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Craniometaphyseal dysplasia: the need for a natural history of disease study

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CRANIOMETAPHYSEAL DYSPLASIA: THE NEED FOR A NATURAL HISTORY OF DISEASE STUDY

by

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DEDICATION

This work is dedicated to Selina, the individual from whose strength my inspiration is derived.

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This study was made possible thanks to the hard work and dedication put forth by the Reichenberger Lab at University of Connecticut Health (UCH), to investigate and educate others on the rare genetic disorder, craniometaphyseal dysplasia.

CRANIOMETAPHYSEAL DYSPLASIA: THE NEED FOR A NATURAL HISTORY OF DISEASE STUDY

MICHAEL ANIL PERSAUD

ABSTRACT

Craniometaphyseal dysplasia (CMD) is a rare genetic skeletal disorder, whose biological understanding is not very well known. The disease manifests itself through bony hypertrophy of the skull base, craniofacial bones, and abnormal morphology of the long bones, present in the carrier of the disease. CMD has been previously determined through genetic analysis to be a result of one of 15 (to date) discovered mutations. Fourteen of those mutations are inherited in an autosomal dominant fashion, via mutations in the *ANKH* gene. One mutation has been discovered to result in CMD through autosomal recessive inheritance, via a locus found in the *connexin 43* gene, coding for gap junction protein alpha-1. As the genetic foundation of CMD has become more clearly understood over time, there has been a lack of similar progress in understanding the clinical manifestations of CMD. To improve our understanding of the clinical characteristics of CMD, we propose a natural history of disease study to be conducted. This study serves as a pilot for this larger scale study, by using a smaller patient population comprised of CMD patient database at the Reichenberger Lab at University of Connecticut Health (UCH), and CMD patients reported in the literature, to understand what is currently known about the clinical manifestations of CMD, and what should be evaluated for further research.

In this study, the existing literature on CMD has been compiled and sorted into distinct groups – created to guide those unfamiliar to the disease through the available information. Secondly, a set of 76 patient cases compiled at the Reichenberger lab at UCH were analyzed to determine what clinical information on CMD has already been collected. Lastly, from an in depth analysis of two specific case files from the Reichenberger Lab CMD patient database it was discovered that blood chemistry levels are an important parameter for analysis in future studies.

From abnormalities in blood chemistry within both cases were found. . In both patients it was found that elevations in serum alkaline phosphatase were present congenitally, and persisted throughout the early childhood years. Specific attention to changing serum alkaline phosphatase concentrations over early childhood development is recommended.

Additionally, from data present in patient case 2, blood urea nitrogen (BUN/creatinine) was found to be highly elevated through early childhood, though eventually slowly decreasing to the upwards bounds of the normal physiological reference range, by the time the patient grew from ages 1 to 12. No BUN/Creatinine data was provided by the first case.

Lastly, from an analysis of the literature, the patient case files at the Reichenberger lab at UCH and an in depth study of two specific patient cases a list of clinical parameters useful for investigation in a full-scale natural history of disease study of CMD is presented.

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LIST OF ABBREVIATIONS

ALP ..... Serum alkaline phosphatase

ANK .....Progressive ankylosis gene

ANKH .....Progressive ankylosis gene human homolog

AR ..... Autosomal recessive

BU .....Boston University

BUN ..... Blood urea nitrogen

CDD ..... Craniodiaphyseal dysplasia

CMD..... Craniometaphyseal dysplasia

CPPD.....Calcium pyrophosphate dihydrate

CN .....Cranial Nerve

CNS .....Central nervous system

CSF..... Cerebrospinal fluid

CT..... Computed tomography

CTD.....Craniotubular dysplasia

Cx43 .....Connexin 43

ePPi.....Extracellular pyrophosphate

FMD .....Frontometaphyseal dysplasia

GJAI ..... Gap junction alpha-1

HA ..... Hydroxyapatite

HGMD.....Human Gene Mutation Database

ICP..... Intracranial pressure

MR..... Magnetic resonance

Pi.....Inorganic phosphate

PC-1.....Plasma cell glycoprotein 1

PCP..... Primary care physician

PPi ..... Intracellular pyrophosphate

PTH ..... Parathyroid hormone

UCH ..... University of Connecticut Health

INTRODUCTION

Craniometaphyseal dysplasia

Craniometaphyseal dysplasia (CMD) is a rare genetic disorder, resulting in abnormalities in bone formation and mineralization of the skeleton. With fewer than 200 reported cases in the literature, CMD is a disease that is extremely rare and little researched.

First identified as its own disease entity by Jackson in 1954 [37], craniometaphyseal dysplasia is a genetic disorder leading to a specific display of bony overgrowth and sclerosis. Most notable is the bony overgrowth of the skull base, hyperostosis of the craniofacial bones, and the characteristic widened metaphyses of the long bones [37]. Individually, each symptom could be explained by a separate respective disease – osteopetrosis (sclerosis of the base of the skull) and metaphyseal dysplasia, as seen in Pyle's Disease (flaring of the metaphyses of the long bones). Jackson found enough clinical evidence, however, to determine the presence of a distinctly separate disease he termed "craniometaphyseal dysplasia." [37]

Currently, there exist over 500 reported different types of rare genetic bone disorders [11]. Within these rare genetic bone disorders, CMD falls in a specific classification of the various craniotubular dysplasias (CTD), a subset of the osteochondrodysplasias. Even within the classification of CTDs, these rare bone diseases contain varying non-specific clinical manifestations, making diagnosis of each of the rare bone disorders complicated and subject to misinterpretation [59]. With an expansive range of skeletal disorders from which a patient may be suffering, it is important to

distinguish each disorder's pathogenesis for proper diagnosis and development of appropriate treatment plans.

Despite over 50 years of clinical studies on CMD, there has never been definitive research investigating CMD for developing both guidelines and standardized procedures for diagnosis and treatment of the disease. For many of the other rare bone diseases, the situation seems to be the same – there is no definitive study informing of the disease, for patients or physicians to refer to. Without CMD guidelines for physicians, patients presenting with clinical signs of CMD may be misdiagnosed due to both physician unfamiliarity with the disease, and the commonalities between CMD and other rare bone disorders. Without a set of guidelines for patients with the disease, patients may not be able to understand progression of the disease, factors for acquiring and transmitting the disease, as well as the varying options for treatment and therapy. Lastly, without detailed knowledge of molecular mechanisms that lead to CMD, pharmaceutical companies do not have a foundation to study molecular targets for development of therapeutics.

To combat this lack of CMD awareness, a natural history study is proposed. A natural history study of CMD seeks to understand the etiology, manifestations and progression of CMD across a sample population. Currently, the Reichenberger Lab at University of Connecticut Health (UCH) in Farmington, CT, is researching the genetics and molecular biology associated with CMD. A relatively large CMD patient population has been screened and the data pooled for use in separate studies. From the collected data, a pilot natural history study of CMD is conducted to compare disease history, disease progression, diagnostic parameters, blood chemistry and genetic composition.

The data from this pilot study will set the foundation for a future large scale natural history study, which may then be used to produce recommendations that may include diagnosis, prognosis of disease progression and treatment - for distribution to both patients with the disease, and physicians who may encounter or diagnose the disease. By conducting this study and developing a report, the physiological and social ramifications of CMD will be better understood, allowing for more accurate differential diagnosis of CMD.

BACKGROUND

Clinical manifestations

Patients diagnosed with craniometaphyseal dysplasia present to the clinic a diverse array of signs and symptoms depending on the severity of the disease, and age of the individual. Craniometaphyseal dysplasia has been reported to occur in both autosomal dominant (AD) and autosomal recessive (AR) forms. It is known that in AD-CMD, the transmission of the genotype from parent to child is complete (further explained in the "Genetics of CMD" section), however, phenotypic expressivity of the disease is demonstrated to vary between patients with identical mutations [62]. CMD does not have a specifically defined pathology, and biochemistry of the disease is not currently completely understood. What is known, however, is that patients diagnosed with CMD develop either some, or all, of a set of noticeable pathognomonic physical characteristics.

Of the clinical manifestations of craniometaphyseal dysplasia, many are apparent through clinical observation. Patients with CMD often present dysmorphic craniofacial features, including: macrocephalus, ocular hypertelorism, wide nasal bridge, frontonasal bossing, prominent mandible, and partial facial nerve paralysis (Figure 1).

