modified or extended the criteria for SRA diagnosis. Such modifications include measurement of alveolar bone level or cementoenamel junction (CEJ) as a reference point to measure anatomical or clinical R/C ratios, use of pre-treatment CBCTs, and "normal" morphology determined as maxillary central incisors with no significant dilacerations or alterations in root shape (Wang et al., 2019; Cutrera et al., 2019). The diagnosis of short root anomalies is made when the fully developed roots are the same length or shorter than the crown and is seen in at least one pair of permanent teeth bilaterally (Edwards et al., 1990; Jakobsson et al., 1973). The diagnosis is also made when other causes of root shortening including resorption from orthodontic treatment, trauma, or developmental disturbance can be ruled out (Lind, 1972; Apajalahti et al., 2002).

3. Teeth affected

Maxillary central incisors are markedly involved in patients diagnosed with short root anomalies though other teeth have also been implicated in literature (Lind, 1972; Apajalahti et al., 1999). Studies have shown a variation in the order of most prominent involvement. However, premolars are often described as the next most diagnosed teeth with short roots (Lind, 1972; Apajalahti et al., 2002; Apajalahti et al., 1999; Ando et al., 1967). In contrast, mandibular central incisors, molars and canines are the least commonly involved (Lind, 1972; Puranik et al., 2015). Affected maxillary central incisors and premolars have been reported to have a distinct, similar radiographic appearance: maxillary central incisors appear plump with rounded apices radiographically and premolars present with blunted apices that

resemble root resorption (Puranik et al., 2015). A case report has been published on a non syndromic SRA case of a 14 year old boy who had signs of generalized short root anomalies in both his permanent and primary dentition. Although the primary teeth were affected, it was especially apparent in the maxillary first primary molars bilaterally and maxillary and mandibular second primary molars. Also reported in the same patient was taurodontism in the mandibular canines and impaction of the permanent maxillary canines bilaterally. This report is of significance as non syndromic occurrences of generalized SRA cases are extremely rare (Venkataraghavan et al., 2014).

4. Prevalence (gender and ethnicity)

Short root anomalies have often been reported to have a 2.4-2.7% prevalence in Caucasians, and have been reported to be as high as 10% in Mongolian and Japanese populations (Lind, 1972; Edwards et al.,1990; Jakobsson et al., 1973; Apajalahti et al., 1999; Ando et al., 1967). There is recent evidence that a higher prevalence of short root anomalies may also exist among Hispanic populations. A recent study that observed 27 patients in a Mexican cohort reported that short root anomalies occur frequently in the Latino population with a strong predilection for anterior teeth**.** A different study that recruited patients with short root anomalies to assess for a possible increased risk for external apical root resorption in orthodontic patients was inclusive of a largely Hispanic population (Cutrera et al., 2019; Puranik et al., 2015). A study that compared root lengths and crown heights of African American, Caucasian, and Hispanic populations reported the Hispanic population to have significantly lower ratios of root lengths and crown heights compared to the other two groups, highlighting that ethnicity may play an important role in establishing specific reference values for diagnosis. In this study that

observed 333 patients consisting of 109 Caucasians, 112 African Americans and 112 Hispanics, the mean R/C ratios varied from 1.8-2.21 for maxillary teeth and 1.83-2.49 for mandibular teeth, with Hispanics representing the significantly lower R/C ratios for most teeth compared to the other two groups. It was also reported that significant differences in R/C ratios existed between African Americans and Caucasians in the upper lateral incisors, lower central incisors, and lower first premolars. The findings of this study suggest that ethnicity may have a stronger influence on the tooth morphology in Hispanic patients presumably due to variations in genes regulating normal root development (Wang et al., 2019).

Short root anomalies appear to have a predilection for females, as studies have reported that the anomaly is three times more common in females than males (Lind, 1972; Jakobsson et al., 1973). It has also been reported that 2.3% of the population have extremely long roots, and boys are more likely to have this presentation with a boy: girl ratio of 5:1 (Jakobsson et al., 1973).

A study analyzing panoramic radiographs of a healthy Finnish population reported that males tend to have higher R/C ratios compared to females, with mean R/C ratios for males ranging from 1.86-2.44 and values ranging from 1.78-2.46 in females. In maxillary and mandibular arches of both boys and girls, highest R/C mean values were found in the second premolars and first premolars, and the lowest R/C mean values reported in the maxillary central incisors, mandibular central incisors, and first molars. The reported mean R/C ratios were significantly larger in males for the permanent maxillary and mandibular central incisors, permanent maxillary lateral incisors, and first and second molars. The results highlight that reference values for R/C ratio assessment should be made separately for males and females to aid in diagnosis (Holtta et al., 2004).

5. Association with other dental anomalies

There is evidence in literature that suggests short root anomalies may be implicated with the presence of other dental anomalies. One study reported a 46% and 33% association with tooth agenesis and ectopic canines, respectively, along with supernumerary teeth and the presence of mesiodens when observing eight SRA affected families. Another study reported that hypodontia, taurodontism, peg shaped, invaginations, and ectopic position frequently occurred in affected teeth other than maxillary central incisors and maxillary first premolars when studying a sample of nearly 2000 panoramic radiographs of healthy young adults (Apajalahti et al., 2002; Apajalahti et al., 1999).

Case reports have made an association with microdontia and SRA presence in the primary dentition of affected individuals (Venkataraghavan et al., 2014). Those with short root anomalies have been reported to be at higher risk for root resorption in the maxillary front teeth, with a study reporting the frequency of root resorption in individuals with a mean R/C of 1.1 as high as 41.1%. In contrast, it has been reported in the same study that individuals with a R/C ratio *higher* than 1.1 has a much lower frequency of resorption at 30%. (Lind, 1972). An additional study reported that 48% of the individuals in the short root group had diagnosed root resorption on one or more maxillary incisor teeth, while no root resorption was found in the individuals belonging to the long root group. This study made comparisons of children either with exceptionally short (short root with R/C < or equal to 1.1) or exceptionally long roots (R/C> or equal to 2.2), with a prevalence of 2.4% and 2.3% respectively. (Holtta et al., 2004).

6. Malocclusion

Several facets of malocclusion have a reported association with short root anomalies. A higher frequency of anterior cross bite has been reported in patients with short root anomalies (Lind, 1972). A higher tendency for crowding in a group of patients with short root anomalies when compared to a group of Caucasian patients with diagnosed long roots has also been reported. In this study, crowding was diagnosed in 22/25 patients with short roots, while only 4/24 subjects were diagnosed with crowding in the long root group (Jakobsson et al., 1973). A pioneer study in 1967 evaluating 300 elementary school children in Japan inferred that biting load naturally tends to be concentrated on the upper central incisors that have had their root formation completed prematurely, and that the shift in biting forces may be responsible for a localized etiology of root shortening rather than a more generalized etiology. A study evaluating 103 pairs of siblings reported that genetic susceptibility of external apical root resorption is unlikely to be related to an individuals' malocclusion (Ando et al., 1967).

7. Association with systemic disturbances

Shortness of the roots have been associated with systemic disturbances including Stevens Johnson syndrome, Down syndrome, severe short limbed dwarfism, and scleroderma, among others (Bajaj et al., 2012; Shaw et al.,1995). In a case report of a patient with severe short limbed dwarfism, in addition to generalized short roots, the dentition also had observed agenesis, conical short roots, and obliterated pulp chambers. Little bone support was noted on radiographs although clinical crowns appeared normal on all affected teeth (Shaw et al., 1995).

A case report on a fifteen- year-old Stevens Johnson syndrome patient reported generalized short roots in her dentition besides the lower first molars and lower central incisors due to a cessation in root development post- acute attack of SJS at age eight. This study is of special note because the generalized short roots observed had marked differences in root lengths (Bajaj et al., 2012). It is also well known that treatment regimens for some childhood cancers have a part in affecting crown and root development. While chemotherapy has been associated with a higher prevalence of enamel defects, including opacities and hypomineralization, radiotherapy has been implicated in producing the most severe defects affecting both crown and root morphology. Such disturbances in root morphology have been reported to include both foreshortening and blunting of the roots. In a study comparing groups receiving either chemotherapy alone, chemotherapy in combination with cranial radiation, or radiotherapy with total body irradiation (TBI) and bone marrow transplant (BMT), it was a statistically significant finding that root surface area was the least in the group receiving TBI/BMT. Further, there was no difference reported in the root surface areas according to the patients' age of cancer diagnosis, providing evidence that the effect of radiotherapy and cancer treatment regimens can affect various age groups at different points of tooth development (Duggal, 2003). Although there is evidence in literature of an association of short root anomalies with systemic disorders and treatment, there are also reports that support isolated diagnoses of short roots. One study reported a 1.3% prevalence of SRA in a population of 2000 healthy university students with no known medical history or concerns, while 10% (30/300) of healthy Japanese school children were diagnosed with short roots in another study (Apajalahti et al., 2002; Ando et al., 1967).

8. Differential diagnosis: trauma, EARR, orthodontic treatment sequelae

When diagnosing short root anomalies, common differential diagnoses include trauma related etiology and external apical root resorption (EARR) secondary to orthodontic treatment. Since the upper front teeth are prone to trauma, short root anomalies in this region can be misdiagnosed as root resorption. Root malformations have been reported as possible sequelae of trauma, and can include root duplication, dilacerations, and partial or complete arrest of root formation (Neto et al., 2013).

External apical root resorption is described as a decrease in root length once full root development has been completed. A study that evaluated two groups with short roots: one group with external root resorption of the maxillary front teeth and the other group with abnormally short roots of the maxillary central incisors, reported that the etiology of the resorption was either the pressure coming from the embedded canines, chronic trauma from orthodontic stress, or from traumatic occlusion (Ando et al., 1967). External apical root resorption in the maxillary central incisors has also been reported to have a high heritability component relative to other teeth, attributed to the fact that these roots are moved greater distances during treatment compared to others (Al-Wawasmi et al., 2003).

The heritability component of EARR was further explored in another study that evaluated 103 sibling pairs and found that siblings experience similar levels of EARR in response to orthodontic treatment (Harris et al., 1997).

Orthodontically induced root resorption is defined as an injury resulting from the pressure applied to the root during tooth movement resulting in localized ischemic necrosis of the PDL in the area of pressure (Weltman et al., 2010). Although histologic studies have reported up to

a 90% occurrence of root resorption in orthodontically treated teeth, in most cases, the resorption is minor and appears less than 2.5mm radiographically. Severe root resorption is defined as either 1/3rd of the original root length or greater than 4 mm, which is seen in 1- 5% of teeth (Weltman et al., 2010). Risks of orthodontic treatment complication depends on a myriad of factors, including treatment duration, force magnitude, direction of movement, method of force, treatment technique, and patient related risk factors including developmental and genetic susceptibility (Weltman et al., 2010; Mavragani et al., 2000). An individual's biochemical or physiological composition has been reported to play an important role in the effects of orthodontic forces influencing EARR, with a heritability component of 60-80% reported in one study (Harris et al., 1997). It has been suggested that although root resorption may occur in patients that have never undergone orthodontic treatment, the incidence is much higher with patients that have had a history of orthodontic treatment (Weltman et al., 2010).

One study reported as high as 1/3rd of orthodontic patients presenting with signs of resorption, while another study found that 13/25 SRA identified individuals had undergone earlier orthodontic treatment (Wang et al., 2019; Harris et al., 1997). Still, it has been reported that having short root anomalies is not necessarily a contraindication for orthodontic treatment. One study reported that the incidence of severe apical root resorption of the incisors after orthodontic treatment was 14.5% (Marques et al., 2010), while another study evaluating CBCT's of SRA patients and control patients found no significant difference in mean values for both root and tooth length after orthodontic treatment (Cutrera et al., 2019). In this study, the mean values for both groups decreased by a range of 0.6-1.3 mm (Cutrera et al., 2019).

9. Orthodontic Considerations

Several considerations and treatment modifications have been described for patients with short root anomalies undergoing orthodontic treatment. Unfavorable root to crown ratios play a large role in the prognosis of orthodontic treatment plans when considering anchoring and the ability of the teeth to withstand force application (Neto et al., 2013). Several factors heavily influence the likelihood of apical root resorption, including the use of compressive forces (instead of tensile forces), apical displacement, longer treatment duration, and the introduction of intrusion and lingual root torque (Chan et al., 2006; Weltman et al., 2010; Han et al., 2005; Parker et al., 1998; Costopoulos et al., 1996). A study using finite element models (FEMs) investigated the stress distribution at the root from orthodontic forces on deviated root shapes including short roots, blunt roots, roots with a bent apex, and roots with a pipette shape. External orthodontic forces were vertical (intrusive) and horizontal (lingual) in nature. A variation in the location of stress concentration was reported for the different study groups. When compared to normal root shapes, roots that were short, bent, or pipette shaped resulted in greater loading of the root, although the loading was concentrated in different parts of the root. In the short root model, it was reported that the decrease in the root-crown ratio might have contributed to enhanced root loading, leading to significant stress concentrated in the *middle* of the root. This study highlights the considerations for root shape prior to orthodontic treatment, as specific shape deviations may lead to a greater loading of the roots with force application (Oyama et al., 2007). It has been suggested that once root resorption is detected, two to three month pauses in force, with a passive arch wire, can minimize further damage. The discontinuous force can be advantageous to allow for the resorbed cementum to heal before force is applied and treatment resumes (Acar et al., 1999). Another method that has been reported is to maintain light,

intermittent forces with longer intervals in between as extensive repair of the cementum occurs between activations that occur over longer intervals (Oppenhiem, 1942; Reitan, 1957). It has also been suggested that periodic radiographs are taken to monitor the movement of teeth with concerns of critical root length (Neto et al, 2013).

10. Genetics

Genetic influence has been reported to contribute significantly in the diagnosis of short roots. In the limited case reports and studies on patients with short roots, a familial occurrence has been established and an autosomal dominant mode of transmission has been suggested (Lind, 1972; Edwards et al., 1990; Jakobsson et al., 1973; Apajalahti et al., 2002; Puranik et al., 2015). In a study evaluating 8 families affected with short root anomalies- in 3/8 families the condition was seen in parent and child, in 2/8 families the condition appeared only in siblings, and in 3/8 families the condition appeared only in the affected individual. In another study, several cases (total of six) were noted to have a familial occurrence (Lind, 1972). One case report evaluating a family of 32 with 7 affected individuals reported an autosomal dominant pattern of inheritance after performing a pedigree analysis (Puranik et al., 2015). A case report on a 10- year-old girl with short roots reported that the patient's dad, paternal uncle, aunt, and two cousins also had short roots while her mom and brother were not affected (Edwards et al., 1990). The genetic susceptibility and origin of root length has also been reported. A study analyzing radiographs of permanent maxillary central incisors of a normal Swedish population of 1038 children reported clinical evidence of root length variation is of genetic origin (Jakobsson et al., 1973). In this study, familial occurrences of root length variation were

reported, with an example of long roots affecting the dentitions of the son, mother and father of a subject as having above average R/C ratios. In addition, the study found that short root anomalies affect girls significantly more often than boys, with a ratio of 2.7: 1.

Specific genes related to matrix metalloproteinases or MMP's and nuclear factor genes have also been reported to have a correlation with patients diagnosed with short roots. Matrix metalloproteinases are a family of structurally related extracellular matrix or cell surface associated enzymes, where activation is typically associated with destruction of tissue and subsequent pathological sequelae. In a study evaluating the gingival crevicular fluid (GCF) of patients with short root anomalies, it was reported that MMP-9 is characteristic in subjects with short roots. The findings of an activation and complex formation of MMP-9 contribute to evidence suggesting that GCF of patients with short roots have low collagenolytic and pathological activity. The presence of MMP-9 is also characteristic to patients with active periodontitis (Apajalahti et al., 2003). Nuclear factor I genes have also been suggested to have a correlation in the development of short roots. Nuclear factor I genes have been previously reported to play a critical role in the development of the brain, lungs, and roots of teeth. The function of odontoblasts is to contribute to the formation of root dentin and crowns of teeth. A mouse study found that disruption of the NFIC gene still allowed for the formation of normal Hertwig's Epithelial Root Sheath (HERS), but disrupted the *differentiation* of odontoblasts. Since the function of NFIC genes are known to play a role in the postnatal stages of tooth development, while crown formation occurs in the embryonic stage, the disruption of the NFIC gene impacted root odontoblast differentiation and did not affect the crown of the tooth. The consequence of disruption in odontoblast formation during

the critical early-stage root formation resulted in the study's findings of decreased cementum and short and abnormal root development (Park et al., 2007).

Chapter II

Significance and Study Approach

Significance

Based on the gender, ethnic, and familial predispositions influencing diagnosis of short root anomalies in available literature, a genetic etiology has been strongly suggested. Because the condition of short roots can affect clinical management and influence best practice for treatment planning in susceptible patients, determining the etiology or etiologies of SRA to facilitate accurate diagnosis is potentially valuable. For instance, establishing an etiology may improve a clinician's understanding of how the application of forces on teeth may lead to the altered response on teeth with short roots, or, conversely, how these responses may cause harm. By improving the understanding of its etiology, we can better understand the disease mechanism and be able to extrapolate predictions on whether applying forces and manipulating the teeth might lead to continued changes in root structure. Further, exploring the molecular mechanisms allows an understanding of whether the condition is largely a developmental process or a progressive, long-term process. If it were a developmental process, clinicians might expect root shortening to be settled around the time development has plateaued, compared to a progressive condition where a worsening of root shortening can be expected. Furthermore, the etiology may provide insights on potentially associated defects or late onset phenotype making monitoring and prevention a possibility. This foundational knowledge can be applied broadly in many facets of dentistry where root to crown ratio must be heavily considered in treatment plan development.

Study Approach

Five families were recruited through the School of Dentistry at the University of Michigan and the Repository at the University of Pittsburgh. Study explanation, pedigree construction,

subject enrollment, clinical examinations and collection of saliva samples were completed under the proper consenting procedure specified in the study protocol. Saliva samples were subjected to genomic DNA isolation and samples from parents and the proband of each family were selected for whole exome sequencing (WES). Following the comparisons between affected and unaffected individuals, a list of prioritized variants were then be subjected to segregation analysis through Sanger sequencing. Assuming all five families have the same phenotype of SRA, sequence data were completed and a list of potential candidate variants from each proband were constructed. Cohort analyses using the list of candidate variants from the pro bands of five families were conducted to filter variants that were not shared by these probands. Following the filtering, the list of variants was compiled. Literature evidence of gene function associated with those variants were reviewed and variants likely associated with the root development were prioritized. Validation experiments were to be conducted based on comparison of variants from additional families with SRA or evaluation of animal models with comparable gene variants. We anticipated that the experimental results would shed light on the etiology of SRA.

Chapter III

Materials & Methods

1. Subject Recruitment and Enrollment

The study protocol and subject consent forms were reviewed and approved by the Institution Review Board at the University of Michigan and the University of Pittsburgh. Five unrelated families with short root anomalies were characterized and recruited. One family was recruited by Dr. Kim-Berman at the University of Michigan. Two families from Chile were recruited by Dr. Vieira at the University of Pittsburgh. Their samples were obtained from the University of Pittsburgh School of Dental Medicine Dental Registry and DNA Repository (IRB#0606091). Two families were recruited by Dr. Hu at the University of Michigan. Study explanation, pedigree construction, subject enrollment, clinical examinations and collection of saliva samples were completed under the proper consenting procedure specified in the approved study protocol.

2. Whole-Exome Sequencing & Bioinformatics Analysis

Non-stimulated saliva sample of 2ml was collected from each participant. Each sample was inspected, coded, then a small aliquot used for genomic DNA isolation following the manufacturer's protocol (Norgen Biotek Corporation, Thorold, ON, Canada). Genomic DNA quality was assessed by 1.5% agarose gel electrophoresis and quantity determined using QubitTM Fluorometer (Thermo Fisher Scientific, Waltham, MA). Samples from parents and proband of each family were selected for whole exome sequencing (WES), and DNA samples from all other family members were used for confirmation analyses. Trio DNA samples following the initial quality control were submitted to Johns Hopkins Center for Inherited Disease Research (CIDR, Baltimore, MD) for WES. Each DNA sample at the concentration of

50 ng/μL, volume of 50 μL, total amount of 2.5 micrograms were plated onto a 96 well plate. A manifesto file with coded sample information and the plated samples were shipped to the CIDR on dry ice. Each sample was genotyped using Illumina QC Array. Once sample aliquoting errors were ruled out and performance potential and genotypes were determined to be appropriate then samples were subjected to WES procedure. Exome capture were completed using the Agilent SureSelect Human All Exon Enrichment System. Using the Illumina HiSeq 2500 (CIDR, Baltimore, MD, paired- end sequencing was generated. Sequencing reads were aligned to the 1000 genomes phase 2 (GRCh37) human genome reference using BWA version 0.7.8 (Li H. 2013). Duplicate reads were flagged with Picard version 1.109. Local realignment around indwells and base call quality score recalibration were performed using the Genome Analysis Toolkit (GATK) (McKenna et al., 2010) version v3.3-0. GATK's reference confidence model workflow was used to perform joint sample genotyping to generate a multi sample VCF file. Variant filtering was done using the Variant Quality Score Recalibration (VQSR) method. Multi-sample VCF files from each family containing variants that were polymorphic among the family members were extracted from the multi sample VCF file derived from the specific cohort with similar phenotypes. All variants in individual VCF files were annotated using VarSeq (Golden Helix, Bozeman, MT) against a variety of data sources including gene annotation, function prediction and frequency information (a cut off value of 0.01 for the minor allele frequency). Following the comparisons between the affected and unaffected individuals, literature review of potential function of the gene variants related to root development, a list of prioritized variants was then subjected to segregation analysis.

3. Segregation Analyses using Sanger Sequencing

The prioritized DNA sequence variations and their segregation with the short root anomaly within each family were assessed by Sanger sequencing. The PCR primers were designed to bracket the candidate variant and the reactions were conducted following established protocols. The PCR amplicons were subjected to Sanger sequencing and the sequencing results were analyzed and compared among the members of each study family.

4. Phenotypic Analyses

Phenotypic comparisons of the five families were performed to determine whether these families can be considered as having similar if not identical clinical presentation of SRA. Families with different presentation of phenotypes may not have the same genetic etiology and should be analyzed separately from the cohort. Phenotypic characteristics that were analyzed for each subject include root length, root width, taurodontism, missing teeth, pulp chamber findings, whether the cases of SRA are localized or generalized, and other pertinent dental anomalies.

Root Length

Root length was analyzed with the use of the "R/C ratio", which measures the root to crown ratio of each tooth. According to Lind, the criteria for classification of short roots is when the root to crown ratio of a tooth is equal to or less than 1.1. The formula used to calculate this ratio is described as "**R/C Ratio= r-m/i-m**" (see Figure 1).

Although Lind describes this classification only for maxillary anterior teeth, the criteria of short roots defined by the root to crown ratio equaling or being less than 1.1 is applied to all types of teeth in this current study, including all incisors, canines, premolars, and molars. Measurements were made using the "Ruler" tool on Adobe Photoshop, with "Pixels" as the measuring unit. All panoramic radiographs were imported into Adobe Photoshop and viewed at 250% with the x-axis on 380 and y-axis set on 240. Once images were set to the correct magnitude, measurements were made using the formula "r-m/i-m" to calculate the root to crown ratio. Two independent observers agreed upon the landmarks used in the formula before measurements were carried out.

Localized vs. Generalized SRA

In the present study, subjects are classified as having localized or generalized cases of short root anomalies based on the percentage of teeth affected. In order to have an absolute denominator to calculate the percentage of affected teeth, 28 was the standard number used for all subjects. Using the AAP 1999 Classification of Periodontal disease guideline, which classifies localized cases as less than 30% of sites and generalized cases as more than 30% of sites affected, the same criteria was used in this study to classify localized and generalized cases of short root anomalies.

Root Width

One of Lind's concurrent findings with the observation of short roots in maxillary anterior teeth was the "onion shape" appearance found in affected teeth. Because the finding of "onion shaped" roots is largely subjective, a modified formula was used to objectively assess whether

affected teeth in the current study have an abnormal root width. This formula is described as "**Rx to Ry/x to y**", which is the ratio of the root width to crown width. Specifically, the width of the root is measured from the middle 1/3rd of the root and the crown width is measured at the CEJ (see Figure 2). Tilk et al. (1979) published a study that measured the mesio-distal width of the roots at the cervical third, middle third, and apical third of 1500 permanent teeth. The quantitative findings of this study were used to develop a criteria for determining if root widths are classified as "wide" or "normal".

For maxillary central incisors, the average root width in the middle third was reported as 5.15 mm with the standard deviation being 0.58 mm (see Figure 4). For the purposes of this study, measurements to 2 standard deviations were calculated. Therefore, 5.15 + 0.58 + 0.58 mm = 6.31 mm and is the number used to calculate the average root width of a maxillary central incisor at the middle third. Similarly, the average root width of the cervical third is measured at 6.22 mm with a standard deviation of 0.51. Therefore, 6.22 + 0.51 + 0.51 = 7.24 mm, which is the number used to calculate the average root width of the maxillary central incisor at the cervical third. By using the formula **"Rx to Ry/x to y"** to apply to these numbers, "Rx to Ry" is equivalent to the root width at the middle third and "x to y" is equivalent to the crown width at the CEJ, or the root width at the cervical third. Therefore, middle 1/3rd at 6.31mm divided by cervical 1/3rd at 7.24 mm is equal to a ratio of 0.87. For the present study, root width ratios of maxillary central incisors **greater than 0.87 are consequently classified as "wide", and anything less than 0.87 is classified as "normal".** In the event that a measurement is two standard deviations below the average, roots are still classified as "normal" in the present study. Measurements of root widths and crown widths were made using the "Ruler" tool on

Adobe Photoshop, with “Pixels” as the measuring unit. All panoramic radiographs were imported into Adobe Photoshop and viewed at 250% with the x-axis on 380 and y-axis set on 240.

The same method was applied to classify root widths of mandibular central incisors as “wide” or “normal”. In this case, the average root width for mandibular central incisors at the middle third is reported at 2.85 mm with a standard deviation of 0.29 (see Figure 3). Therefore, the calculated average root width at the middle 1/3rd with 2 standard deviations is 2.85 mm + 0.29mm + 0.29mm = 3.43 mm. The average root width of the mandibular central incisors at the cervical third is reported at 3.44 mm with a standard deviation of 0.29mm. Therefore, the calculated average root width at the cervical 1/3rd with 2 standard deviations is 3.44 mm + 0.29 mm + 0.29 mm= 4.02 mm. The root width ratio is determined by dividing the middle 1/3rd root width of 3.43 mm by the cervical 1/3rd root width of 4.02, which is equal to a root width ratio of 0.85. In the present study, any root width ratio of a mandibular central incisor higher than 0.85 is therefore classified as “wide”, and any root width ratio of less than 0.85 is classified as “normal”. Measurements were made using the “Ruler” tool on Adobe Photoshop, with “Pixels” as the measuring unit. All panoramic radiographs were imported into Adobe Photoshop and viewed at 250% with the x-axis on 380 and y-axis set on 240. Measurements were repeated a second time, two weeks after the initial measurements were taken, in order to calculate intra-class correlation.

Taurodontism

In the present study, taurodontism in first and second molars of subjects were objectively classified based on the modified formula described by MacDonald et al (2019). MacDonald et al cited an article published in 1978 by Shifman and Chanannel who were the first to describe "variables" of a tooth used to define a "Taurodont Index". The formula used to classify whether a molar is a taurodont is: **(Variable 1/Variable 2) x 100**, with Variable 1 being the lowest point of the roof of the pulp chamber to the highest point of the floor of the pulp chamber while Variable 2 is defined as the lowest point of the roof of the pulp chamber to the apex of the longest root. Using the Taurodont Index, if the calculated number is less than 20, the root shape is classified as "normal". If the calculated number is between 20 to 30, it is classified as hypotaurodont. If the calculated number is between 30 and 40, the tooth is classified as a mesotaurodont, and if the taurodont index is any higher than 40, the tooth is classified as a hypertaurodont. For the purpose of this study, only teeth that are classified as mesotaurodont or hypertaurodont are classified as taurodonts in the phenotypic analysis. Measurements of the "variables" that determine the "Taurodont Index" number were made using the "Ruler" tool on Adobe Photoshop, with "Pixels" as the measuring unit. All panoramic radiographs were imported into Adobe Photoshop and viewed at 250% with the x-axis on 380 and y-axis set on 240. The landmarks used in the formula for each subject were agreed upon by two independent observers before measurements were carried out.

Pulpal findings, Missing Teeth/Agenesis, Other Dental Anomalies

The observation of pulp stones, missing teeth/agenesis, and other dental anomalies were subjectively noted in each subject and agreed upon by two independent observers. Because

phenotypic analyses are done using only panoramic radiographs, ratios are needed for objective findings. Therefore, if ratios are not able to be determined, only subjective findings can be reported.

Combining the Genotypic and Phenotypic Analyses

Following the whole exome sequencing, data from five families with similar phenotype of SRA were compiled, sequence variations annotated and a list of potential candidate variants from each proband was constructed. A cohort analyses using the list of candidate variants from probands of five families was conducted which allowed filtering of variants not shared by these probands. Following this filtering, the list of potential variants was reviewed and prioritized based on the functional impact of these variants. Validation experiments involving comparison of variants from additional families or evaluation of the dentition from animal models with comparable genetic variants or mutations were performed.

Chapter IV

Results

1. Subject Recruitment and Enrollment

Family One (USSO): Family one was a two-generation Caucasian family, with a total of 5 subjects recruited for the study. The mother was reported to have short roots, while the father was unaffected with normal tooth morphology. At the time of enrollment, they had two daughters and one son, age 14, 8, and 7 respectively. Proband (labeled II:1) was a 14 year old girl who's affected with short roots, while second daughter/proband's sister (labeled II:2) was an 8 year old girl who's also affected by short roots. The only male offspring, labeled II:3, is unaffected. Pedigree, oral photographs, and radiographs of subject II:1 is presented in Figure 6., and pedigree, oral photographs, and radiographs of subject II:2 is presented in Figure 7.

Family Two (USRE): Family two was a three-generation Caucasian family, with a total of 4 subjects recruited for the study: grandmother, mother, son/half brother, and daughter/half sister. The family pedigree was constructed by report. At the time of enrollment, both mother (II:2) and grandmother (I:2) reported short roots. Radiographic confirmation of short roots was obtained from proband's mother (II:2) and proband (III:5) only. Grandmother, mother, and daughter/half sister also reported to have hypodontia. The son/half brother (labeled III:4) is unaffected. Pedigree, available clinical photos, and panoramic radiographs for subjects II:2 and III:5 are presented in Figure 8.

Family Three (KRMO): Family three was a two-generation Korean family, with a total of 4 subjects recruited for the study: father, mother, daughter, and son. Proband (labeled II:2) was the only one affected with short roots, while father, mother, and son remain unaffected. Following the genetic analysis, the parents were found to be related (illustrated with the double

line between mother and father on the pedigree). Pedigree and panoramic radiographs for proband (subject II:2), unaffected father (subject I:1), and unaffected mother (subject I:2) are presented in Figure 9.

Family Four (CHII): Family four was a two-generation Chilean family, with a total of 3 subjects recruited for the study: mother, son, and second son (labeled I:2, II:1, and II:2, respectively). Proband (II:2) was the only affected person with short roots who's tooth 28 is congenitally missing. Pedigree and panoramic radiograph for proband (subject II:2) is presented in Figure 10.

Family Five (CHC): The proband was part of a five-generation Chilean family and was the only one affected with short roots (labeled V:1). The proband has maxillary canine (11) impaction, and agenesis of several teeth. Pedigree, clinical photos, and panoramic radiographs for proband (subject V:1) are presented in Figure 11.

2. Whole-Exome Sequencing & Bioinformatics Analysis

Whole exome sequencing was performed on 15 subjects from those five families with SRA. Initial QC screening of all samples demonstrated sufficient quality of the genomic DNA and correct subject relationship and gender. A total of 16 samples were subjected (one sample yielded no data due to poor sequencing performance) to sequencing, which yielded an average sequencing depth of 89.2x (USSOAB had no data released thus was not included in this calculation) and the average number of raw sequence reads was 129,580,435 with 125-bp

sequencing length. Following the standard WES analysis pipeline that were optimized in our lab, a list of heterozygous and homozygous variants with SIFT score, PolyPhen-2 score, Combined Annotation Dependent Depletion (CADD), Alt Allele Frequency (AAF from genomAD), and dbSNP 154 was compiled for each of the five families (Tables 1-5). These list of variants were cross checked and the affected genes common to two or more families were extracted and listed in Table 6.

There were no variants involving the same gene present in all five families.

PLEKHG5, pleckstrin homology domain-containing family G member 5, plays a role in angiogenesis through regulation of endothelial cells chemotaxis. It affects also the migration, adhesion, and matrix/bone degradation in macrophages and osteoclasts. CHII proband has a novel heterozygous inframe deletion NM_020631.6:c.2163_2165delGGA:p.Glu723del and the USSO proband has novel compound heterozygous frameshift mutations NM_020631.6:c.2164_2165delGA:p.Glu722Glyfs*63 and NM_020631.6:c.2163delG:p.Glu722Argfs*43. Due to limited experimental data, how these changes impact function of PLEKHG5 cannot be deduced.

DENND4B is a guanine nucleotide exchange factor which may activate Rab10 to promote the exchange of GDP to GTP, converting inactive GDP-bound Rab proteins into their active GTP-bound form. USRE proband has a heterozygous frameshift mutation NM_014856.3:c.2703_2730del:p.Gln902Serfs*38 while USSO II:2 has the same mutation. There is no reported data on expression and potential function of DENND4B during tooth and

tooth root development.

IGFN1, immunoglobulin-like and fibronectin type III domain-containing protein , mediates homophilic cell adhesion via plasma membrane adhesion molecules, retina layer formation and synapse assembly. USSO II:2 has a heterozygous frameshift variant NM_001164586.2:c.1664_1665delAA:p.Lys555Serfs*61 while CHC proband has a heterozygous missense variant NM_001164586.2:c.10154G>A:p.Ser3385Asn which is rare (AAF 0.0017) and is predicted to be damaging. However, the functional significance of IGFN1 and tooth root development cannot be predicted based on limited scientific data.

ATXN7 encodes a transcription coactivator that mediates the interaction of STAGA complex with the CRX and is involved in CRX-dependent gene activation and it is necessary for microtubule cytoskeleton stabilization. Proband of family CHC has a novel heterozygous 3 base-pair inframe deletion NM_001377405.1:c.116_118delAGC:p.Gln39del while proband of USRE has a heterozygous 3 base-pair insertion NM_001377405.1:c.123_125dupGCC:p.Pro43dup (rs1553686135, AAF 0.00570556). ATXN7 involvement in tooth root development is unclear.

DCHS2 encodes a calcium-dependent cell-adhesion protein. Proband of family CHC has a novel 4 base-pair deletion resulting in frameshift NM_001358235.2:c.4019-769_4019-766delCAAA. Proband of family USRE has a single base change that resulted in a stop again NM_001358235.2:c.5392C>T:p.Arg1798Ter (rs150179829, AAF 0.00134781). Both variants are likely damaging to the function of DCHS2, however, its impact on tooth development is

unknown.

ATXN1 encodes a chromatin-binding factor that repress Notch signaling in the absence of Notch intracellular domain by acting as a CBF1 corepressor. In concert with CIC and ATXN1L, ATXN1 involves in brain development. Proband of CHII has an inframe 3bp insertion NM_001128164.2:c.666_668dupGCA:p.Gln225dup while USRE proband has also a heterozygous 3bp insertion NM_001128164.2:c.624_626dupGCA:p.Gln208dup (rs193922926). The significance of these variants on tooth root development cannot be determined.

HLA-DRB1 in complex with the alpha chain HLA-DRA, displays antigenic peptides on professional antigen presenting cells (APCs) for recognition by alpha-beta T cell receptor (TCR) on HLA-DRB1-restricted CD4-positive T cells. This guides antigen-specific T-helper effector functions, both antibody-mediated immune response and macrophage activation, to ultimately eliminate the infectious agents and transformed cells. CHII proband has a homozygous frameshift variant NM_002124.4:c.295delinsCGG:p.Gln99Argfs*31 and USSO has a heterozygous frameshift variant NM_002124.4:c.294delG:p.Glu98Aspfs*31. HLA-DRB1 function in tooth development has not been determined.

PODXL encodes a protein that acts as a pro-adhesive molecule, enhancing the adherence of cells to immobilized ligands, increasing the rate of migration and cell-cell contacts in an integrin-dependent manner and induces the formation of apical actin-dependent microvilli. It governs a positive regulation of cell-cell adhesion mediated by integrin. Both probands from

the Chilean families carried the heterozygous novel inframe insertion variant, NM_001018111.3:c.78_83dupGTCGCC:p.Pro30_Ser31dup while USSO II:2 has a heterozygous frameshift mutation NM_001018111.3:c.70_85del:p.Pro24Argfs*138.

RP1L1, retinitis pigmentosa 1-like 1 protein, is required for the differentiation of photoreceptor cells. It plays a role in the organization of outer segment of rod and cone photoreceptors. Proband of USSO has a heterozygous stop gain mutation NM_178857.6:c.4054G>T:p.Glu1352Ter while KRMO proband has a heterozygous frameshift variant NM_178857.6:c.324_325insT:p.Pro109Serfs*29. There is no literature evidence of RP1L1 involving in tooth development.

PKHD1L1 has a molecular function involving in signaling receptor activity of the immune response and sensory perception of sound. USSO proband has a missense mutation NM_177531.6:c.442A>G:p.Ile148Val that is damaging while CHII proband has a splice donor site mutation NM_177531.6:c.6507+1G>A that has been reported to have an alternative allele frequency of 0.00767286 (rs72687022).

ZDHHC16, palmitoyltransferase, is required during embryonic heart development and cardiac function, possibly involved in apoptotic process, cellular damage to DNA damage stimulus and telencephalon development. USSO proband has a heterozygous missense mutation NM_198046.3:c.117G>C:p.Trp39Cys (rs766784631) that is rare (AAF 0.00001599) and predicted to be damaging and USRE proband also has a missense heterozygous mutation NM_198046.3:c.973G>A:p.Gly325Ser (rs377074050) that is also rare (AAF 0.00001989) and damaging. *ZDHHC16* expression during tooth development is unknown.

KRT76 were identified in two of the five families. Probands of the KRMO and CHC have an identical inframe deletion mutation NM_015848.4:c.1639_1641delAGT:p.Ser547del or rs370657661 which has a AAF of 0.00128743. The putative functions of *KRT76* include cornification, keratinization, pigmentation, cytoskeleton organization, and sebaceous gland development. KRT76 is typically present in epidermis. Among the many keratins expressed in the oral cavity, KRT76 has been previously reported to be the topmost down regulated gene amongst all differentially expressed genes (Ambatipudi et al., 2012). In a study conducted by Ambatipudi et al. (2013), who examined the differential expression of KRT76 in human and hamster oral precancerous and cancerous lesions, it was reported that a loss of KRT76 is sufficient to cause hyperplasia in the oral cavity of the mice. A possible theory on why KRT76 loss may contribute to cancer development is that it contributes to a barrier defect in the epithelium, allowing it to be more exposed to potential carcinogens. The same study reported that there was a strong association of reduced KR76 expression with increased risk of oral precancerous lesions and oral squamous cell carcinoma development. Its potential role in development of tooth root, a mesenchyme-derived structure, is not immediately clear. However, understanding that existing studies are able to correlate downregulation of KRT76 gene with pertinent oral findings may be important to form potential associations with presence of short roots in future studies.

EP400 is a component of the NuA4 histone acetyltransferase complex which is involved in transcriptional activation of select genes principally by acetylation of nucleosomal histones H4 and H2A. It binds to DNA, ATP and chromatin to impact helicase, nucleosome-dependent ATPase activity. An inframe insertion heterozygous variant was identified in CHC,

NM_015409.5:c.8223_8225dupGCA:p.Gln2748dup. While an inframe deletion heterozygous variant was found in USRE proband, NM_015409.5:c.8223_8225delGCA:p.Gln2748del (rs528214697). Functional impact of these novel variants is unclear.

ZIC5 is essential for neural crest development, converting cells from an epidermal fate to a neural crest cell fate. Probands from CHC and CHII carried a heterozygous inframe deletion NM_033132.5:c.1176_1178delGCC:p.Pro400del mutation.

NIPA1 encodes a transmembrane transporter of Mg^{2+}, although it can also transport other divalent cations such as Fe^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+} and Co^{2+} but to a much less extent. Proband of family KRMO has a heterozygous inframe deletion NM_144599.5:c.42_47delGGCGGC:p.Ala15_Ala16del (rs531550505, AAF 0.000346947) while families CHC and CHII shared the same novel heterozygous inframe deletion NM_144599.5: c.39_41delGGC:p.Ala16del. The deleted amino acid Alanine is located in the topological domain of this transporter presumably interacts with the ion ligand. The speculated functional impact of these deletions concentrates on ligand binding. There is no literature evidence of this transporter regulating tooth root development.

LMF1, lipase maturation factor 1, involves in the maturation of specific proteins in the endoplasmic reticulum. Required for maturation and transport of active lipoprotein lipase (LPL) through the secretory pathway. USSO proband has a heterozygous missense variant NM_022773.4:c.1567C>T:p.Arg523Cys while CHII proband has a stop gain heterozygous mutation NM_022773.4:c.1431C>T:p.Asn477= (rs772646362). Both variants are rare, AAF of

0.00003467 and 0.00011285, respectively, and their potential impact on LMF1 function is unknown.

DNAH3 encodes Dynein axonemal heavy chain 3 which is a force generating protein of respiratory cilia. It produces force towards the minus ends of microtubules. Dynein has ATPase activity. Both CHC and CHII probands carried a heterozygous variant NM_017539.2:c.2724G>C:p.Arg908Ser (rs117470111, AAF0.00624139) which is predicted to be damaging.

THAP11 encodes a transcriptional repressor that plays a central role for embryogenesis and the pluripotency of embryonic stem (ES) cells. It is a sequence-specific DNA-binding factor that represses gene expression in pluripotent ES cells by directly binding to key genetic loci and recruiting epigenetic modifiers. Proband of CHC has a heterozygous inframe deletion NM_020457.3:c.597_611delAGAGGGCGCAGCCGC: p.Glu200_Ala204del (rs750317616, AAF 2.84322E-05), proband of CHII has a novel heterozygous inframe deletion NM_020457.3:c.394_396delCAG:p.Gln132del, while proband of USRE has a homozygous frameshift mutation NM_020457.3:exon 1:c.369delG:p.Gln123Hisfs*42 (rs111586870). Proband KRMO has compound heterozygous mutation NM_020457.3:c.363_364delGC:p.Gln122Thrfs*117 and NM_020457.3:c.366_369delACAG:p.Gln122Hisfs*42. All mutation sites are located between functionally important regions, motifs, and domains. There is no literature evidence on how these mutations may impact the transcriptional repression of *THAP11*. However, four out of five porbands with rare frameshift variants that are potentially damaging to the gene function is

interesting. There is no literature evidence of this gene expression during tooth or tooth root development. Important experiments to carry out will include gene expression study by detection of mRNA transcripts during tooth development using both the in situ riboprobes and Reverse transcription polymerase chain reaction (RT-PCR).

VEZF1 is a transcription factor specifically binds to the CT/GC-rich region of the interleukin-3 promoter and mediates tax transactivation of IL-3.USSO proband has compound heterozygous frameshift variants NM_007146.3:c.1044delG:p.Gln348Hisfs*9 and NM_007146.3:c.1041_1042delGC:p.Gln348Alafs*27 while proband of CHC has a heterozygous inframe insertation variant NM_007146.3:c.1032_1034dupGCA:p.Gln354dup.

ZSCAN30, zinc finger and SCAN domain-containing protein 30, may be involved in transcriptional regulation. Family CHC proband has a heterozygous missense variant NM_001112734.4:c.1186C>T:p.Arg396Trp and CHII proband also has a heterozygous stop gain mutation NM_001112734.4:c.325C>T:p.Arg109Ter. No literature evidence supporting the involvement of this gene in tooth root development.

CACNA1A encodes voltage-dependent P/Q type calcium channel subunit alpha-1A. The voltage-sensitive calcium channels (VSCC) mediate the entry of calcium ions into excitable cells and are also involved in a variety of calcium-dependent processes, including muscle contraction, hormone or neurotransmitter release, gene expression, cell motility, cell division and cell death. Both probands of family CHC and CHII carried a heterozygous variant

NM_001127221.2:c.*161_*166delCAGCAG resulting in inframe deletion which will likely be damaging to the gene function.

ANKLE1 encodes an endonuclease that probably plays a role in the DNA damage response and DNA repair. Proband of CHC has a heterozygous variant NM_152363.6:c.*132_*139delGTGTGTGT (rs5853575, AAF 0.0092908) resulting in a frameshift mutation, while proband of USRE also carried a heterozygous variant NM_152363.6:c.*141_*145delTGTGT resulting in a frameshift mutation as well.

Given the diverse genetic background of the study families, we had anticipated a short list of variants that is shared among the affected. Unfortunately, the majority of the variants were shared by only two families making them unlikely to be the causative variants. The most logical candidate *THAP11* is selected for further investigation because of potentially significant, functionally damaging variants identified in four study families with Hispanic, Asian and Caucasian background.

3. Segregation Analyses using Sanger Sequencing

When selecting candidate variants for segregation analyses, factors including number of probands from the study families, number of affected from the study families, literature evidence, animal models depicting gene function were carefully assessed and reviewed. The sequencing results of additional affected individual III:2 from USSO (Family 1), unaffected III:3 from USSO (Family 1), unaffected individual II:2 from KRMO (Family 3), and unaffected individual III:1 from USRE (Family 2) were referenced when filtering candidate

variants identified from 5 study probands. Given the WES data and literature evidence, there were no logical variants or candidate genes that can be targeted for segregation analyses.

4. Phenotypic Analysis

Localized and generalized cases of short root anomalies

Out of the seven subjects that underwent phenotypic analysis, four were classified as localized cases based on having less than 30% of the dentition affected by short root anomalies. Among the four subjects that were classified as having localized short root anomalies, two of the subjects were from the same family while the other two subjects were from a different family. The remaining three subjects were classified as generalized short root anomaly cases based on more than 30% of the dentition being affected. Among the three subjects with generalized cases of short root anomalies, none of them belonged to the same family (Table 7).

Root length (short roots)

The most frequently affected teeth with short roots in this study are maxillary central incisors (Table 8), maxillary first premolars (Table 11) and maxillary second premolars (Table 12). These teeth appeared to affect subjects with both localized and generalized cases. Maxillary first molars were affected in one subject with a localized case and one subject with a generalized case (Table 13).

Short roots were also noted in maxillary lateral incisors, maxillary canines, maxillary second molars, mandibular central incisors, mandibular lateral incisors, mandibular canines,

mandibular first premolars, mandibular second premolars, mandibular first molars, and mandibular second molars, but only appeared to affect cases classified as having generalized short root anomalies (Table 9, 10, 14, 15, 16, 17, 18, 19, 20, and 21).

Root width

Wide root widths affecting the maxillary central incisors, determined by whether root to crown ratio was greater than 0.87, affected five subjects, two of which were classified as localized cases of SRA and three of which were classified as generalized cases of SRA. In all five affected subjects, wide root widths of both central incisors (#8 and 9) were noted (see Table 22).

Wide root widths were also determined on mandibular central incisors in three subjects, one of which was classified as having localized SRA while the other two cases were classified as generalized cases of SRA (Table 23). Wide root width was determined if the tooth had a R/C ratio of >0.85. In the localized case (Family 1 Subject II:1), only #25 was noted to have a wide root width. In one generalized case (Family 3- Subject II:2), both mandibular central incisors were noted to have wide root widths (#24, 25) while the other affected generalized case (Family 5 Subject V:1) was noted to have wide root width in #25 only.

Taurodontism

For the purposes of this study, only mesotaurodont and hypertaurodont classifications were noted to be significant as having taurodontism for the purposes of this study.

Maxillary taurodontism was noted in three cases, two of which were noted in localized cases of SRA and one noted in a generalized case of SRA. Worth noting is that the two affected subjects (both localized cases) belong to the same family (Family 1- Subjects II:1 and II:2). In all three cases, both second molars (#2 and #15) were affected (Table 24).
Mandibular taurodontism was less common, and was only noted in Family 1- Subject II:2, who is classified as a localized case of SRA. In this case, the mandibular second molars were affected (Table 25).

Other Dental Anomalies

The presence of a peg lateral was determined by the ratio of mesial-distal (M-D) width dimension of central incisors compared to ratio of M-D dimension of lateral incisors. A normal central incisor (CI) M-D width to lateral incisor (LI) M-D width ratio should equal 1.3. A central incisor (CI) M-D width to lateral incisor (L1) M-D width ratio of 1.6 was observed in one subject. Based on this criteria, a peg lateral was observed in one subject with a localized case of short root anomalies (Table 26).

Missing teeth/agenesis was also noted in several of the subjects. Four subjects were noted to exhibit agenesis/ have missing teeth. Of the four subjects, only one subject was classified as a localized case while the other three were ones having generalized cases of SRA. In Family 2 Subject III:5 (localized SRA case), #7 was noted to be missing. In Family 4 Subject II:2, #16 was noted to be missing. In Family 5 Subject V:1, #7, 10, and 26 were also noted as missing (Table 29). It is important to note that we did not include missing third molars as missing teeth in our study.

Pulp stones were noted in three subjects. In Family 1 Subject II:1, pulp stones were noted in maxillary molars #3, 14, and 15. In Family 3 Subject II:2, pulp stone was noted in mandibular molar #30. In Family 5 Subject V:1, pulp stones was noted in maxillary molar #3. Other significant pulpal findings include the presence of what appears to be generalized pulpal obliteration in Family 3 Subject II:2, who was classified as having a generalized case of short root anomaly (Table 29).

Several other dental anomalies were noted during phenotypic analysis of our subjects. In Family 2 Subject III:5, classified as having a localized case of short roots, we also noted a peg lateral on #10 and invaginatus on #8. In Family 4 Subject II:2, hypercementosis was noted on the roots of #3, 14, 18, 30, and 31. In Family 5 Subject V:1, ectopic eruption of upper left maxillary canine (#11) was noted (see Table 29).

Reliability in Measurements

I. Intraclass Correlation Coefficient (ICC)

Intraclass correlation coefficient at $p<0.05$ was calculated for root length and crown length measurements taken at time one (T1) and time two (T2) across all subjects and tooth types. Most measurements had an ICC of 0.8 or higher, which represents a good-strong correlation between repeated measurements. Measurements with an ICC of ≥ 0.7 but < 0.8 represent a fair correlation, and this was noted in the root length measurements for #8 and #9 and the crown length measurement for #9. One measurement, the crown length of tooth #14, had an ICC of 0.591, which represents a less than ideal correlation between the measurements taken at both time points.

Intraclass correlation coefficient was also calculated for root width and crown width measurements of central incisors taken at time one (T1) and time two (T2) across all subjects. All measurements had an ICC of 0.9 and above, which represents a very strong correlation between repeated measurements (See Table 29).

II. Bland Altman Plots

Several Bland-Altman plots were created to illustrate the reliability of repeated measurements taken.

In Figure 12, a Bland-Altman plot depicting root length taken the first time (R1) compared to root length taken the second time (R2) across all subjects and tooth types found that most measurements (all but four), are within five pixels of each other.
In Figure 13, a Bland-Altman plot depicting crown length taken the first time (C1) compared to crown length taken the second time (C2) across all subjects and tooth types found that most data points tend to cluster around the "mean", with limited points that appear as outliers.

In Figure 14, a Bland-Altman plot depicting root width measurements of maxillary central incisors taken the first time (RW1) compared to root width measurements taken the second time (RW2) across all subjects found that all measurements are within one pixel of each other. The data appears scattered due to the limited number of data points illustrated in the plot. Similarly, in Figure 15, a Bland-Altman plot depicting root width of mandibular central incisors taken the first time (RW1) compared to root width measurements taken the second time (RW2) across all subjects found that most measurements cluster around the "mean", and

are within one pixel of each other. Due to the limited number of data points, data appears scattered although data points are within an acceptable range of error.

In Figure 16 and 17, Bland-Altman plots depicting crown width of maxillary central incisors and mandibular central incisors taken the first time (CW1) compared to crown width measurements taken the second time (CW2) across all subjects found that most measurements cluster around the mean and are within 1-1.5 pixels of each other, with the exception of a few outlier data points.

In Figure 18 and 19, Bland-Altman plots depicting Variable 1 measurements (to calculate Taurodont Index) of maxillary first and second molars taken at time point one (V1_1) and time point two (V1_2) across all subjects found that most data points cluster around the mean, which are within two pixels of each other.

In Figures 20 and 21, Bland-Altman plots depicting Variable 2 measurements (to calculate Taurodont Index) of maxillary first and second molars taken at time point one (V2_1) and time point two (V2_2) across all subjects found that most data points cluster around the mean, which are within three pixels of each other.

Chapter V

Discussion

1. Findings

This study represents a comprehensive genetic and phenotypic evaluation of five families (seven subjects total) with non-syndromic short root anomalies. The aim of this study was to define clinical features and identify specific gene mutations that are associated with short root anomalies, and to determine the potential association between clinical features and genetic mutations in order to provide scientific evidence for oral health providers to enhance diagnosis and management of patients with short root anomalies.

Among the five families in this study, affected probands in USSO (Family 1) and USRE (Family 2) presented with localized short roots while probands of KRMO (Family 3), CHC (Family 4) and CHII (Family 5) families have generalized short roots. The number of participants recruited from the study families was small, which limited the power to identify potential candidate variants. Family USSO is the only family with affected individuals in both the parents' and the children's generation, which was very important for filtering variants to narrow down potential causality. The other four families have simplex cases making determining the mode of inheritance difficult.

I. Genotypic Analysis

There were no variants involving the same gene present in five families. Four out of five probands have genetic variants in THAP11. All variants are located between functionally important regions, motifs, and domains. How these variants may impact the function of *THAP11* cannot be predicted. Variants of gene *KRT76* were identified in two of the five families. Probands of the KRMO and CHC have an identical inframe deletion mutation which

was interesting as these two families are diverse in their genetic background. This alternative allele, rs370657661, has a frequency of 0.00128743 in the general population.

There were 20 additional unique genes with potentially damaging variants shared by at least two of the five study families. Each unique gene annotation and function was reviewed. We cross checked those variants with the variants from the sequencing results of additional affected individual (III:2) in USSO family, and not a single one of those genes was identified among the list of variants of USSO III:2 producing no logical candidates for further segregation analysis. Given this finding, our decision was made to focus the study on 1) careful analysis of all data sets to ensure accuracy, and 2) active recruitment of additional families with SRA with both generalized and localized cases.

II. Phenotypic Analysis

Determining Reliability in Measurements

Reliability of measurements was evaluated in two different ways. First, all measurements taken (root length, crown length, root width, crown width, variables for determining taurodontism, and mesial-distal widths to calculate ratio between central and lateral incisor for peg lateral determination) were repeated twice, spaced two weeks apart. The measurements taken at time point one and time point two were then used to calculate intraclass correlation coefficients (ICC) for each measurement. A two-way mixed effects model was used where subjects are random and the measurer effect is fixed. The intraclass correlation coefficients were done using an absolute agreement definition, where p value was < 0.05.

An ICC value of 0.9 and above is classified as having a strong correlation, value of 0.8-0.9 is classified as having a good correlation, 0.7-0.8 as having fair correlation, and any ICC equal to 0.5 or below is classified as having poor correlation.

The second way to evaluate reliability was to create a series of Bland-Altman plots for the different measurements (root length, crown length, root width, crown width, variables used to calculate Taurodont Index, values to calculate ratio for peg lateral) to illustrate whether repeated measurements tend to "cluster" around a mean, which would be interpreted that most measurements are within an acceptable range of error. Alternatively, if repeated measurements vary significantly from the measurements taken at time point one, this would be illustrated by data appearing more scattered on the plots and would suggest that the methodology to gather data may not be as accurate.

Out of the seven subjects that underwent phenotypic analysis, four were classified as localized cases based on having less than 30% of the dentition affected by short root anomalies. The remaining 3 subjects were classified as generalized short root anomaly cases based on more than 30% of the dentition being affected. All subjects in the present study self-reported a non-contributory medical history. As non-syndromic occurrences of generalized short root anomalies are rare, the finding of three out of seven of our subjects being considered as having generalized short root anomalies is of significance.

The most frequently affected teeth with short roots in this study are maxillary central incisors, maxillary first premolars and maxillary second premolars. These teeth appeared to affect subjects with both localized and generalized cases. This finding supports previously reported studies that short root anomalies typically affect maxillary central incisors, and premolars are

often described as the next most diagnosed tooth type with short roots (Lind, 1972; Apajalahti et al., 2002; Apajalahti et al., 1999; Ando et al., 1967; Puranik et al., 2015). Maxillary first molars were affected in one subject with a localized case (Family 2 Subject II:2), and one subject with a generalized case (Family 5, Subject V:1).

Short roots were also noted in maxillary lateral incisors, maxillary canines, maxillary second molars, mandibular central incisors, mandibular lateral incisors, mandibular canines, mandibular first premolars, mandibular second premolars, mandibular first molars, and mandibular second molars, but only appeared to affect cases classified as having generalized short root anomalies. This finding is supported by previous studies that found the tooth types that are least affected by short root anomalies include mandibular central incisors, molars, and canines (Lind, 1972; Puranik et al., 2015).

The characteristic of a "plump, onion shape" root appearance in affected maxillary central incisors as described by Lind in 1972 was quantified in the present study. Wide root widths affecting the maxillary central incisors, determined by whether root to crown ratio was greater than 0.87, affected five subjects, two of which were classified as localized cases of SRA and three of which were classified as generalized cases of SRA. In all five affected subjects, wide root widths of both central incisors (#8 and 9) were noted. Our findings, using a formula to quantifiably assess the "wide root width" or "plump, onion shape" characteristic supported Lind's (1972) report that maxillary central incisors affected by short roots tend to have this characteristic shape.

Wide root widths were also determined on mandibular central incisors in three subjects, one of which was classified as having localized SRA while the other two cases were classified as generalized cases of SRA. Wide root width was determined if the tooth had a R/C ratio of >0.85. In the localized case (Family 1 Subject II:1), only #25 was noted to have a wide root width. In one generalized case (Family 3- Subject II:2), both mandibular central incisors were noted to have wide root widths (#24, 25) while the other affected generalized case (Family 5 Subject V:1) was noted to have wide root width in #25 only. The finding of a wide root width or characteristic "plump, onion shape" in affected mandibular central incisors with short roots has not been reported previously.

Taurodontism is identified based on radiographic assessment and affected teeth typically take on a rectangular shape tapering towards the roots. A typical presentation of taurodont-affected teeth include an exceedingly large pulp chamber, absence of the cervical constriction, and significantly shorter roots (Dineshshankar et al., 2014; Shifman and Chanannel, 1978). Only mesotaurodont and hypertaurodont classifications were noted to be significant as having taurodontism for the purposes of this study. Maxillary taurodontism was noted in three cases, two of which were noted in localized cases of SRA and one noted in a generalized case of SRA. Worth noting is that the two affected subjects (both localized cases) belong to the same family (Family 1- Subjects II:1 and II:2). In all three cases, both second molars (#2 and #15) were affected. Mandibular taurodontism was less common, and was only noted in Family 1- Subject II:2, who is classified as a localized case of SRA. In this case, the mandibular second molars were affected (#18 and #31). Our findings from this study support previous studies that have suggested taurodontism and short roots may occur together (Apajahlati et al. 2002;

Apajahlati et al., 1999). In our present study, taurodontism was only considered and measurements made for all maxillary and mandibular first and second molar tooth types. However, a previous case report found that taurodontism in the mandibular canines occurred in a patient who had non-syndromic generalized short roots (Venkataraghavan et al., 2014). Therefore, taurodontism for more than just molar tooth types may be considered for exploration in future studies.

Missing teeth/agenesis was noted in several of the subjects. Four subjects were noted to exhibit agenesis/ have missing teeth. Of the four subjects, only one subject was classified as a localized case while the other three were ones having generalized cases of SRA. In Family 2 Subject III:5 (localized SRA case), #7 was noted to be missing. In Family 4 Subject II:2, #16 was noted to be missing. In Family 5 Subject V:1, #7, 10, and 26 were also noted as missing. It is important to note that we did not include missing third molars as missing teeth in our study. The observation of agenesis/missing teeth phenotype in several of our subjects gives the impression that short root phenotypes may coexist with the finding of agenesis. The findings of agenesis in several of our affected subjects is supported by previous studies that have reported a significant association between short roots and tooth agenesis, with one study even reporting as high as a 46% association (Apajalahti et al., 2002; Apajalahti et al., 1999, Shaw et al., 1995).

Pulp stones were noted in three subjects. In Family 1 Subject II:1, pulp stones were noted in maxillary molars #3, 14, and 15. In Family 3 Subject II:2, pulp stone was noted in mandibular molar #30. In Family 5 Subject V:1, pulp stones was noted in maxillary molar #3. Other significant pulpal findings include the presence of what appears to be generalized pulpal obliteration in Family 3 Subject II:2, who was classified as having a generalized case of short

root anomaly. A previous case report of a patient with severe short limbed dwarfism reported the observation of obliterated pulp chambers in addition to generalized short roots and agenesis (Shaw et al., 1995). Interestingly, our subject (Family 3 Subject II:2) was found to also have generalized short roots and pulpal obliteration. However, our subject has reported no significant health concerns and is therefore considered as having a non-syndromic case of short root anomalies.

Several other dental anomalies were noted during phenotypic analysis of our subjects. In Family 2 Subject III:5, classified as having a localized case of short roots, we also noted a peg lateral on #10 and invaginatus on #8. In Family 4 Subject II:2, hypercementosis was noted on the roots of #3, 14, 18, 30, and 31. In Family 5 Subject V:1, ectopic eruption of upper left maxillary canine (#11) was noted. These findings are consistent with previous studies that report the incidence of peg shaped teeth, invaginations, ectopically positioned teeth, mesiodens, and other dental anomalies are higher in patients with affected short root anomalies (Apajalahti et al., 2002; Apajalahti et al., 1999).

Summary of Phenotypic Conclusions

1. The most frequently affected teeth with short roots in this study are maxillary central incisors, maxillary first premolars, and maxillary second premolars. These teeth appeared to affect subjects with both localized and generalized cases. This supports previous studies that report these tooth types as most commonly affected by SRA.

2. Other tooth types appeared to be affected by short roots, but were only seen in subjects with generalized cases of short root anomalies. These teeth include maxillary lateral incisors, maxillary canines, maxillary second molars, mandibular central incisors, mandibular lateral incisors, mandibular canines, mandibular first premolars, mandibular second premolars, mandibular first molars, and mandibular second molars.

3. Our findings, using a formula to quantifiably assess the "wide root width" or "plump, onion shape" characteristic supported Lind (1972)'s report that maxillary central incisors affected by short roots tend to have this characteristic shape.

4. Wide root widths were seen in mandibular central incisors in three subjects, one who had a localized case of SRA while the other two cases were classified as generalized cases of SRA. The finding of a wide root width or characteristic "plump, onion shape" in affected mandibular central incisors with short roots has not been reported previously.

5. Our findings from the study support previous studies that have suggested taurodontism and short roots may occur together.

6. Missing teeth/agenesis was noted in four of seven subjects. Of the four subjects, only one subject was classified as a localized case of SRA while the other three were generalized cases.

7. Pulp stones were noted in one subject with a localized case of SRA, and in two subjects with generalized cases of SRA. Generalized pulpal obliteration was noted in one subject with a generalized case of short roots.

8. Several other dental anomalies were noted during phenotypic analysis of all our affected subjects. These include peg shaped teeth, invaginations, hypercementosis, and ectopically positioned teeth.

There appears to be a phenotypic difference between localized and generalized SRA. Localized SRA is generally limited to maxillary teeth, specifically, maxillary central incisors and 1st and 2nd premolars. The results seem to indicate that SRA localized and generalized cases may have different etiologies, therefore, representing different disease entities.

2. Limitations

The study has a significant limitation that includes having a small number of study families with a limited number of available family members for enrollment. Our study participants were recruited from the United States and Chile. The small number of participants drawn from two different countries limits the generalizability of our results to a particular population. It is also difficult to determine the relative size of the genetic effect, as there are many known factors, which can be sources of variation in disease risk such as environmental effects, which may vary across the different geographic regions. Due to the limited number of study participants, it is also difficult to draw correlations with the potential role of ethnicities and gender and the prevalence of short root anomalies as reported in previous literature. Further, only two of the five families had more than one affected family member for analysis. Therefore, it is difficult to draw conclusions or comment on the mode of inheritance based on the limited number of recruited subjects. It is important to note, however, that both subjects in Family 1 USSO had localized cases and both subjects in Family 2 USRE also had localized cases. Therefore, it is possible that there is a genetic component in determining whether short root anomalies present similarly within the same family.

Another limitation is in the inconsistencies with quality and number of radiographs, clinical photos, and diagnostic information obtained across the families/subjects. For instance, some available radiographs were not of reasonable quality to allow for measurements and this was noted in our raw data tables. Additionally, we used panoramic radiographs rather than periapical radiographs for phenotype characterizations. If periapical radiographs were used instead, we might expect more precise measurements when compared to measurements taken

from a panoramic radiograph. Phenotypic assessment was carefully completed with the data and diagnostic information available. Two independent raters agreed on whether various phenotypic features were present or not, but neither raters were able to complete individual clinical examinations to confirm or negate agreed findings. Only one rater completed measurements at time point 1 and time point 2 (two weeks later), therefore measurements have only intra-rater reliability but no external validity or inter-rater reliability. Information on dental history and past dental treatment was also not readily available to us, so it is unclear whether some teeth were congenitally missing or due to past treatment rendered. Due to the inconsistencies in diagnostic information, it is possible that additional clinical features may be present and undocumented in this study.

The absence of a comprehensive and standard medical assessment across all subjects may have led to an inaccurate diagnosis of non-syndromic short root anomalies. All subjects are presumed to be healthy based on self-reporting, however, it is possible that medical histories reported were not complete or accurate. This would be especially significant in determining whether there is a mutual etiology between subjects that exhibit localized cases of short roots versus those with generalized cases of short root anomalies. It is possible that subjects in our study population identified as having non-syndromic short root anomalies have subclinical characteristics that would suggest a systemic etiology.

3. Future Directions

From this pilot study, we learned that a systematic approach to define specific phenotype associated with each SRA case is critical. It is our impression that SRA localized and

generalized cases may have different etiologies, therefore, representing different disease entities. The contributions made by this study included the establishment of an assessment scheme for phenotype characterization. Additionally, those five fully characterized study families are valuable resources for cohort analysis when additional SRA families are recruited and their sequence data available for comparisons.

As with all genetic disorders, the quest to determine causality has to begin with proper characterization of phenotype, which will allow distinguishing cases with similar phenotypic presentations but different disease entities. The more consistently accurate the clinical diagnosis can be determined for a case, the more likely it will be to determine the genetic etiology of the case.

We considered this study a pilot study with objectives to establish the 1) enrollment and characterization protocol, 2) phenotypic assessment scheme, and 3) sequencing data analysis strategies. The logical follow up study will include enrollment and characterization of additional families with SRA, both generalized and localized types, and cohort analysis of sequencing data from all affected individuals who presented with similar if not the same clinical phenotypic features of SRA.

Chapter VI

Conclusion

In this study, there were no shared variants in genes across the subjects across the five affected families. We identified variants of gene *THAP11* in four of the five probands which are rare sequence variants that may impact gene function. However, further study of the expression of *THAP11* during tooth development is necessary in order to determine its role on tooth root formation. Additionally, we found 22 additional unique genes with potentially damaging variants shared by at least two of the five study families. However, after cross checking these candidates with the sequencing results of additional affected individuals in Family 1, we were unable to produce any logical candidates for further segregation analysis. Because this is a pilot study in exploring the genetic etiology of short root anomalies, there is ongoing active recruitment for subsequent studies of subjects with both localized and generalized expression of short roots. Findings from genotypic analysis in subsequent studies may be able to reference and draw from the identification of unique genes and variants reported in this current study.

We were able to develop a protocol for phenotypic analysis of our subjects across the families that can be referenced in subsequent studies/future directions. We were able to apply existing criteria from previous literature, make modifications to existing indices, and create quantifiable metrics to characterize phenotypic characteristics such as classifying localized vs. generalized cases, short root length, wide root widths, taurodontism, and other significant dental anomalies.

In subjects with localized cases of short root anomalies, we observed the anomaly in maxillary central incisors, first premolars, second premolars, and first molars. Other tooth types were

affected by short roots, but this was only seen in subjects with generalized cases of short roots. We also observed that the characteristic of wide root widths affected both maxillary and mandibular central incisors in both localized and generalized cases of short root anomalies. Taurodontism was noted in the second molars of subjects with both localized and generalized cases of short roots. Other dental anomalies, such as agenesis, peg lateral, invaginatus, ectopic eruption, and pulpal findings, were noted in subjects with both localized and generalized cases of short roots, supporting previous literature that suggests short root anomalies tend to co-exist with other dental anomalies.

Phenotypic variability among the subjects and families recruited in our study suggest that there may be multiple causative genetic and epigenetic factors at play that determine whether short root anomalies are expressed in a localized or generalized manner, and additional studies should continue to explore these etiologies.