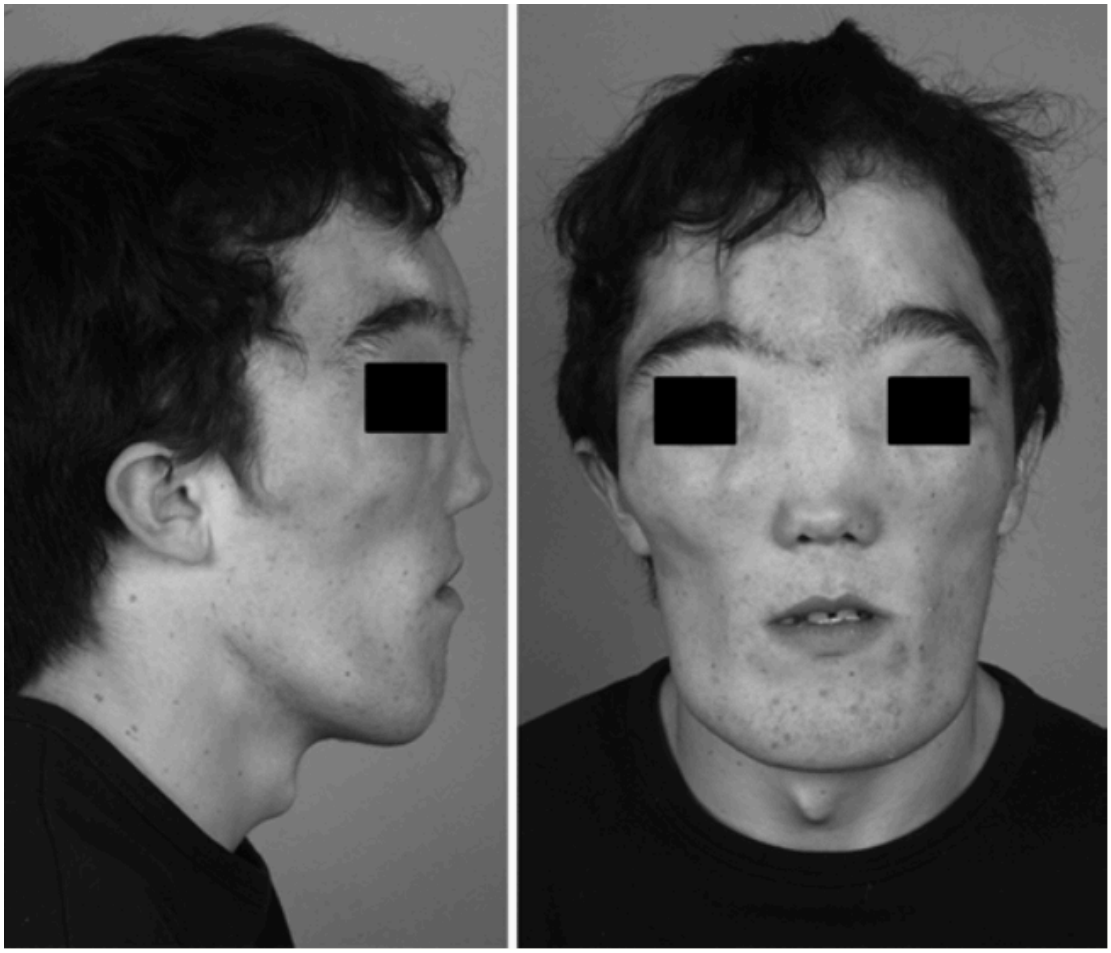

Figure 1: Clinical presentation of craniometaphyseal dysplasia.

Depicted above is a 21 year old male patient diagnosed with craniometaphyseal dysplasia. In this image, examples of the dysmorphic facial features of CMD patients are shown. From the frontal profile (R) ocular hypertelorism is noticeable, as well as a very wide nasal bridge, flattening out the center of his face. From the side profile (L) frontonasal bossing can be seen, as well as the prominence of his mandible. Macrocephalus is also noticeable in the patient, where the cranial circumference exceeds the normal physiological range.

(Figure taken from [38])

Clinical manifestations of craniometaphyseal dysplasia may relate to the length of time an individual has lived with the disease, the severity of the disease (whether AD or

AR form), and how their body has compensated to the disease. In younger patients such as infants, CMD can present itself through nasal obstruction due to sclerosis of the nasal choanae [72]. As children get older, narrowing of the cranial foramen caused by bone overgrowth (hyperostosis) can compress nerves and result in gradually degrading visual and auditory perception, as well as partial facial paralysis or facial palsy [74].

Along with changes in formation of the craniofacial bones, CMD is also responsible for flaring in the metaphyses of long bones. This is commonly referred to as the "Erlenmeyer flask" shaping of long bone metaphyses, where the metaphyses appears to be widened on both ends, this reducing the length of long bone diaphysis. In a study of CMD comparing wild type mice and mice with a knock-in mutation (Phe377 del) in *ANKH*, it was found that mice containing the *ANKH* mutation displayed more trabeculation within the diaphyses of the long bones, however, less trabeculation in the metaphyses [14]. Differences in disease characteristics and pathophysiological manifestations between the mouse model and AD-CMD patients containing the same mutation (Phe377del) are thought to be due to phenotypic thresholds specific to each species, dependent on lifestyle and lifespan.

Though primarily a skeletal disorder, patients may enter the clinic presenting with neurological symptoms. CMD's increased bone deposition leads to the progressive thickening of the craniofacial skeleton throughout life. Foramina within the skull reduce in size and compress cranial nerves. Often, patients present with lost or reduced bilateral vision and bilateral hearing due to the obstruction of the optic, oculomotor, trochlear and abducens nerves (Cranial Nerve (CN) II, CN III, CN IV, CN VI) and vestibulocochlear

nerve (CN VIII), respectively [64]. Additionally, patients may also present with partial paralysis of the facial muscles, due to compression of the facial nerve (CN VII). If left untreated, these neurological deficits can become severe, leading to: partial or complete bilateral blindness and/or deafness, motor deficits, facial pain and facial palsy [38]. Severe neurological complications in CMD are found more often in patients diagnosed later in life, where surgical or therapeutic intervention has not been provided at an earlier age.

Alongside increased pressure on the cranial nerves due to narrowing of cranial foramina, exists the risk of increased pressure on the entire brainstem and spinal cord, due to narrowing of the foramen magnum. This condition is referred to as a Chiari I malformation, and is sometimes found syndromic with CMD. In Chiari I malformation, crowding within the lower base of the skull can result in the cerebellar tonsils pushing through the skull base and onto the spinal canal. These herniated tonsils will block the flow of cerebrospinal fluid (CSF). The resulting increased intracranial pressure (ICP) will exacerbate the cerebellar tonsils to move even further down, increasing pressure on the brainstem and cervical spinal cord. Symptoms of Chiari I malformations present in late childhood as severe headaches, dizziness and in severe cases, paraplegia of the arms and reduced sensation of the hands [7].

Diagnostic considerations

Currently, there are no published guidelines for the diagnosis of craniometaphyseal dysplasia. Information on the disease is sparse, and thus many physicians may be unaware of the disease for consideration in diagnosis. In the literature,

initial diagnosis of CMD has been made at a wide range of ages [21, 46]. Diagnosis may be further complicated in some patients with prior misdiagnosis of separate rare skeletal disorders or other ailments. Differential diagnosis of craniometaphyseal dysplasia reported in the literature has been made in subjects ranging from infancy to adulthood.

Due to the overlapping clinical manifestations of many rare bone diseases, diagnosis of CMD cannot be made based on clinical observation alone. Diagnosis of CMD is made predominantly based on patient bone structure, seen through computed tomography (CT) and magnetic resonance (MR) imaging. Specifically, within CMD patients, physicians must identify the presence of both a thickened craniofacial skeleton, and flared metaphyses within the long bones (Figure 2 & Figure 3). Since either hyperostosis of the cranial bones or flared metaphyses alone are not specific to any one rare bone disease, differential diagnosis between these diseases can become difficult for physicians [62]. It is important that both conditions are displayed via radiographic imaging for the diagnosis to be made. Additional less common radiographic findings include sclerosis along both the skull sutures, and the petrous region of the temporal bone [49].

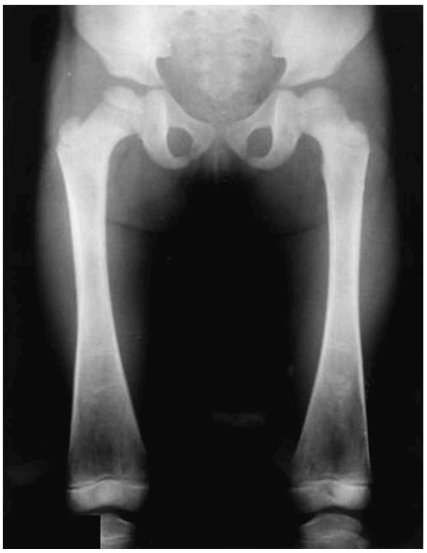

Figure 2: Flared metaphyses of long bones in craniometaphyseal dysplasia.

CT imaging of both femurs in a 4-year-old female patient diagnosed with CMD. Note the flared "Erlenmeyer flask" shape of the femoral metaphyses.