Chapter VII

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Chapter VIII

Tables

Table 1A. Candidate heterozygous variants in II:2 USSONO (affected 2nd child) of Family 1

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
1	1007255	G	C	*RNF223*	missense	exon	NM_001205252.2:c.692C>G:p.Pro231Arg	Damaging (0.006)	N/A	19.47	0.00420806	rs534034872
1	6529186	TC	-	*PLEKHG5*	frameshift	exon	NM_020631.6:c.2164_2165delGA:p.Glu722Glyfs*63	N/A	N/A	N/A	N/A	N/A
1	6529188	C	-	*PLEKHG5*	frameshift	exon	NM_020631.6:c.2163delG:p.Glu722Argfs*43	N/A	N/A	N/A	N/A	N/A
1	33960171	T	G	*ZSCAN20*	missense	exon	NM_001377376.1:c.2227T>G:p.Cys743Gly	N/A	Probably damaging (0.978)	26.3	0.00046361	rs35642856
1	36752142	A	G	*THRAP3*	missense	exon	NM_005119.4:c.311A>G:p.Tyr104Cys	Damaging (0.002)	Probably damaging (0.998)	26.7	N/A	N/A
1	87045902	ACCTACT	-	*CLCA4*	frameshift	exon	NM_012128.4:c.2634_2640delACCTACT:p.Pro879Leufs*24	N/A	N/A	N/A	N/A	N/A
1	89448809	T	C	*RBMXL1*	missense	exon	NM_001162536.3:c.701A>G:p.Tyr234Cys	Damaging (0)	Probably damaging (0.996)	24	0.00382183	rs139713926
1	114340502	C	T	*RSBN1*	missense	exon	NM_018364.5:c.860G>A:p.Arg287His	Damaging (0.009)	Probably damaging (0.999)	23.7	0.00125507	rs41283514
1	144930589	C	T	*PDE4DIP*	missense	exon	NM_001350520.1:c.1120G>A:p.Val374Ile	N/A	N/A	N/A	0.00000398	rs782810306
1	153907279	CTGCTGCT GCTGCTG CTGCTGCT GCTGT	-	*DENND4B*	frameshift	exon	NM_014856.3:c.2703_2730del:p.Gln902Serfs*38	N/A	N/A	N/A	N/A	N/A
1	155019738	T	C	*DCST1*	missense	exon	NM_152494.4:c.1562T>C:p.Ile521Thr	Damaging (0.002)	Probably damaging (1)	26.1	0.00021076	rs139043706
1	160168756	C	A	*CASQ1*	missense	exon	NM_001231.5:c.897C>A:p.Phe299Leu	Damaging (0.017)	Probably damaging (1)	25.2	0.00002386	rs751160652
1	200867572	T	A	*INAVA*	missense	exon	NM_001142569.3:c.44T>A:p.Ile15Asn	Damaging (0)	Probably damaging (0.998)	27.9	0.00751174	rs41269923
1	201175684	AA	-	*IGFN1*	frameshift	exon	NM_001164586.2:c.1664_1665delAA:p.Lys555Serfs*61	N/A	N/A	N/A	0.00020927	rs778348997

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
2	50574009	CGC	-	*NRXN1*	inframe deletion	exon	NM_001135659.3:c.3485-109903_3485-109901delGCG	N/A	N/A	N/A	0.00090727	rs750165040
2	233272091	C	T	*ALPG*	missense	exon	NM_031313.3:c.280C>T:p.Pro94Ser	Damaging (0.012)	Probably damaging (0.994)	23.3	0.00653363	rs146482704
2	237076427	GGCGGCG GC	-	*GBX2*	inframe deletion	exon	NM_001485.4:c.180_188delGCCGCCGCC:p.Pro61_Pro63del	N/A	N/A	N/A	0.00229971	rs545073704
3	37458926	G	A	*C3orf35*	missense	exon	NM_178339.2:c.169G>A:p.Gly57Ser	N/A	N/A	N/A	0.00416312	rs150550724
3	101370529	T	A	*ZBTB11*	splice acceptor	intron	NM_014415.4:c.2645-2A>T	N/A	N/A	35	N/A	rs1488357348
3	121342050	A	G	*FBXO40*	missense	exon	NM_016298.4:c.1774A>G:p.Ser592Gly	Damaging (0.003)	Probably damaging (0.998)	25.1	N/A	rs1325477603
4	1656925	C	T	*FAM53A*	missense	exon	NM_001013622.3:c.662G>A:p.Arg221His	Damaging (0.007)	Probably damaging (1)	23.4	0.00006097	rs370251492
4	89020529	G	A	*ABCG2*	missense	exon	NM_004827.3:c.1439C>T:p.Pro480Leu	Damaging (0.002)	Probably damaging (0.985)	24.6	0.00011619	rs202192122
5	54456860	CA	-	*GPX8*	frameshift	exon	NM_001008397.4:c.245_246delCA:p.Thr82Argfs*59	N/A	N/A	N/A	N/A	rs1283449938
6	7231682	C	T	*RREB1*	missense	exon	NM_001003699.4:c.3350C>T:p.Pro1117Leu	Damaging (0.03)	Probably damaging (1)	22.2	0.00015756	rs780852600
6	26638306	T	C	*ZNF322*	missense	exon	NM_024639.5:c.476A>G:p.Glu159Gly	Damaging (0.009)	Probably damaging (0.966)	24.7	0	rs1327527568
6	31324494	AGGT	-	*HLA-B*	frameshift	exon	NM_005514.8:c.311_314delACCT:p.Asn104Serfs*46	N/A	N/A	N/A	N/A	rs746718295
6	31914966	G	A	*CFB*	missense	exon	NM_001710.6:c.481G>A:p.Gly161Arg	Damaging (0.002)	Probably damaging (1)	28.7	0.00005277	rs781667620
6	32551962	C	-	*HLA-DRB1*	frameshift	exon	NM_002124.4:c.294delG:p.Glu98Aspfs*31	N/A	N/A	N/A	N/A	N/A
6	44329579	C	T	*SPATS1*	missense	exon	NM_001372081.1:c.424C>T:p.Arg142Cys	Damaging (0.002)	Probably damaging (1)	29.3	0.00161095	rs145942181

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
6	131186722	A	C	*EPB41L2*	missense	exon	NM_001431.4:c.2783T>G:p.Ile928Ser	Damaging (0)	Probably damaging (1)	29.7	0.00001193	rs149169560
7	99654642	G	A	*ZSCAN21*	missense	exon	NM_145914.3:c.13G>A:p.Val5Ile	Damaging (0.004)	Probably damaging (0.976)	20.8	0.00134934	rs141613498
7	107601664	G	A	*LAMB1*	missense	exon	NM_002291.3:c.2096C>T:p.Thr699Met	Damaging (0.014)	Probably damaging (1)	25.5	0.00001591	rs565604685
7	131241034	ACGGCGACGGCGACGG	-	*PODXL*	frameshift	exon	NM_001018111.3:c.70_85del:p.Pro24Argfs*138	N/A	N/A	N/A	N/A	N/A
8	10467554	C	A	*RP1L1*	stop gained	exon	NM_178857.6:c.4054G>T:p.Glu1352Ter	N/A	N/A	34	N/A	N/A
8	110396323	A	G	*PKHD1L1*	missense	exon	NM_177531.6:c.442A>G:p.Ile148Val	Damaging (0.021)	Probably damaging (0.995)	22.9	N/A	N/A
9	17135271	-	G	*CNTLN*	frameshift	exon	NM_017738.4:c.214dupG:p.Ala72Glyfs*51	N/A	N/A	N/A	0.00180671	rs578214691
9	98270634	-	GA	*PTCH1*	frameshift	exon	NM_000264.5:c.10_11insTC:p.Ala4Valfs*77	N/A	N/A	N/A	N/A	N/A
9	133302750	G	A	*HMCN2*	missense	exon	NM_001291815.2:c.12418G>A:p.Ala4140Thr	N/A	N/A	11.27	0.00167844	rs553134249
9	136804316	G	A	*VAV2*	missense	exon	NM_001134398.2:c.230C>T:p.Thr77Ile	Damaging (0.014)	Probably damaging (1)	26.4	N/A	N/A
9	139731827	G	A	*RABL6*	missense	exon	NM_024718.5:c.839G>A:p.Arg280His	Damaging (0.002)	Probably damaging (1)	25.7	0.00305479	rs200704265
10	1284303	C	T	*ADARB2*	missense	exon	NM_018702.4:c.1252G>A:p.Gly418Ser	Damaging (0.004)	Probably damaging (1)	24.2	0.00450774	rs146452150
10	16996468	GA	-	*CUBN*	frameshift	exon	NM_001081.4:c.4774_4775delTC:p.Ser1592Leufs*38	N/A	N/A	N/A	N/A	N/A
10	45799077	A	C	*OR13A1*	missense	exon	NM_001004297.3:c.794T>G:p.Val265Gly	Damaging (0)	Probably damaging (1)	23.6	N/A	N/A
10	50530703	G	A	*C10orf71*	missense	exon	NM_001135196.2:c.113G>A:p.Arg38Gln	Damaging (0.008)	Probably damaging (1)	26	0.00011682	rs199898708

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
10	50960753	-	G	*OGDHL*	frameshift	exon	NM_018245.3:c.409dupC:p.Leu137Profs*21	N/A	N/A	N/A	N/A	rs1363127112
10	75675086	T	C	*PLAU*	missense	exon	NM_002658.6:c.1048T>C:p.Tyr350His	Damaging (0.002)	Probably damaging (1)	25	0.00391449	rs72816325
10	88681437	C	T	*BMPR1A*	missense	exon	NM_004329.3:c.1327C>T:p.Arg443Cys	Damaging (0)	Probably damaging (1)	31	0.00067597	rs35619497
10	99211549	G	C	*ZDHHC16*	missense	exon	NM_198046.3:c.117G>C:p.Trp39Cys	Damaging (0.017)	Probably damaging (1)	23.4	0.00001599	rs766784631
11	5373519	G	T	*OR51B6*	missense	exon	NM_001004750.1:c.782G>T:p.Arg261Met	Damaging (0)	Probably damaging (1)	26	0.00084418	rs145119951
11	51411918	C	T	*OR4A5*	missense	exon	NM_001005272.3:c.478G>A:p.Val160Met	N/A	N/A	N/A	0.00711745	rs150995059
11	57003767	C	T	*APLNR*	missense	exon	NM_005161.5:c.712G>A:p.Glu238Lys	N/A	Probably damaging (0.986)	23.8	0.00002789	rs745562987
11	73753106	A	G	*C2CD3*	missense	exon	NM_015531.6:c.5653T>C:p.Ser1885Pro	Damaging (0.012)	Probably damaging (0.993)	26.5	0.00261032	rs142277857
11	101833267	AAA	-	*CEP126*	inframe deletion	exon	NM_020802.4:c.1501_1503delAAA:p.Lys501del	N/A	N/A	N/A	0.00442368	rs529950842
11	111958677	A	G	*SDHD*	missense	exon	NM_003002.4:c.149A>G:p.His50Arg	Damaging (0)	Probably damaging (0.985)	23.4	0.00660542	rs11214077
12	2224449	G	A	*CACNA1C*	missense	exon	NM_000719.7:c.109G>A:p.Gly37Arg	Damaging (0)	Probably damaging (1)	26	0.00346439	rs34534613
12	91449302	C	A	*KERA*	missense	exon	NM_007035.4:c.757G>T:p.Gly253Cys	Damaging (0)	Probably damaging (0.996)	26.4	N/A	N/A
13	100953715	T	G	*PCCA*	missense	exon	NM_000282.4:c.1067T>G:p.Val356Gly	Damaging (0)	Probably damaging (1)	32	N/A	N/A
13	103395207	T	A	*CCDC168*	missense	exon	NM_001146197.3:c.7840A>T:p.Asn2614Tyr	N/A	N/A	5.691	N/A	N/A
14	73989211	G	A	*HEATR4*	stop gained	exon	NM_001220484.1:c.646C>T:p.Arg216Ter	N/A	N/A	35	0.00034639	rs149734041

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
14	100069631	GTG	-	*CCDC85C*	inframe deletion	exon	NM_001144995.2:c.664_666delCAC:p.His222del	N/A	N/A	N/A	0.00075000	rs1412395126
15	42364046	T	C	*PLA2G4D*	missense	exon	NM_178034.4:c.1499A>G:p.Glu500Gly	Damaging (0)	Probably damaging (0.999)	28	0.00145224	rs146739833
15	49584639	A	G	*GALK2*	missense	exon	NM_002044.4:c.872A>G:p.His291Arg	Damaging (0.021)	Probably damaging (0.99)	25.5	0.00005969	rs777255659
15	57731371	G	C	*CGNL1*	missense	exon	NM_032866.5:c.1174G>C:p.Ala392Pro	Damaging (0.003)	Probably damaging (1)	27.1	0.00000398	rs1412714858
16	336701	CT	-	*PDIA2*	frameshift	exon	NM_006849.4:c.1391_1392delTC:p.Leu464Glnfs*13	N/A	N/A	N/A	0.00696536	rs201624048
16	904669	G	A	*LMF1*	missense	exon	NM_022773.4:c.1567C>T:p.Arg523Cys	Damaging (0.014)	Probably damaging (0.996)	31	0.00003467	rs758116895
16	2835406	C	G	*PRSS33*	missense	exon	NM_152891.3:c.484G>C:p.Val162Leu	Damaging (0)	Probably damaging (0.967)	24.1	0.00005878	rs769756854
16	3406185	T	C	*OR2C1*	missense	exon	NM_012368.3:c.245T>C:p.Leu82Pro	Damaging (0)	Probably damaging (1)	26.3	0.00080732	rs149625259
16	86612826	CGGAGGCGCAGC	-	*FOXL1*	inframe deletion	exon	NM_005250.3:c.505_516delCAGCCGGAGGCG:p.Gln169_Ala172del	N/A	N/A	N/A	0.00007878	rs749019038
16	90103716	G	A	*GAS8*	missense	exon	NM_001481.3:c.833G>A:p.Arg278His	Damaging (0.003)	Probably damaging (1)	25.7	0.00768169	rs117053233
17	4722498	C	T	*PLD2*	missense	exon	NM_002663.5:c.2293C>T:p.Arg765Trp	Damaging (0.003)	Probably damaging (1)	25.9	0.00001195	rs746497873
17	6555534	A	G	*C17orf100*	missense	exon	NM_001105520.2:c.301A>G:p.Ser101Gly	N/A	Probably damaging (0.994)	24.3	0.00211861	rs200915782
17	6905131	G	A	*ALOX12*	splice donor	intron	NM_000697.3:c.1161+1G>A	N/A	N/A	34	0.00000399	rs756762345
17	38249487	G	A	*NR1D1, THRA*	missense	exon	NM_021724.5:c.1694C>T:p.Thr565Met, NM_003250.6:c.1325G>A:p.Arg442His	Damaging, Damaging (0)	Probably damaging, Probably damaging (1)	25.1, 25.1	0.00003189	rs201684407

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
17	51901797	G	A	*KIF2B*	missense	exon	NM_032559.5:c.1403G>A:p.Gly468Glu	Damaging (0)	Probably damaging (1)	28.6	0.00658759	rs116955880
17	56056607	C	-	*VEZF1*	frameshift	exon	NM_007146.3:c.1044delG:p.Gln348Hisfs*9	N/A	N/A	N/A	0.00036898	rs746343850
17	56056609	GC	-	*VEZF1*	frameshift	exon	NM_007146.3:c.1041_1042delGC:p.Gln348Alafs*27	N/A	N/A	N/A	0.00009867	rs775689252
17	60469326	C	T	*EFCAB3*	stop gained	exon	NM_173503.4:c.295C>T:p.Arg99Ter	N/A	N/A	33	0.00773503	rs61751980
18	32843992	G	A	*ZSCAN30*	stop gained	exon	NM_001112734.4:c.325C>T:p.Arg109Ter	N/A	N/A	33	0.00019167	rs201609495
18	44625640	G	A	*KATNAL2*	missense	exon	NM_001387690.1:c.1238G>A:p.Arg413His	Damaging (0)	Probably damaging (1)	32	0.00000399	rs770137252
19	632849	C	T	*POLRMT*	missense	exon	NM_005035.4:c.178G>A:p.Val60Met	N/A	Probably damaging (0.967)	19.55	0.00345162	rs139758373
19	4550183	G	A	*SEMA6B*	missense	exon	NM_032108.4:c.1223C>T:p.Ala408Val	N/A	Probably damaging (1)	28.3	0.00106998	rs142864702
19	17397500	TTTG	-	*ANKLE1*	frameshift	exon	NM_152363.6:c.*140_*143delTTGT	N/A	N/A	N/A	N/A	N/A
19	55591633	G	T	*EPS8L1*	missense	exon	NM_133180.3:c.416G>T:p.Gly139Val	Damaging (0.024)	Probably damaging (1)	26.6	0.00025060	rs371608469
19	55865253	-	G	*COX6B2*	frameshift	exon	NM_144613.5:c.194dupC:p.Leu66Alafs	N/A	N/A	N/A	0.00170866	rs576407591
19	58213743	G	A	*ZNF154*	stop gained	exon	NM_001085384.3:c.574C>T:p.Arg192Ter	N/A	N/A	24.3	0.00912947	rs74939505
19	58907569	AGG	-	*RNF225*	inframe deletion	exon	NM_001195135.2:c.132_134delGGA:p.Glu45del	N/A	N/A	N/A	0.00348905	rs747436338
20	56793724	G	A	*ANKRD60*	missense	exon	NM_001304369.1:c.865C>T:p.Pro289Ser	Damaging (0.037)	N/A	23.7	0.00677881	rs41275658
20	62493176	-	GGCCAGCTCGCTAGCTACGCGCT	*ABHD16B*	frameshift	exon	NM_080622.4:c.287_309dup:p.His104Serfs*23	N/A	N/A	N/A	0.00374866	rs539544290

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
22	39537403	G	C	*CBX7*	missense	exon	NM_175709.5:c.152C>G:p.Pro51Arg	Damaging (0.016)	Probably damaging (0.992)	24.5	N/A	N/A
22	44527492	C	T	*PARVB*	missense	exon	NM_013327.5:c.502C>T:p.Arg168Trp	Damaging (0)	Probably damaging (0.999)	26.9	0.00002400	rs144931273
X	228249	-	TGGA	*GTPBP6*	frameshift	exon	NM_012227.3:c.272_275dupTCCA	N/A	N/A	N/A	0.00005214	rs748253388
X	48419200	G	A	*TBC1D25*	missense	exon	NM_002536.4:c.1904G>A:p.Arg635His	Damaging (0.012)	Probably damaging (0.989)	23.6	0.00967106	rs41307344

Table 1B. Candidate homozygous variants in II:2 USSONO (affected 2nd child) of Family 1 USSO

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
5	79950708	TGCAGCGGCC	-	*MSH3*	frameshift	exon	NM_002439.5:c.162_171delTGCAGCGGCC:p.Ala55Glnfs*22	N/A	N/A	N/A	N/A	N/A
6	31324525	CCT	-	*HLA-B*	disruptive inframe deletion	exon	NM_005514.8:c.281_283delAGG:p.Gln94_Ala95delinsPro	N/A	N/A	N/A	N/A	N/A
11	1651643	C	-	*KRTAP5-5*	frameshift	exon	NM_001001480.2:c.573delC:p.Tyr192Thrfs	N/A	N/A	N/A	N/A	N/A
21	46057625	TGTCTGCTGTGTGCCC	-	*KRTAP10-10*	frameshift	exon	NM_181688.3:c.291_306del:p.Val98Serfs	N/A	N/A	N/A	N/A	N/A

Table 2A. Candidate heterozygous variants in II:2 (affected mother) of Family 2 (USRE)

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
1	12067224	C	T	*MFN2*	missense	exon	NM_014874.4:c.1987C>T:p.Arg663Cys	Damaging (0.001)	Probably damaging (1)	27.1	0.00017501	rs369762154
1	37291244	C	T	*GRIK3*	missense	exon	NM_000831.4:c.1714G>A:p.Ala572Thr	Damaging (0.001)	Probably damaging (1)	26.1	0.00000411	rs1463357168
1	45965038	GATACAGCCACTGCTACAGAAGAAACAGATGCACAGGCCAATGAGCTCTGTGGAGACCCCAAGAG	-	*CCDC163*	frameshift	exon	NM_001102601.3:c.92_156del:p.Pro31Leufs*9	N/A	N/A	N/A	0.00210472	N/A
1	53793513	CAGCA	-	*LRP8*	frameshift	exon	NM_004631.5:c.68_72delTGCTG:p.Leu23Profs*11	N/A	N/A	N/A	N/A	N/A
1	55282719	C	T	*LEXM*	missense	exon	NM_001110533.2:c.1108C>T:p.Arg370Trp	Damaging (0.017)	Probably damaging (1)	21.6	0.00015910	rs146890884
1	84962054	G	A	*RPF1*	splice donor	intron	NM_025065.7:c.1008+1G>A	N/A	N/A	34	N/A	N/A
1	109839501	C	T	*MYBPHL*	missense	exon	NM_001010985.3:c.634G>A:p.Gly212Ser	Damaging (0)	Probably damaging (1)	25.6	0.00031018	rs140827712
1	153233536	-	CGG	*LORICRIN*	inframe insertion	exon	NM_000427.3:c.121_123dupGGC:p.Gly41dup	N/A	N/A	N/A	0.00277080	rs770195151
1	153907279	CTGCTGCTGCTGCTGCTGCTGCTGCTGT	-	*DENND4B*	frameshift	exon	NM_014856.3:c.2703_2730del:p.Gln902Serfs*38	N/A	N/A	N/A	N/A	N/A
1	155150602	G	A	*TRIM46*	missense	exon	NM_025058.5:c.1034G>A:p.Arg345His	Damaging (0.012)	Probably damaging (0.996)	25.6	0.00108926	rs143154805
1	171605478	G	A	*MYOC*	stop gained	exon	NM_000261.2:c.1102C>T:p.Gln368Ter	N/A	N/A	37	0.00110977	rs74315329
1	186375340	G	A	*ODR4*	missense	exon	NM_017847.6:c.1126G>A:p.Glu376Lys	Damaging (0.003)	Probably damaging (0.996)	27	0.00058734	rs201764660
1	206858676	-	GCC	*MAPKAPK2*	inframe insertion	exon	NM_032960.4:c.114_116dupGCC:p.Pro40dup	N/A	N/A	N/A	0.00240874	rs782493788

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
1	207760805	G	A	*CR1*	missense	exon	NM_000651.6:c.5605G>A:p.Gly1869Arg	Damaging (0)	Probably damaging (1)	22.9	0.00032112	rs191896925
2	1204916	G	T	*SNTG2*	missense	exon	NM_018968.4:c.719G>T:p.Arg240Met	Damaging (0)	Probably damaging (1)	35	N/A	rs201301696
2	9013424	C	T	*MBOAT2*	missense	exon	NM_138799.4:c.697G>A:p.Val233Ile	Damaging (0.003)	Probably damaging (0.99)	23.2	0.00466075	rs34573615
2	11931913	T	C	*LPIN1*	splice donor	intron	NM_145693.4:c.1606-127T>C	N/A	N/A	22.1	N/A	N/A
2	75118042	C	T	*HK2*	missense	exon	NM_000189.5:c.2728C>T:p.Arg910Cys	Damaging (0)	Probably damaging (1)	29.1	0.00000398	rs372862075
2	144709553	T	C	*GTDC1*	missense	exon	NM_001376312.2:c.1289A>G:p.Tyr430Cys	Damaging (0.01)	Probably damaging (1)	25.7	N/A	rs902414666
2	175202200	GCG	-	*SP9*	inframe deletion	exon	NM_001145250.2:c.1407_1409delGGC:p.Ala470del	N/A	N/A	N/A	0.00082528	rs746223726
2	176944986	CCG	-	*EVX2*	inframe deletion	exon	NM_001080458.1:c.1278_1280delCGG:p.Gly428del	N/A	N/A	N/A	0.00234082	rs765360165
2	196602773	G	A	*DNAH7*	missense	exon	NM_018897.3:c.11947C>T:p.Arg3983Trp	Damaging (0)	Probably damaging (1)	27.5	0.00829924	rs114621989
2	203019499	G	A	*KIAA2012*	missense	exon	NM_001277372.4:c.2012G>A:p.Arg671His	Damaging (0.007)	Probably damaging (1)	23.3	0.00015569	rs182994074
2	241500286	G	-	*DUSP28*	frameshift	exon	NM_001370465.2:c.185delG:p.Gly62Alafs*39	N/A	N/A	N/A	N/A	N/A
3	51422742	-	GGA	*MANF*	initiator codon	exon	NM_006010.6:c.-2_1dupGGA:p.Met1insArg	N/A	N/A	N/A	0	rs1328383326, rs1553620634
3	63898391	-	GCC	*ATXN7*	inframe insertion	exon	NM_001377405.1:c.123_125dupGCC:p.Pro43dup	N/A	N/A	N/A	0.00570556	rs1553686135
3	157840045	G	A	*RSRC1*	stop gained	exon	NM_001271838.2:c.152G>A:p.Trp51Ter	N/A	N/A	38	N/A	N/A
3	183906916	C	T	*ABCF3*	missense	exon	NM_018358.2:c.1018C>T:p.Arg340Trp	Damaging (0)	Probably damaging (1)	29.4	0.00149310	rs150245456

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
5	14465657	T	C	*TRIO*	missense	exon	NM_007118.4:c.5671T>C:p.Ser1891Pro	Damaging (0.044)	Probably damaging (0.999)	25.8	0.00032610	rs138840811
5	16179589	CGG	-	*MARCHF11*	inframe deletion	exon	NM_001102562.3:c.94_96delCCG:p.Pro32del	N/A	N/A	N/A	0	rs1017917272
5	98109867	GCT	-	*RGMB*	inframe deletion	exon	NM_001366508.1:c.109_111delCTG:p.Leu37del	N/A	N/A	N/A	0.00469968	rs770214076
5	140032593	GA	-	*IK*	frameshift	exon	NM_006083.4:c.281_282delAG:p.Glu94Alafs*21	N/A	N/A	N/A	0.00009555	rs747924967
5	150723155	C	A	*SLC36A2*	missense	exon	NM_181776.3:c.260G>T:p.Gly87Val	Damaging (0)	Probably damaging (1)	25.1	0.00935063	rs77010315
6	16327916	-	TGC	*ATXN1*	inframe insertion	exon	NM_001128164.2:c.624_626dupGCA:p.Gln208dup	N/A	N/A	N/A	N/A	rs193922926
6	42930898	C	G	*CNPY3-GNMT, GNMT*	stop gained	exon	NM_001318857.2:c.357C>G:p.Tyr119Ter, NM_018960.6:c.540C>G:p.Tyr180Ter	N/A	N/A	34	0.00000399	rs147746130
6	43190330	C	T	*CUL9*	missense	exon	NM_015089.4:c.6983C>T:p.Ser2328Phe	Damaging (0.001)	Probably damaging (0.986)	24.8	0.00044699	rs41274936
6	109837169	C	G	*AK9*	missense	exon	NM_001145128.3:c.3956G>C:p.Arg1319Pro	Damaging (0)	Probably damaging (1)	28.2	0.00010171	rs369214077
6	159184508	G	A	*SYTL3*	missense	exon	NM_001242394.2:c.1690G>A:p.Asp564Asn	Damaging (0)	Probably damaging (1)	25	0.00004374	rs548865352
7	21582969	GAG	-	*DNAH11*	inframe deletion	exon	NM_001277115.2:c.118_120delGAG:p.Glu40del	N/A	N/A	N/A	0.00014851	rs754826899
7	100680297	-	C	*MUC17*	frameshift	exon	NM_001040105.2:c.5600dupC:p.Ala1868Cysfs*16	N/A	N/A	N/A	N/A	N/A
7	141765516	C	T	*MGAM*	missense	exon	NM_001365693.1:c.4655C>T:p.Thr1552Met	Damaging (0.041)	N/A	19.89	0.00172398	rs190699321
7	148851263	C	T	*ZNF398*	missense	exon	NM_170686.3:c.251C>T:p.Thr84Ile	Damaging (0.003)	Probably damaging (1)	23.3	N/A	N/A
7	155595750	-	GCC	*SHH*	inframe insertion	exon	NM_000193.4:c.1231_1233dupGGC:p.Gly411dup	N/A	N/A	N/A	0	rs769920627

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
5	14465657	T	C	*TRIO*	missense	exon	NM_007118.4:c.5671T>C:p.Ser1891Pro	Damaging (0.044)	Probably damaging (0.999)	25.8	0.00032610	rs138840811
5	16179589	CGG	-	*MARCHF11*	inframe deletion	exon	NM_001102562.3:c.94_96delCCG:p.Pro32del	N/A	N/A	N/A	0	rs1017917272
5	98109867	GCT	-	*RGMB*	inframe deletion	exon	NM_001366508.1:c.109_111delCTG:p.Leu37del	N/A	N/A	N/A	0.00469968	rs770214076
5	140032593	GA	-	*IK*	frameshift	exon	NM_006083.4:c.281_282delAG:p.Glu94Alafs*21	N/A	N/A	N/A	0.00009555	rs747924967
5	150723155	C	A	*SLC36A2*	missense	exon	NM_181776.3:c.260G>T:p.Gly87Val	Damaging (0)	Probably damaging (1)	25.1	0.00935063	rs77010315
6	16327916	-	TGC	*ATXN1*	inframe insertion	exon	NM_001128164.2:c.624_626dupGCA:p.Gln208dup	N/A	N/A	N/A	N/A	rs193922926
6	42930898	C	G	*CNPY3-GNMT, GNMT*	stop gained	exon	NM_001318857.2:c.357C>G:p.Tyr119Ter, NM_018960.6:c.540C>G:p.Tyr180Ter	N/A	N/A	34	0.00000399	rs147746130
6	43190330	C	T	*CUL9*	missense	exon	NM_015089.4:c.6983C>T:p.Ser2328Phe	Damaging (0.001)	Probably damaging (0.986)	24.8	0.00044699	rs41274936
6	109837169	C	G	*AK9*	missense	exon	NM_001145128.3:c.3956G>C:p.Arg1319Pro	Damaging (0)	Probably damaging (1)	28.2	0.00010171	rs369214077
6	159184508	G	A	*SYTL3*	missense	exon	NM_001242394.2:c.1690G>A:p.Asp564Asn	Damaging (0)	Probably damaging (1)	25	0.00004374	rs548865352
7	21582969	GAG	-	*DNAH11*	inframe deletion	exon	NM_001277115.2:c.118_120delGAG:p.Glu40del	N/A	N/A	N/A	0.00014851	rs754826899
7	100680297	-	C	*MUC17*	frameshift	exon	NM_001040105.2:c.5600dupC:p.Ala1868Cysfs*16	N/A	N/A	N/A	N/A	N/A
7	141765516	C	T	*MGAM*	missense	exon	NM_001365693.1:c.4655C>T:p.Thr1552Met	Damaging (0.041)	N/A	19.89	0.00172398	rs190699321
7	148851263	C	T	*ZNF398*	missense	exon	NM_170686.3:c.251C>T:p.Thr84Ile	Damaging (0.003)	Probably damaging (1)	23.3	N/A	N/A
7	155595750	-	GCC	*SHH*	inframe insertion	exon	NM_000193.4:c.1231_1233dupGGC:p.Gly411dup	N/A	N/A	N/A	0	rs769920627

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7	157333411	C	A	*PTPRN2*	missense	exon	NM_002847.5:c.3045G>T:p.Gln1015His	Damaging (0)	Probably damaging (1)	26.7	0.00603877	rs61757813
8	133053823	C	A	*OC90*	missense	exon	NM_001080399.3:c.293G>T:p.Gly98Val	Damaging (0)	Probably damaging (1)	26.3	0.00077995	rs200563917
9	139964552	GCG	-	*SAPCD2*	inframe deletion	exon	NM_178448.4:c.359_361delCGC:p.Pro120del	N/A	N/A	N/A	0	rs930150269
10	72462171	G	A	*ADAMTS14*	missense	exon	NM_080722.4:c.626G>A:p.Arg209Gln	Damaging (0.008)	Probably damaging (1)	29.4	0.00083053	rs141259412
10	88259879	T	C	*WAPL*	missense	exon	NM_015045.5:c.1121A>G:p.Gln374Arg	Damaging (0.005)	Probably damaging (0.996)	26.6	0.00056187	rs140780773
10	99215755	G	A	*ZDHHC16*	missense	exon	NM_198046.3:c.973G>A:p.Gly325Ser	Damaging (0)	Probably damaging (1)	31	0.00001989	rs377074050
10	103919000	AGC	-	*NOLC1*	inframe deletion	exon	NM_004741.5:c.672_674delCAG:p.Ser227del	N/A	N/A	N/A	0.00001209	N/A
10	135371369	T	C	*SYCE1*	missense	exon	NM_001143764.3:c.373A>G:p.Arg125Gly	Damaging (0.009)	Probably damaging (0.994)	33	0.00209173	rs141668584
11	640058	CCCGGGGTCCCTGCG	-	*DRD4*	disruptive inframe deletion	exon	NM_000797.4:c.809_823delCCCGGGGTCCCTGCG:p.Pro270_Gly275delinsArg	N/A	N/A	N/A	N/A	N/A
11	640070	GCGGCCCCGAC	-	*DRD4*	frameshift	exon	NM_000797.4:c.821_831delGCGGCCCCGAC:p.Cys274Leufs	N/A	N/A	N/A	N/A	N/A
11	640081	TGTGCGCCCGCCGCGCCCAGCCTCCCCCAGGACCCCTGC	-	*DRD4*	inframe deletion	exon	NM_000797.4:c.832_870del:p.Cys278_Cys290del	N/A	N/A	N/A	N/A	N/A
11	640090	GCCGCGCCCAGCCTCCCCCAGGACCCCTGCGGCCCCGACTGTGCGCCCC	-	*DRD4*	frameshift	exon	NM_000797.4:c.841_889del:p.Ala281Profs*49	N/A	N/A	N/A	N/A	N/A
11	640099	A	-	*DRD4*	frameshift	exon	NM_000797.4:c.850delA:p.Ser284Alafs*62	N/A	N/A	N/A	N/A	N/A

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
11	1213048	-	AGCACAACCTCTGGTCCTGGAACTACTCCCAGCCCTGTTCCCACCACCAGCACAACCTCTGCTCCTATAACC	*MUC5AC*	inframe insertion	exon	NM_001304359.2:c.2230_2231ins(72):p.Pro743_Thr744ins24	N/A	N/A	N/A	N/A	N/A
11	1578747	C	-	*DUSP8*	frameshift	exon	NM_004420.3:c.879delG:p.Leu294Trpfs*7	N/A	N/A	N/A	N/A	N/A
11	7981706	-	TTTGGT	*NLRP10*	inframe insertion	exon	NM_176821.4:c.1448_1453dupACCAAA:p.Gln484_Ser485insAsnGln	N/A	N/A	N/A	0.00012726	rs764641531
11	61724367	ACA	-	*BEST1*	inframe deletion	exon	NM_004183.4:c.536_538delACA:p.Asn179del	N/A	N/A	N/A	0.00001591	rs775979290
12	1005682	C	T	*WNK1*	missense	exon	NM_018979.4:c.6029C>T:p.Pro2010Leu	Damaging (0.001)	Probably damaging (0.99)	25.1	0.00002388	rs764595104
12	10124287	G	A	*CLEC12A*	splice donor	intron	NM_138337.6:c.91+1G>A	N/A	N/A	32	0.00135684	rs148336258
12	53298582	C	A	*KRT8*	missense	exon	NM_002273.4:c.184G>T:p.Gly62Cys	Damaging (0.001)	Probably damaging (1)	22.2	0.00485043	rs11554495
12	108169270	A	G	*ASCL4*	missense	exon	NM_203436.3:c.275A>G:p.Tyr92Cys	Damaging (0)	Probably damaging (1)	32	0.00001112	rs1019945493
12	112167753	G	A	*ACAD10*	missense	exon	NM_025247.6:c.1387G>A:p.Asp463Asn	Damaging (0)	Probably damaging (1)	25.6	0.00404514	rs36046440
12	121432028	G	A	*HNF1A*	missense	exon	NM_000545.8:c.775G>A:p.Val259Ile	Damaging (0.017)	Probably damaging (0.994)	26.2	0.00001204	rs778074427
12	132547094	CAG	-	*EP400*	inframe deletion	exon	NM_015409.5:c.8223_8225delGCA:p.Gln2748del	N/A	N/A	N/A	N/A	rs528214697
13	25671804	GCT	-	*PABPC3*	inframe deletion	exon	NM_030979.3:c.1482_1484delTGC:p.Ala496del	N/A	N/A	N/A	0.00000800	rs150143049

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13	52343385	G	A	*DHRS12*	missense	exon	NM_001377533.1:c.604C>T:p.Arg202Cys	Damaging (0.004)	Probably damaging (0.999)	2.996	0.00009484	rs138384725
13	99378690	T	C	*SLC15A1*	missense	exon	NM_005073.4:c.35A>G:p.Tyr12Cys	Damaging (0)	Probably damaging (1)	26.1	0.00074437	rs144970844
14	63779773	C	T	*GPHB5*	missense	exon	NM_145171.4:c.259G>A:p.Glu87Lys	N/A	N/A	22.6	0.00688262	rs140208070
15	74032299	G	T	*INSYN1*	missense	exon	NM_001039614.3:c.841C>A:p.Pro281Thr	Damaging (0)	Probably damaging (1)	26.3	0.00004793	rs149206275
16	5105348	A	G	*C16orf89*	missense	exon	NM_001098514.3:c.767T>C:p.Met256Thr	Damaging (0.005)	Probably damaging (0.992)	24	N/A	rs943414474
16	11933743	C	A	*RSL1D1*	missense	exon	NM_015659.3:c.955G>T:p.Asp319Tyr	Damaging (0.024)	Probably damaging (0.996)	16.63	0.00720286	rs34999527
16	19710890	C	T	*VPS35L*	missense	exon	NM_020314.7:c.2713C>T:p.Arg905Cys	Damaging (0.008)	Probably damaging (1)	28.5	0.00880383	rs150300279
16	57509069	C	T	*DOK4*	missense	exon	NM_001330556.2:c.352G>A:p.Asp118Asn	Damaging (0.011)	Probably damaging (0.996)	22.8	0.00013528	rs111834942
17	17697096	G	-	*RAI1*	frameshift	exon	NM_030665.4:c.834delG:p.Gln278Hisfs*86	N/A	N/A	N/A	N/A	rs1296939152
17	17697098	AGC	-	*RAI1*	inframe deletion	exon	NM_030665.4:c.870_872delGCA:p.Gln291del	N/A	N/A	N/A	N/A	N/A
17	17697101	AGCAGCAGCAGCAG	-	*RAI1*	frameshift	exon	NM_030665.4:c.839_852delAGCAGCAGCAGCAG:p.Gln280Profs*104	N/A	N/A	N/A	N/A	N/A
17	38975104	-	GCTGCCGCCGCCGTATCCGCCGCCGGAGCT	*KRT10*	inframe insertion	exon	NM_000421.5:c.1654_1683dup:p.Gly556_Gly565dup	N/A	N/A	N/A	N/A	rs776920005
17	39623282	G	A	*KRT32*	missense	exon	NM_002278.3:c.296C>T:p.Thr99Ile	Damaging (0)	Probably damaging (1)	25	0.00149517	rs117304287
17	39968014	A	G	*P3H4*	missense	exon	NM_006455.3:c.154T>C:p.Trp52Arg	Damaging (0)	Probably damaging (0.989)	28.5	0.00000754	rs546832339

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18	61306960	G	-	*SERPINB4*	frameshift	exon	NM_002974.4:c.520delC:p.Leu174Trpfs*4	N/A	N/A	N/A	0.00122361	rs554627371
19	1567642	GGC	-	*MEX3D*	inframe deletion	exon	NM_203304.4:c.414_416delGCC:p.Pro139del	N/A	N/A	N/A	0.00222985	rs773372717
19	7019299	G	A	*MBD3L2B*	missense	exon	NM_001364674.2:c.488C>T:p.Pro163Leu	N/A	N/A	8.931	0.00624507	rs377522880
19	17397501	TTGTG	-	*ANKLE1*	frameshift	exon	NM_152363.6:c.*141_*145delTGTGT	N/A	N/A	N/A	N/A	N/A
19	33149861	G	A	*ANKRD27*	missense	exon	NM_032139.3:c.61C>T:p.Arg21Cys	Damaging (0.009)	Probably damaging (0.999)	27.2	0.00477561	rs147502994
19	36002371	-	CCACTGCTGCCG	*DMKN*	inframe insertion	exon	NM_033317.5:c.849_860dupCGGCAGCAGTGG:p.Gly288_Gly291dup	N/A	N/A	N/A	N/A	rs760401945
19	41173895	CTGT	-	*NUMBL*	frameshift	exon	NM_004756.5:c.1305_1308delACAG:p.Gln435Hisfs*149	N/A	N/A	N/A	N/A	N/A
19	41173898	TTGCTGTTGC	-	*NUMBL*	frameshift	exon	NM_004756.5:c.1296_1305delGCAACAGCAA:p.Gln432Hisfs*150	N/A	N/A	N/A	N/A	N/A
19	46274249	GGC	-	*DMPK*	inframe deletion	exon	NM_004409.5:c.1715_1717delGCC:p.Arg572del	N/A	N/A	N/A	0.00002070	rs1233738068
19	52034549	C	A	*SIGLEC6*	missense	exon	NM_001245.7:c.292G>T:p.Asp98Tyr	Damaging (0.001)	Probably damaging (1)	17.63	0.00880648	rs62617068
20	2464234	G	A	*ZNF343*	missense	exon	NM_024325.6:c.1373C>T:p.Thr458Met	Damaging (0.01)	Probably damaging (0.999)	14.08	0.00358725	rs146214742
20	31647696	G	T	*BPIFB3*	splice acceptor	intron	NM_182658.3:c.387-1G>T	N/A	N/A	33	0.00761933	rs11697151
21	40190408	C	A	*ETS2*	missense	exon	NM_005239.6:c.649C>A:p.Leu217Ile	Damaging (0.007)	Probably damaging (0.966)	13.35	0.00735136	rs61735785
21	45107549	G	A	*RRP1B*	missense	exon	NM_015056.3:c.1294G>A:p.Glu432Lys	N/A	Probably damaging (0.978)	16.56	0.00009292	rs575488096
21	45919715	G	C	*TSPEAR*	missense	exon	NM_144991.3:c.1961C>G:p.Ser654Cys	Damaging (0.021)	Probably damaging (1)	27.3	0.00000476	rs782551483

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
18	61306960	G	-	*SERPINB4*	frameshift	exon	NM_002974.4:c.520delC:p.Leu174Trpfs*4	N/A	N/A	N/A	0.00122361	rs554627371
19	1567642	GGC	-	*MEX3D*	inframe deletion	exon	NM_203304.4:c.414_416delGCC:p.Pro139del	N/A	N/A	N/A	0.00222985	rs773372717
19	7019299	G	A	*MBD3L2B*	missense	exon	NM_001364674.2:c.488C>T:p.Pro163Leu	N/A	N/A	8.931	0.00624507	rs377522880
19	17397501	TTGTG	-	*ANKLE1*	frameshift	exon	NM_152363.6:c.*141_*145delTGTGT	N/A	N/A	N/A	N/A	N/A
19	33149861	G	A	*ANKRD27*	missense	exon	NM_032139.3:c.61C>T:p.Arg21Cys	Damaging (0.009)	Probably damaging (0.999)	27.2	0.00477561	rs147502994
19	36002371	-	CCACTGCTGCCG	*DMKN*	inframe insertion	exon	NM_033317.5:c.849_860dupCGGCAGCAGTGG:p.Gly288_Gly291dup	N/A	N/A	N/A	N/A	rs760401945
19	41173895	CTGT	-	*NUMBL*	frameshift	exon	NM_004756.5:c.1305_1308delACAG:p.Gln435Hisfs*149	N/A	N/A	N/A	N/A	N/A
19	41173898	TTGCTGTTGC	-	*NUMBL*	frameshift	exon	NM_004756.5:c.1296_1305delGCAACAGCAA:p.Gln432Hisfs*150	N/A	N/A	N/A	N/A	N/A
19	46274249	GGC	-	*DMPK*	inframe deletion	exon	NM_004409.5:c.1715_1717delGCC:p.Arg572del	N/A	N/A	N/A	0.00002070	rs1233738068
19	52034549	C	A	*SIGLEC6*	missense	exon	NM_001245.7:c.292G>T:p.Asp98Tyr	Damaging (0.001)	Probably damaging (1)	17.63	0.00880648	rs62617068
20	2464234	G	A	*ZNF343*	missense	exon	NM_024325.6:c.1373C>T:p.Thr458Met	Damaging (0.01)	Probably damaging (0.999)	14.08	0.00358725	rs146214742
20	31647696	G	T	*BPIFB3*	splice acceptor	intron	NM_182658.3:c.387-1G>T	N/A	N/A	33	0.00761933	rs11697151
21	40190408	C	A	*ETS2*	missense	exon	NM_005239.6:c.649C>A:p.Leu217Ile	Damaging (0.007)	Probably damaging (0.966)	13.35	0.00735136	rs61735785
21	45107549	G	A	*RRP1B*	missense	exon	NM_015056.3:c.1294G>A:p.Glu432Lys	N/A	Probably damaging (0.978)	16.56	0.00009292	rs575488096
21	45919715	G	C	*TSPEAR*	missense	exon	NM_144991.3:c.1961C>G:p.Ser654Cys	Damaging (0.021)	Probably damaging (1)	27.3	0.00000476	rs782551483

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
22	24035173	G	C	*RGL4*	missense	exon	NM_153615.2:c.691G>C:p.Asp231His	Damaging (0)	Probably damaging (1)	21.5	0.00005465	rs780423673
22	25603079	G	A	*CRYBB3*	missense	exon	NM_004076.5:c.536G>A:p.Arg179His	Damaging (0.018)	Probably damaging (0.978)	25.2	0.00027215	rs149232677
22	38074614	G	A	*LGALS1*	missense	exon	NM_002305.4:c.214G>A:p.Glu72Lys	Damaging (0)	Probably damaging (1)	32	0.00143227	rs139136100
22	38142320	GGC	-	*TRIOBP*	inframe deletion	exon	NM_001039141.3:c.5322+5301_5322+5303delCGG	N/A	N/A	N/A	0.00714286	rs953748818
22	38478666	C	G	*SLC16A8*	splice donor	intron	NM_013356.3:c.214+1G>C	N/A	N/A	34	0.00484040	rs77968014
X	27480000	G	A	*PPP4R3C*	missense	exon	NM_207319.4:c.1414C>T:p.His472Tyr	N/A	N/A	0.488	0.00183455	rs184393870
X	99662437	G	C	*PCDH19*	missense	exon	NM_001184880.2:c.1159C>G:p.Arg387Gly	Damaging (0.002)	Probably damaging (0.992)	26.2	0.00000552	rs201266898

Table 2B. Candidate homozygous variants in II:2 (affected mother) of Family 2 (USRE).

Table ?-2 Candidate homozygous variants in II:2 (affected mother) of Family Reiber USRE

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
16	67876826	G	-	*THAP11*	frameshift	exon	NM_020457.3:exon1:c.369delG:p.Gln123Hisfs*42	N/A	N/A	N/A	N/A	rs111586870

Table 3A. Candidate heterozygous variants in II:2 KRMOSO (affected 2nd child) of Family 3 (KRMO).

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
1	10699141	TCG	-	*CASZ1*	inframe deletion	exon	NM_001079843.3:c.5136_5138delCGA:p.Asp1712del	N/A	N/A	N/A	0.00024810	rs201089181
2	80136918	A	C	*CTNNA2*	missense	exon	NM_001282597.3:c.1051A>C:p.Asn351His	Damaging (0.032)	Probably damaging (0.984)	24.2	0.00124086	rs1185995811
3	183525839	G	A	*YEATS2*	missense	exon	NM_018023.5:c.4033G>A:p.Val1345Met	Damaging (0.007)	Probably damaging (0.999)	28	0.00003473	rs761313198
4	85556475	T	-	*CDS1*	frameshift	exon	NM_001263.4:c.789delT:p.Phe263Leufs*6	N/A	N/A	N/A	0.00095610	rs759091263
4	146031253	A	T	*ABCE1*	splice acceptor	intron	NM_002940.3:c.406-2A>T	N/A	N/A	35	0.00005834	rs1316787321
5	140503331	TGGTGACCA	-	*PCDHB4*	disruptive inframe deletion	exon	NM_018938.4:c.1751_1759delTGGTGACCA:p.Leu584_Lys587delinsGln	N/A	N/A	N/A	N/A	N/A
5	140503344	GGTGG	-	*PCDHB4*	frameshift	exon	NM_018938.4:c.1764_1768delGGTGG:p.Val589Glyfs*84	N/A	N/A	N/A	N/A	N/A
9	79635239	GGC	-	*FOXB2*	inframe deletion	exon	NM_001013735.1:c.687_689delGGC:p.Ala234del	N/A	N/A	N/A	0.00073410	rs750048680
12	53162773	ACT	-	*KRT76*	inframe deletion	exon	NM_015848.4:c.1639_1641delAGT:p.Ser547del	N/A	N/A	N/A	0.00128743	rs370657661
13	95363659	-	AG	*SOX21*	frameshift	exon	NM_007084.4:c.645_646insCT:p.Ala216Leufs*21	N/A	N/A	N/A	N/A	rs1594515327
13	95363660	-	G	*SOX21*	frameshift	exon	NM_007084.4:c.644dupC:p.Ala216Glyfs	N/A	N/A	N/A	N/A	N/A
13	95363664	-	A	*SOX21*	frameshift	exon	NM_007084.4:c.640_641insT:p.Ala214Valfs	N/A	N/A	N/A	0	rs1477819900
13	95363666	-	GCGGGAAGAAGACGCGTGCGGCG	*SOX21*	frameshift	exon	NM_007084.4:c.638_639ins(23):p.Ala216Argfs*28	N/A	N/A	N/A	N/A	N/A
14	50605490	G	T	*SOS2*	missense	exon	NM_006939.4:c.2798C>A:p.Thr933Lys	Damaging (0.001)	Probably damaging (0.986)	24.8	0.00006086	rs1482259721
15	23086365	GCCGCC	-	*NIPA1*	inframe deletion	exon	NM_144599.5:c.42_47delGGCGGC:p.Ala15_Ala16del	N/A	N/A	N/A	0.00034695	rs531550505
16	67876820	GC	-	*THAP11*	frameshift	exon	NM_020457.3:c.363_364delGC:p.Gln122Thrfs*117	N/A	N/A	N/A	0	rs1491456602
16	67876823	ACAG	-	*THAP11*	frameshift	exon	NM_020457.3:c.366_369delACAG:p.Gln122Hisfs*42	N/A	N/A	N/A	0.00000412	rs773652130
17	18576348	CGC	-	*FOXO3B*	inframe deletion	exon	NM_001368135.1:c.145_147delGCG:p.Ala49del	N/A	N/A	N/A	0.00515448	rs761206346
19	36279042	A	C	*ARHGAP33*	missense	exon	NM_001366178.1:c.3575A>C:p.His1192Pro	Damaging (0)	Probably damaging (0.997)	24.2	0.00014807	rs1346720614
19	50029305	C	T	*FCGRT*	missense	exon	NM_001136019.3:c.1027C>T:p.Leu343Phe	Damaging (0.003)	Probably damaging (1)	23.3	0.00000400	rs146734541

Table 3B. Candidate homozygous variants in II:2 KRMOSO (affected 2nd child) of Family 3 (KRMO).

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
2	27665623	GGCTGCTGGTGAGCGC	-	*KRTCAP3*	splice donor	exon	NM_173853.4:c.209_213+11del	N/A	N/A	N/A	0.00007764	rs754004983
2	28050464	G	-	*RBKS*	frameshift	exon	NM_022128.3:c.765delC:p.Thr256Glnfs	N/A	N/A	N/A	0.00097354	rs372614067
8	10480388	-	A	*RP1L1*	frameshift	exon	NM_178857.6:c.324_325insT:p.Pro109Serfs*29	N/A	N/A	N/A	0.00201036	rs138816053
12	11461223	G	A	*PRB4*	stop gained	exon	NM_002723.6:c.694C>T:p.Gln232Ter	N/A	N/A	35	0.00290437	rs77514395
19	2271386	C	T	*OAZ1*	missense	exon	NM_004152.3:c.149C>T	N/A	Probably damaging (0.976)	24.9	0.00538330	rs28384673

Table 4A. Candidate heterozygous variants in II:2 (affected 2nd child) of Family 4 (CHII).

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
1	2460945	GCG	-	*HES5*	inframe deletion	exon	NM_001010926.4:c.462_464delCGC:p.Ala155del	N/A	N/A	N/A	0	rs559739405
1	3683159	A	C	*CCDC27*	missense	exon	NM_152492.3:c.1513A>C:p.Asn505His	Damaging (0.004)	Probably damaging (0.999)	25.7	0.00849718	rs144089943
1	6529186	TCC	-	*PLEKHG5*	inframe deletion	exon	NM_020631.6:c.2163_2165delGGA:p.Glu723del	N/A	N/A	N/A	N/A	N/A
1	20827431	G	A	*MUL1*	missense	exon	NM_024544.3:c.811C>T:p.Arg271Cys	Damaging (0.007)	Probably damaging (1)	29.9	0.00004380	rs774965777
1	47184700	G	A	*EFCAB14*	5 prime UTR premature start codon gain	UTR5	NM_014774.3:c.-941C>T:p.Met1ext-314	N/A	N/A	N/A	N/A	rs562442365
1	63270841	A	T	*ATG4C*	splice acceptor	intron	NM_032852.4:c.77-2A>T	N/A	N/A	35	0.00002510	rs768565980
1	147127275	C	T	*ACP6*	splice donor	intron	NM_016361.5:c.647+1G>A	N/A	N/A	32	N/A	N/A
1	230907799	C	T	*CAPN9*	missense	exon	NM_006615.3:c.829C>T:p.Arg277Trp	Damaging (0)	Probably damaging (1)	26.9	0.00199648	rs28359655
1	247921467	A	-	*OR1C1*	frameshift	exon	NM_012353.3:c.242delT:p.Met81Argfs*2	N/A	N/A	N/A	0.00000401	rs1434822523
2	71738977	G	A	*DYSF*	missense	exon	NM_003494.4:c.383G>A:p.Gly128Glu	Damaging (0.002)	Probably damaging (1)	26.2	0.00713247	rs34997054
2	105708941	G	A	*MRPS9*	missense	exon	NM_182640.3:c.734G>A:p.Arg245Gln	Damaging (0.049)	Probably damaging (1)	25.7	0.00373328	rs116793449
2	140990879	T	C	*LRP1B*	missense	exon	NM_018557.3:c.13676A>G:p.Asn4559Ser	Damaging (0.013)	Probably damaging (0.997)	24.6	0.00173494	rs148848707
2	192225453	T	G	*MYO1B*	missense	exon	NM_001130158.3:c.659T>G:p.Leu220Arg	Damaging (0)	Probably damaging (1)	33	N/A	N/A
2	211019134	A	G	*KANSL1L*	missense	exon	NM_152519.4:c.173T>C:p.Leu58Pro	Damaging (0.003)	Probably damaging (1)	24.2	0.00000401	rs777147277

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
2	220349156	T	A	*SPEG*	missense	exon	NM_005876.5:c.6971T>A:p.Ile2324Asn	Damaging (0.001)	Probably damaging (0.996)	27.1	0.00061294	rs201170917
3	9822175	T	C	*TADA3*	missense	exon	NM_006354.4:c.1165A>G:p.Asn389Asp	Damaging (0.043)	Probably damaging (0.993)	25.2	N/A	N/A
3	42739011	T	C	*HHATL*	missense	exon	NM_020707.4:c.854A>G:p.Asp285Gly	Damaging (0.028)	Probably damaging (0.987)	24.4	N/A	N/A
3	100995553	C	G	*IMPG2*	missense	exon	NM_016247.4:c.538G>C:p.Glu180Gln	Damaging (0.046)	Probably damaging (0.999)	15.52	N/A	N/A
3	105586417	G	T	*CBLB*	missense	exon	NM_170662.5:c.5C>A:p.Ala2Glu	Damaging (0.001)	Probably damaging (0.996)	31	0.00005571	rs780651060
3	113329939	A	G	*SIDT1*	missense	exon	NM_017699.3:c.1805A>G:p.Tyr602Cys	Damaging (0.008)	Probably damaging (1)	28.3	0.00712797	rs34023543
3	148857965	C	T	*HPS3*	missense	exon	NM_032383.5:c.392C>T:p.Pro131Leu	Damaging (0.002)	Probably damaging (1)	24.6	0.00020289	rs577273287
3	184910069	T	A	*EHHADH*	missense	exon	NM_001966.4:c.2117A>T:p.Asn706Ile	Damaging (0.006)	Probably damaging (0.966)	24.4	0.00442986	rs56056620
3	194790601	G	C	*XXYLT1*	missense	exon	NM_152531.5:c.1025C>G:p.Pro342Arg	Damaging (0.006)	Probably damaging (0.999)	26.1	0.00028065	rs199593762
4	8608506	C	T	*CPZ*	stop gained	exon	NM_001014447.3:c.949C>T:p.Gln317Ter	N/A	N/A	48	0.00042217	rs146935182
4	122737523	G	A	*EXOSC9*	splice acceptor	intron	NM_005033.3:c.1157-1G>A	N/A	N/A	33	0.00000536	rs1361964123
4	153268225	T	A	*FBXW7*	splice acceptor	intron	NM_001349798.2:c.585-2A>T	N/A	N/A	35	0.00020535	rs1319900991
5	31532534	G	C	*C5orf22*	missense	exon	NM_018356.3:c.35G>C:p.Arg12Pro	Damaging (0.002)	Probably damaging (1)	27.1	0.00555161	rs76256365
5	45695973	CGC	-	*HCN1*	inframe deletion	exon	NM_021072.4:c.221_223delGCG:p.Gly74del	N/A	N/A	N/A	0.00361834	rs747975797
5	140175321	G	A	*PCDHA2*	missense	exon	NM_018905.3:c.772G>A:p.Gly258Arg	Damaging (0)	Probably damaging (1)	28.9	N/A	N/A

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
5	178418555	C	A	*GRM6*	missense	exon	NM_000843.4:c.727G>T:p.Val243Phe	Damaging (0.001)	Probably damaging (0.979)	24.6	0.00501791	rs17078894
6	16327874	-	TGC	*ATXN1*	inframe insertion	exon	NM_001128164.2:c.666_668dupGCA:p.Gln225dup	N/A	N/A	N/A	N/A	N/A
6	24456744	G	A	*GPLD1*	missense	exon	NM_001503.4:c.1130C>T:p.Pro377Leu	Damaging (0.02)	Probably damaging (1)	25.1	0.00001607	rs755522021
6	28240063	G	T	*ZSCAN26*	missense	exon	NM_001023560.4:c.364G>T:p.Val122Phe	N/A	Probably damaging (0.983)	23	N/A	N/A
6	32191671	-	AGCAGC	*NOTCH4*	inframe insertion	exon	NM_004557.4:c.30_35dupGCTGCT:p.Leu15_Leu16dup	N/A	N/A	N/A	N/A	N/A
6	43406448	G	A	*ABCC10*	missense	exon	NM_001198934.2:c.2042G>A:p.Arg681Gln	Damaging (0.004)	Probably damaging (1)	26.2	0.00000398	rs760324037
6	44238518	CGG	-	*TMEM151B*	inframe deletion	exon	NM_001137560.2:c.54_56delCGG:p.Gly21del	N/A	N/A	N/A	0	rs1307963698
6	56879978	G	A	*BEND6*	missense	exon	NM_152731.3:c.346G>A:p.Glu116Lys	Damaging (0)	Probably damaging (0.998)	25.6	0.00002805	rs368001280
6	116381963	G	T	*FRK*	5 prime UTR premature start codon gain	UTR5	NM_002031.3:c.-489C>A:p.Met1ext-163	N/A	N/A	N/A	N/A	rs190251355
6	168440826	C	G	*KIF25*	missense	exon	NM_030615.4:c.576C>G:p.His192Gln	Damaging (0.004)	Probably damaging (1)	14.52	0.00089195	rs6928620
7	5428205	G	A	*TNRC18*	missense	exon	NM_001080495.3:c.1250C>T:p.Pro417Leu	Damaging (0.038)	Probably damaging (0.997)	24.8	N/A	rs1202132284
7	44153772	AGGAGGAGA	-	*AEBP1*	inframe deletion	exon	NM_001129.5:c.3390_3398delGGAGGAGAA:p.Lys1133_Glu1135del	N/A	N/A	N/A	0.00024674	rs765774763
7	80293761	G	A	*CD36*	missense	exon	NM_001001547.3:c.649G>A:p.Gly217Arg	Damaging (0)	Probably damaging (1)	32	0.00029907	rs200067322
7	107696101	A	C	*LAMB4*	missense	exon	NM_007356.3:c.3731T>G:p.Val1244Gly	Damaging (0.003)	Probably damaging (0.979)	24.3	0.00834714	rs147992634
7	123267310	C	T	*ASB15*	stop gained	exon	NM_001290258.2:c.844C>T:p.Arg282Ter	N/A	N/A	36	0.00963725	rs116956332

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
7	131241036	-	GGCGAC	*PODXL*	inframe insertion	exon	NM_001018111.3:c.78_83dupGTCGCC:p.Pro30_Ser31dup	N/A	N/A	N/A	N/A	N/A
7	143049017	C	T	*CLCN1*	stop gained	exon	NM_000083.3:c.2926C>T:p.Arg976Ter	N/A	N/A	38	0.00027882	rs142539932
8	17823868	C	T	*PCM1*	missense	exon	NM_001352632.2:c.3224C>T:p.Thr1075Met	Damaging (0.002)	Probably damaging (1)	27.1	0.00002008	rs769856435
8	90983460	G	A	*NBN*	missense	exon	NM_002485.5:c.643C>T:p.Arg215Trp	Damaging (0.003)	Probably damaging (1)	25.9	0.00251140	rs34767364
8	109458492	AG	-	*EMC2*	frameshift	exon	NM_014673.5:c.40+2565_40+2566delAG	N/A	N/A	N/A	0.00103119	rs751876197
8	110464510	G	A	*PKHD1L1*	splice donor	intron	NM_177531.6:c.6507+1G>A	N/A	N/A	33	0.00767286	rs72687022
8	124195352	G	T	*FAM83A*	stop gained	exon	NM_032899.6:c.256G>T:p.Gly86Ter	N/A	N/A	35	0.00371674	rs148011353
8	144998464	C	T	*PLEC*	missense	exon	NM_000445.5:c.5714G>A:p.Arg1905Gln	Damaging (0.041)	Probably damaging (0.999)	26	0.00035663	rs373617951
9	3898759	G	A	*GLIS3*	missense	exon	NM_001042413.2:c.2060C>T:p.Ser687Phe	Damaging (0)	Probably damaging (1)	27.9	0.00027443	rs374929970
9	82337405	G	T	*TLE4*	missense	exon	NM_007005.6:c.2026G>T:p.Ala676Ser	Damaging (0)	Probably damaging (0.986)	26.5	0.00000802	rs748463718
9	136213468	G	A	*MED22*	missense	exon	NM_133640.5:c.50C>T:p.Ser17Phe	Damaging (0.001)	Probably damaging (0.999)	25.7	0.00000798	rs782341842
9	139354251	C	T	*SEC16A*	missense	exon	NM_014866.2:c.5149G>A:p.Asp1717Asn	Damaging (0.019)	Probably damaging (1)	24.8	0.00067934	rs202232995
10	5043747	C	G	*AKR1C2*	missense	exon	NM_001354.6:c.211G>C:p.Asp71His	Damaging (0.001)	Probably damaging (0.994)	23.1	0.00244669	rs142672563
10	24762833	G	A	*KIAA1217*	missense	exon	NM_019590.5:c.1523G>A:p.Arg508His	Damaging (0)	Probably damaging (1)	27.9	0.00550986	rs41279868
10	49928168	C	A	*WDFY4*	missense	exon	NM_020945.2:c.347C>A:p.Ala116Glu	Damaging (0.002)	Probably damaging (1)	25.2	0.00126458	rs147299795

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
10	90353729	C	T	*LIPJ*	missense	exon	NM_001010939.2:c.157C>T:p.His53Tyr	Damaging (0)	Probably damaging (1)	23.5	N/A	rs1354680538
10	90486553	G	A	*LIPK*	missense	exon	NM_001080518.2:c.107G>A:p.Ser36Asn	Damaging (0.026)	Probably damaging (0.965)	25.1	0.00228067	rs55788049
10	99218456	C	T	*MMS19*	missense	exon	NM_022362.5:c.3086G>A:p.Gly1029Asp	Damaging (0)	Probably damaging (1)	26.1	0.00508162	rs36023427
10	124593424	G	A	*CUZD1*	missense	exon	NM_022034.6:c.1415C>T:p.Pro472Leu	Damaging (0.002)	Probably damaging (0.999)	23.1	N/A	rs868153899
11	376424	TCC	-	*B4GALNT4*	inframe deletion	exon	NM_178537.5:c.1302_1304delCCT:p.Phe434del	N/A	N/A	N/A	N/A	N/A
11	4105953	A	C	*STIM1*	missense	exon	NM_001382567.1:c.1525A>C:p.Thr509Pro	N/A	N/A	N/A	0.00145627	rs553648008
11	4108060	G	A	*STIM1*	missense	exon	NM_001382567.1:c.1634+287G>A	N/A	N/A	16.27	0.00148441	rs562406813
11	7669736	T	A	*PPFIBP2*	missense	exon	NM_003621.5:c.1765T>A:p.Leu589Ile	Damaging (0)	Probably damaging (0.998)	23	0.00479633	rs149157151
11	57087830	G	A	*TNKS1BP1*	missense	exon	NM_033396.3:c.451C>T:p.Pro151Ser	Damaging (0)	Probably damaging (1)	25.5	N/A	N/A
11	67766704	G	A	*UNC93B1*	missense	exon	NM_030930.4:c.626C>T:p.Pro209Leu	Damaging (0.006)	Probably damaging (0.981)	23.1	0.00387625	rs144399212
11	83544716	G	T	*DLG2*	missense	exon	NM_001142699.3:c.1663C>A:p.Leu555Met	Damaging (0.001)	Probably damaging (1)	23.8	0.00004399	rs775296170
12	1963174	G	A	*CACNA2D4*	missense	exon	NM_172364.5:c.2189C>T:p.Ala730Val	Damaging (0.004)	Probably damaging (0.99)	24.3	0.00833091	rs181994120
12	4543446	G	A	*FGF6*	missense	exon	NM_020996.3:c.562C>T:p.Arg188Trp	Damaging (0.001)	Probably damaging (1)	26.2	0.00011929	rs142642694
12	7045254	C	T	*ATN1*	missense	exon	NM_001007026.2:c.824C>T:p.Pro275Leu	Damaging (0.003)	Probably damaging (0.993)	21.6	0.00048059	rs201165264
12	7045904	CAGCAGCAG	-	*ATN1*	inframe deletion	exon	NM_001007026.2:c.1500_1508delGCAGCAGCA:p.Gln500_Gln502del	N/A	N/A	N/A	N/A	N/A

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
12	49998302	G	C	*FAM186B*	stop gained	exon	NM_032130.3:c.116C>G:p.Ser39Ter	N/A	N/A	32	N/A	N/A
12	50745201	C	-	*FAM186A*	frameshift	exon	NM_001145475.3:c.5414delG:p.Gly1805Glufs*35	N/A	N/A	N/A	N/A	N/A
12	104089412	C	T	*STAB2*	missense	exon	NM_017564.10:c.3460C>T:p.Arg1154Trp	Damaging (0.002)	Probably damaging (1)	27.6	0.00005567	rs200109142
12	122459834	G	ATG	*BCL7A*	5 prime UTR premature start codon gain	UTR5	NM_001024808.3:c.-164delinsATG:p.Met1ext-53	N/A	N/A	N/A	N/A	N/A
12	124333367	G	A	*DNAH10*	missense	exon	NM_001372106.1:c.6040G>A:p.Val2014Met	Damaging (0)	Probably damaging (1)	26.7	0.00779475	rs75173589
13	20038622	G	A	*TPTE2*	stop gained	exon	NM_199254.3:c.715C>T:p.Gln239Ter	N/A	N/A	37	0.00109216	rs139121187
13	30829754	T	-	*KATNAL1*	splice acceptor	intron	NM_032116.5:c.324-2delA	N/A	N/A	N/A	N/A	N/A
13	31543068	G	T	*TEX26*	missense	exon	NM_152325.3:c.693G>T:p.Lys231Asn	Damaging (0.003)	Probably damaging (0.998)	23.5	0.00611337	rs9533168
13	42873889	C	T	*AKAP11*	missense	exon	NM_016248.4:c.1007C>T:p.Pro336Leu	Damaging (0)	Probably damaging (1)	25.6	0.00025619	rs200013198
13	100622674	GGC	-	*ZIC5*	inframe deletion	exon	NM_033132.5:c.1182_1184delGCC:p.Pro400del	N/A	N/A	N/A	N/A	N/A
14	24646412	-	AGC	*REC8*	inframe insertion	exon	NM_001048205.2:c.687_689dupAGC:p.Ala230dup	N/A	N/A	N/A	0.00123854	rs370800103
15	23086371	GCC	-	*NIPA1*	inframe deletion	exon	NM_144599.5:c.39_41delGGC:p.Ala16del	N/A	N/A	N/A	N/A	N/A
15	23685467	-	CCGCA	*GOLGA6L2*	frameshift	exon	NM_001304388.2:c.2155_2156insTGCGG:p.Arg719Metfs	N/A	N/A	N/A	0.00044340	rs1566736761
15	23685469	-	TT	*GOLGA6L2*	frameshift	exon	NM_001304388.2:c.2153_2154insAA:p.Ser720Aspfs	N/A	N/A	N/A	0	rs772623131
16	919042	G	A	*LMF1*	stop gained	exon	NM_022773.4:c.1431C>T:p.Asn477=	N/A	N/A	N/A	0.00011285	rs772646362
16	3736085	C	T	*TRAP1*	missense	exon	NM_016292.3:c.383G>A:p.Arg128His	Damaging (0)	Probably damaging	27.8	0.00314243	rs61758086

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
									(1)			
16	21098323	C	G	*DNAH3*	missense	exon	NM_017539.2:c.2724G>C:p.Arg908Ser	Damaging (0.001)	Probably damaging (0.999)	23.6	0.00624139	rs117470111
16	67876827	CAG	-	*THAP11*	inframe deletion	exon	NM_020457.3:c.394_396delCAG:p.Gln132del	N/A	N/A	N/A	N/A	N/A
16	88496681	G	A	*ZNF469*	missense	exon	NM_001367624.2:c.2803G>A:p.Glu935Lys	Damaging (0.022)	Probably damaging (0.978)	22	0.00534628	rs117995699
17	7948640	G	T	*ALOX15B*	missense	exon	NM_001141.3:c.934G>T:p.Ala312Ser	Damaging (0.002)	Probably damaging (1)	22.6	0.00348460	rs138027535
17	38078803	T	G	*ORMDL3*	stop lost	exon	NM_139280.4:c.462A>C:p.Ter154Cysext*39	N/A	N/A	17.71	N/A	N/A
17	39552773	-	GCA	*KRT31*	inframe insertion	exon	NM_002277.3:c.485_487dupTGC:p.Leu162dup	N/A	N/A	N/A	0.00051713	rs565048074
17	45895692	G	A	*OSBPL7*	missense	exon	NM_145798.3:c.541C>T:p.Arg181Trp	Damaging (0.002)	Probably damaging (0.995)	23.4	0.00358584	rs35767160
17	66872845	C	G	*ABCA8*	splice acceptor	intron	NM_001288985.2:c.4200-1G>C	N/A	N/A	33	0.00558659	rs184510635
18	3071878	C	T	*MYOM1*	missense	exon	NM_003803.4:c.4718G>A:p.Arg1573Gln	Damaging (0.005)	Probably damaging (1)	28.3	0.00757906	rs117342470
18	7231276	G	T	*LRRC30*	missense	exon	NM_001105581.2:c.140G>T:p.Gly47Val	Damaging (0.013)	Probably damaging (1)	23.8	N/A	N/A
18	9256640	AAC	-	*ANKRD12*	inframe deletion	exon	NM_015208.5:c.3377_3379delCAA:p.Thr1126del	N/A	N/A	N/A	0.00317939	rs547937894
18	32833713	G	A	*ZSCAN30*	missense	exon	NM_001112734.4:c.1186C>T:p.Arg396Trp	Damaging (0.004)	Probably damaging (1)	23.8	0.00099076	rs146290259
18	55103865	C	T	*ONECUT2*	missense	exon	NM_004852.3:c.917C>T:p.Pro306Leu	N/A	Probably damaging (0.959)	23.5	0.00011489	rs541827118
19	374336	C	T	*THEG*	missense	exon	NM_016585.5:c.394G>A:p.Ala132Thr	Damaging (0)	Probably damaging (1)	23.2	0.00000399	rs1323171785

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
19	5844658	TC	-	*FUT3*	frameshift	exon	NM_000149.4:c.192_193delGA:p.Ile65Profs*53	N/A	N/A	N/A	N/A	N/A
19	13318694	CTGCTG	-	*CACNA1A*	inframe deletion	exon	NM_001127221.2:c.*161_*166delCAGCAG	N/A	N/A	N/A	N/A	N/A
19	15550454	C	T	*WIZ*	missense	exon	NM_001371589.1:c.1351G>A:p.Ala451Thr	N/A	N/A	0.267	0.00046735	rs556521095
19	40520799	CCC	-	*ZNF546*	inframe deletion	exon	NM_178544.5:c.1622_1624delCCC:p.Thr541del	N/A	N/A	N/A	0.00214539	rs540685002
19	45153113	G	C	*PVR*	missense	exon	NM_006505.5:c.460G>C:p.Val154Leu	Damaging (0.018)	Probably damaging (0.986)	10.12	0.00183589	rs35959395
19	54973653	C	T	*LENG9*	missense	exon	NM_001301782.2:c.1057G>A:p.Glu353Lys	N/A	Probably damaging (0.99)	24.2	0.00644936	rs35752413
19	56015533	A	G	*SSC5D*	missense	exon	NM_001144950.2:c.2785+2965A>G	N/A	N/A	2.097	0.00157987	rs114532247
19	56515319	A	C	*NLRP5*	missense	exon	NM_153447.4:c.300A>C:p.Glu100Asp	Damaging (0.036)	Probably damaging (0.998)	9.549	0.00018054	rs199871361
20	61956811	G	A	*COL20A1*	missense	exon	NM_020882.4:c.3313G>A:p.Gly1105Arg	Damaging (0)	Probably damaging (0.997)	26.2	0.00047633	rs201493226
21	31768494	C	A	*KRTAP13-1*	stop gained	exon	NM_181599.3:c.90C>A:p.Tyr30Ter	N/A	N/A	27.6	0.00218744	rs1985418
22	22221712	-	CCG	*MAPK1*	inframe insertion	exon	NM_002745.5:c.17_19dupCGG:p.Ala7dup	N/A	N/A	N/A	N/A	N/A
22	42262949	GCA	-	*SREBF2*	inframe deletion	exon	NM_004599.4:c.221_223delGCA:p.Ser74del	N/A	N/A	N/A	0.00012742	rs143615881
22	50900099	C	T	*SBF1*	missense	exon	NM_002972.4:c.2692G>A:p.Asp898Asn	Damaging (0.031)	Probably damaging (0.979)	25.6	N/A	N/A

Table 4B. Candidate homozygous variants in II:2 (affected 2nd child) of Family 4 (CHII).