(Image taken from [43])

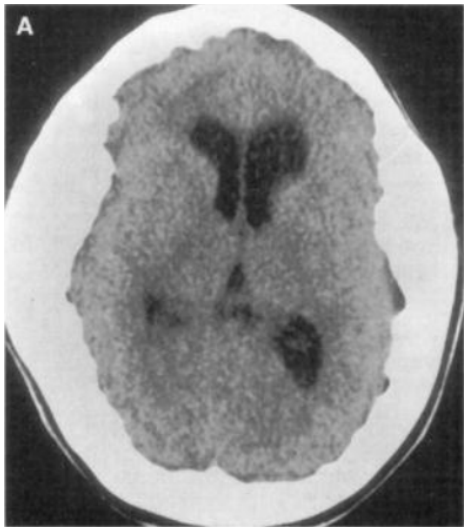

Figure 3: Thickened craniofacial skeleton in craniometaphyseal dysplasia.

CT image identifying the thickened cranium of a 15-year-old female patient with craniometaphyseal dysplasia. A significant thickening of the cranium is noticeable, decreasing the volume available for the enclosed brain.

(Image taken from [20])

Differential diagnosis is extremely important between rare skeletal disorders, because of their many overlapping clinical manifestations and characteristics[24]. Knowing specifically which of the rare skeletal disorders a patient should be diagnosed with is important for predicting the disease progression. One disorder popularly confused with CMD is craniodiaphyseal dysplasia (CDD). CDD is associated with a widening of the diaphyses instead of metaphyses, causing long bones to take on a cylindrical shape resulting from sclerosis of the cortical bone [6]. Another disorder commonly misdiagnosed as CMD, is metaphyseal dysplasia or Pyle's disease. The important distinction with Pyle's disease is that unlike CMD, hyperostosis of the skull base rarely occurs. A third disease subject to misdiagnosis is frontometaphyseal dysplasia (FMD). FMD contains similar manifestations as CMD, however it also exhibits hypertrophy of the supraorbital ridges, deficient mandibular growth, and both narrow ribs and pelvis [60]. FMD patients develop alterations of the digits, vertebrae, iliac crest and pelvis, in addition to CMD's characteristic craniofacial and long bone deformations [60]. Along with help for differential diagnosis, CT and MR imaging are also encouraged to analyze the impact the disease has made on the patient's central nervous system (CNS) and cranial vasculature.

When used alongside clinical observations, molecular diagnosis is a valuable tool to confirm CMD. Prenatal diagnosis of CMD can be made by genetic analysis, however this is only possible if the mutation runs within the family (not reliable for sporadic cases). With known mutations already discovered and listed in the Human Gene

Mutation Database (HGMD), cross-referencing mutations may help confirm CMD when making a differential diagnosis. Occasionally, ultrasound scans in utero may also be indicative towards the presence of CMD, however this is not as reliable for diagnosis, when compared to genetic and biomolecular analysis [87].

Genetics of CMD

Craniometaphyseal dysplasia is a genetically inherited disease [55, 62]. Craniometaphyseal dysplasia has been reported to occur in both autosomal dominant (AD) and autosomal recessive (AR) forms.

The AD form of CMD appears more commonly in the literature and historically has been mistakenly reported as the less severe form of the two. The dominant inheritance of AD-CMD has been proven to be dissimilar to the recessive inheritance of the related skeletal disorder metaphyseal dysplasia, or Pyle's Disease [27]. In the AD form of CMD it has been found that mutations in cytoplasmic regions close to the C-terminus of the human *ANK* gene (*ANKH*) product result in CMD [62]. AD-CMD and mutations of the *ANKH* gene have been linked to the chromosome region 5p15.2-p14.1 occurring usually between exon 7 and exon 10 of the *ANKH* gene, relating to transmembrane regions close to the C-terminus of the transmembrane protein [55, 62]. Separate mutations of the *ANKH* gene have also been reported in cases of chondrocalcinosis, and in CMD patients with comorbid chondrocalcinosis [2]. The distinguishable difference, however, seems to be that mutations in *ANKH* exons 7-10 cause CMD, whereas mutations in exons 1 and 2 lead to chondrocalcinosis [21].

The *ANK* gene is highly conserved amongst vertebrates, exemplifying its importance through genetic inheritance [32]. The ANK protein resulting as the *ANK* gene product has not yet been definitively discovered in terms of topology and protein structure. Though the *ANKH* protein has not been crystallized, several computer model predictions exist [45]. There is currently debate regarding the number of transmembrane loops in available computer models of the *ANKH* protein. From murine studies of the homologous mouse *ANK* gene, it is known that the *ANKH* protein acts as a pyrophosphate transporter, moving pyrophosphate across the plasma membrane from the intracellular to extracellular domain [32]. Though this study concentrates on the role of *ANKH* in bone formation, *ANK* expression has also been identified in neuronal pathways within the brain [86].

Regulation between extracellular pyrophosphate (ePPi), intracellular pyrophosphate (PPi) and inorganic phosphate (Pi) is important in regulating bone mineral content homeostasis. Normal ePPi at physiological levels will inhibit mineralization, whereas low ePPi will increase hydroxyapatite (HA) deposition. High ePPi levels as seen in chondrocalcinosis patients can increase formation of calcium pyrophosphate dehydrate (CPPD) in joints [15]. A buildup of ePPi can be detrimental in leading to ectopic mineralization in joints and the formation of osteophytes in joints [29]. Along with *ANK*, bone content and phosphate levels are additionally regulated by proteins PC-1, (plasma cell glycoprotein 1) which creates PPi from nucleoside phosphates (NTP), and TNAP (tissue non-specific alkaline phosphatase), which hydrolyzes ePPi to Pi. Deficiency in any of these three genes or gene products have been shown to produce abnormal bone

mineral content, due to their participation in regulating the ePPi and PPi physiological homeostasis [15].

Independent mouse studies of the murine *ANK* homolog have been conducted to show the significance of *ANK* mutations in producing physiology similar to humans diagnosed with CMD. Comparisons were made between CMD patients containing a single point mutation in the *ANKH* gene (Phe377del), and mice treated to have a knock-out (KO) mutation of their *ANKH* gene. It was found that in humans with mutated *ANKH* and mice with knock-out *ANK*, there are increases in skull thickness, narrowing of the foramen magnum leading to compression on the brainstem, and a decrease of bone trabeculae found in the metaphyses of the femur [29]. Absent from the mouse KO-*ANK* condition, however, are the hypertrophy of nasal choanae, blocking of nasal sinuses, ocular hypertelorism, and flaring of metaphyses of the long bones, such as the femur – conditions all found in CMD patients with mutated *ANKH* genes. From this, it has been concluded that the human homolog of *ANK* (*ANKH*) may have a separate function (other than PPi transport) [15].

Several *ANKH* gene mutations in CMD patients have been reported in the literature. Most mutations occur on exons 7-10 of the gene [55, 62]. A list of so far discovered mutations in *ANKH* resulting in the AD form of CMD is shown in Table 1.

Table 1: Genetic mutations of *ANKH* protein resulting in CMD.
Tabulated below is a list of all genetic mutations in *ANKH* currently known to result in the development of AD-CMD. Additionally listed is a mutation in *connexin 43*, which has been documented as being a causing factor for the development of AR-CMD.
* The mutation in *connexin 43* (gap junction protein, alpha-1) is associated with the autosomal dominant form of CMD [33].

Location	DNA change	Amino Acid change	Reference
Exon 7	c.853-870del	p.V285_Y290del	[20]
	c. 936T>C, c.938C>G, c.942_953del	p.Y290Q, W292-E295del	[88]
	c.942T>C	p.W292R	[55]
Exon 8	c.1059T>C	p.C331R	[55]
	c.1001T>G	p.L334R	[45]
Exon 9	c.1015T>C	p.C339R	[45]
	c.1192_1194delCCT	p.S375del	[55, 62]
	c.1127_1129delTCT	p.F376del	[62]
	c.1196_1198 del CTT	p.F377del	[55, 62]
Intron 9	c.1210-4 A>G	p.380_V381insAla	[55]
Exon 10	c.1139_1141insCAG	p.380insA	[55, 62]
	c.1233G>A	p.G389R	[55]
	c.1172T>C	p.L391P	[45]
	c.1178-1189del	p.T393K396	[20]
Connexin 43*	c.716G>A	p.R239Q	[33]

Presence of any one of the autosomal dominant genetic mutations will confirm the inheritance of CMD from parent to child. Another importance of referencing the HGMD when conducting genetic analysis is to help distinguish AD vs. AR forms of CMD. Due

to the variety of phenotypes presented in CMD patients, it is not obviously clear which form of CMD a patient may display, by clinical observation alone.

The AR form of CMD has been mapped to chromosome 6q21-2 [36]. Mutations in the *connexin 43* gene (*Cx43*) have been identified as a causative factor in AR-CMD development [33]. *Cx43* codes the gene product for protein gap junction alpha-1 (GJA1) [33].