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
4	140811086	GCTGCT GCTGCT	T	*MAML3*	frameshift	exon	NM_018717.5:c.1493_1504delinsA:p.Gln499Alafs*24	N/A	N/A	N/A	N/A	N/A
4	147560470	-	GGC	*POU4F2*	inframe insertion	exon	NM_004575.3:c.198_200dupCGG:p.Gly68dup	N/A	N/A	N/A	N/A	N/A
6	32551961	G	CCG	*HLA-DRB1*	frameshift	exon	NM_002124.4:c.295delinsCGG:p.Gln99Argfs*31	N/A	N/A	N/A	N/A	N/A
X	54321213	G	A	*WNK3*	missense	exon	NM_020922.5:c.1466C>T:p.Thr489Met	Damaging (0.017)	Probably damaging (0.994)	22	0.00002198	rs373983548
X	132351417	C	T	*TFDP3*	missense	exon	NM_016521.3:c.871G>A:p.Asp291Asn	Damaging (0.003)	Probably damaging (0.995)	18.56	0.00000547	rs762072463

Table 5A. Candidate heterozygous variants in V:1 (affected 1st child) of Family 5 (CHC).

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
1	25256279	GCC	-	*RUNX3*	inframe deletion	exon	NM_004350.3:c.79_81delGGC:p.Gly27del	N/A	N/A	N/A	0.00532476	rs532454563
1	47138637	C	T	*TEX38*	missense	exon	NM_001145474.4:c.130C>T:p.Arg44Trp	Damaging (0.001)	Probably damaging (1)	23.8	0.00267304	rs142885686
1	63789537	-	CCCTACGGCCGC	*FOXD3*	inframe insertion	exon	NM_012183.3:c.818_829dupGCCCCTACGGCC:p.Arg273_Gly276dup	N/A	N/A	N/A	0.00053057	rs532147202
1	65321246	G	A	*JAK1*	missense	exon	NM_002227.4:c.1594C>T:p.Arg532Cys	Damaging (0)	Probably damaging (0.998)	27.6	0.00000802	rs773295685
1	151006651	G	A	*PRUNE1*	missense	exon	NM_021222.3:c.1303G>A:p.Glu435Lys	Damaging (0)	Probably damaging (1)	25.7	0.00002387	rs771792256
1	197297974	GATGGAATT	-	*CRB1*	inframe deletion	exon	NM_201253.3:c.498_506delAATTGATGG:p.Ile167_Gly169del	N/A	N/A	N/A	0.00062486	rs398124615
1	201190827	G	A	*IGFN1*	missense	exon	NM_001164586.2:c.10154G>A:p.Ser3385Asn	Damaging (0.016)	Probably damaging (0.99)	26	0.00178550	rs138889052
1	207195520	G	A	*C1orf116*	missense	exon	NM_023938.6:c.1589C>T:p.Pro530Leu	Damaging (0)	Probably damaging (1)	24.7	0.00176866	rs114867640
1	248097597	A	T	*OR2AJ1*	missense	exon	NM_001355235.2:c.527A>T:p.His176Leu	Damaging (0)	N/A	22.3	N/A	N/A
2	27499675	G	A	*DNAJC5G*	missense	exon	NM_173650.3:c.79G>A:p.Gly27Ser	Damaging (0)	Probably damaging (0.998)	24.4	0.00450601	rs61754191
2	179615060	C	T	*TTN*	missense	exon	NM_001267550.2:c.11311+2791G>A	Damaging (0)	Probably damaging (1)	21.1	0.00127231	rs143253411
3	12046186	C	T	*SYN2*	missense	exon	NM_133625.6:c.161C>T:p.Ala54Val	N/A	N/A	8.553	0	rs747241247
3	15116371	C	T	*RBSN*	missense	exon	NM_022340.4:c.1273G>A:p.Gly425Arg	Damaging (0.028)	Probably damaging (0.979)	23.3	0.00464762	rs144008665
3	47040833	G	A	*NBEAL2*	missense	exon	NM_015175.3:c.3572G>A:p.Arg1191Gln	Damaging (0.021)	Probably damaging (0.974)	23.6	0.00000412	rs762921961

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
3	48895168	A	G	*SLC25A20*	missense	exon	NM_000387.6:c.881T>C:p.Leu294Pro	Damaging (0.001)	Probably damaging (1)	29.5	0.00000399	rs749560455
3	58252948	G	A	*ABHD6*	missense	exon	NM_001320126.2:c.152G>A:p.Arg51His	Damaging (0.013)	Probably damaging (0.958)	22.1	0.00029440	rs150907072
3	63898367	GCA	-	*ATXN7*	inframe deletion	exon	NM_001377405.1:c.116_118delAGC:p.Gln39del	N/A	N/A	N/A	N/A	N/A
3	65425564	-	CTG	*MAGI1*	inframe insertion	exon	NM_001033057.2:c.1258_1260dupCAG:p.Gln421dup	N/A	N/A	N/A	N/A	N/A
3	99513112	C	T	*COL8A1*	missense	exon	NM_020351.4:c.367C>T:p.Arg123Cys	Damaging (0.029)	Probably damaging (0.998)	25.9	0.00001227	rs745489693
3	107520125	A	G	*BBX*	missense	exon	NM_001142568.3:c.2735A>G:p.His912Arg	Damaging (0)	Probably damaging (0.999)	25.1	0.00000400	rs747821182
3	120952486	G	A	*STXBP5L*	missense	exon	NM_001308330.2:c.1135G>A:p.Val379Met	Damaging (0.022)	Probably damaging (0.981)	25.2	0.00540985	rs61996323
3	129120623	A	G	*EFCAB12*	missense	exon	NM_207307.3:c.1532T>C:p.Leu511Pro	Damaging (0)	Probably damaging (1)	27.5	0.00091865	rs114112678
3	130733118	A	C	*ASTE1*	missense	exon	NM_014065.4:c.1823T>G:p.Phe608Cys	Damaging (0.011)	Probably damaging (0.999)	27.1	0.00031456	rs149138190
3	149260194	CTG	-	*WWTR1*	inframe deletion	exon	NM_015472.6:c.697_699delCAG:p.Gln233del	N/A	N/A	N/A	0.00002854	rs748784098
3	157099066	C	T	*VEPH1*	missense	exon	NM_001167912.2:c.1006G>A:p.Asp336Asn	Damaging (0.017)	Probably damaging (0.996)	24.4	0.00371136	rs147644993
3	179322703	A	C	*NDUFB5*	missense	exon	NM_002492.4:c.100A>C:p.Thr34Pro	Damaging (0.011)	Probably damaging (0.994)	23.3	0.00957569	rs35399127
4	647921	G	A	*PDE6B*	missense	exon	NM_000283.4:c.905G>A:p.Gly302Asp	Damaging (0)	Probably damaging (1)	25.5	0.00066250	rs146646008
4	15005329	CCCGGACCTTCCACA	-	*CPEB2*	inframe deletion	exon	NM_001177382.2:c.1037_1051delACCTTCCACACCCGG:p.Asp346_Pro350del	N/A	N/A	N/A	0.00847950	rs554793227
4	22425950	G	A	*ADGRA3*	missense	exon	NM_145290.4:c.1469C>T:p.Ala490Val	Damaging (0.005)	Probably damaging (1)	28.6	N/A	N/A

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
4	37903835	T	G	*TBC1D1*	missense	exon	NM_015173.4:c.119T>G:p.Leu40Arg	Damaging (0)	Probably damaging (1)	29.1	N/A	N/A
4	68784885	C	A	*TMPRSS11A*	missense	exon	NM_001114387.2:c.758G>T:p.Arg253Ile	Damaging (0.001)	Probably damaging (1)	25.1	0.00497546	rs138567771
4	85418864	GGC	-	*NKX6-1*	inframe deletion	exon	NM_006168.3:c.516_518delGCC:p.Pro173del	N/A	N/A	N/A	0.00564794	rs750148249
4	186380129	G	A	*CCDC110*	stop gained	exon	NM_152775.4:c.1612C>T:p.Gln538Ter	N/A	N/A	37	0.00036564	rs185078759
5	33637759	C	T	*ADAMTS12*	missense	exon	NM_030955.4:c.1811G>A:p.Arg604Gln	Damaging (0)	Probably damaging (1)	29.5	0.00001593	rs778897770
5	55206398	G	A	*IL31RA*	missense	exon	NM_139017.6:c.1540G>A:p.Glu514Lys	Damaging (0.029)	Probably damaging (0.957)	21.6	0.00114530	rs113369650
5	113697881	GCC	-	*KCNN2*	inframe deletion	exon	NM_021614.4:c.59_61delCGC:p.Pro20del	N/A	N/A	N/A	N/A	rs566655645
5	121786667	G	C	*SNCAIP*	missense	exon	NM_005460.4:c.2125G>C:p.Glu709Gln	Damaging (0.001)	Probably damaging (0.999)	24.6	0.00805880	rs55712196
5	137515500	C	T	*KIF20A*	missense	exon	NM_005733.3:c.131C>T:p.Ser44Phe	Damaging (0.001)	Probably damaging (1)	27.4	0.00288061	rs150704301
5	145393493	C	T	*SH3RF2*	missense	exon	NM_152550.4:c.928C>T:p.Arg310Trp	Damaging (0.002)	Probably damaging (0.996)	24.6	0.00065299	rs149514957
5	145895608	A	C	*GPR151*	missense	exon	NM_194251.3:c.69T>G:p.Phe23Leu	Damaging (0.01)	Probably damaging (0.982)	24.1	0.00073950	rs144066680
6	31106502	-	C	*PSORS1C1*	frameshift	exon	NM_014068.3:c.118dupC:p.His40Profs*3	N/A	N/A	N/A	N/A	N/A
6	32497960	C	AAC	*HLA-DRB5*	frameshift	exon	NM_002125.4:c.42delinsGTT:p.Leu15Phefs*2	N/A	N/A	N/A	N/A	N/A
6	43612947	G	C	*RSPH9*	missense	exon	NM_152732.5:c.112G>C:p.Asp38His	Damaging (0.043)	Probably damaging (0.999)	29.8	N/A	N/A
6	96054069	C	T	*MANEA*	missense	exon	NM_024641.4:c.1177C>T:p.Arg393Cys	Damaging (0.008)	Probably damaging (0.987)	21.8	0.00106592	rs150823996

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
7	1608858	C	T	*PSMG3*	missense	exon	NM_032302.4:c.118G>A:p.Gly40Arg	Damaging (0.009)	Probably damaging (0.998)	24.8	N/A	N/A
7	15725801	TGG	-	*MEOX2*	inframe deletion	exon	NM_005924.5:c.225_227delCCA:p.His80del	N/A	N/A	N/A	N/A	N/A
7	72298713	C	T	*TYW1B*	5 prime UTR premature start codon gain	UTR5	NM_001145440.3:c.-59G>A:p.Met1ext-19	N/A	N/A	N/A	N/A	rs7791253
7	72395666	C	T	*POM121*	missense	exon	NM_001387691.1:c.7C>T:p.Pro3Ser	Damaging (0)	Probably damaging (1)	21.4	0.00287045	rs559447703
7	72395811	C	T	*POM121*	missense	exon	NM_001387691.1:c.152C>T:p.Ala51Val	Damaging (0)	Probably damaging (0.999)	14.35	0.00242515	rs530947859
7	90894758	G	A	*FZD1*	missense	exon	NM_003505.2:c.563G>A:p.Arg188His	Damaging (0.001)	Probably damaging (1)	32	0.00001605	rs757226244
7	95039293	AAC	-	*PON2*	inframe deletion	exon	NM_000305.3:c.613_615delGTT:p.Val205del	N/A	N/A	N/A	0.00033028	rs574854691
7	131241036	-	GGCGAC	*PODXL*	inframe insertion	exon	NM_001018111.3:c.78_83dupGTCGCC:p.Pro30_Ser31dup	N/A	N/A	N/A	N/A	N/A
7	133863343	C	T	*LRGUK*	missense	exon	NM_001365700.3:c.1196C>T:p.Pro399Leu	Damaging (0.018)	Probably damaging (1)	26.4	0.00397156	rs61749957
7	154760693	CTG	-	*PAXIP1*	inframe deletion	exon	NM_007349.4:c.1216_1218delCAG:p.Gln406del	N/A	N/A	N/A	0.00065287	rs753071824
8	17513515	A	T	*MTUS1*	missense	exon	NM_001363057.2:c.2965T>A:p.Tyr989Asn	Damaging (0.001)	Probably damaging (1)	24.5	0.00000801	rs1208199286
8	36780142	G	A	*KCNU1*	missense	exon	NM_001031836.3:c.2731G>A:p.Ala911Thr	Damaging (0.001)	Probably damaging (1)	25.7	0.00176217	rs189989959
8	61777765	C	T	*CHD7*	missense	exon	NM_017780.4:c.8267C>T:p.Thr2756Met	Damaging (0.002)	Probably damaging (0.979)	25	0.00000414	rs757521506
8	68965355	ATT	-	*PREX2*	inframe deletion	exon	NM_024870.4:c.967_969delATT:p.Ile323del	N/A	N/A	N/A	N/A	N/A
8	69445325	C	G	*C8orf34*	missense	exon	NM_052958.4:c.1046C>G:p.Pro349Arg	Damaging (0.002)	Probably damaging	23.4	0.00463419	rs77860035

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
									(0.958)			
8	120850563	G	A	*DSCC1*	missense	exon	NM_024094.3:c.1009C>T:p.Arg337Cys	Damaging (0)	Probably damaging (1)	28.8	0.00001612	rs772562797
8	121210089	A	C	*COL14A1*	missense	exon	NM_021110.4:c.632A>C:p.His211Pro	Damaging (0.006)	Probably damaging (0.996)	27.2	N/A	rs1213022756
8	139691859	C	-	*COL22A1*	splice donor	intron	NM_152888.3:c.3072+1delG	N/A	N/A	N/A	0.00000398	rs767448103
8	144822092	C	T	*IQANK1*	missense	exon	NM_001381874.1:c.149C>T:p.Ser50Leu	N/A	N/A	6.055	0.00900111	rs149608943
8	145107924	A	G	*OPLAH*	missense	exon	NM_017570.5:c.2980T>C:p.Ser994Pro	N/A	Probably damaging (0.989)	25.5	N/A	rs1587552539
9	35562439	-	G	*FAM166B*	frameshift	exon	NM_001164310.3:c.677dupC:p.Leu227Thrfs*14	N/A	N/A	N/A	0.00926861	rs746052643
9	35906602	-	CA	*HRCT1*	frameshift	exon	NM_001039792.2:c.318_319insAC:p.His107Thrfs	N/A	N/A	N/A	N/A	rs1564066721
9	79999551	-	TGA	*VPS13A*	inframe insertion	exon	NM_033305.3:c.9189+2575_9189+2577dupTGA	N/A	N/A	N/A	N/A	N/A
9	107589238	C	G	*ABCA1*	missense	exon	NM_005502.4:c.2328G>C:p.Lys776Asn	Damaging (0.009)	Probably damaging (0.998)	25.2	0.00327030	rs138880920
9	126139182	CCT	-	*CRB2*	inframe deletion	exon	NM_173689.7:c.3714_3716delCCT:p.Leu1239del	N/A	N/A	N/A	0.00048795	rs767432277
9	137779251	G	A	*FCN2*	missense	exon	NM_004108.3:c.932G>A:p.Arg311Gln	Damaging (0.027)	Probably damaging (1)	24	0.00443456	rs76267164
9	140087031	-	TCCTCCTCCTCCTCCTCC	*TPRN*	inframe insertion	exon	NM_001128228.3:c.1821_1838dup:p.Glu616_Glu621dup	N/A	N/A	N/A	N/A	N/A
10	3185636	T	G	*PITRM1*	missense	exon	NM_014889.4:c.2590A>C:p.Asn864His	Damaging (0.002)	Probably damaging (1)	23.9	0.00000401	rs1254078551
10	15713620	T	C	*ITGA8*	missense	exon	NM_003638.3:c.829A>G:p.Thr277Ala	Damaging (0.012)	Probably damaging (1)	25.1	0.00001994	rs767285224

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10	50824106	C	T	*CHAT*	missense	exon	NM_020549.5:c.287-442C>T	Damaging (0)	N/A	5.182	0.00940674	rs41306415
10	51584644	A	G	*NCOA4*	missense	exon	NM_001145263.2:c.743A>G:p.Asn248Ser	N/A	N/A	N/A	0.00522382	rs117257055
10	51584910	G	A	*NCOA4*	missense	exon	NM_001145263.2:c.1009G>A:p.Val337Met	N/A	N/A	N/A	0.00061320	rs146543857
10	51585223	G	A	*NCOA4*	missense	exon	NM_001145263.2:c.1322G>A:p.Gly441Glu	N/A	N/A	N/A	0.00485510	rs146205784
10	95121243	C	T	*MYOF*	stop gained	exon	NM_013451.4:c.2940G>A:p.Trp980Ter	N/A	N/A	39	0.00006411	rs201932767
10	106139973	C	T	*CFAP58*	stop gained	exon	NM_001008723.2:c.1360C>T:p.Gln454Ter	N/A	N/A	37	0.00058313	rs145302969
11	1103207	G	T	*MUC2*	missense	exon	NM_002457.4:c.7951G>T:p.Asp2651Tyr	N/A	N/A	N/A	N/A	N/A
11	14501248	G	A	*COPB1*	missense	exon	NM_001144061.2:c.1225C>T:p.Leu409Phe	Damaging (0.002)	Probably damaging (1)	28.4	0.00000477	rs760195782
11	35463134	C	T	*PAMR1*	missense	exon	NM_001001991.3:c.928G>A:p.Val310Met	N/A	Probably damaging (0.988)	6.693	0.00009549	rs141335779
11	48285770	C	T	*OR4X1*	missense	exon	NM_001004726.1:c.358C>T:p.Arg120Cys	Damaging (0.006)	Probably damaging (1)	21.1	0.00608128	rs79872488
11	65325329	-	CAGCAG	*LTBP3*	inframe insertion	exon	NM_001130144.3:c.97_102dupCTGCTG:p.Leu34_Leu35dup	N/A	N/A	N/A	N/A	N/A
11	72539468	G	A	*ATG16L2*	missense	exon	NM_033388.2:c.1537G>A:p.Asp513Asn	Damaging (0)	Probably damaging (0.995)	25.8	0.00001591	rs759829275
11	102643636	C	A	*MMP10*	missense	exon	NM_002425.3:c.1168G>T:p.Ala390Ser	Damaging (0.011)	Probably damaging (0.999)	25.2	0.00119047	rs139168339
11	108160480	T	G	*ATM*	missense	exon	NM_000051.4:c.4388T>G:p.Phe1463Cys	Damaging (0.001)	Probably damaging (1)	28.8	0.00155256	rs138327406
11	111784401	G	A	*HSPB2*	missense	exon	NM_001541.4:c.331G>A:p.Gly111Ser	Damaging (0)	Probably damaging (1)	27	0.00705307	rs4252589

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11	113803108	A	C	*HTR3B*	missense	exon	NM_006028.4:c.466A>C:p.Ser156Arg	Damaging (0.004)	Probably damaging (0.975)	16.75	0.00438429	rs72466469
11	117222649	-	A	*CEP164*	frameshift	exon	NM_014956.5:c.347dupA:p.Glu117Glyfs*88	N/A	N/A	N/A	N/A	N/A
11	118307385	C	T	*KMT2A*	missense	exon	NM_001197104.2:c.158C>T:p.Ala53Val	Damaging (0)	Probably damaging (0.961)	18.64	0.00256410	rs9332747
11	126276006	C	T	*ST3GAL4*	missense	exon	NM_001254757.2:c.-45C>T	N/A	N/A	N/A	0.00500593	rs190884962
12	49416536	C	T	*KMT2D*	missense	exon	NM_003482.4:c.16175G>A:p.Arg5392His	Damaging (0.001)	Probably damaging (0.999)	27.2	N/A	rs1592099456
12	50232444	-	TAACA	*BCDIN3D*	frameshift	exon	NM_181708.3:c.589_590insTGTTA:p.Pro197Leufs*44	N/A	N/A	N/A	N/A	N/A
12	52284540	-	GGCCCA	*ANKRD33*	inframe insertion	exon	NM_182608.4:c.830_835dupCCCAGG:p.Ala277_Gln278dup	N/A	N/A	N/A	0.00602495	rs528277578
12	53162773	ACT	-	*KRT76*	inframe deletion	exon	NM_015848.4:c.1639_1641delAGT:p.Ser547del	N/A	N/A	N/A	0.00128743	rs370657661
12	53491528	GCT	-	*IGFBP6*	inframe deletion	exon	NM_002178.3:c.39_41delGCT:p.Leu14del	N/A	N/A	N/A	0.00032034	rs749537120
12	103352181	-	GCAGCA GCAGCA	*ASCL1*	inframe insertion	exon	NM_004316.4:c.175_186dupCAGCAGCAGCAG:p.Gln59_Gln62dup	N/A	N/A	N/A	N/A	N/A
12	110234451	C	T	*TRPV4*	missense	exon	NM_021625.5:c.1211G>A:p.Arg404His	Damaging (0)	Probably damaging (1)	29.5	0.00002786	rs377257364
12	132547097	-	CAG	*EP400*	inframe insertion	exon	NM_015409.5:c.8223_8225dupGCA:p.Gln2748dup	N/A	N/A	N/A	N/A	N/A
13	100622680	GGC	-	*ZIC5*	inframe deletion	exon	NM_033132.5:c.1176_1178delGCC:p.Pro400del	N/A	N/A	N/A	N/A	N/A
13	100635011	-	CCA	*ZIC2*	inframe insertion	exon	NM_007129.5:c.716_718dupACC:p.His239dup	N/A	N/A	N/A	N/A	N/A
14	24568323	C	T	*PCK2*	missense	exon	NM_004563.4:c.730C>T:p.Arg244Trp	Damaging (0)	Probably damaging (1)	23.4	0.00224287	rs75497728

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14	76644266	C	T	*GPATCH2L*	stop gained	exon	NM_017926.4:c.1053-65C>T	N/A	N/A	22.5	0.00469119	rs117516637
14	94394792	G	A	*FAM181A*	missense	exon	NM_138344.5:c.347G>A:p.Arg116Gln	Damaging (0.004)	Probably damaging (1)	29.3	0.00400074	rs139972787
15	23086371	GCC	-	*NIPA1*	inframe deletion	exon	NM_144599.5:c.39_41delGGC:p.Ala16del	N/A	N/A	N/A	N/A	N/A
15	29346251	G	A	*APBA2*	missense	exon	NM_001353788.2:c.164G>A:p.Ser55Asn	Damaging (0)	Probably damaging (0.998)	23.2	0.00526756	rs142678624
15	58853075	A	G	*LIPC*	missense	exon	NM_000236.3:c.1064A>G:p.Gln355Arg	Damaging (0.001)	Probably damaging (0.988)	23.5	0.00421401	rs140272400
15	65425745	-	CGC	*PDCD7*	inframe insertion	exon	NM_005707.2:c.373_375dupGCG:p.Ala125dup	N/A	N/A	N/A	0	rs566766250
15	86213034	A	G	*AKAP13*	missense	exon	NM_007200.5:c.5074A>G:p.Met1692Val	Damaging (0.038)	Probably damaging (0.982)	23.1	N/A	N/A
15	90347148	G	C	*ANPEP*	missense	exon	NM_001150.3:c.1265C>G:p.Ala422Gly	Damaging (0.016)	Probably damaging (0.996)	27.5	0.00215197	rs144282919
15	90904421	A	G	*ZNF774*	missense	exon	NM_001004309.3:c.1358A>G:p.His453Arg	Damaging (0)	Probably damaging (0.996)	24.3	0.00855782	rs191257274
15	91769554	C	T	*SV2B*	missense	exon	NM_001323032.3:c.61C>T:p.Arg21Cys	Damaging (0.004)	Probably damaging (0.958)	23	0.00005573	rs141522931
15	99511760	T	-	*PGPEP1L*	frameshift	exon	NM_001167902.2:c.376delA:p.Arg126Glufs	N/A	N/A	N/A	0.00194603	rs375574170
16	461507	G	A	*DECR2*	missense	exon	NM_020664.4:c.808G>A:p.Gly270Arg	Damaging (0.001)	Probably damaging (1)	25.6	0.00014399	rs202106031
16	2548287	C	G	*TBC1D24*	missense	exon	NM_001199107.2:c.1032C>G:p.Ile344Met	Damaging (0.004)	Probably damaging (1)	15.11	0.00000410	rs769656369
16	21098323	C	G	*DNAH3*	missense	exon	NM_017539.2:c.2724G>C:p.Arg908Ser	Damaging (0.001)	Probably damaging (0.999)	23.6	0.00624139	rs117470111
16	67877046	GCAGCCG CAGAGGG C	-	*THAP11*	inframe deletion	exon	NM_020457.3:c.597_611delAGAGGGCGCAGCCGC:p.Glu200_Ala204del	N/A	N/A	N/A	0.00002843	rs750317616

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16	82069091	G	A	*HSD17B2*	missense	exon	NM_002153.3:c.62G>A:p.Gly21Glu	Damaging (0.004)	Probably damaging (0.997)	18.97	N/A	N/A
16	85689989	-	GAGCGC	*GSE1*	inframe insertion	exon	NM_014615.5:c.1041_1046dupCGAGCG:p.Arg351_Glu352dup	N/A	N/A	N/A	N/A	N/A
16	89265821	G	A	*SLC22A31*	missense	exon	NM_001384763.1:c.283C>T:p.Arg95Cys	N/A	N/A	4.528	0.00215380	rs184407156
17	1388997	G	C	*MYO1C*	stop gained	exon	NM_001080779.2:c.76-1400C>G	N/A	N/A	16.12	N/A	N/A
17	4009087	T	G	*ZZEF1*	missense	exon	NM_015113.4:c.1294A>C:p.Met432Leu	Damaging (0.017)	Probably damaging (0.982)	25.9	N/A	N/A
17	4837662	T	C	*GP1BA*	missense	exon	NM_000173.7:c.1763T>C:p.Val588Ala	Damaging (0)	Probably damaging (0.991)	22.9	0.00026881	rs201408072
17	8017832	G	A	*ALOXE3*	missense	exon	NM_021628.3:c.650C>T:p.Thr217Met	Damaging (0.001)	Probably damaging (0.977)	22.1	0.00013519	rs200646727
17	37619279	T	A	*CDK12*	missense	exon	NM_016507.4:c.955T>A:p.Tyr319Asn	Damaging (0)	Probably damaging (0.999)	28.7	N/A	N/A
17	40824339	G	A	*PLEKHH3*	missense	exon	NM_024927.5:c.841C>T:p.Arg281Trp	Damaging (0.017)	Probably damaging (1)	26.1	0.00867818	rs200210041
17	56056617	-	TGC	*VEZF1*	inframe insertion	exon	NM_007146.3:c.1032_1034dupGCA:p.Gln354dup	N/A	N/A	N/A	N/A	N/A
17	77808250	-	GTG	*CBX4*	inframe insertion	exon	NM_003655.3:c.1189_1191dupCAC:p.His400dup	N/A	N/A	N/A	N/A	N/A
17	78397337	C	T	*ENDOV*	missense	exon	NM_173627.5:c.421C>T:p.His141Tyr	Damaging (0)	Probably damaging (1)	25.5	0.00768913	rs41299812
18	48703543	T	G	*MEX3C*	missense	exon	NM_016626.5:c.1158A>C:p.Glu386Asp	Damaging (0.005)	Probably damaging (0.999)	23.4	N/A	N/A
18	76752627	GCA	-	*SALL3*	inframe deletion	exon	NM_171999.4:c.646_648delCAG:p.Gln216del	N/A	N/A	N/A	0.00026627	rs759024614
19	615947	CCG	-	*HCN2*	inframe deletion	exon	NM_001194.4:c.2162_2164delCGC:p.Pro721del	N/A	N/A	N/A	0.00045786	rs527536363

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19	1506031	G	A	*ADAMTSL5*	missense	exon	NM_213604.3:c.1399C>T:p.Arg467Trp	Damaging (0.005)	Probably damaging (1)	23.8	0.00507794	rs144153954
19	10665794	G	A	*KRI1*	missense	exon	NM_023008.5:c.1750C>T:p.Arg584Trp	Damaging (0.002)	Probably damaging (0.997)	22.3	0.00001591	rs143447983
19	11558344	GAG	-	*PRKCSH*	inframe deletion	exon	NM_002743.3:c.966_968delGGA:p.Glu325del	N/A	N/A	N/A	N/A	N/A
19	13318694	CTGCTG	-	*CACNA1A*	inframe deletion	exon	NM_001127221.2:c.*161_*166delCAGCAG	N/A	N/A	N/A	N/A	N/A
19	17397457	GTGTGTGT	-	*ANKLE1*	frameshift	exon	NM_152363.6:c.*132_*139delGTGTGTGT	N/A	N/A	N/A	0.00929080	rs58535756
19	40876131	G	A	*PLD3*	missense	exon	NM_012268.4:c.665G>A:p.Arg222His	Damaging (0)	Probably damaging (1)	32	0.00002796	rs765630414
19	45818809	C	T	*CKM*	missense	exon	NM_001824.5:c.395G>A:p.Arg132His	Damaging (0.03)	Probably damaging (0.96)	24.8	0.00017776	rs147574145
19	49657893	-	TCC	*HRC*	inframe insertion	exon	NM_002152.3:c.600_602dupGGA:p.Glu204dup	N/A	N/A	N/A	N/A	N/A
19	52217174	C	T	*HAS1*	missense	exon	NM_001297436.2:c.1240G>A:p.Val414Met	Damaging (0.008)	Probably damaging (0.996)	23.9	0.00412496	rs45625331
19	54780707	GAG	-	*LILRB2*	inframe deletion	exon	NM_001080978.4:c.1432_1434delCTC:p.Leu478del	N/A	N/A	N/A	0.00049832	rs749421385
20	3672046	C	T	*SIGLEC1*	missense	exon	NM_023068.4:c.4532G>A:p.Cys1511Tyr	Damaging (0)	Probably damaging (1)	25.7	0.00248470	rs143489222
20	4705364	C	T	*PRND*	missense	exon	NM_012409.4:c.167C>T:p.Pro56Leu	Damaging (0.032)	Probably damaging (0.968)	22.9	0.00806221	rs35453518
20	37580879	G	C	*FAM83D*	missense	exon	NM_030919.3:c.1474G>C:p.Gly492Arg	Damaging (0.002)	Probably damaging (0.986)	22.6	N/A	rs1334017436
20	37634861	T	C	*DHX35*	missense	exon	NM_021931.4:c.1084T>C:p.Phe362Leu	Damaging (0.003)	Probably damaging (0.998)	29.5	N/A	rs879112653
20	60697785	G	C	*LSM14B*	missense	exon	NM_144703.3:c.63G>C:p.Gln21His	Damaging (0.004)	Probably damaging (0.975)	25.2	0.00000994	rs779722984

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21	43504286	G	A	*UMODL1*	missense	exon	NM_001004416.3:c.412G>A:p.Asp138Asn	Damaging (0.005)	Probably damaging (1)	24.1	0.00198413	rs74796076
21	46047494	T	C	*KRTAP10-9*	missense	exon	NM_198690.3:c.406T>C:p.Cys136Arg	Damaging (0)	Probably damaging (0.997)	22	0.00070976	rs201991753
22	29446789	C	T	*ZNRF3*	missense	exon	NM_001206998.2:c.2620C>T:p.Arg874Trp	Damaging (0.001)	Probably damaging (0.997)	23.8	0.00000862	rs747893029
22	50658165	G	A	*TUBGCP6*	missense	exon	NM_020461.4:c.4223C>T:p.Ala1408Val	Damaging (0)	Probably damaging (0.958)	2.570	0.00293792	rs142798996
22	51153407	G	A	*SHANK3*	missense	exon	NM_001372044.2:c.2383G>A:p.Ala795Thr	N/A	N/A	22.2	0.00014248	rs376095125
X	26236047	C	T	*MAGEB5*	missense	exon	NM_001271752.1:c.629C>T:p.Thr210Ile	N/A	N/A	3.544	N/A	N/A
X	66766363	GGCGGCGGCGGC	-	*AR*	inframe deletion	exon	NM_000044.6:c.1409_1420delGCGGCGGCGGCG:p.Gly470_Gly473del	N/A	N/A	N/A	N/A	N/A

Table 5B. Candidate homozygous variants in V:1 (affected 1st child) of Family 5 (CHC).

Chr	Position	Ref	Alt	Gene names	Sequence ontology	Gene region	Base and amino acid change	SIFT	Polyphen2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
4	155244406	TTTG	-	*DCHS2*	frameshift	exon	NM_001358235.2:c.4019-769_4019-766delCAAA	N/A	N/A	N/A	N/A	N/A
10	125780754	G	-	*CHST15*	frameshift	exon	NM_001270764.2:c.1347+18delC	N/A	N/A	N/A	N/A	N/A

Table 6. Shared candidate variants across all five SRA families. Each gene that has variants in more than one family was collected in this table.