To inherit AR-CMD, both parents must carry a mutated recessive gene, and there is a resulting 25% chance of inheritance to the offspring. The presence of an AR form of CMD is an even more rare find than AD-CMD and contains a variation of severity of clinical manifestations. An important distinction between AD and AR forms of the disease, is that the disease is apparent only if both alleles carry causative variants while heterozygous offspring appear physically normal. In contrast, AD-CMD has a 50% chance to be passed on the next generation and a mutation in a single allele is sufficient to cause the disease [55, 62].

Recently, there has been a novel discovery in the genetics of AR-CMD. A mutation in the C-terminus of GJA1 coding for *connexin 43* (*Cx43*) has been discovered in 6 subjects of 3 different families, resulting in AR-CMD [33]. Separate mutations within *Cx43* are known to result in at least 7 other bone disorders, which indicates that *connexin 43* plays an important role during skeletal development and bone maintenance [33].

The third and final way CMD can be acquired in an individual is through spontaneous genetic mutation [88]. Somatic mutations have not yet been described in

CMD patients. It appears that mutations occur early during development and affect the germline therefore they can be transmitted to the next generation. DNA testing for CMD has been performed from both saliva and blood, suggesting that de novo mutations may affect the germline.

Historic literature reporting on CMD patients diagnosed through clinical presentation, generally do not contain genotyping data. Since genotyping is not included, there is no concrete way to determine that the patients carried CMD specifically, opposed to a separate but similar bone remodeling disorder. Careful consideration must be used when examining the historic literature of CMD, due to the lack of provided genetic evidence.

Currently, the biochemical and genetic foundation behind craniometaphyseal dysplasia is currently not completely understood. This is due partially to the rarity of the disease – with over 100 cases mentioning "craniometaphyseal" or "cranio-metaphyseal" in the literature cataloged in Pubmed/Pubmed Central [48]. No epidemiological data is currently available, however estimating from the published literature we can assume there are only a few thousand patients worldwide.

Treatment options

The age of patients from the literature diagnosed with CMD have ranged from infancy to adulthood. With diagnosis occurring at different ages, physicians detect the disease at varying levels of severity and disease progression. Thus, treatment options must be catered specifically to each patient based on: the severity of disease within the

patient, the patient's age, how each patient's body has specifically compensated for the disease, and each patient's individual goals for disease treatment.

Surgical treatment is often suggested to combat neurological issues caused as a result of CMD. Commonly reported procedures include enlarging cranial foramina to decrease compression on affected cranial nerves and vasculature penetrating through the cranium [38]. One example is enlarging the foramen magnum used to reduce pressure on the brainstem, used for CMD patients with comorbid Chiari I malformation. Most surgical procedures, to assuage pressure on nerves and vasculature passing through cranial foramen, are conducted via an extracranial route. This extracranial approach is generally taken due to difficulty penetrating the abnormally thickened skull tissue seen in CMD patients. The abnormal skull tissue can reach diameters of 50mm or more, compared to the 6-7mm thickness of skull tissue found in normal physiology [38]. Even in extracranial procedures, surgical intervention may become difficult due to the increased hardness of patient skulls, causing resulting abrasion of surgical instruments.

An option available to some patients is to reduce the skull thickness to within the normal physiological range via a reduction cranioplasty. Whether or not a patient can undergo this procedure varies, and the decision is made at the craniofacial surgeon's discretion. Patients suffering from CMD do not contain a homogenous density within their craniofacial bones, as found in normal physiology [38]. This means surgeons cannot take a simple approach to reduce the skull thickness consistently from the outside in, to a desired depth. Choosing to undergo reduction cranioplasty includes life-threatening risks, such as passing through the skull and into the dura mater leading to

brain hemorrhages. Additionally, within the thick bone often lays copious vasculature. Removing a significant amount of bone presents the possibility of reducing the brain's blood supply through bone, thus risking the patient undergoing a potential stroke. Deciding whether or not to undergo a cranioplasty reduction surgery procedure varies on a patient-to-patient basis, where compensation for nerve and vasculature compression and amount of bone vasculature may vary significantly between CMD patients.

Surgical options to assuage CMD complications are not limited to treating only neural deficits. Complications with nasal breathing may also be surgically repaired. Through extracranial surgery, the nasal cavity may be widened, to ease patient breathing. This approach can help treat patients of CMD in their infant years, when nasal breathing may be difficult due to early hyperplasia of the nasal choanae. Reduced nasal breathing is often compensated in the individual by mouth breathing, however there is a report of severe choanal stenosis in an infant CMD patient, resulting in cardiopulmonary arrest [17]. This is increasingly important in neonates, since the epiglottis and soft palate are close to the tongue, thus reducing compensatory mouth breathing, and increasing risk of asphyxia if choanal atresia is severe enough [17].

Mandibular prognathism is another common facial disfiguration caused by CMD, where the mandible can be overgrown and extend further ventral than the maxilla. Surgical and hormonal corrections can be performed to change facial aesthetics by skeletal contouring [46]. This procedure includes high doses of calcitonin initially to inhibit bone formation, and later high doses of calcitriol to induce an increase in bone

resorption [46]. This attempt of a hormonal calicitonin/ calcitriol treatment, did not lead to any significant positive result in the patient.

Many patients suffering from hydrocephalus as a result of CMD have been successfully treated as well. Hydrocephalus is a condition in which there is an increased intracranial pressure (ICP) due to an abnormal amount of CSF buildup in the ventricles of the brain. Hydrocephalus and ventriculomegaly are often treated with surgical intervention. In severe cases, immediate cannulation and draining of CSF from the ventricles can rapidly reduce pressure on the brain and brainstem. Often times the accumulation of CSF will subsequently return, thus a more permanent solution includes implanting a surgical shunt to drain the CSF from skull, into the abdominal cavity [64].

With the dominance of surgical intervention, various non-surgical interventions are much less popular as supplemental or independent treatment options for CMD patients. Hormonal treatment regiments include calcitonin therapy and calcitriol treatment with a low calcium diet [46]. Calcitonin therapy acts to suppress osteoclasts, thus inhibiting osteoblast bone formation through the RANK/RANK-L feedback system. Studies contradict this approach due to abnormal osteoclast formation and functioning [15]. Thus inhibiting osteoclasts by calcitonin therapy, may have unwarranted detrimental effects.

Calcitriol (1,25-dihydroxyvitamin D3) treatment includes an additional lifestyle change of introducing and maintaining a low calcium diet, in addition to hormonal therapy. The low calcium diet will promote hypocalcemia, thus inhibiting osteoclast activation from monocyte precursors. The low calcium diet works alongside calcitriol

induced increases in bone resorption, to slow the progression of excess bone formation. This approach has been reported to delay the progression of skull ossification in two cases of infant female patients [40, 63]. The efficacy of this method is questionable, due to the vast variation of phenotype progression in CMD patients. Larger studies would be needed to show hormonal therapy's efficacy in comparison to the more predominantly used surgical intervention for palliative treatment of CMD patients.

As with surgical intervention, due to the wide range of phenotypic variation between CMD patients, whether or not to undergo hormonal therapy should be made based on a patient-to-patient basis. Considerations include factors such as delayed dentition, bone strength, composition and density, and baseline hormone levels [67].

Comparatively, surgical decompression is more effective than hormonal approaches in improving neurological deficits resulting from bony overgrowth in the cranial foramen of CMD patients. Surgical removal of bony overgrowth within the cranial foramen will begin decompression of the cranial nerves, reducing the regression of their sensory content. Removing parts of the skull bone to reduce pressure on the brainstem and spinal cord, as well as increasing space available for the cerebellum has been shown to increase sensory perception and motor function within patients as quickly as 6 weeks post-surgery [66].

While surgical intervention and hormonal therapy have been discussed, overlooked supplements to these approaches, are the various types of physical and occupational therapies available. Physical therapy can help to stimulate facial muscles in cases of facial nerve palsy secondary to CMD. Additionally, physical therapy can help

improve motor function of extremities, which may be reduced due to sclerosis of the skull base cranial foramina [20]. Present in many CMD patients, are concurrent debilitation in sensory responses and motor function. Occupational therapy can allow some subjects with CMD to develop lifestyle adaptations to compensate for debilities caused by the disease, increasing their quality of life. This includes therapies for coping with blindness and deafness, which can be secondary effects of CMD [3].

Currently, no treatment or combination of treatments for CMD has been indicated curative. Patients are only offered palliative solutions to help reduce the physiological impact of CMD, and occupational therapy to help accommodate for the lifestyle difficulties that CMD patients face.

SPECIFIC AIMS

The overall objective of this report is to compile and organize the known information on craniometaphyseal dysplasia, in order to conduct a natural history of disease pilot study of the rare skeletal bone disease, CMD. Doing so will set a precedent for understanding the foundation and progression of CMD, and relay this information to research groups interested in conducting a full scale natural history study of CMD.

To compile the information necessary to conduct a pilot study, and direct a future natural history of disease study, this project is designed through the accomplishments of three specific aims:

SPECIFIC AIM 1: Compile an organized collection of publications to use as a starting point for a natural history study for CMD.

In the current literature, there is no single definitive study investigating the etiology, clinical manifestations, and timeline of progression of the disease. Additionally, there are no reports compiling the information from a large number of CMD cases. Our current understanding of CMD relies on publications based on cases. Different studies investigate different parameters of the disorder, making it hard to directly compare phenotypic progression of CMD. This study seeks to streamline the presentation of information on CMD, through the presentation of a compendium of publications pertaining to specific characteristics of the disease. In doing so, future groups seeking to learn more about CMD may refer to this compendium for guidance on where to look for information.

SPECIFIC AIM 2: Assess the available data on the CMD patient population available in the Reichenberger Lab, to determine what clinical information is already available.

Currently, a patient population of CMD subjects with information of genetic mutations and clinical data for individual cases is available in the Reichenberger Lab at University of Connecticut Health (UCH) in Farmington, CT (unpublished). Genetic data, along with the existing clinical data are assembled in a database. Clinical correlations are made through comparison of compiled data. Understanding what clinical information is already available within the database of CMD patient cases at the Reichenberger Lab is crucial for later deciding which clinical qualities will be of most interest for further investigation. Additionally, cataloging information is already present will help determine what additional information remains to be collected from these existing patients, when planning for a larger scale natural history of disease study for CMD.

SPECIFIC AIM 3: Analyze existing collected clinical data to determine which parameters may be of interest for further investigation in a large scale natural history study of CMD.

Once the CMD patient population data within the Reichenberger Lab at UCH has been examined to determine which pertinent recorded clinical information is available for each patient, clinical information will be analyzed. It is important to understand any preexisting trends in the data, as they will help to set a foundation for what information

should be focused on for collection in a future full scale CMD natural history of disease study Additionally, clinical data published in the literature may also be used in comparison with the subjects from the Reichenberger Lab CMD patient database, for analyzing trends.

Between the literature, and the CMD patient population database at the Reichenberger Lab, these three aims seek to discover what information on CMD is currently available, and how this information can be incorporated in directing a future natural history of disease study for CMD. Research groups interested in further developing a full scale natural history of disease study for CMD will be able to use the work from this project as a roadmap.

METHODS

SPECIFIC AIM 1: Compile an organized collection of publications to use as a starting point for a natural history study for CMD.

For this project, the literature was sorted and subsequently organized into succinct categories containing information specific to individual characteristics of the disease. Publications were collected by searching for "craniometaphyseal," or "craniometa-physeal," within the PubMed and PubMed Central databases. Despite over 120 publications returned from the search, the database only provided links to online copies of original publications for roughly 90 of the reported studies of CMD. The publications able to be collected directly from PubMed/PubMed Central were used in this study, as these are the publications that can be publically found by future research groups seeking to further investigate CMD. The remaining publications are most likely only available in print or through intralibrary loans. These publications were not included due to regional limitation, along with the temporal restraints placed on completing this thesis.

The literature was grouped to provide a basis for researchers to understand: the diagnosis, known biochemistry, and genetic basis of CMD. In doing so, eight major categories of published research were organized for presentation to the general public interested in learning more of CMD:

1. **Genetic mutations resulting in CMD**
2. **Molecular mechanisms of CMD**
3. **CMD case reports**
4. **CMD differential diagnosis**
5. **Surgical & medical therapy for CMD**
6. **2º Abnormalities resulting from CMD**

7. **Familial studies of CMD**
8. **CMD overview and literature review**

The categories of publication groups are defined to help address the key information necessary in understanding CMD. Understanding these foundations of CMD individually is critical to gain a gestalt understanding of the disease. Additionally, in categorizing the literature into groups, publications with outdated information or possibly incorrect information can be more easily identified. Progressing within a specific category of papers in chronological order, one may find a publication with a novel discovery contradicting and outdating the discoveries of a previous paper.

Since the disease is not well known, all publications found on CMD during this study were included in the research compendium. In doing so, any research group interested in further investigating CMD can make their own autonomous conclusions regarding which studies provide beneficial information, and publications for which the information has been outdated.

SPECIFIC AIM 2: Assess the available data on the CMD patient population available in the Reichenberger Lab, to determine what clinical information is already available.

The CMD patient population collected so far at the Reichenberger Lab at UCH exists of 76 different cases, collected previously for studies relating to the underlying genetic basis of CMD. Though many of the case files within the CMD patient database at the Reichenberger Lab were generated with the goal of determining genetic mutations

leading to the disease, clinical information is present as well, however, scarce and variable for many cases.

Within the clinical information available in the subject database, availability of the data varies between subjects. The available clinical information for each of the individual cases has been listed, with attention towards clinical parameters deemed important by their relativity of occurrences in previously published CMD studies. This is valuable in creating an inventory of clinical data already available within the CMD patient database, thus identifying which information still needs to be collected from these patients if they are to be a part of a future natural history of disease study of CMD.

All cases in the CMD patient database, were individually determined as to whether or not they contain information for the following parameters:

1. Patient Information
2. Referring Physician Contact Information
3. Tissue Sample (available)
4. Blood Chemistry (some)
5. Growth Information
6. Skull Diameter Measurements
7. Visual Acuity
8. Auditory Acuity
9. Clinical Symptoms
10. Radiological Data
11. Surgical and or Hormonal Treatment Information
12. *ANKH* Mutation Present
13. *Connexin 43* Present
14. Diet and Exercise Information

To avoid incorporating data from patients with other skeletal disorders that aren't CMD, only patients with known mutations resulting in CMD (mutations in *ANKH* and connexin 43) are included in this study. Patients not identified as having mutations in *ANKH* or *Cx43* are disregarded for this pilot study, as they may possibly have a bone

disorder with similar clinical manifestations or mutations outside the exons that were sequenced for the study. Though reducing population size for inclusion in a future natural history of disease study for CMD, these exclusions increases precision of the study, by reducing confounding information from patients with other potential non-CMD skeletal disorders.

Personal health information is de-identified in all tables and database files for this study. Identifiable information is kept under lock in the Reichenberger Lab at UCH as per approved IRB protocol.

SPECIFIC AIM 3: Analyze existing collected clinical data to determine which parameters may be of interest for further investigation in a large scale natural history study of CMD.

To evaluate trends within data of the CMD database the inventory created from the second aim was used to identify the subject cases containing the most available and relevant clinical data.

Two patients with detailed clinical data available were used as examples of comparison for this proposed analysis. Details of the two patient cases are shown below in Table 2 [Reichenberger, unpublished].

Table 2: Demographic information of two patient cases used for comparison in pilot CMD natural history of disease study. [Reichenberger, unpublished].
The two cases were chosen such that a comparison of disease progression could be made from 2003 onwards in two CMD patients of: similar age

	CASE 1	**CASE 2**
Sex	Female	Male
Year of Birth	2003	2003
Mutation (in ANKH)	p.Ser375del	p.Phe376del
Race	Asian	Whitej

These two cases were chosen on the basis that they contain the most information available in the CMD database and are of similar age. Using these two subjects for comparison, allows clinical changes and disease progression over time to be compared more accurately as it removes the confounding variables associated with the changes in disease progression over age.

Using the two cases, varying quantitative and qualitative clinical measurements and clinical observations were compared. The comparisons were made both amongst the two CMD patient cases, as well as alongside the baseline clinical measurements and clinical observations of a non-disease standard. Clinical parameters chosen for analysis were based on the clinical measurements commonly used in identified CMD publications. This ranged from qualitative measurements such as changes in bone hypertrophy between yearly checkups, to quantitative measurements of blood chemistry. The results from these comparisons in association with the clinical measurements reported in the literature

were ultimately used to compile a list of pertinent clinical parameters for further investigation in future studies of CMD.

RESULTS

SPECIFIC AIM 1: Compile an organized collection of publications to use as a starting point for a natural history study for CMD.

After analyzing the available CMD literature, important publications were compiled and categorized within eight main categories:

1. **Genetic mutations resulting in CMD**
2. **Molecular mechanisms of CMD**
3. **CMD patient case reports**
4. **CMD differential diagnosis**
5. **Surgical & medical therapy for CMD**
6. **Secondary phenotype resulting from CMD**
7. **Familial studies of CMD**
8. **CMD overview and literature review**

These publications are sorted and organized below in Table 3. All publications listed below were obtained via PubMed/PubMed Central. Some of the older publications on CMD were listed, however, only publications that were electronically available were obtained.