Individual	Chr	Position	Ref	Alt	Gene names	Zygosity	Sequence Ontology	Gene Region	Base and amino acid change	SIFT	Polyphen 2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
CHII, II:2	1	6529186	TCC	-	*PLEKHG5*	Het	inframe deletion	exon	NM_020631.6:c.2163_2165delGGA:p.Glu723del	N/A	N/A	N/A	N/A	N/A
USSO, II:2	1	6529186	TC	-	*PLEKHG5*	Het	frameshift	exon	NM_020631.6:c.2164_2165delGA:p.Glu722Glyfs*63	N/A	N/A	N/A	N/A	N/A
USSO, II:2	1	6529188	C	-	*PLEKHG5*	Het	frameshift	exon	NM_020631.6:c.2163delG:p.Glu722Argfs*43	N/A	N/A	N/A	N/A	N/A
USRE, II:2	1	153907279	CTGCTGCTGCTGCTGCTGCTGCTGCTGCTGT	-	*DENND4B*	Het	frameshift	exon	NM_014856.3:c.2703_2730del:p.Gln902Serfs*38	N/A	N/A	N/A	N/A	N/A
USSO, II:2	1	153907279	CTGCTGCTGCTGCTGCTGCTGCTGCTGCTGT	-	*DENND4B*	Het	frameshift	exon	NM_014856.3:c.2703_2730del:p.Gln902Serfs*38	N/A	N/A	N/A	N/A	N/A
USSO, II:2	1	201175684	AA	-	*IGFN1*	Het	frameshift	exon	NM_001164586.2:c.1664_1665delAA:p.Lys555Serfs*61	N/A	N/A	N/A	0.00020927	rs778348997
CHC, V:1	1	201190827	G	A	*IGFN1*	Het	missense	exon	NM_001164586.2:c.10154G>A:p.Ser3385Asn	Damaging (0.016)	Probably damaging (0.99)	26	0.00178550	rs138889052
CHC, V:1	3	63898367	GCA	-	*ATXN7*	Het	inframe deletion	exon	NM_001377405.1:c.116_118delAGC:p.Gln39del	N/A	N/A	N/A	N/A	N/A
USRE, II:2	3	63898391	-	GCC	*ATXN7*	Het	inframe insertion	exon	NM_001377405.1:c.123_125dupGCC:p.Pro43dup	N/A	N/A	N/A	0.00570556	rs1553686135
USRE, II:2	4	155226252	G	A	*DCHS2*	Het	stop gained	exon	NM_001358235.2:c.5392C>T:p.Arg1798Ter	N/A	N/A	41	0.00134781	rs150179829

Individual	Chr	Position	Ref	Alt	Gene names	Zygosity	Sequence Ontology	Gene Region	Base and amino acid change	SIFT	Polyphen 2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
CHC, V:1	4	155244406	TTTG	-	*DCHS2*	Homo	frameshift	exon	NM_001358235.2:c.4019-769_4019-766delCAAA	N/A	N/A	N/A	N/A	N/A
CHII, II:2	6	16327874	-	TGC	*ATXN1*	Het	inframe insertion	exon	NM_001128164.2:c.666_668dupGCA:p.Gln225dup	N/A	N/A	N/A	N/A	N/A
USRE, II:2	6	16327916	-	TGC	*ATXN1*	Het	inframe insertion	exon	NM_001128164.2:c.624_626dupGCA:p.Gln208dup	N/A	N/A	N/A	N/A	rs193922926
CHII, II:2	6	32551961	G	CCG	*HLA-DRB1*	Homo	frameshift	exon	NM_002124.4:c.295delinsCGG:p.Gln99Argfs*31	N/A	N/A	N/A	N/A	N/A
USSO, II:2	6	32551962	C	-	*HLA-DRB1*	Het	frameshift	exon	NM_002124.4:c.294delG:p.Glu98Aspfs*31	N/A	N/A	N/A	N/A	N/A
USSO, II:2	7	131241034	ACGGCGACGGCGACGG	-	*PODXL*	Het	frameshift	exon	NM_001018111.3:c.70_85del:p.Pro24Argfs*138	N/A	N/A	N/A	N/A	N/A
CHC, V:1	7	131241036	-	GGCGAC	*PODXL*	Het	inframe insertion	exon	NM_001018111.3:c.78_83dupGTCGCC:p.Pro30_Ser31dup	N/A	N/A	N/A	N/A	N/A
CHII, II:2	7	131241036	-	GGCGAC	*PODXL*	Het	inframe insertion	exon	NM_001018111.3:c.78_83dupGTCGCC:p.Pro30_Ser31dup	N/A	N/A	N/A	N/A	N/A
USSO, II:2	8	10467554	C	A	*RP1L1*	Het	stop gained	exon	NM_178857.6:c.4054G>T:p.Glu1352Ter	N/A	N/A	34	N/A	N/A
KRMO, II:2	8	10480388	-	A	*RP1L1*	Homo	frameshift	exon	NM_178857.6:c.324_325insT:p.Pro109Serfs*29	N/A	N/A	N/A	0.00201036	rs138816053
USSO, II:2	8	110396323	A	G	*PKHD1L1*	Het	missense	exon	NM_177531.6:c.442A>G:p.Ile148Val	Damaging (0.021)	Probably damaging (0.995)	22.9	N/A	N/A

Individual	Chr	Position	Ref	Alt	Gene names	Zygosity	Sequence Ontology	Gene Region	Base and amino acid change	SIFT	Polyphen 2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
CHII, II:2	8	110464510	G	A	*PKHD1L1*	Het	splice donor	intron	NM_177531.6:c.6507+1G>A	N/A	N/A	33	0.00767286	rs72687022
USSO, II:2	10	99211549	G	C	*ZDHHC16*	Het	missense	exon	NM_198046.3:c.117G>C:p.Trp39Cys	Damaging (0.017)	Probably damaging (1)	23.4	0.00001599	rs766784631
USRE, II:2	10	99215755	G	A	*ZDHHC16*	Het	missense	exon	NM_198046.3:c.973G>A:p.Gly325Ser	Damaging (0)	Probably damaging (1)	31	0.00001989	rs377074050
KRMO, II:2	12	53162773	ACT	-	*KRT76*	Het	inframe deletion	exon	NM_015848.4:c.1639_1641delAGT:p.Ser547del	N/A	N/A	N/A	0.00128743	rs370657661
CHC, V:1	12	53162773	ACT	-	*KRT76*	Het	inframe deletion	exon	NM_015848.4:c.1639_1641delAGT:p.Ser547del	N/A	N/A	N/A	0.00128743	rs370657661
USRE, II:2	12	132547094	CAG	-	*EP400*	Het	inframe deletion	exon	NM_015409.5:c.8223_8225delGCA:p.Gln2748del	N/A	N/A	N/A	N/A	rs528214697
CHC, V:1	12	132547097	-	CAG	*EP400*	Het	inframe insertion	exon	NM_015409.5:c.8223_8225dupGCA:p.Gln2748dup	N/A	N/A	N/A	N/A	N/A
CHII, II:2	13	100622674	GGC	-	*ZIC5*	Het	inframe deletion	exon	NM_033132.5:c.1182_1184delGCC:p.Pro400del	N/A	N/A	N/A	N/A	N/A
CHC, V:1	13	100622680	GGC	-	*ZIC5*	Het	inframe deletion	exon	NM_033132.5:c.1176_1178delGCC:p.Pro400del	N/A	N/A	N/A	N/A	N/A
KRMO, II:2	15	23086365	GCCGCC	-	*NIPA1*	Het	inframe deletion	exon	NM_144599.5:c.42_47delGGCGGC:p.Ala15_Ala16del	N/A	N/A	N/A	0.00034695	rs531550505
CHC, V:1	15	23086371	GCC	-	*NIPA1*	Het	inframe deletion	exon	NM_144599.5:c.39_41delGGC:p.Ala16del	N/A	N/A	N/A	N/A	N/A

Individual	Chr	Position	Ref	Alt	Gene names	Zygosity	Sequence Ontology	Gene Region	Base and amino acid change	SIFT	Polyphen 2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
CHII, II:2	15	23086371	GCC	-	*NIPA1*	Het	inframe deletion	exon	NM_144599.5:c.39_41delGGC:p.Ala16del	N/A	N/A	N/A	N/A	N/A
USSO, II:2	16	904669	G	A	*LMF1*	Het	missense	exon	NM_022773.4:c.1567C>T:p.Arg523Cys	Damaging (0.014)	Probably damaging (0.996)	31	0.00003467	rs758116895
CHII, II:2	16	919042	G	A	*LMF1*	Het	stop gained	exon	NM_022773.4:c.1431C>T:p.Asn477=	N/A	N/A	N/A	0.00011285	rs772646362
CHC, V:1	16	21098323	C	G	*DNAH3*	Het	missense	exon	NM_017539.2:c.2724G>C:p.Arg908Ser	Damaging (0.001)	Probably damaging (0.999)	23.6	0.00624139	rs117470111
CHII, II:2	16	21098323	C	G	*DNAH3*	Het	missense	exon	NM_017539.2:c.2724G>C:p.Arg908Ser	Damaging (0.001)	Probably damaging (0.999)	23.6	0.00624139	rs117470111
KRMO, II:2	16	67876820	GC	-	*THAP11*	Het	frameshift	exon	NM_020457.3:c.363_364delGC:p.Gln122Thrfs*117	N/A	N/A	N/A	0.00000000	rs1491456602
KRMO, II:2	16	67876823	ACAG	-	*THAP11*	Het	frameshift	exon	NM_020457.3:c.366_369delACAG:p.Gln122Hisfs*42	N/A	N/A	N/A	0.00000412	rs773652130
USRE, II:2	16	67876826	G	-	*THAP11*	Homo	frameshift	exon	NM_020457.3:exon1:c.369delG:p.Gln123Hisfs*42	N/A	N/A	N/A	N/A	rs111586870
CHII, II:2	16	67876827	CAG	-	*THAP11*	Het	inframe deletion	exon	NM_020457.3:c.394_396delCAG:p.Gln132del	N/A	N/A	N/A	N/A	N/A
CHC, V:1	16	67877046	GCAGCCGCAGAGGGC	-	*THAP11*	Het	inframe deletion	exon	NM_020457.3:c.597_611delAGAGGGCGCAGCCGC:p.Glu200_Ala204del	N/A	N/A	N/A	0.00002843	rs750317616
USSO, II:2	17	56056607	C	-	*VEZF1*	Het	frameshift	exon	NM_007146.3:c.1044delG:p.Gln348Hisfs*9	N/A	N/A	N/A	0.00036898	rs746343850

Individual	Chr	Position	Ref	Alt	Gene names	Zygosity	Sequence Ontology	Gene Region	Base and amino acid change	SIFT	Polyphen 2	CADD	Alt allele freq (gnomAD Exomes)	dbSNP 154
USSO, II:2	17	56056609	GC	-	*VEZF1*	Het	frameshift	exon	NM_007146.3:c.1041_1042delGC:p.Gln348Alafs*27	N/A	N/A	N/A	0.00009867	rs775689252
CHC, V:1	17	56056617	-	TGC	*VEZF1*	Het	inframe insertion	exon	NM_007146.3:c.1032_1034dupGCA:p.Gln354dup	N/A	N/A	N/A	N/A	N/A
CHII, II:2	18	32833713	G	A	*ZSCAN30*	Het	missense	exon	NM_001112734.4:c.1186C>T:p.Arg396Trp	Damaging (0.004)	Probably damaging (1)	23.8	0.00099076	rs146290259
USSO, II:2	18	32843992	G	A	*ZSCAN30*	Het	stop gained	exon	NM_001112734.4:c.325C>T:p.Arg109Ter	N/A	N/A	33	0.00019167	rs201609495
CHC, V:1	19	13318694	CTGCTG	-	*CACNA1A*	Het	inframe deletion	exon	NM_001127221.2:c.*161_*166delCAGCAG	N/A	N/A	N/A	N/A	N/A
CHII, II:2	19	13318694	CTGCTG	-	*CACNA1A*	Het	inframe deletion	exon	NM_001127221.2:c.*161_*166delCAGCAG	N/A	N/A	N/A	N/A	N/A
CHC, V:1	19	17397457	GTGTGTGT	-	*ANKLE1*	Het	frameshift	exon	NM_152363.6:c.*132_*139delGTGTGTGT	N/A	N/A	N/A	0.00929080	rs58535756
USSO, II:2	19	17397500	TTTG	-	*ANKLE1*	Het	frameshift	exon	NM_152363.6:c.*140_*143delTTGT	N/A	N/A	N/A	N/A	N/A
USRE, II:2	19	17397501	TTGTG	-	*ANKLE1*	Het	frameshift	exon	NM_152363.6:c.*141_*145delTGTGT	N/A	N/A	N/A	N/A	N/A

Table 7. Localized and generalized cases of short root anomalies (SRA), number of teeth affected and number of teeth present among subjects of five families. In order to have an absolute denominator to calculate the percentage of affected teeth, 28 was the standard number used for "# of Teeth Present" in all subjects. Localized cases are %SRA less than 30% and generalized cases are %SRA are more than 30%.

Subject	# of Teeth Affected	# of Teeth Present	% SRA	Localized	Generalized
Family 1- Subject II:1	5	28	18	Y	-
Family 1- Subject II:2	4	28	14	Y	-
Family 2- Subject II:2	8	28	29	Y	-
Family 2- Subject III:5	3	28	11	Y	-
Family 3- Subject II:2	10	28	36	-	Y
Family 4- Subject II:2	22	28	79	-	Y
Family 5- Subject V:1	17	28	61	-	Y

Y represents the presence of either a localized or generalized classification.

Table 8. Root to crown ratio (R/C) of maxillary central incisors. Unit of measure is in pixels. Short root anomalies are defined as a R/C ratio of ≤ 1.1. All calculated R/C ratios < 1.1 are marked with an asterisk.

Root Length (Maxillary)	Tooth #	Root Length (Pixels)	Crown Length (Pixels)	R/C Ratio Length
Central Incisors				
Subject				
Family 1- Subject II:1	8	50	36	1.4
	9	49	44	1.1*
Family 1- Subject II:2	8	35	45	0.8*
	9	38	56	0.8*
Family 4- Subject II:2	8	31	60	0.5*
	9	33	63	0.5*
Family 3- Subject II:2	8	23	31	0.7*
	9	22	30	0.7*
Family 2- Subject II:2	8	39	39	1.0*
	9	37	41	0.9*
Family 2- Subject III:5	8	51	40	1.3
	9	48	42	1.2
Family 5- Subject V:1	8	33	41	0.8*
	9	35	42	0.8*

* represents the presence of a R/C ratio of ≤ 1.1 and short root anomaly classification

Table 9. Root to crown ratio (R/C) of maxillary lateral incisors. Unit of measure is in pixels. Short root anomalies are defined as a R/C ratio of ≤ 1.1. All calculated R/C ratios < 1.1 are marked with an asterisk.

Root Length (Maxillary)	Tooth #	Root Length (Pixels)	Crown Length (Pixels)	R/C Ratio Length
Lateral Incisors				
Subject				
Family 1- Subject II:1	7	50	39	1.3
	10	50	43	1.2
Family 1- Subject II:2	7	59	44	1.3
	10	61	42	1.5
Family 4- Subject II:2	7	30	55	0.6*
	10	39	39	1.0*
Family 3- Subject II:2	7	24	25	1.0*
	10	28	22	1.3
Family 2- Subject II:2	7	47	39	1.2
	10	52	40	1.3
Family 2- Subject III:5	7	N/A	N/A	N/A
	10	47	32	1.5
Family 5- Subject V:1	7	N/A	N/A	N/A
	10	N/A	N/A	N/A

* represents the presence of a R/C ratio of ≤ 1.1 and short root anomaly classification
N/A represents teeth that are not present and therefore no calculation can be made

Table 10. Root to crown ratio (R/C) of maxillary canines. Unit of measure is in pixels. Short root anomalies are defined as a R/C ratio of ≤ 1.1. All calculated R/C ratios < 1.1 are marked with an asterisk.

Root Length (Maxillary)	Tooth #	Root Length (Pixels)	Crown Length (Pixels)	R/C Ratio Length
Canines				
Subject				
Family 1- Subject II:1	6	86	58	1.5
	11	84	61	1.4
Family 1- Subject II:2	6	88	50	1.8
	11	84	45	1.9
Family 4- Subject II:2	6	52	53	1.0*
	11	55	52	1.1*
Family 3- Subject II:2	6	31	27	1.2
	11	32	25	1.3
Family 2- Subject II:2	6	N/A	N/A	N/A
	11	N/A	N/A	N/A
Family 2- Subject III:5	6	48	35	1.4
	11	61	37	1.7
Family 5- Subject V:1	6	39	47	0.8*
	11	15	17	0.9*

* represents the presence of a R/C ratio of ≤ 1.1 and short root anomaly classification
N/A represents teeth that are not present and therefore no calculation can be made

Table 11. Root to crown ratio (R/C) of maxillary first premolars. Unit of measure is in pixels. Short root anomalies are defined as a R/C ratio of ≤ 1.1. All calculated R/C ratios < 1.1 are marked with an asterisk.

Root Length (Maxillary)	Tooth #	Root Length (Pixels)	Crown Length (Pixels)	R/C Ratio Length
First Premolars				
Subject				
Family 1- Subject II:1	5	39	40	1.0*
	12	30	40	0.8*
Family 1- Subject II:2	5	51	38	1.3
	12	57	38	1.5
Family 4- Subject II:2	5	40	44	0.9*
	12	39	46	0.9*
Family 3- Subject II:2	5	30	29	1.0*
	12	31	25	1.2
Family 2- Subject II:2	5	30	36	0.8*
	12	33	41	0.8*
Family 2- Subject III:5	5	23	25	0.9*
	12	44	37	1.2
Family 5- Subject V:1	5	35	42	0.8*
	12	21	31	0.7*

* represents the presence of a R/C ratio of ≤ 1.1 and short root anomaly classification

Table 12. Root to crown ratio (R/C) of maxillary second premolars. Unit of measure is in pixels. Short root anomalies are defined as a R/C ratio of ≤ 1.1. All calculated R/C ratios < 1.1 are marked with an asterisk.

Root Length (Maxillary)	Tooth #	Root Length (Pixels)	Crown Length (Pixels)	R/C Ratio Length
Second Premolars				
Subject				
Family 1- Subject II:1	4	38	35	1.1*
	13	40	37	1.1*
Family 1- Subject II:2	4	40	39	1.0*
	13	42	38	1.1*
Family 4- Subject II:2	4	45	43	1.1*
	13	44	44	1.0*
Family 3- Subject II:2	4	30	28	1.1*
	13	28	20	1.4
Family 2- Subject II:2	4	38	34	1.1*
	13	30	36	0.8*
Family 2- Subject III:5	4	37	33	1.1*
	13	32	36	0.9*
Family 5- Subject V:1	4	33	37	0.9*
	13	35	34	1.0*

* represents the presence of a R/C ratio of ≤ 1.1 and short root anomaly classification

Table 13. Root to crown ratio (R/C) of maxillary first molars. Unit of measure is in pixels. Short root anomalies are defined as a R/C ratio of ≤ 1.1. All calculated R/C ratios < 1.1 are marked with an asterisk.

Root Length (Maxillary)	Tooth #	Root Length (Pixels)	Crown Length (Pixels)	R/C Ratio Length
First Molars				
Subject				
Family 1- Subject II:1	3	63	35	1.8
	14	51	44	1.2
Family 1- Subject II:2	3	64	38	1.7
	14	67	36	1.9
Family 4- Subject II:2	3	53	44	1.2
	14	56	41	1.4
Family 3- Subject II:2	3	33	23	1.4
	14	29	21	1.4
Family 2- Subject II:2	3	44	40	1.1*
	14	43	39	1.1*
Family 2- Subject III:5	3	51	35	1.5
	14	37	30	1.2
Family 5- Subject V:1	3	43	40	1.1*
	14	62	41	1.5

* represents the presence of a R/C ratio of ≤ 1.1 and short root anomaly classification

Table 14. Root to crown ratio (R/C) of maxillary second molars. Unit of measure is in pixels. Short root anomalies are defined as a R/C ratio of ≤ 1.1. All calculated R/C ratios < 1.1 are marked with an asterisk.

Root Length (Maxillary)	Tooth #	Root Length (Pixels)	Crown Length (Pixels)	R/C Ratio Length
Second Molars				
Subject				
Family 1- Subject II:1	2	59	38	1.6
	15	58	37	1.6
Family 1- Subject II:2	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A
Family 4- Subject II:2	2	49	46	1.1*
	15	52	40	1.3
Family 3- Subject II:2	2	47	48	1.0*
	15	17	20	0.9*
Family 2- Subject II:2	2	59	39	1.5
	15	62	35	1.8
Family 2- Subject III:5	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A
Family 5- Subject V:1	2	44	45	1.0*
	15	39	35	1.1*

* represents the presence of a R/C ratio of ≤ 1.1 and short root anomaly classification
N/A represents teeth that are either not present or not fully erupted and therefore no calculation can be made

Table 15. Root to crown ratio (R/C) of mandibular central incisors. Unit of measure is in pixels. Short root anomalies are defined as a R/C ratio of ≤ 1.1. All calculated R/C ratios < 1.1 are marked with an asterisk.

Root Length (Mandibular)	Tooth #	Root Length (Pixels)	Crown Length (Pixels)	R/C Ratio Length
Central Incisors				
Subject				
Family 1- Subject II:1	24	61	38	1.6
	25	59	39	1.5
Family 1- Subject II:2	24	55	38	1.5
	25	64	39	1.6
Family 4- Subject II:2	24	34	38	0.9*
	25	32	35	0.9*
Family 3- Subject II:2	24	21	20	1.0*
	25	25	20	1.3
Family 2- Subject II:2	24	N/A	N/A	N/A
	25	43	28	1.5
Family 2- Subject III:5	24	32	23	1.4
	25	29	24	1.2
Family 5- Subject V:1	24	30	30	1.0*

* represents the presence of a R/C ratio of ≤ 1.1 and short root anomaly classification
N/A represents teeth that are not present and therefore no calculation can be made

Table 16. Root to crown ratio (R/C) of mandibular lateral incisors. Unit of measure is in pixels. Short root anomalies are defined as a R/C ratio of ≤ 1.1. All calculated R/C ratios < 1.1 are marked with an asterisk.

Root Length (Mandibular)	Tooth #	Root Length (Pixels)	Crown Length (Pixels)	R/C Ratio Length
Lateral Incisors				
Subject				
Family 1- Subject II:1	23	63	44	1.4
	26	72	41	1.8
Family 1- Subject II:2	23	60	41	1.5
	26	66	41	1.6
Family 4- Subject II:2	23	41	45	0.9*
	26	41	47	0.9*
Family 3- Subject II:2	23	27	19	1.4
	26	27	20	1.4
Family 2- Subject II:2	23	43	33	1.3
	26	46	33	1.4
Family 2- Subject III:5	23	41	33	1.2
	26	50	32	1.6
Family 5- Subject V:1	23	29	33	0.9*
	26	N/A	N/A	N/A

* represents the presence of a R/C ratio of ≤ 1.1 and short root anomaly classification
N/A represents teeth that are not present and therefore no calculation can be made

Table 17. Root to crown ratio (R/C) of mandibular canines. Unit of measure is in pixels. Short root anomalies are defined as a R/C ratio of ≤ 1.1. All calculated R/C ratios < 1.1 are marked with an asterisk.

Root Length (Mandibular)	Tooth #	Root Length (Pixels)	Crown Length (Pixels)	R/C Ratio Length
Canines				
Subject				
Family 1- Subject II:1	22	61	42	1.6
	27	58	44	1.3
Family 1- Subject II:2	22	83	43	1.9
	27	87	45	1.9
Family 4- Subject II:2	22	50	53	0.9*
	27	56	52	1.1*
Family 3- Subject II:2	22	27	22	1.2
	27	29	21	1.4
Family 2- Subject II:2	22	45	36	1.3
	27	49	41	1.2
Family 2- Subject III:5	22	44	39	1.1*
	27	56	40	1.4
Family 5- Subject V:1	22	35	30	1.2
	27	37	33	1.1*

* represents the presence of a R/C ratio of ≤ 1.1 and short root anomaly classification

Table 18. Root to crown ratio (R/C) of mandibular first premolars. Unit of measure is in pixels. Short root anomalies are defined as a R/C ratio of ≤ 1.1. All calculated R/C ratios < 1.1 are marked with an asterisk.

Root Length (Mandibular)	Tooth #	Root Length (Pixels)	Crown Length (Pixels)	R/C Ratio Length
First Premolars				
Subject				
Family 1- Subject II:1	21	57	40	1.4
	28	63	38	1.7
Family 1- Subject II:2	21	72	36	2.0
	28	64	39	1.6
Family 4- Subject II:2	21	42	42	1.0*
	28	40	45	0.9*
Family 3- Subject II:2	21	29	23	1.3
	28	30	23	1.3
Family 2- Subject II:2	21	N/A	N/A	N/A
	28	N/A	N/A	N/A
Family 2- Subject III:5	21	42	32	1.3
	28	40	33	1.2
Family 5- Subject V:1	21	37	33	1.1*
	28	36	35	1.0*

* represents the presence of a R/C ratio of ≤ 1.1 and short root anomaly classification
N/A represents teeth that are not present and therefore no calculation can be made

Table 19. Root to crown ratio (R/C) of mandibular second premolars. Unit of measure is in pixels. Short root anomalies are defined as a R/C ratio of ≤ 1.1. All calculated R/C ratios < 1.1 are marked with an asterisk.

Root Length (Mandibular)	Tooth #	Root Length (Pixels)	Crown Length (Pixels)	R/C Ratio Length
Second Premolars				
Subject				
Family 1- Subject II:1	20	50	38	1.3
	29	62	40	1.6
Family 1- Subject II:2	20	48	36	1.3
	29	64	39	1.6
Family 4- Subject II:2	20	38	43	0.9*
	29	37	42	0.9*
Family 3- Subject II:2	20	23	22	1.0*
	29	27	20	1.4
Family 2- Subject II:2	20	48	33	1.5
	29	38	29	1.3
Family 2- Subject III:5	20	39	29	1.3
	29	37	32	1.2
Family 5- Subject V:1	20	41	34	1.2
	29	30	35	0.9*

* represents the presence of a R/C ratio of ≤ 1.1 and short root anomaly classification

Table 20. Root to crown ratio (R/C) of mandibular first molars. Unit of measure is in pixels. Short root anomalies are defined as a R/C ratio of ≤ 1.1. All calculated R/C ratios < 1.1 are marked with an asterisk.

Root Length (Mandibular)	Tooth #	Root Length (Pixels)	Crown Length (Pixels)	R/C Ratio Length
First Molars				
Subject				
Family 1- Subject II:1	19	56	36	1.6
	30	60	37	1.6
Family 1- Subject II:2	19	59	32	1.8
	30	64	35	1.8
Family 4- Subject II:2	19	46	48	1.0*
	30	56	40	1.4
Family 3- Subject II:2	19	30	23	1.3
	30	32	22	1.5
Family 2- Subject II:2	19	51	39	1.3
	30	57	36	1.6
Family 2- Subject III:5	19	69	33	2.1
	30	63	33	1.9
Family 5- Subject V:1	19	66	40	1.7
	30	64	42	1.5

* represents the presence of a R/C ratio of ≤ 1.1 and short root anomaly classification

Table 21. Root to crown ratio (R/C) of mandibular second molars. Unit of measure is in pixels. Short root anomalies are defined as a R/C ratio of ≤ 1.1. All calculated R/C ratios < 1.1 are marked with an asterisk.

Root Length (Mandibular)	Tooth #	Root Length (Pixels)	Crown Length (Pixels)	R/C Ratio Length
Second Molars				
Subject				
Family 1- Subject II:1	18	48	34	1.4
	31	55	31	1.8
Family 1- Subject II:2	18	N/A	N/A	N/A
	31	N/A	N/A	N/A
Family 4- Subject II:2	18	44	40	1.1*
	31	43	43	1.0*
Family 3- Subject II:2	18	27	23	1.2
	31	24	22	1.1*
Family 2- Subject II:2	18	59	36	1.6
	31	51	34	1.3
Family 2- Subject III:5	18	N/A	N/A	N/A
	31	N/A	N/A	N/A
Family 5- Subject V:1	18	29	24	1.2
	31	49	38	1.3

* represents the presence of a R/C ratio of ≤ 1.1 and short root anomaly classification
N/A represents teeth that are not present and therefore no calculation can be made

Table 22. Root width is determined by the R/C ratio, where "R" is represented by the root width at the middle third of the root, and "C" is represented by the root width at the cervical third of the root. Root width ratios of maxillary central incisors ≥ 0.87 are classified as "wide", and anything < 0.87 is classified as "normal".

Root Width (Maxillary Central Incisors)	Tooth #	Root Width at the Middle 1/3rd of the root (Pixels)	Crown Width at the Cervical 1/3rd of the root (Pixels)	R/C Ratio for Width
Subject				
Family 1- Subject II:1	8	22	28	0.79
	9	23	28	0.82
Family 1- Subject II:2	8	42	43	0.98*
	9	40	44	0.91*
Family 2- Subject II:2	8	34	37	0.92*
	9	32	36	0.89*
Family 2- Subject III:5	8	26	32	0.81
	9	27	32	0.84
Family 3- Subject II:2	8	20	19	1.05*
	9	21	20	1.05*
Family 4- Subject II:2	8	33	36	0.92*
	9	35	40	0.88*
Family 5- Subject V:1	8	39	43	0.91*
	9	40	42	0.95*

***** represents a R/C ratio (for width) greater than 0.87 and a "wide" root classification

Table 23. Root width is determined by the R/C ratio, where "R" is represented by the root width at the middle third of the root, and "C" is represented by the root width at the cervical third of the root. Root width ratios of mandibular central incisors ≥ 0.85 are classified as "wide", and anything < 0.85 is classified as "normal".

Root Width (Mandibular Central Incisors)	Tooth #	Root Width at the Middle 1/3rd of the root (Pixels)	Crown Width at the Cervical 1/3rd of the root (Pixels)	R/C Ratio for Width
Subject				
Family 1- Subject II:1	24	16	22	0.73
	25	17	20	0.85*
Family 1- Subject II:2	24	20	25	0.80
	25	22	26	0.84
Family 2- Subject II:2	24	N/A	N/A	N/A
	25	17	21	0.81
Family 2- Subject III:5	24	11	15	0.73
	25	11	17	0.65
Family 3- Subject II:2	24	12	14	0.86*
	25	11	13	0.85*
Family 4- Subject II:2	24	17	22	0.77
	25	14	19	0.74
Family 5- Subject V:1	24	26	32	0.81
	25	33	34	0.97*

* represents a R/C ratio (for width) greater than 0.85 and a "wide" root classification
N/A represents teeth that are not present and therefore no calculation can be made

Table 24. The presence of taurodontism in the maxillary molars across all subjects as determined by the Taurodont Index, which is represented by the formula Variable 1/Variable 2 as described by MacDonald et al. (26). Variable 1 represents the lowest point of the roof of the pulp chamber to the highest point of the floor of the pulp chamber. Variable 2 is defined as the lowest point of the roof of the pulp chamber to the apex of the longest root of the molar. If the taurodont index is ≥30-40, the molar is classified as a mesotaurodont. If the taurodont index is ≥40, the molar is classified as a hypertaurodont. If the taurodont index is < 30, the molar is classified as normal.

Taurodont (Maxillary Molars)	Tooth #	Variable 1 (Pixels)	Variable 2 (Pixels)	Taurodont Index
Subject				
Family 1- Subject II:1	2	19	57	33*
	3	16	57	28
	14	16	57	24
	15	16	47	34*
Family 1- Subject II:2	2	27	47	57**
	3	16	70	23
	14	17	59	29
	15	31	49	63**
Family 2- Subject II:2	2	10	67	15
	3	10	54	19
	14	5	49	10
	15	9	61	15
Family 2- Subject III:5	3	10	58	17
	14	8	53	15
Family 3- Subject II:2	2	41	26	15
	3	3	31	10
	14	3	29	10
	15	5	30	17
Family 4- Subject II:2	2	22	53	42**
	3	6	53	11
	14	5	51	10
	15	26	51	51**
Family 5- Subject V:1	N/A	N/A	N/A	N/A

* represents mesotaurodont classification
** represents hypertaurodont classification
N/A represents teeth that are not diagnostic for measurement on available radiographs

Table 25. The presence of taurodontism in the mandibular molars across all subjects as determined by the Taurodont Index, which is represented by the formula Variable 1/Variable 2 as described by MacDonald et al. (26). Variable 1 represents the lowest point of the roof of the pulp chamber to the highest point of the floor of the pulp chamber. Variable 2 is defined as the lowest point of the roof of the pulp chamber to the apex of the longest root of the molar. If the taurodont index is ≥30-40, the molar is classified as a mesotaurodont. If the taurodont index is ≥40, the molar is classified as a hypertaurodont. If the taurodont index is < 30, the molar is classified as normal.

Taurodont (Mandibular Molars)	Tooth #	Variable 1 (Pixels)	Variable 2 (Pixels)	Taurodont Index
Subject				
Family 1- Subject II:1	18	11	44	25
	19	11	56	19
	30	11	63	17
	31	13	57	23
Family 1- Subject II:2	18	24	50	48**
	19	8	64	13
	30	9	73	12
	31	19	46	41**
Family 2- Subject II:2	18	4	65	8
	19	6	65	9
	30	4	52	8
	31	4	50	8
Family 2- Subject III:5	19	8	69	12
	30	7	66	11
Family 3- Subject II:2	18	5	30	17
	19	2	32	6
	30	2	32	6
	31	3	29	10
Family 4- Subject II:2	18	10	43	23
	19	13	55	24
	30	11	51	22
	31	11	48	23
Family 5- Subject V:1	N/A	N/A	N/A	N/A

** represents hypertaurodont classification
N/A represents teeth that are not diagnostic for measurement on available radiographs

Table 26. Presence of peg lateral was determined by the ratio of mesial-distal (M-D) width dimension of central incisors compared to ratio of M-D dimension of lateral incisors. A normal central incisor (CI) M-D width to lateral incisor (LI) M-D width ratio should equal 1.3. CI/LI ratios greater than 1.3 determines small lateral incisor/peg lateral classification.

Peg Lateral	Tooth # (CI)	M-D Width (Pixels)	Tooth # (LI)	M-D Width (Pixels)	CI/LI Ratio
Subject					
Family 1- Subject II:1	8	37	7	28	1.3
	9	41	10	31	1.3
Family 1- Subject II:2	8	50	7	38	1.3
	9	47	10	35	1.3
Family 2- Subject II:2	8	42	7	35	1.2
	9	40	10	34	1.2
Family 2- Subject III:5	8	36	7	N/A	N/A
	9	36	10	22	1.6*
Family 3- Subject II:2	8	22	7	17	1.3
	9	23	10	18	1.3
Family 4- Subject II:2	8	38	7	32	1.2
	9	41	10	31	1.3
Family 5- Subject V:1	8	42	7	N/A	N/A
	9	44	10	N/A	N/A

* represents a CI/LI ratio significantly > 1.3 and peg lateral classification
N/A represents teeth that are not present and therefore no ratio calculation can be made

Table 27. Measurements for root length and crown length measurements taken at time one (T1) and time two (T2) across all subjects and tooth types.