Table 3A: Categorized published literature on craniometaphyseal dysplasia.
A: Genetic mutations resulting in CMD & molecular mechanisms of CMD.
All publications cited in Table 3 are reduced to only cite the primary author and year. The full citations of these publications are detailed in the bibliography

Genetic mutations resulting in CMD	Molecular mechanisms of CMD
1. Chen et al., 2013 [12]	1. Stains & Civitelli, 2015 [70]
2. Hu et al., 2013 [33]	2. Chen et al., 2014 [11]
3. Kato et al., 2013 [39]	3. Chen et al., 2013 [12]
4. Dutra et al., 2012 [21]	4. Las Heras et al., 2012 [47]
5. Huang et al., 2011 [34]	5. Moochhala, 2012 [50]
6. Protera et al., 2011 [59]	6. Zhao et al., 2012 [89]
7. Chen et al., 2011 [15]	7. Chen et al., 2011 [15]
8. Kornak et al., 2010 [45]	8. Collins et al., 2011 [18]
9. Zajac et al., 2010 [88]	9. Xu et al., 2011 [84]
10. Chen et al., 2009 [14]	10. Kim et al., 2010 [41]
11. Wang et al., 2009 [79]	11. Neunaber et al., 2010 [54]
12. Reichenberger et al., 2001 [62]	12. Oca et al., 2010 [57]
13. Nurnberg et al., 2001 [55]	13. Skubutyte et al., 2010 [69]
14. Chandler et al., 2001 [10]	14. Carr et al., 2009 [9]
15. Iughetti et al., 2000 [36]	15. Chen et al., 2009 [14]
16. Nurnberg et al., 1997 [56]	16. Kirsch et al., 2009 [44]
17. Reichenberger et al., 1993 [61]	17. Wang et al., 2009 [79]
	18. Moochhala et al., 2008 [51]
	19. Wang et al., 2008 [78]
	20. Wang et al., 2007 [80]
	21. Gurley et al., 2006 [29]
	22. Wang et al., 2005 [81]
	23. Pendleton et al., 2002 [58]
	24. Guo et al., 2001 [28]
	25. Yamamato et al., 1993 [85]

Table 3B: Categorized published literature on craniometaphyseal dysplasia. B: CMD patient case reports, CMD differential diagnosis, surgical & medical therapy for CMD, and 2º Abnormalities resulting from CMD.
All publications cited in Table 3 are reduced to only cite the primary author and year. The full citations of these publications are detailed in the bibliography.

CMD patient case reports	**CMD differential diagnosis**
1. Taggart et al., 2014 [72] 2. Esteves et al., 2014 3. Tanigawa et al., 2011 [74] 4. Rivero-Garvia et al., 2011 [64] 5. Lamazza et al., 2009 [46] 6. Vasu et al., 2006 [77] 7. Kim et al., 2005 [43] 8. Shepard et al., 2003 [67] 9. McKay & Filov, 2002 [48] 10. Feingold et al., 1999 [26] 11. Boltshauser et al., 1996 [5] 12. Hudgins et al., 1987 [35] 13. Nema et al., 1974 [53] 14. Carlson & Harris, 1972 [8]	1. Chen et al., 2014 [11] 2. Waterval et al., 2014 [82] 3. Lamezza et al., 2009 [29] 4. Faden et al., 2009 [24] 5. Baynam et. al., 2009 [2] 6. Cai et al., 2008 [7] 7. Richards et al., 1996 [63] 8. Beighton, 1995 [3] 9. Reichenberger et al., 1993 [61] 10. Reardon et al., 1991 [60] 11. Gorlin et al., 1969 [27] 12. Jackson et al., 1954 [37]
Surgical & medical therapy for CMD	**2º Abnormalities resulting from CMD**
1. Twigg et al., 2015 [76] 2. Kim et al., 2013 [42] 3. Rivero-Garvia et al., 2011 [64] 4. Juergens et al., 2011 [38] 5. Sewell, 2008 [66] 6. Singhal, 2008 [68] 7. Ahmed et al., 2006 [1] 8. Kim et al., 2005 [43] 9. Shepard et al., 2003 [67] 10. Day et al., 1997 [20] 11. Cheung et al., 1997 [16] 12. Satoh et al., 1994 [65] 13. Fanconi et al., 1988 [25] 14. Key et al., 1988 [40]	1. Chen et al., 2014 [13] 2. Dutra et al., 2013 [22] 3. Tanaka et al., 2013 [73] 4. Chida et al., 2011 [17] 5. Collins et al., 2011 [18] 6. Sun et al., 2011 [71] 7. Singhal, 2008 [68] 8. Sewell, 2008 [66] 9. Cai et al., 2008 [7] 10. Vasu et al., 2006 [77] 11. Mintz & Veliz, 2004 [49] 12. Pendleton et al., 2002 [58] 13. Hayashibara et al., 2000 [31] 14. Elcioglu & Hall, 1998 [23] 15. Boltshauser et al., 1996 [5] 16. Haverkamp et al., 1996 [30] 17. Fanconi et al., 1988 [25] 18. Hudgins et al., 1987 [35]

Table 3C: Categorized published literature on craniometaphyseal dysplasia.
C: Familial studies of CMD & CMD overview & Literature review.
All publications cited in Table 3 are reduced to only cite the primary author and year. The full citations of these publications are detailed in the bibliography, correlated to their citation.

Familial studies of CMD	CMD overview & literature review
1. Hu et al., 2013 [33] 2. Kato et al., 2013 [39] 3. Protera et al., 2011 [59] 4. Morava et al., 2011 [52] 5. Baynam et. al., 2009 [2] 6. Pendleton et al., 2002 [58] 7. Iughetti et al., 2000 [36] 8. Tinschert & Braun, 1998 [75] 9. Nurnberg et al., 1997 [56] 10. Beighton et al., 1995 [3] 11. Beighton et al., 1979 [4] 12. Carlson & Harris, 1972 [8]	1. Chen et al., 2014 [11] 2. Waterval et al., 2014 [82] 3. Cai et al., 2008 [7] 4. Shepard et al., 2003 [67] 5. Reichenberger et al., 1993 [61] 6. Brueton et al., 1990 [6] 7. Cooper et al., 1974 [19]

SPECIFIC AIM 2: Assess the available data on the CMD patient population available in the Reichenberger Lab, to determine what clinical information is already available.

Of the available patients in the CMD patient database at the Reichenberger Lab at UCH, a total of 76 individual patient cases were found eligible for consideration for incorporation in a future natural history of disease study, through confirmation of a sequenced *ANKH* or *CX43* mutation. Since mutations in these genes are the only known mutations in the literature and the Human Gene Mutation Database (HGMD) identified to result in CMD development, patients with similar clinical symptoms but without genetic confirmation were excluded. This was done to avoid enrolling patients who may have

clinical symptoms similar to CMD, but a differential diagnosis of a non-CMD bone disorder.

Of the 76 patient cases, each case was scrutinized for clinical information ascertained during a previous genetic study. A survey of available pre-existing clinical information within the CMD patient database is displayed in Table 4.

Table 4: Clinical information present in case files within the CMD Database at the Reichenberger Lab at University of Connecticut Health.
The clinical information for identification was determined on the basis of what was most commonly investigated and reported in the previously published CMD literature.

Clinical Parameters of Interest	Patients
Patient Information	26
Referring Physician Contact Information	54
Tissue Sample (available)	7
Blood Chemistry (some)	4
Growth Information	6
Skull Diameter Measurements	6
Visual Acuity	5
Auditory Acuity	6
Clinical Symptoms	10
Radiological Data	11
Surgery and/or Hormonal Treatment Information	10
ANKH Mutation Present	65
Connexin 43 Mutation Present	11
Diet & Exercise Information	1
Total Patient Cases Considered	**76**

Within these categories, the available information varies by each patient. No case contains a full set of clinical data spanning over the subject's lifespan. Available information was also found to be dependent on the varying documentation styles of different physicians, and which clinical tests were conducted for each patient. After documenting the clinical information present within the database, two cases (Table 3) were determined suitable for comparison in this pilot study.

SPECIFIC AIM 3: Analyze existing collected clinical data to determine which parameters may be of interest for further investigation in a large scale natural history study of CMD.

Case 1

Case 1 is a 13 year old girl with CMD. She was born to two healthy nonconsanguinous parents of Asian descent. In 2003, she was delivered vaginally with a nuchal cord complication, and was noted as hypovolemic shortly after birth. The subject was immediately resuscitated via bag and mask ventilation and transferred to the ICU to normal recovery. At birth, she was measured with a head circumference of 33cm (39^{h} percentile,) birth weight of 2740g (27th percentile) and birth length of 48cm (42nd percentile). At birth, the subject was confirmed responsive to both sight and hearing. The subject also was identified to exhibit mild nasal flaring during respiration immediately after birth, and identified as anemic, though hemodynamically stable upon discharge.

Within the first postnatal months, the subject exhibited frontal bossing, biparietal widening and moderate ocular telorism and a wide flat nasal bridge. It was noticed by her parents that there was blinking in one eye more than the other, and when presented to her PCP, pallor of the optic discs was discovered. In her first year, CT scans confirmed bony sclerosis in subcortacoid space of sulci (involving skull base and calvariae) resembling the common trait of thickening of the skull base seen in CMD patients. Worsening of bone hypertrophy around the skull base and optic nerve helped to explain visual issues.