Measurements of Root Length and Crown Length at T1 and T2		T1		T2	
Subject	Tooth #	R1 (Pixels)	C1 (Pixels)	R2 (Pixels)	C2 (Pixels)
Family 1, Subject II:1	2	59	38	55	37
	3	63	35	53	36
	4	38	35	37	34
	5	39	40	39	44
	6	86	58	81	56
	7	50	39	54	47
	8	50	36	46	39
	9	49	44	44	42
	10	50	43	54	44
	11	84	61	79	57
	12	30	40	37	44
	13	40	37	40	41
	14	51	44	52	43
	15	58	37	61	45
	18	48	34	48	37
	19	56	36	61	39
	20	50	38	49	34
	21	57	40	59	36
	22	61	42	66	40
	23	63	44	66	37
	24	61	38	63	33
	25	59	39	63	32
	26	72	41	71	40
	27	58	44	65	42
	28	63	38	66	36
	29	62	40	62	37
	30	60	37	60	42
	31	55	31	53	34
Family 1, Subject II:2	3	64	38	66	37
	4	40	39	41	37
	5	51	38	51	42
	6	88	50	80	54
	7	59	44	53	44
	8	35	45	35	46
	9	38	46	38	46
	10	61	42	54	42

	11	84	45	85	50
	12	57	38	53	41
	13	42	38	43	39
	14	67	36	62	44
	19	59	32	63	32
	20	48	36	51	33
	21	72	36	68	36
	22	83	43	86	40
	23	60	41	64	39
	24	55	38	58	35
	25	64	39	64	38
	26	66	41	65	40
	27	87	45	87	41
	28	64	39	70	35
	29	64	39	55	30
	30	64	35	71	34
Family 2- Subject II:2	2	59	39	59	37
	3	44	40	46	42
	4	38	34	38	34
	5	30	36	33	37
	7	47	39	50	37
	8	39	39	40	40
	9	37	41	36	40
	10	52	40	52	40
	12	33	41	33	40
	13	30	36	29	35
	14	43	39	44	38
	15	62	35	60	34
	18	59	36	58	32
	19	51	39	51	37
	20	48	33	50	30
	22	45	36	42	36
	23	43	33	45	30
	25	43	28	37	30
	26	46	33	46	30
	27	49	41	49	37
	29	38	29	37	25
	30	57	36	57	35
	31	51	34	54	30
Family 2, Subject III:5	3	51	35	50	31
	4	37	33	36	32
	5	23	25	24	22

	6	48	35	53	39
	8	51	40	54	39
	9	48	42	49	40
	10	47	32	49	31
	11	61	37	61	36
	12	44	37	39	34
	13	32	36	34	32
	14	37	30	45	32
	19	69	33	63	32
	20	39	29	38	33
	21	42	32	42	31
	22	44	39	42	40
	23	41	33	43	30
	24	32	23	37	26
	25	29	24	36	30
	26	50	32	49	30
	27	56	40	55	38
	28	40	33	45	32
	29	37	32	41	33
	30	63	33	59	30
Family 3, Subject II:2	2	27	26	28	26
	3	33	23	34	24
	4	30	28	27	25
	5	30	29	28	26
	6	31	27	28	25
	7	24	25	23	25
	8	23	31	26	24
	9	22	30	26	25
	10	28	22	27	20
	11	32	25	31	23
	12	31	25	33	21
	13	28	20	31	20
	14	29	21	29	22
	15	17	20	18	21
	18	27	23	23	24
	19	30	23	27	22
	20	23	22	23	19
	21	29	23	28	21
	22	27	22	27	20
	23	27	19	29	19
	24	21	20	20	17
	25	25	20	25	19

	26	27	20	25	18
	27	29	21	24	20
	28	30	23	27	21
	29	27	20	25	19
	30	32	22	30	21
	31	24	22	27	22
Family 4, Subject II:2	2	49	46	52	41
	3	53	44	49	44
	4	45	43	44	49
	5	40	44	42	49
	6	52	53	52	55
	7	30	55	33	48
	8	31	60	46	49
	9	33	63	46	46
	10	39	39	37	40
	11	55	52	56	52
	12	39	46	44	46
	13	44	44	46	48
	14	56	41	56	42
	15	52	40	51	48
	18	44	40	42	39
	19	46	48	47	48
	20	38	43	40	43
	21	42	42	46	43
	22	50	53	57	53
	23	41	45	38	44
	24	34	38	35	38
	25	32	35	35	34
	26	41	47	43	48
	27	56	52	58	50
	28	40	45	41	44
	29	37	42	39	41
	30	56	40	50	40
	31	43	43	46	38
Family 5, Subject V:1	2	44	45	43	47
	3	43	40	53	37
	4	33	37	33	34
	5	35	42	37	40
	6	39	47	39	44
	8	33	41	44	40
	9	35	42	37	38
	11	15	17	14	15

	12	21	31	20	30
	13	35	34	37	39
	14	62	41	48	41
	15	39	35	38	40
	18	29	24	28	22
	19	66	40	60	46
	20	41	34	36	33
	21	37	33	36	33
	22	35	30	35	33
	23	29	33	27	29
	24	30	30	30	28
	25	28	31	30	29
	27	37	33	38	35
	28	36	35	37	34
	29	30	35	30	33
	30	64	42	62	37
	31	49	38	47	34

Table 28. Measurements for root width (RW) and crown width (CW) taken at time one (T1) and time two (T2) across all subjects and tooth types.

ICC for Root Width and Crown Width		T1		T2	
Subject	**Tooth #**	**RW1 (Pixels)**	**CW1 (Pixels)**	**RW2 (Pixels)**	**CW2 (Pixels)**
Family 1, Subject II:1	8	22	28	22	28
	9	23	28	25	31
	24	16	22	18	22
	25	17	20	17	21
Family 1, Subject II:2	8	42	43	41	40
	9	40	44	40	43
	24	20	25	20	24
	25	22	26	20	25
Family 2, Subject II:2	8	34	37	33	37
	9	32	36	33	37
	25	17	21	16	20
Family 2, Subject III:5	8	26	32	24	34
	9	27	32	26	32
	24	11	15	12	16
	25	11	17	12	17
Family 3, Subject II:2	8	20	19	20	19
	9	21	20	21	22
	24	12	14	12	13
	25	11	13	11	13
Family 4, Subject II:2	8	33	36	35	39
	9	35	40	35	40
	24	17	22	17	21
	25	14	19	17	22
Family 5, Subject V:1	8	39	43	40	44
	9	40	42	40	43
	24	26	32	30	34
	25	33	34	28	30

Table 29. Intraclass correlation coefficient (ICC) calculated for root length (R) and crown length (C) measurements across all tooth types. Intraclass correlation coefficient (ICC) calculated for crown width (CW) and root width (RW) measurements on maxillary and mandibular central incisors only. An ICC value of 0.9 and above represents a strong intraclass correlation. An ICC value of 0.8-0.89 represents good intraclass correlation. An ICC value of 0.7 to 0.79 represents a fair intraclass correlation. An ICC value of 0.5 and below represents a poor intraclass correlation. This is a two-way mixed effects model where people effects are random and measures effects are fixed.

Tooth Number	ICC for Root Length (R1, R2)	ICC for Crown Length (C1, C2)	ICC for Root Width (RW1, RW2)	ICC for Crown Width (CW1, CW2)
2	0.983	0.945	N/A	N/A
3	0.847	0.949	N/A	N/A
4	0.965	0.883	N/A	N/A
5	0.981	0.920	N/A	N/A
6	0.980	0.969	N/A	N/A
7	0.965	0.884	N/A	N/A
8	0.722	0.822	0.989	0.977
9	0.785	0.701	0.993	0.983
10	0.951	0.992	N/A	N/A
11	0.997	0.985	N/A	N/A
12	0.928	0.940	N/A	N/A
13	0.959	0.918	N/A	N/A
14	0.860	0.591	N/A	N/A
15	0.995	0.836	N/A	N/A
18	0.989	0.945	N/A	N/A
19	0.949	0.948	N/A	N/A
20	0.966	0.911	N/A	N/A
21	0.986	0.963	N/A	N/A
22	0.982	0.980	N/A	N/A
23	0.983	0.919	N/A	N/A
24	0.987	0.925	0.993	0.986
25	0.967	0.841	0.937	0.947
26	0.997	0.984	N/A	N/A
27	0.985	0.962	N/A	N/A
28	0.973	0.960	N/A	N/A
29	0.961	0.864	N/A	N/A
30	0.947	0.902	N/A	N/A
31	0.974	0.869	N/A	N/A

Table 30. Summary table describing phenotypic characteristics observed in subjects with localized and generalized short root anomalies.

Phenotype	Localized SRA Cases (n=4)	Generalized SRA cases (n=3)
Root Length (Short Roots)	Seen in: Maxillary central incisors, first premolars, second premolars, first molars	Seen in: Maxillary central incisors, lateral incisors, canines, first premolars, second premolars, first molars, second molars. Mandibular central incisors, lateral incisors, canines, first premolars, second premolars, first molars, second molars.
Wide Root Width (Central Incisors only)	Seen in: Maxillary central incisors, Mandibular central incisors	Seen in: Maxillary central incisors Mandibular central incisors
Taurodontism (Molars only)	Seen in: Maxillary second molars, Mandibular second molars	Seen in: Maxillary second molars
Peg Lateral	Present in one subject	Not observed
Agenesis	Seen in: Lateral incisor	Seen in: Maxillary lateral incisors, canines Mandibular central incisor, lateral incisor, first premolar, second premolar
Other Dental Anomalies	Dens invaginatus Pulp stones	Pulpal obliteration Hypercementosis Ectopic eruption Pulp stones

Chapter IX

Figures

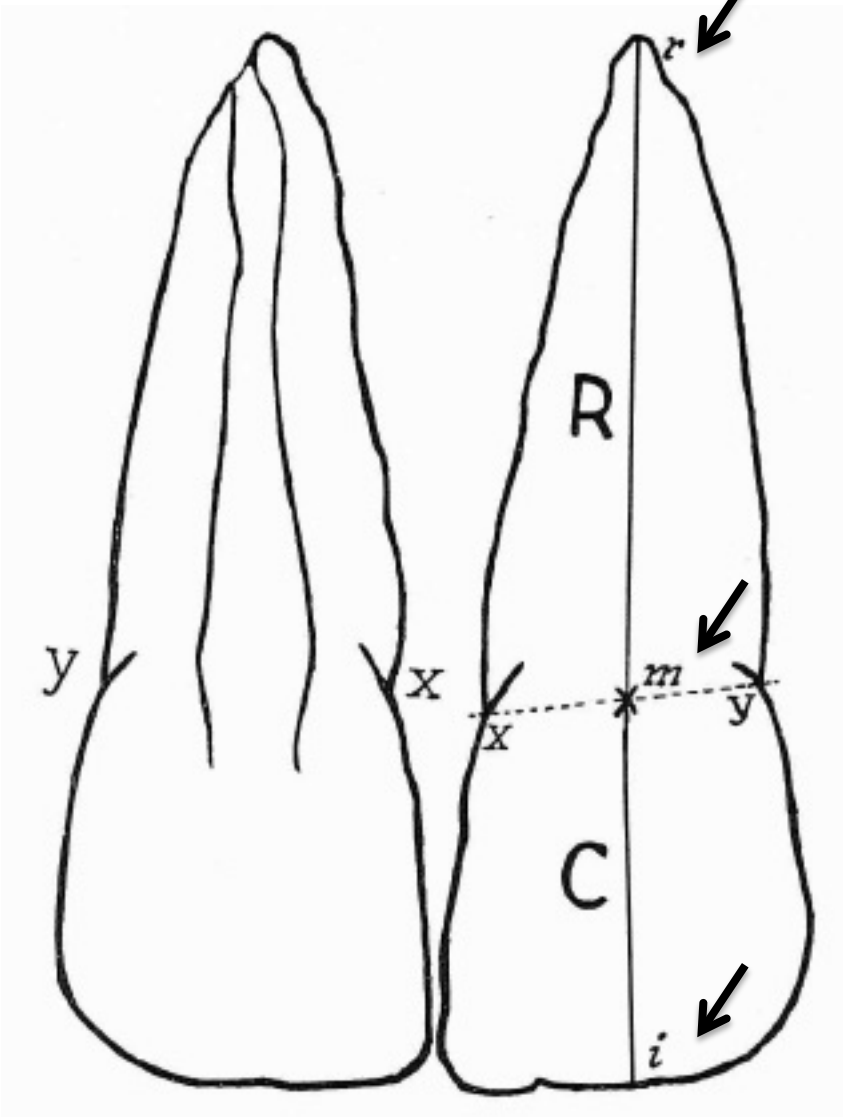


Figure 1. Modified diagram from Figure 5. of Lind article (25) to depict anatomical landmarks used to measure relative root length (R/C). Points of intersection between the outer contours of the root and their crown, x and y are connected by a straight line. Root length (R) is measured from the midpoint of this line, (m), to the apex (r), and crown height (C) is measured from (m) to the middle of the incisal edge (i).

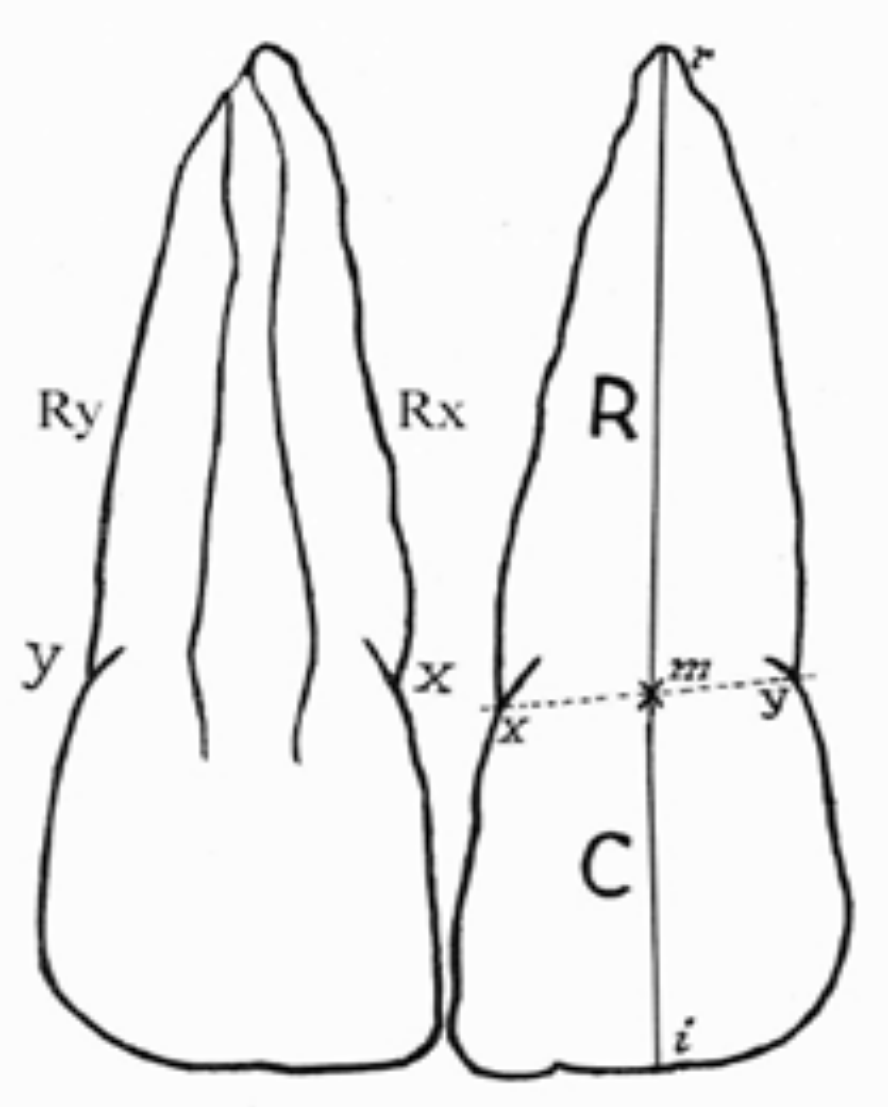


Figure 2. Modified diagram from Figure 5. of Lind article (25) to depict "Ry" and "Rx", landmarks used to measure root width at the middle 1/3rd of the root. The formula used to measure root width is the distance from Rx to Ry divided by the distance from x to y. "Rx to Ry" is equivalent to the root width at the middle third while the distance from x to y represents the crown width at the level of the CEJ. Root width ratios of maxillary central incisors greater than 0.87 are classified as "wide", and anything less than 0.87 is classified as "normal". Root width ratios of mandibular central incisors greater than 0.85 are classified as "wide", and anything less than 0.85 is classified as "normal".

Tooth	Cervical	Middle third	Apical third
Central incisor	3.44 ± 0.29	2.85 ± 0.29	1.92 ± 0.31
	2.87 to 4.01	2.28 to 3.42	1.31 to 2.53
	2.60 to 4.30	2.30 to 3.50	1.40 to 2.60

Figure 3. Root widths of mandibular teeth. Adapted from Table 2 of Tilk et al. article (38) describing average root widths of mandibular central incisors at the cervical third, middle third, and apical third. Individual standard deviations noted for the three width areas.

Tooth	Cervical	Middle third	Apical third
Central incisor	6.22 ± 0.51*	5.15 ± 0.58	3.87 ± 0.54
	5.22 to 7.22†	4.01 to 6.29	2.80 to 4.94
	5.20 to 7.80‡	3.70 to 6.60	2.50 to 4.90

Figure 4. Root widths of maxillary teeth. Adapted from Table 1 from Tilk et al. article (38) describing average root widths of maxillary central incisors at the cervical third, middle third, and apical third. Individual standard deviations noted for the three width areas. All values are expressed in mm.
*Mean and standard deviation
+95% confidence interval
++Range

Root shape	Taurodont index
Normal (cynodont)	Less than 20
Hypo-taurodont	From 20 to up to 30
Meso-taurodont	Over 30 to up to 40
Hyper-taurodont	Greater than 40

Figure 5. Taurodont Index adapted from Table 1. of MacDonald et al. article (26). Classification of type of taurodont (hypo-, meso-, and hyper-) with corresponding taurodont index. Taurodont index is defined by the formula: (Variable 1/Variable 2) x 100. Variable 1 represents the lowest point of the root of the pulp chamber to the highest point of the floor of the pulp chamber. Variable 2 is defined as the lowest point of the roof of the pulp chamber to the apex of the longest root of the tooth. If the calculated taurodont index is less than 20, the root shape is classified as "normal". If the calculated number is between 20 to 30, it is classified as hypotaurodont. If the calculated number is between 30 and 40, the tooth is classified as a mesotaurodont, and if the taurodont index is any higher than 40, the tooth is classified as a hypertaurodont. For the purpose of this study, only teeth that are classified as mesotaurodont or hypertaurodont are classified as taurodonts in the phenotypic analysis.

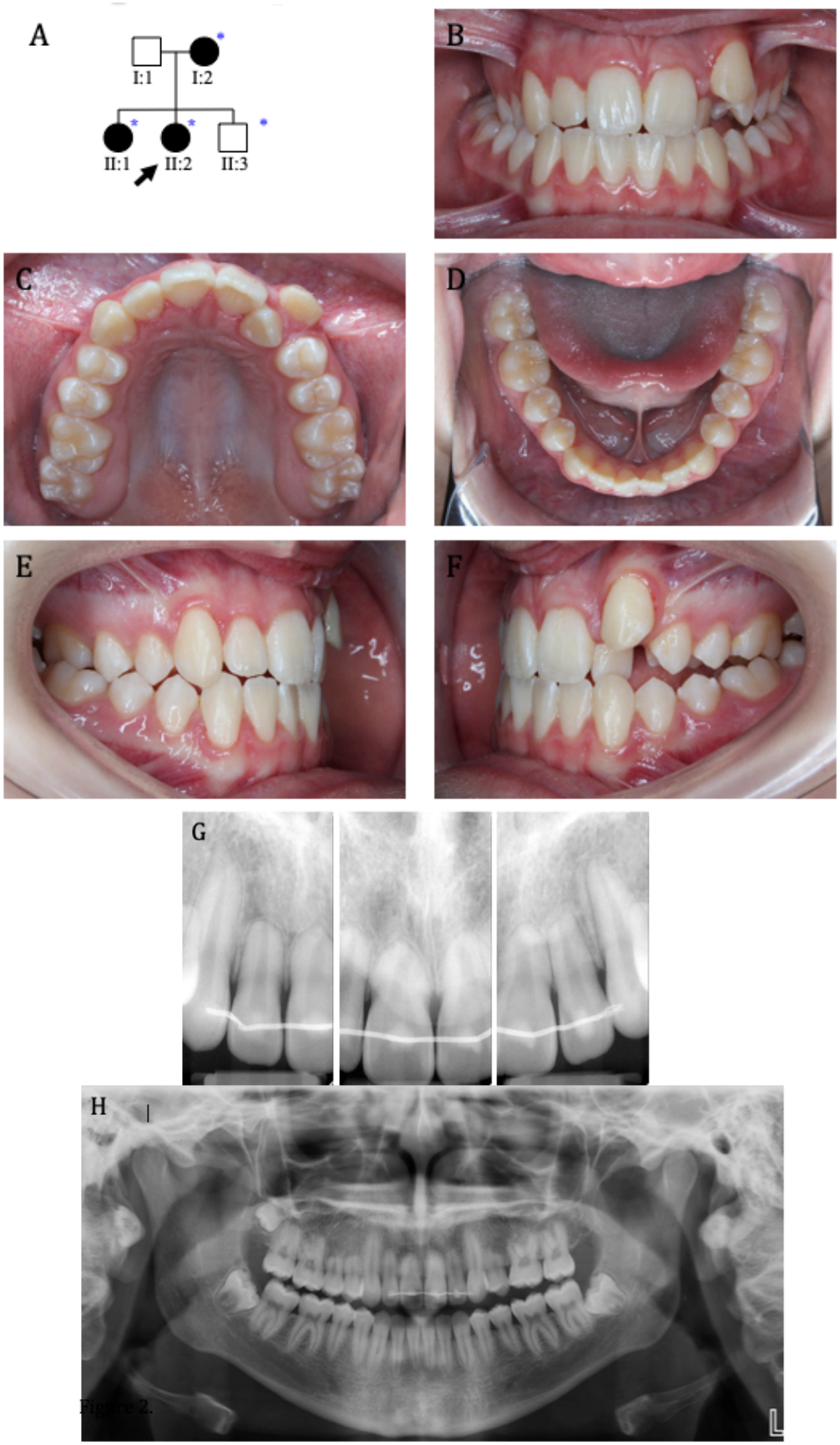


Figure 6. Pedigree, oral photographs, and radiographs of subject II:1(age 14) in Family one below. Localized short roots noted on #4, 5, 9, 12, 13. Wide root width noted on #25, taurodontism on #2, 15, and pulp stones noted in #3, 14, 15.

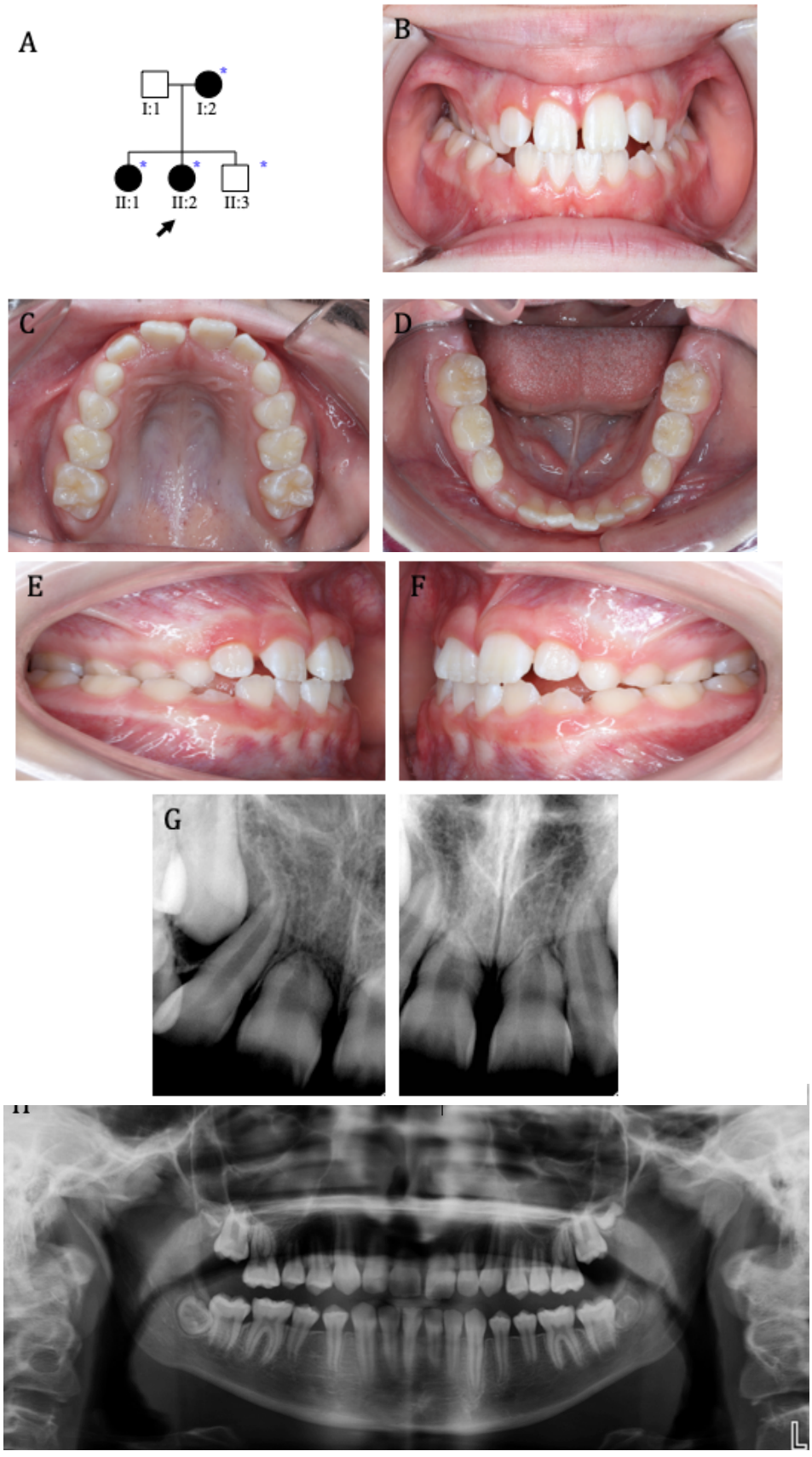


Figure 7. Pedigree, oral photographs, and radiographs of subject II:2 (age 8) in Family one below. Localized short roots noted on #4, 8, 9, 13. Wide root width noted on #8 and #9, taurodontism noted on #2, 15, 18, 31, and pulp stone identified on #30.

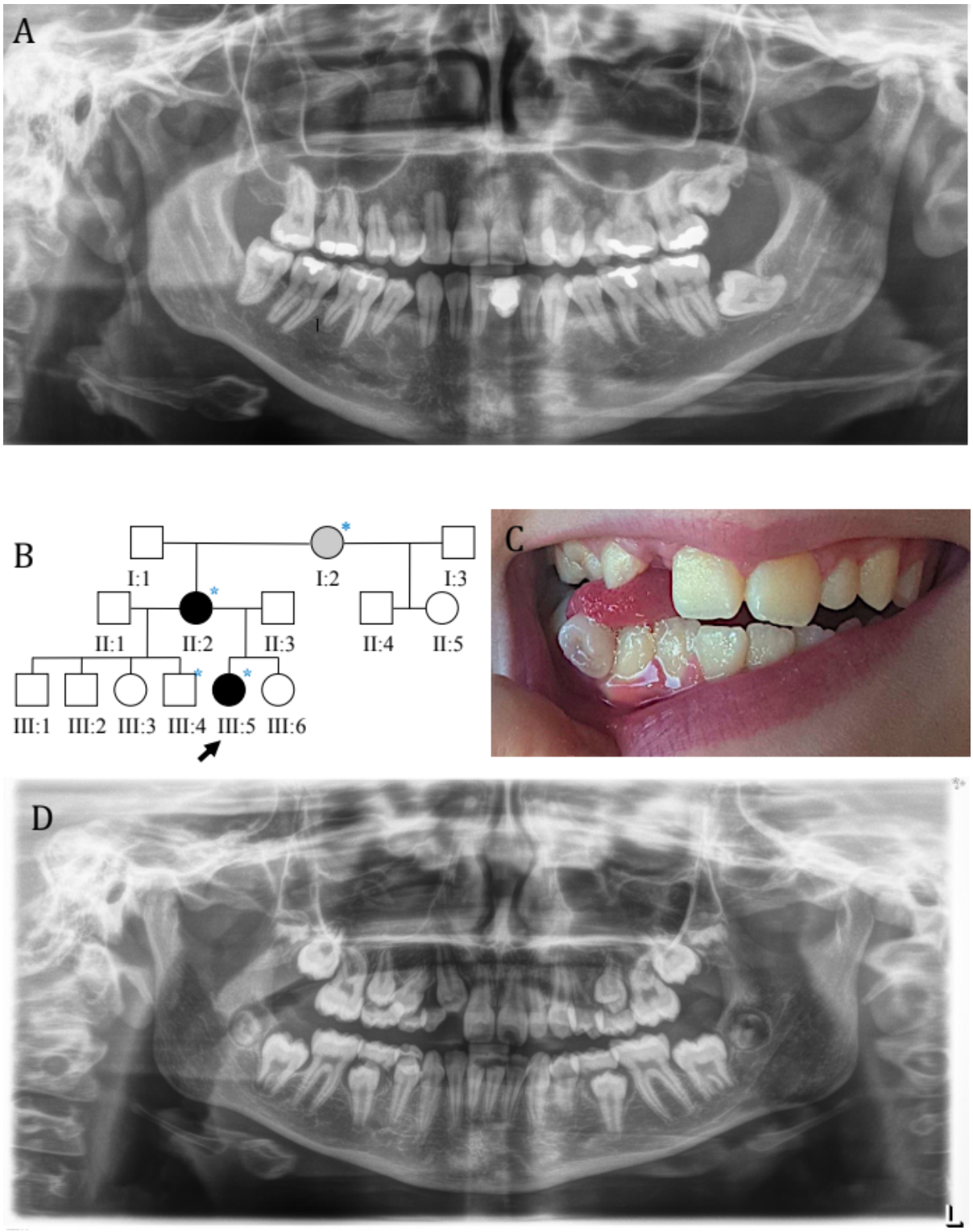


Figure 8. Panoramic radiograph of subject II:2 in Family Two noted in box "A". Localized short roots noted on #3, 4, 5, 8, 9, 12, 13, 14. Wide root width noted on #8, 9. Box "B" illustrates the pedigree of both affected subjects II:2 and III:5 in Family Two. Box "C" is a clinical oral photograph of subject III:5. Box "D" is a panoramic radiograph of subject III:5 from Family Two. Localized short roots noted on #4, 5, and 13. Agenesis of #7, invaginatus of #8, peg lateral of #10, and pulp stone noted on #3.

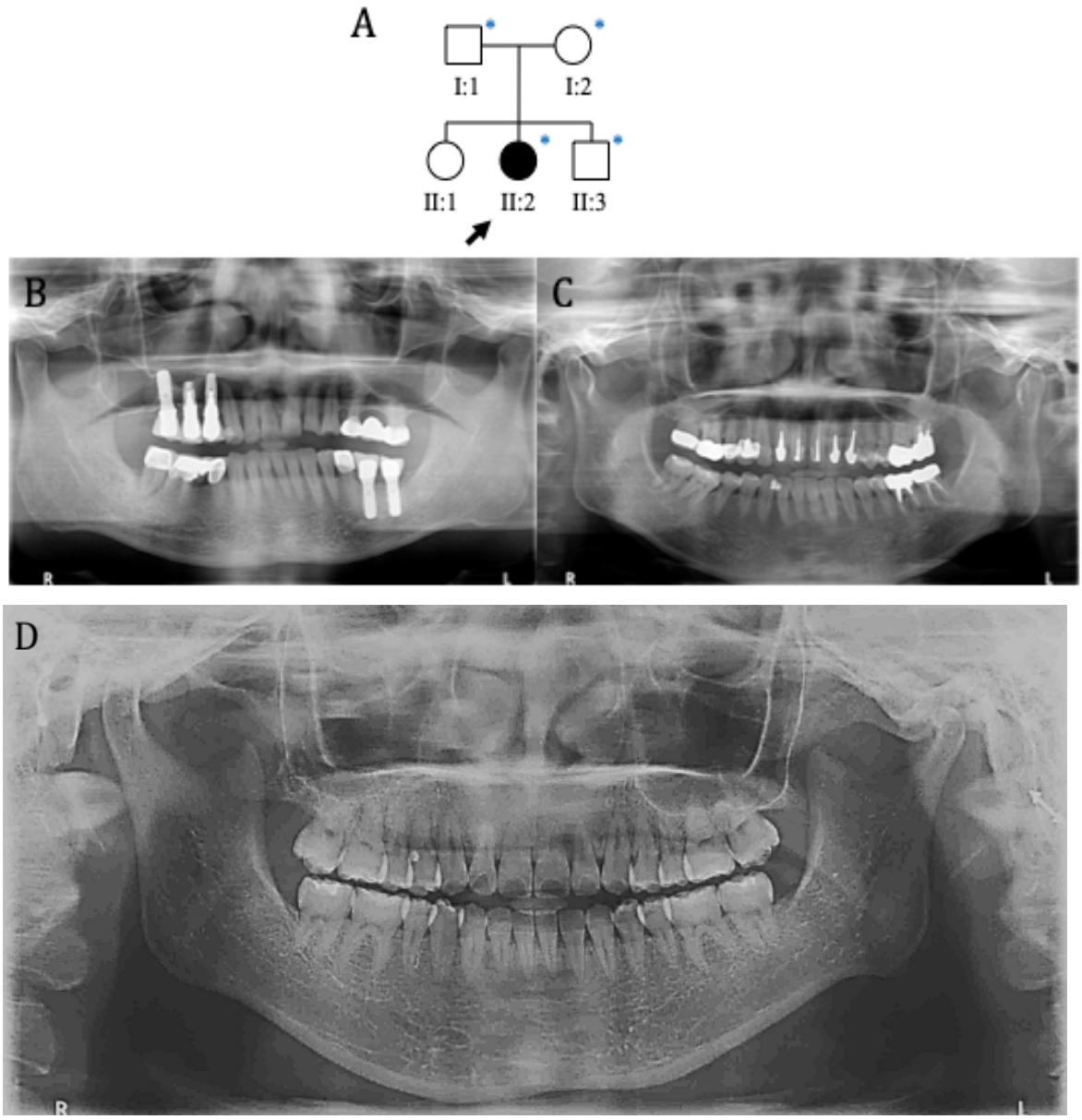


Figure 9. Pedigree and panoramic radiograph for subject II:2 in Family Three (boxes A and D). Generalized short roots noted on #2, 4, 5, 7, 8, 9, 15, 20, 24, 31. Wide root width noted on #8 and #9 and generalized obliteration of pulp noted on panoramic radiograph. Box "B" is the panoramic radiograph of subject I:1 (unaffected father), and Box "C" is the panoramic radiograph of subject I:2 (unaffected mother). Blue asterisks denote members recruited in this study.

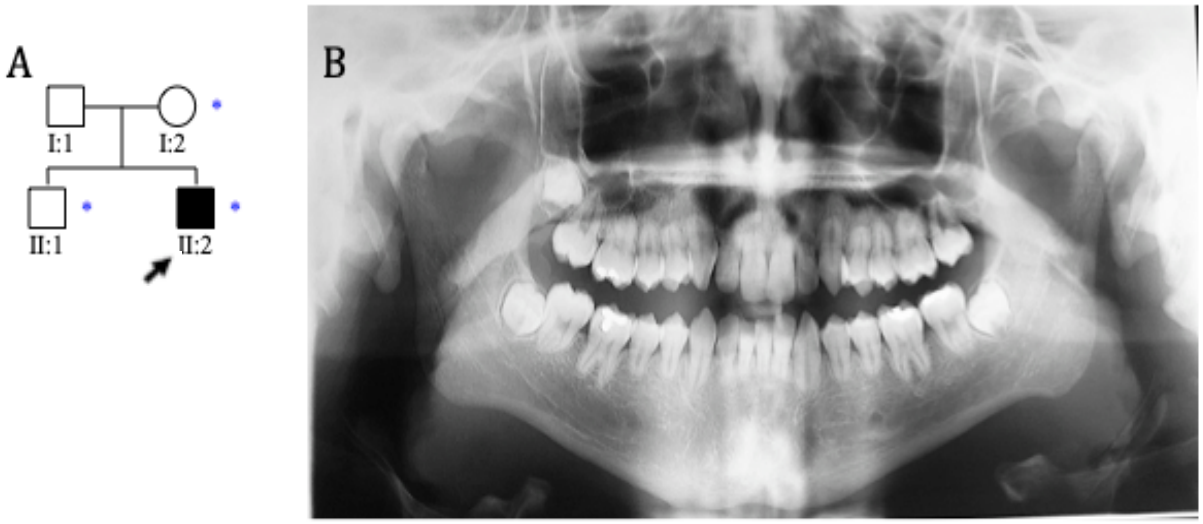


Figure 10. Pedigree and panoramic radiograph for subject II:2 in Family Four below. Generalized short roots noted on #2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 18, 19, 20, 21, 22, 23, 26, 27, 28, 29, 31. Wide root widths noted on #8 and #9, taurodontism noted on #2 and #15, and hypercementosis noted on #3, 14, 18, 30, and 31.

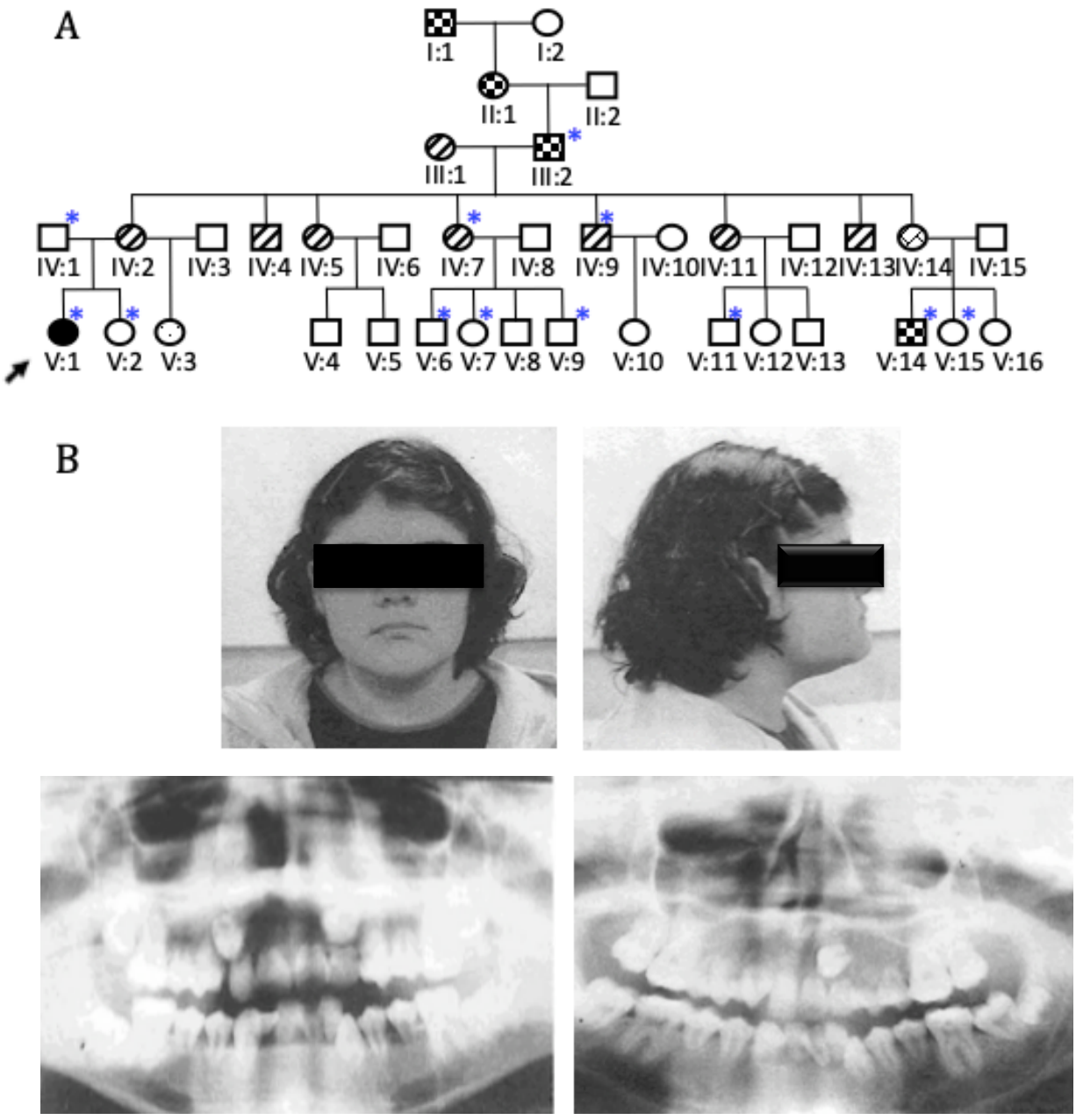


Figure 11. Pedigree, clinical photos, and radiographs of subject V:1 in Family Five. Generalized short roots noted on #2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 15, 21, 23, 24, 25, 28, 29. Wide root width noted on #8, 9, and 25. Agenesis of #7, 10, 26, and ectopic eruption noted of #11.

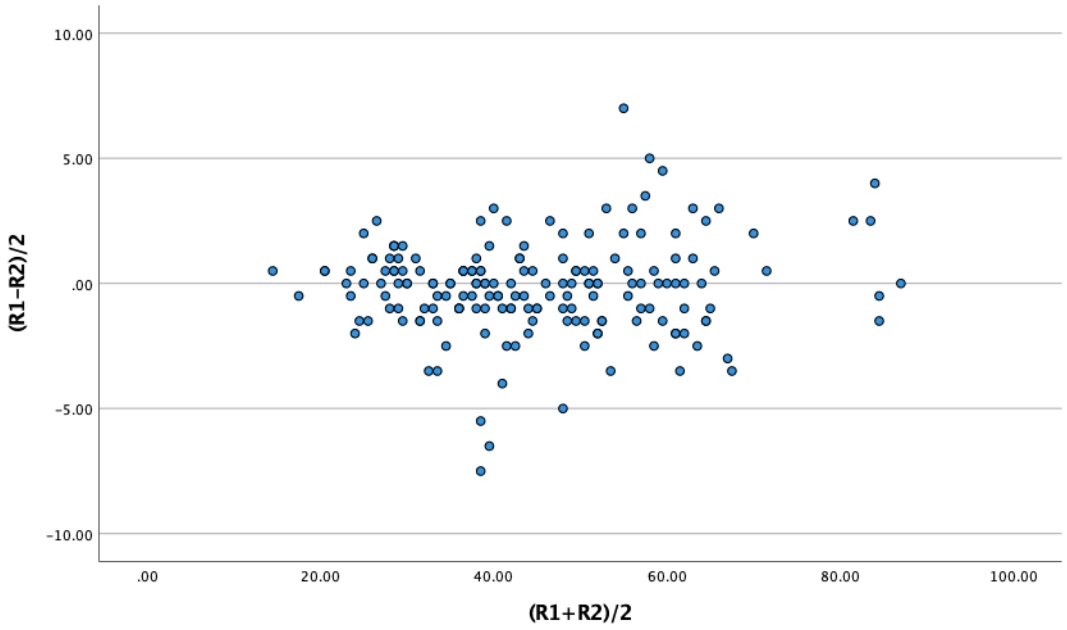


Figure 12. Bland-Altman plot depicting root length taken the first time =R1 compared to root length taken the second time=R2 across all 7 subjects and tooth types. All measurements, with the exception of four measurements, are within 5 pixels of each other. X-axis represents the average of R1 and R2 while the Y-axis represents the difference between R1 and R2.

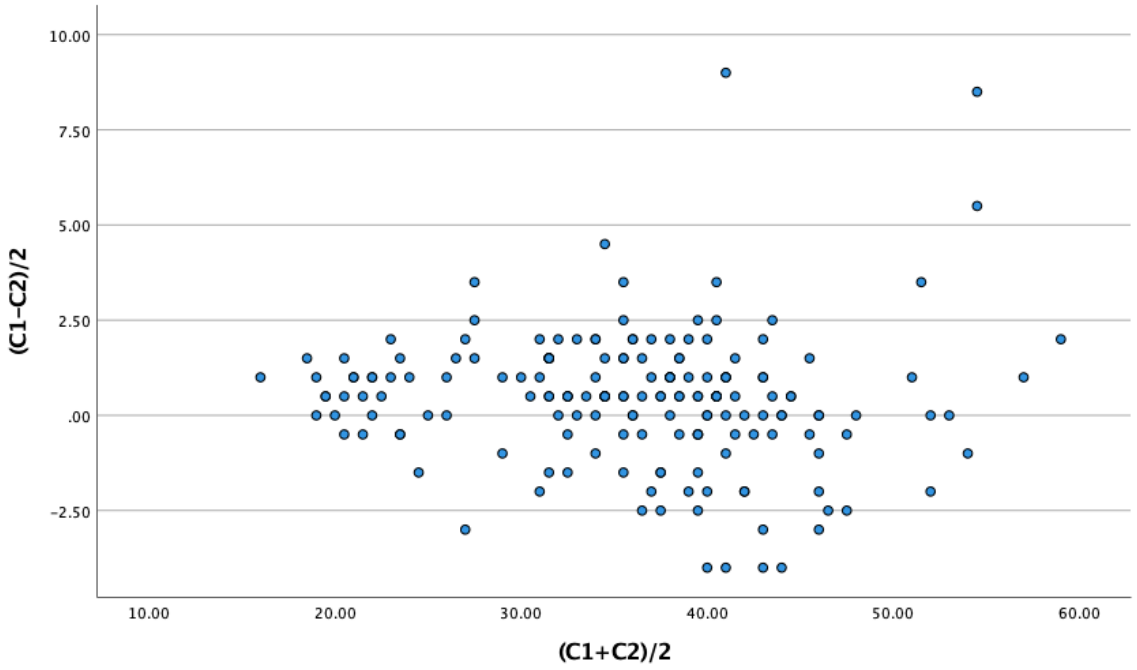


Figure 13. Bland-Altman plot depicting crown length taken the first time =C1 compared to crown length taken the second time=C2 across all seven subjects and tooth types. X-axis represents the average of C1 and C2 while the Y-axis represents the difference between C1 and C2.

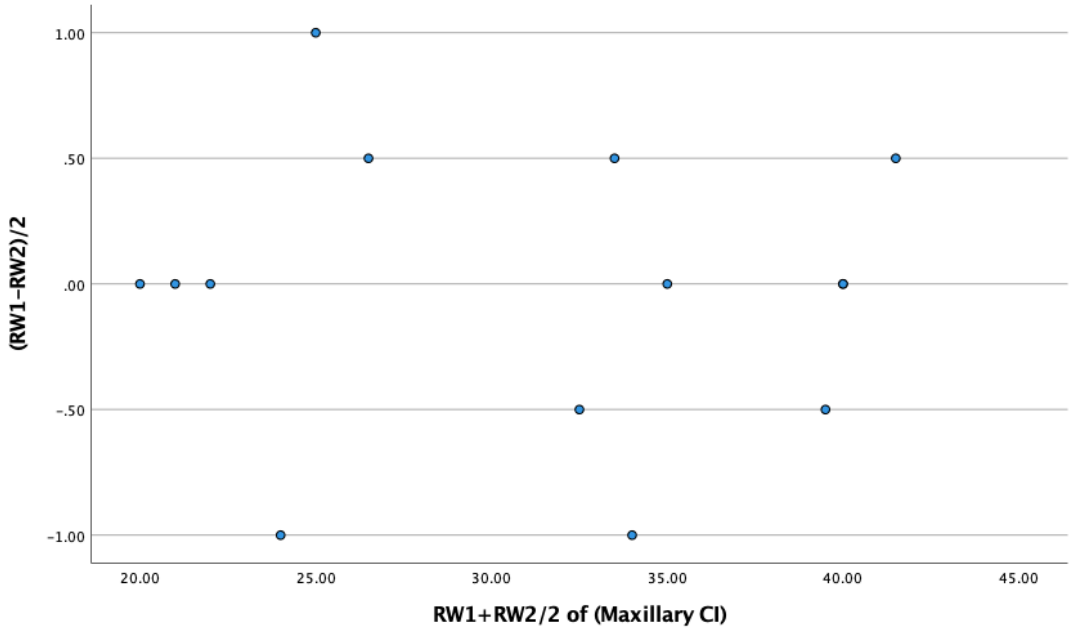


Figure 14. Bland-Altman plot depicting root width taken the first time =RW1 compared to crown length taken the second time=RW2 across all seven subjects for maxillary central incisors. All measurements are within 1 unit (pixel) of each other. X-axis represents the average of RW1 and RW2 while the Y-axis represents the difference between RW1 and RW2. Data appears scattered due to small amount of data points (only root widths of central incisors were measured).

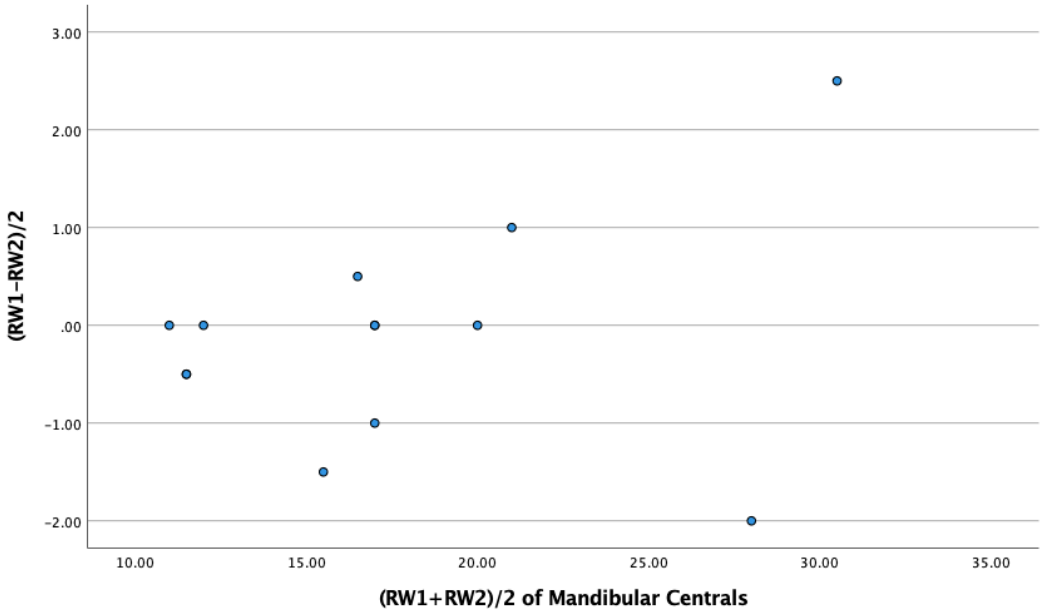


Figure 15. Bland-Altman plot depicting root width taken the first time =RW1 compared to root width taken the second time=RW2 of mandibular central incisors across all seven subjects. X-axis represents the average of RW1 and RW2 while the Y-axis represents the difference between RW1 and RW2. Data appears scattered due to small amount of data points (only root widths of central incisors were measured).

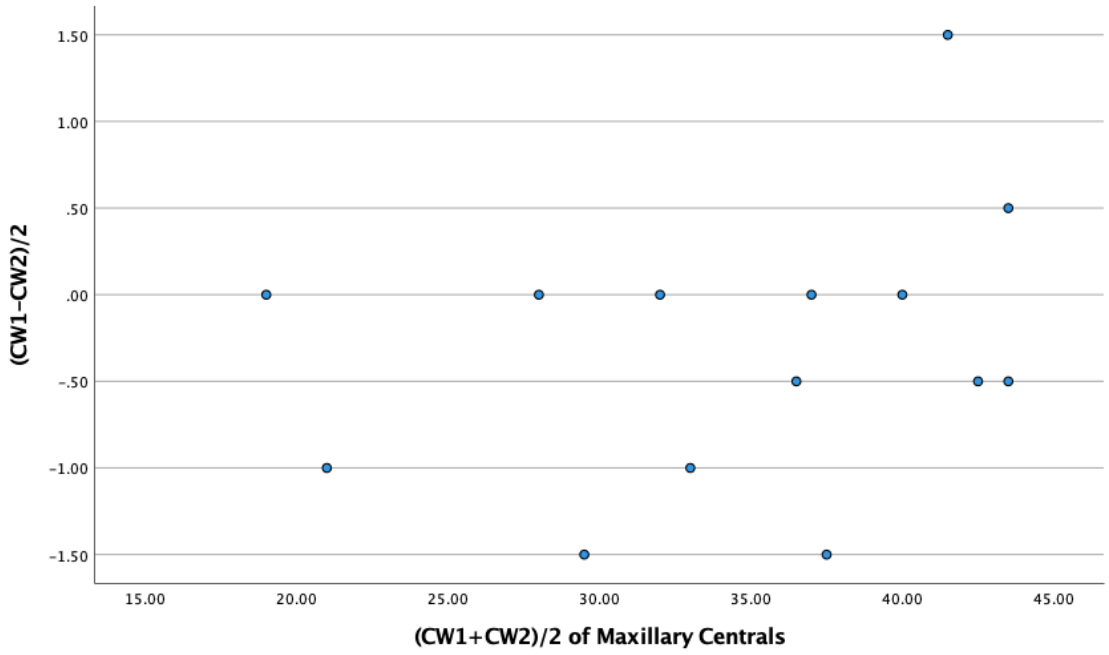


Figure 16. Bland-Altman plot depicting crown width taken the first time =CW1 compared to crown width taken the second time=CW2 of maxillary central incisors across all seven subjects. X-axis represents the average of CW1 and CW2 while the Y-axis represents the difference between CW1 and CW2.

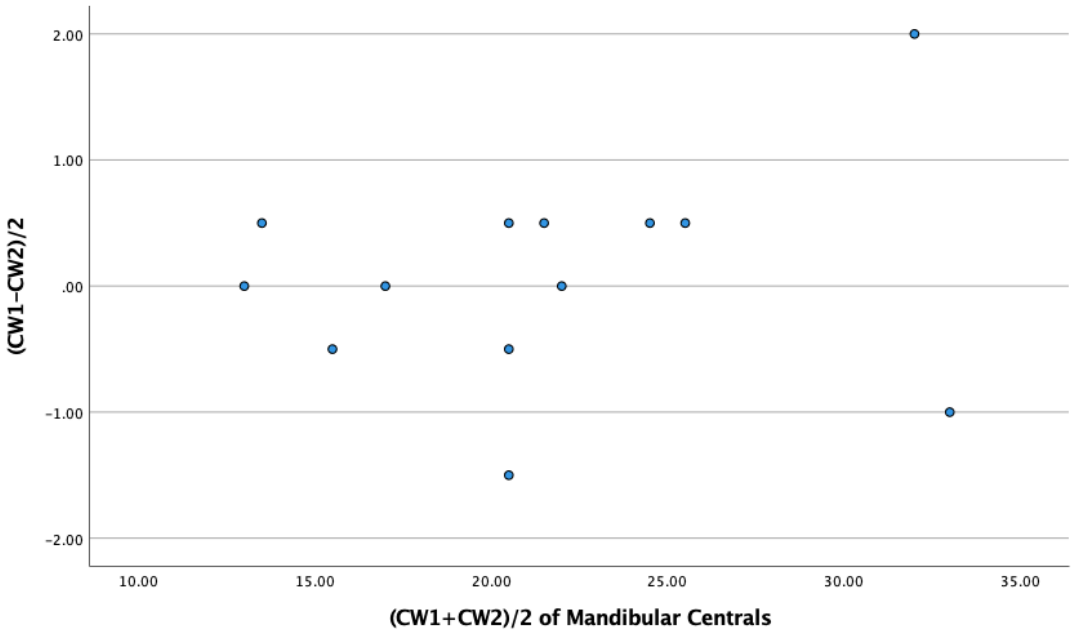


Figure 17. Bland-Altman plot depicting crown width taken the first time =CW1 compared to crown width taken the second time=CW2 of mandibular central incisors across all seven subjects. X-axis represents the average of CW1 and CW2 while the Y-axis represents the difference between CW1 and CW2.

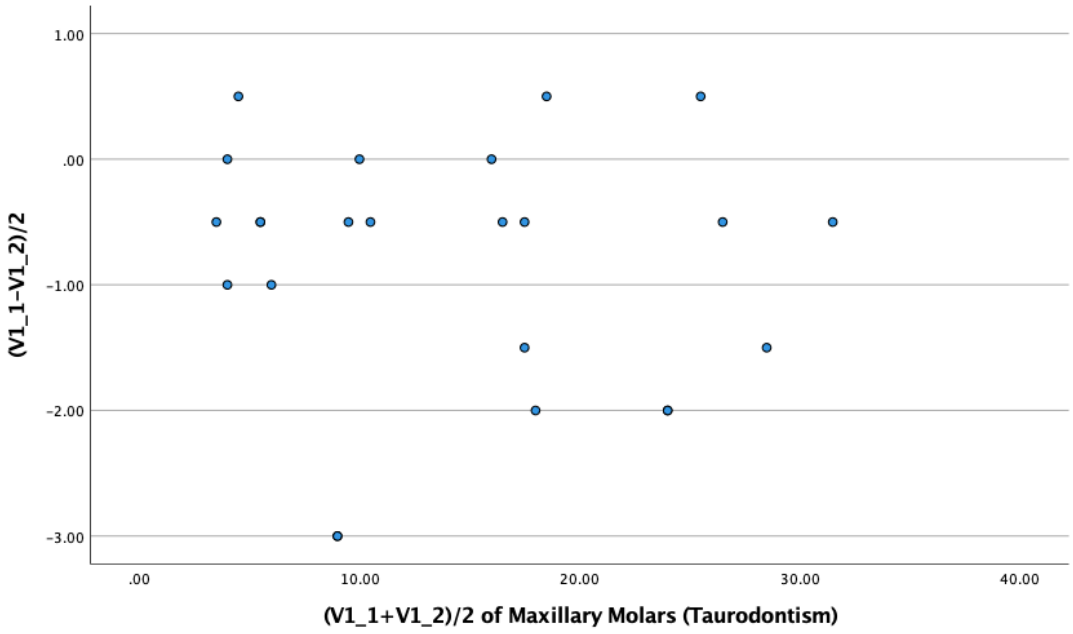


Figure 18. Bland-Altman plot depicting Variable 1 (one of the variables to assess Taurodont Index) taken the first time =V1_1 compared to Variable 1 taken the second time=V1_2 of maxillary first and second molars across all seven subjects. X-axis represents the average of V1_1 and V1_2 while the Y-axis represents the difference between V1_1 and V1_2.

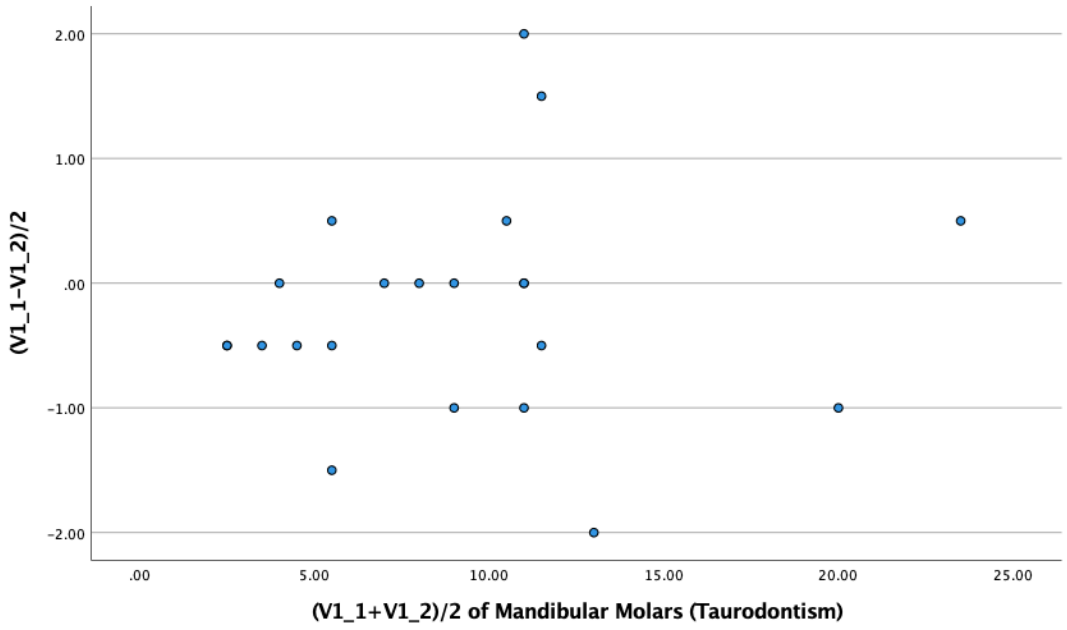


Figure 19. Bland-Altman plot depicting Variable 1 (one of the variables to assess Taurodont Index) taken the first time =V1_1 compared to Variable 1 taken the second time=V1_2 of mandibular first and second molars across all seven subjects. X-axis represents the average of V1_1 and V1_2 while the Y-axis represents the difference between V1_1 and V1_2.

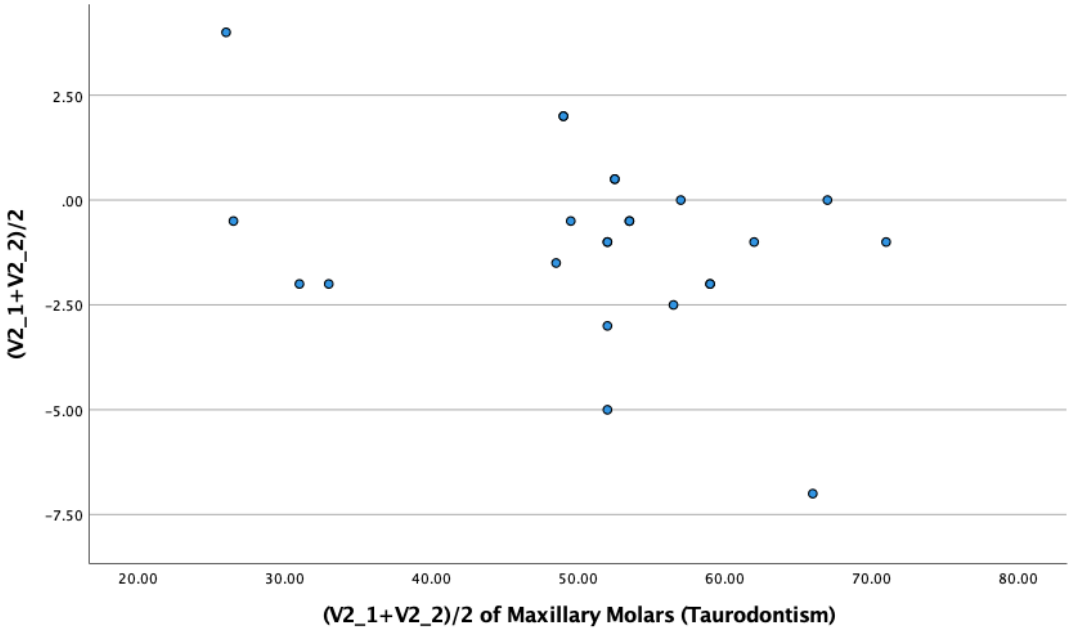


Figure 20. Bland-Altman plot depicting Variable 2 (one of the variables to assess Taurodont Index) taken the first time =V2_1 compared to Variable 2 taken the second time=V2_2 of maxillary first and second molars across all seven subjects. X-axis represents the average of V2_1 and V2_2 while the Y-axis represents the difference between V2_1 and V2_2.

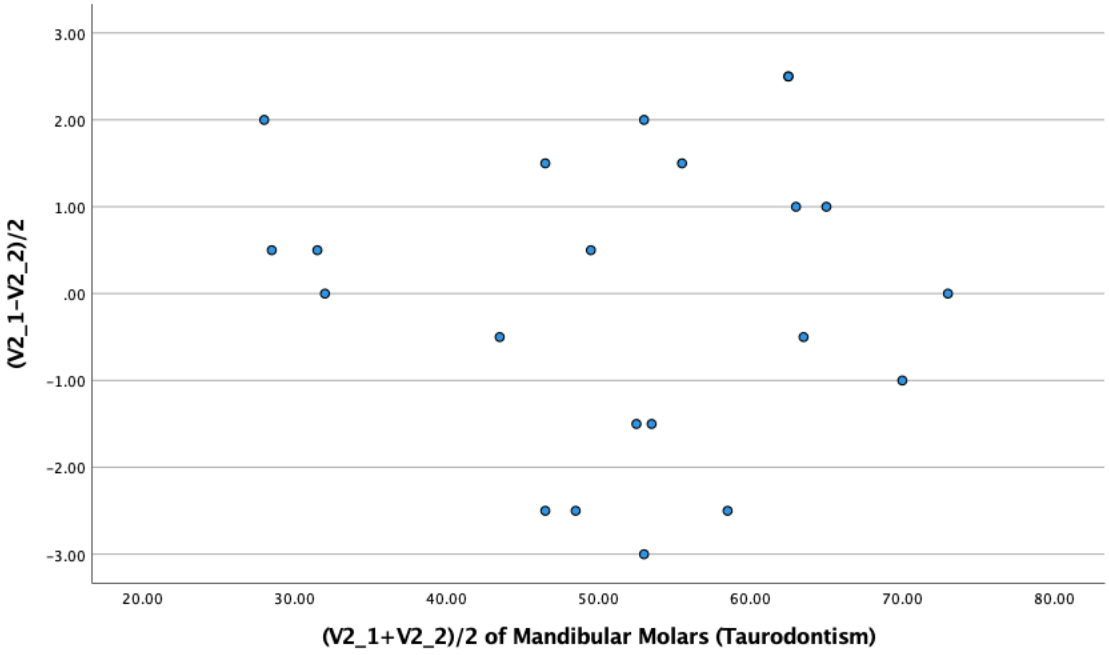


Figure 21. Bland-Altman plot depicting Variable 2 (one of the variables to assess Taurodont Index) taken the first time =V2_1 compared to Variable 2 taken the second time=V2_2 of mandibular first and second molars across all seven subjects. X-axis represents the average of V2_1 and V2_2 while the Y-axis represents the difference between V2_1 and V2_2.

Chapter X

Appendices

APPENDICES

Appendix A: Institutional Review Board- University of Michigan

UNIVERSITY OF MICHIGAN
eResearch.umich.edu
Medical School Institutional Review Board (IRBMED) • 2800 Plymouth Road, Building 520, Suite 3214, Ann Arbor, MI 48109-2800 • phone (734) 763 4768 • fax (734) 763 9603 • irbmed@umich.edu

To: Dr. James Simmer

FROM:

Michael	Geisser
Alan	Sugar
Robertson	Davenport

Cc:

Paul	Benke
Jan Ching Chun	Hu
James	Simmer
Hera	Kim-Berman
Paul	Edwards
Curtis	Rogers

Subject: Scheduled Continuing Review [CR00088040] Approved for [H03-00001835-M1]

SUBMISSION INFORMATION:
Study Title: Proteomics and Genetics of Enamel and Dentin
Full Study Title (if applicable): Genetics of disorders affecting tooth structure, number, morphology, and eruption.
Study eResearch ID: H03-00001835-M1
SCR eResearch ID: CR00088040
SCR Title: H03-00001835-M1_Continuing Review - Thu Apr 8 11:42:17 EDT 2021
Date of this Notification from IRB: 5/6/2021
Date Approval for this SCR: 5/6/2021
Review: Expedited
Expiration Date: Approval for this application expires on **11:59 p.m. on** 5/5/2022
UM Federal Assurance: FWA00004969 (For the current FWA expiration date, please visit the UM HRPP Webpage)
OHRP IRB Registration Number(s): IRB00001996

Approved Risk Level(s) as of this Continuing Report:

Name	Risk Level
H03-00001835-M1	No more than minimal risk

Continuing Review Required: Yes

NOTICE OF IRB APPROVAL AND CONDITIONS:
The IRBMED has reviewed and approved the scheduled continuing review (SCR) to the study referenced above. The IRB determined that the proposed research continues to conform with applicable guidelines, State and federal

regulations, and the University of Michigan's Federalwide Assurance (FWA) with the Department of Health and Human Services (HHS). You must conduct this study in accordance with the description and information provided in the approved application and associated documents.

APPROVAL PERIOD AND EXPIRATION:
The approval period for this study is listed above. Please note the expiration date. If the approval lapses, you may not conduct work on this study until appropriate approval has been re-established, except as necessary to eliminate apparent immediate hazards to research subjects. Should the latter occur, you must notify the IRB Office as soon as possible.

RENEWAL/TERMINATION:
The IRB has determined that annual review and approval is required for this research. At least two months prior to the expiration date, you should submit a continuing review application either to renew or terminate the study. Failure to allow sufficient time for IRB review may result in a lapse of approval that may also affect any funding associated with the study.

IMPORTANT REMINDERS AND ADDITIONAL INFORMATION FOR INVESTIGATORS

APPROVED STUDY DOCUMENTS:
You must use any date-stamped versions of recruitment materials and informed consent documents available in the eResearch workspace (referenced above). Date-stamped materials are available in the "Currently Approved Documents" section on the "Documents" tab.

AMENDMENTS:
All proposed changes to the study (e.g., personnel, procedures, or documents), must be approved in advance by the IRB through the amendment process, except as necessary to eliminate apparent immediate hazards to research subjects or others. Should the latter occur, you must notify the IRB Office as soon as possible.

AEs/ORIOs:
You must inform the IRB of adverse events (AEs) and other reportable information and occurrences (ORIOs) according to your IRB's required reporting timetable (IRBMED and IRB-HSBS/Flint/Dearborn).

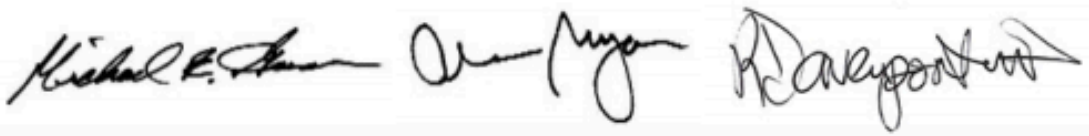
UNANTICIPATED PROBLEMS INVOLVING RISKS TO SUBJECTS OR OTHERS (UPIRSOs or UaPs)
Investigators must continue to inform the IRB via eResearch submission of any potential Unanticipated Problems (UaPs or UPIRSOs) that come to the attention of the study team. Unanticipated Problems meet all of the following criteria:

1. **Unexpected** (in terms of nature, severity, or frequency);
2. **Related or possibly related to participation in the research**; and
3. Suggests that the research places subjects or others at **a greater risk of harm** than was previously known or recognized.

See U-M HRPP Operations Manual Part 12.III.B.1.a. Routine AEs and ORIOs after Termination need not be reported.

SUBMITTING VIA eRESEARCH:
You can access the online forms for continuing review, amendments, and AE/ORIO reporting in the eResearch workspace for this approved study, referenced above.

MORE INFORMATION:
You can find additional information about UM's Human Research Protection Program (HRPP) in the Operations Manual and other documents available at: http://research-compliance.umich.edu/human-subjects.



Michael Geisser **Alan Sugar** **Robertson Davenport**

Appendix B: Institutional Review Board- University of Pittsburgh

University of Pittsburgh
Institutional Review Board

3500 Fifth Avenue
Ground Level
Pittsburgh, PA 15213
(412) 383-1480
(412) 383-1508 (fax)

MEMORANDUM

TO: Alexandre R. Viera, DDS, MS, PhD

FROM: Christopher Ryan, PhD, Vice Chair

DATE: April 19, 2012

SUBJECT: IRB #0606091: University of Pittsburgh School of Dental Medicine Dental Registry and DNA Repository

Your renewal of the above-referenced proposal has received expedited review and approval by the Institutional Review Board under 45 CFR 46.110 (3,5).

Please include the following information in the upper right-hand corner of all pages of the consent form:

Approval Date: April 19, 2012
Renewal Date: May 12, 2013
University of Pittsburgh
Institutional Review Board
IRB #0606091

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. The IRB Reference Manual (Chapter 3, Section 3.3) describes the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Event Coordinator at 412-383-1504.

The protocol and consent forms, along with a brief progress report must be resubmitted at least **one month prior** to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh), FWA00003567 (Magee-Womens Health Corporation), FWA00003338 (University of Pittsburgh Medical Center Cancer Institute).

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

CR:kh