In her first year, radiographic imaging also confirmed the patient's middle ear cavities obliterated by bony thickening of the otic canal and a resultant narrowed internal auditory canal. As audiology tests reported decreased auditory acuity in the subject, at six months of age, she was given hearing aids to help improve her hearing capacity. By the end of her first year, Case 1 was diagnosed with a skeletal dysplasia, though an exact diagnosis was decided to be made later after additional testing.

Though not given the confirmed differential diagnosis of CMD, by age 1, physical therapy was provided for Case 1. Therapy included visual stimulations to increase motor coordination, in conjunction with the sight of her one working eye. Massage therapy was used to help stimulate facial nerves once partial CN VII paralysis was observed, to prevent complete facial palsy. Humidifiers with bronchodilators were also used to help assuage nasal breathing, working against the bony hypertrophy of the nasal choanae.

In the subject's second year after birth, she was measured with a: skull circumference of 53.75cm (97^{th} percentile) weight of 35 lbs (90^{th} percentile) and height of 38.25 inches (90^{th} percentile).

In her third year of life subarachnoid spaces over convexities and interhemispheric fissure were noted to be smaller than the year before, due once again to continued bone hypertrophy. Additionally skull, mandible and proximal cervical spine were found sclerotic with diffuse marrow expansion, internal auditory canals and middle ear were declared stenotic, skull base foramina were narrowed and mastoid sinuses were found to be non-pneumatized. All resulting symptoms were determined to be due to bony sclerosis. Additional displacement of cerebellar tonsils 12mm below her foramen magnum, with associated pointing of tonsils and mild cervicomedullar compression, showed evidence of severe type 1 Chiari malformation.

In her fourth year, interestingly, clinical reports indicated thickening of calvarium and facial bones had remained unchanged over the past year. Voids and dural sinuses were additionally noted to remain unchanged, halting the progressive compression seen in previous years.

Radiographic imaging of the characteristic "Erlenmeyer Flask" shape of her femur was documented, displaying the possibility of an initial misdiagnosis of CDD. At age 10, the subject's blood was sent to the Reichenberger Lab, for participation in a genetic study. After genetic sequencing, the patient was confirmed to contain the Ser375del mutation of ANKH, specific to CMD development.

By the fifth year, the subject underwent her first recorded vision test, with results of: OD 20/800 (right), OU (20/2100) (left), OS (20/800) both. This confirmed the subject to be blind in her left eye, however still able to process some vision in her right eye, which remains her only visual support.

Palliative therapy for Case 1 included two surgical interventions. At age 6, a ventriculo-peritoneal shunt was inserted to drain excess CSF from the cranium to the peritoneum, helping alleviate recurring headache believed to be caused by high ICP resultant of the subject's increased bone mass and reduced sinuses. At age 10, a craniotomy was performed to reduce pressure from the hypertrophied skull on the patient's cerebellum - pressure which had been restricting motor function of the subject's upper extremities. At the same time, a C1lamenectomy was performed to reduce pressure on the cerebellar tonsils pushed below the skull caused by the patient's Chiari 1 malformation. Case 1 also underwent early childhood intervention, with both physical therapy and occupational therapy.

In her yearly blood chemistry tests, it has been noted that Case 1 has recurrently had high cholesterol, which could be associated to diet or extraneous genetic factors. Additionally, after being released from the hospital as anemic post-birth, Case 1 has displayed low hemoglobin and/or hematocrit counts for 6 out of the 7 years in which her case has identifiable clinical blood chemistry measurements. Additionally, the subject reports high BUN/Creatinine levels in 4 out of 7 years in which her case has blood chemistry measurements. These observations are displayed in Table 5.

Table 5: Significant blood chemistry trends seen in Case 1.
Shown are values of the trends in blood chemistry indicated above. Clinical blood chemistry values measured in the subject are reported on top, while reference range values are reported parenthetically below it. Reference ranges are provided by the individual labs conducting the blood work exams.

* Indicates that values were not provided in the patient's clinical case file.

Case 1 Clinical Blood Work Values of Interest

Age	ALP (U/L) (ref. range)	BUN/Creatinine (ref. range)	Hemoglobin (g/dL) (ref. range)	Hematocrit (%) (ref. range)
0	1107 (40-350)	*	13.2 (12.8-20.4)	39.1 (50-76)
1	1338 (25-500)	63 (8-27)	11.4 (10-14)	32.7 (30-42)
2	705 (100-400)	40 (8-27)	12.3 (10-14)	36 (30-42)
3	482 (100-400)	38 (8-27)	11.9 (11.2-14.3)	34.9 (32-44)
4	468 (96-297)	48 (6-22)	11.9 (11.5- 14.0)	34.7 (34-42)
5	303 (93-309)	47 (6-22)	11.6 (11.7-15.5)	32.9 (35-45)
6	330 (96-297)	43 (6-22)	11.6 (11.7- 15.5)	32.9 (35-45)
7	351 (184-415)	normal (*)	12.7 (11.5-15.5)	37.7 (35-45)
8	427 (100-400)	30 (9-25)	12.6 (11.7-15)	38.5 (34.8-43.5)
9	316 (100-400)	28 (9-25)	11.7 (11.7- 15.7)	38.1 (34.8-45.8)
10	434 (104-471)	24 (9-25)	11.6 (11.5- 15.5)	36.3 (35-45)
11	306 (104-471)	* (6-22)	11.9 (11.7- 15.7)	36.3 (34.8-45.8)
12	227 (134-439)	24 (9-25)	9.9 (11.7- 15.7)	32.8 (34.8-45.8)

The subject has been described as a friendly, very social, and helpful and intelligent child. At the age of 5 she was shown to have oral comprehension skills at a third grade level. The subject's lifestyle includes dietary restrictions of beef and pork for religious reasons. She enjoys drinking two cups of tea (with milk) a day. Of normal intelligence, she currently attends the New York Institute for the Blind and participates in school social activities and exercise during physical education. Her main communication is through speech and is taught in both print and braille. The subject has minimal exercise outside of school, only on long walks running errands or doing family activities at local parks.

Case 2

Case 2 is a 12 year old boy born to two nonconsanguinous parents in 2003. Shortly after birth, it was noted the subject had thick effusion in middle ear, no air space in the right tympanic membrane and an eardrum and incus overlap in right ear. In his first year after birth, he has been reported to often catch a cold (presents stuffy nose).

In the subject's second year, he presented with sclerosis of the calvarium, and a dense sclerosis of the skull base (much more dense than that around the calvarium). The subject also displayed sclerotic ribs but an unaffected vertebrae. The nasal choanae were shown to be obstructed. Despite the vertebrae not being affected by bony overgrowth, in radiographic images there was confirmed sclerosis and thickening in the shaft of the patient's long bones. Along with the constant stuffy nose, telecanthus was observed, leading to increased excretion from the subject's tear ducts, though pupils were shown to

be normal. To ameliorate the effect, bilateral nasolacrimal duct intubation was introduced to help clear the tear ducts.

Additionally in the second year CT scans were important in displaying an increased skull thickness and skull sclerosis. There was no intracranial calcification and coronal and sagittal sutures were not completely fused. Paranasal sinuses were described as being hypoplastic. Internal auditory canal shape was shown to be within the normal range, however similarly to case 1, there was hypoplasia of mastoids and paranasal sinuses. Finally in Case 2's second year, bilateral to moderate hearing loss was found with no response to 70 dB in each ear.

By Case 2's second year, an additional 40 dB hearing loss was found in the subject's right ear. Facial morphology had evolved into significant hypertelorism. In contrast to Case 1's childhood development involving more speech communication. Case 2 was taught sign language. By Case 2's second year, he was able to comprehend 30-40 known words, and able to replicate back 5-10 words. In terms of diet and exercise, Case 2 was a picky eater as a child and exercise was not significant.

By the age of 3, Case 2's progression of clinical manifestations of CMD seemed to be steadily increasing. Right temporal bone overgrowth and right side bony overgrowth encroaching into the small internal auditory canal results in a 40dB loss in his right ear. Nasal cavity constriction and Class 2 occlusion with an open bite was also reported, common to Case 1.

In Case 2's 4th year, initial signs of a Chiari I malformation were found. Additionally, the subject's calvariae became homogeneous in density of trabecular bone compared to 2006 where there was reported to be a more "ground-glass density".

The 5th year, however, the signs of a Chiari I malformation had increased, with the cerebellar tonsils being 4mm below the foramen magnum, still less than Case 1 had shown, with her Chiari I malformation in her third year.

During the patient's 10th year, it appeared that his thickening of both the calvarium and the optic canals have stabilized, thus in the subject's 9th year, there was no acute intracranial pressure leading to damage – unlike Case 1 who needed the ventriculoperitoneal shunt to alleviate increased ICP. Similar to Case 1, the patient's paranasal sinuses are filled in due to expansile bony hypertrophy.

In the 13th and final year of patient's recorded clinical data (2015), the calvarial and facial structures have stopped changing significantly. The mastoid air cells appear abnormal, normal inner ear anatomy and internal auditory canal remained the same, middle ear cavities overlap and are not well delineated. Cerebellar tonsils also stopped encroaching downwards below the foramen magnum at 4mm. Additionally, the primary care physician identified no definitive change in bone morphology from 2014, and according to the audiologist there was no change in hearing from 2014.

Blood Chemistry Comparison

When comparing the clinical data between the two cases. A trend was noticed within the blood work. Compared to standard blood hormone levels normalized for sex

and age, both patients exhibited high serum alkaline phosphatase (ALP) content during early childhood. While still abnormally high, the variance between the subject serum alkaline phosphatase level and the standardized serum alkaline phosphatase level decreased over time. Serum alkaline phosphatase levels are shown in Figure 4.

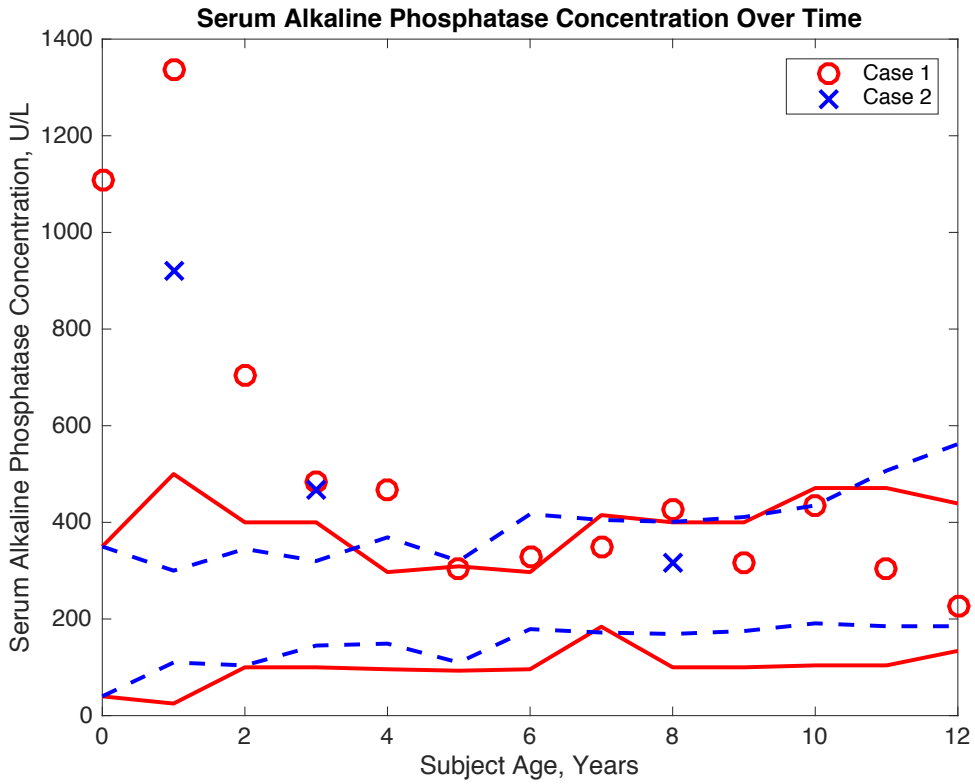

Figure 4: Serum alkaline phosphatase levels in both cases over time
Reference values for normal ranges of serum alkaline phosphatase are provided by the laboratories responsible for each blood test. Normal reference values for male subjects fall within the dashed lines, while normal reference values for female subjects fall within the solid lines. For years in which there was no recorded blood work on file for each of the two cases, reference values supplied by the Mayo Clinic are being used.
Note: Global reference values supplied by the Mayo Clinic are established for subjects over 4 years (see Appendix). For under 4 years, reference values are provided by the laboratory conducting the blood work tests.

Despite there not being birth data for Case 2, for Case 1, clinical measurements of alkaline phosphatase isoenzymes were reported. These concentrations are reported in Figure 5.

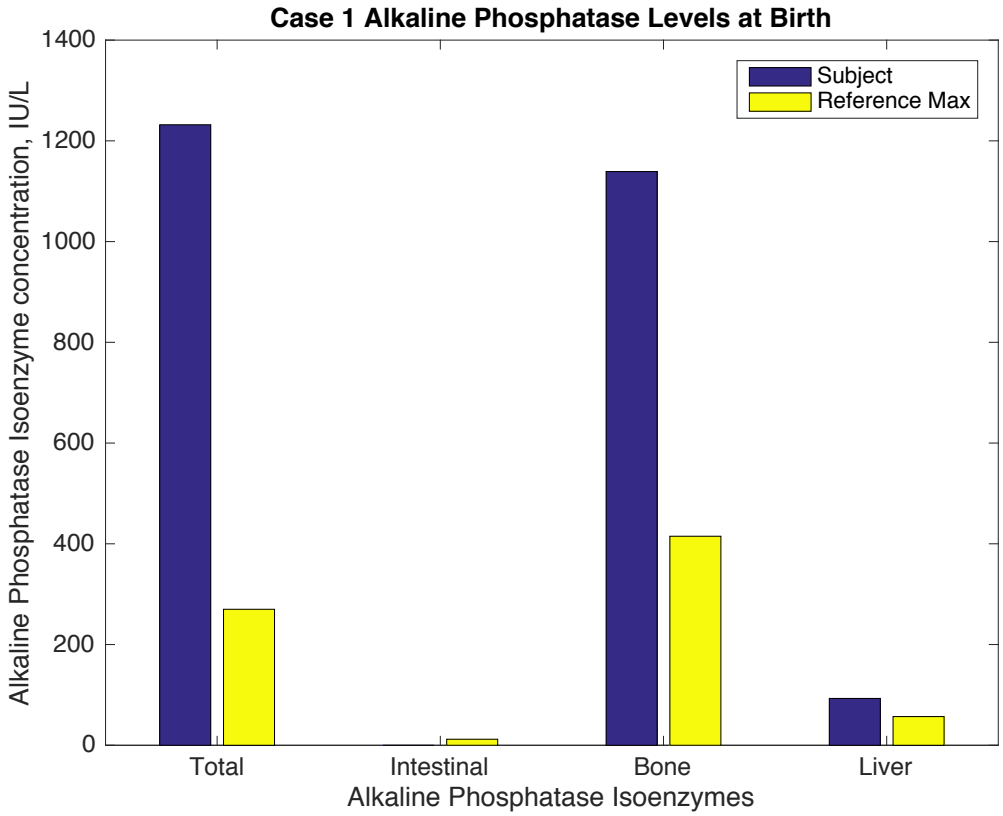

Figure 5: Alkaline phosphatase isoenzyme levels present at birth.
Clinical data indicating the alkaline phosphatase isoenzyme levels present at birth was collected for the patient in Case 1. The concentration of bone alkaline phosphatase is shown to be almost three times more than the maximum reference value for bone alkaline phosphatase at birth.

High serum alkaline phosphatase levels in CMD patients have also been indicated in the literature. In one study of a 3-year-old male alkaline phosphatase levels were 1138 IU/L, compared to the normal control range of 115-391 IU/L [85]. A table of CMD

patients with reported serum alkaline phosphatase levels from the literature is shown below (Table 6).

Table 6: Serum alkaline phosphatase levels in CMD patients as found in the literature.
Historically reported serum alkaline phosphatase levels are tabulated below. In most CMD cases, the serum alkaline phosphatase (ALP) levels are either elevated, or within the upper region of the reference rage.
* Indicates value not provided in the publication

Age (years)	Sex	Recorded ALP (U/L)	ALP normal reference range (U/L)	Notes	Reference
1	Male	2417-3002	58-400	Elevated	[83]
3	Male	*	*	Normal	[33]
4	Female	392 (IU/L)	*	Elevated	[42]
25	Female	51.1	*	*	[73]
1-2	Female	427-1944	150-400	Elevated	[21]
<1 (7 mo.)	Female	4168 (IU/L)	*	Elevated	[17]
6	Male	533 (IU/L)	125-410 (IU/L)	Elevated	[45]
36	Male	*	*	Normal	[46]
45	Male	*	*	Normal	[49]
<1 (4 wk.)	Female	1839	117-390	Elevated	[67]
2	Female	559	*	Elevated	[63]
3	Male	1138	115-391	Elevated	[85]

Not enough clinical data was found within the Case 2 clinical file to compare BUN/creatinine measurements with that of Case 1. An interesting trend was noticed in an elevation of BUN/creatinine, first recorded at age 1, which gradually decreased with age, despite still being higher than the referred range. This is tabulated in Table 5, however a visual representation is also provided in Figure 6.

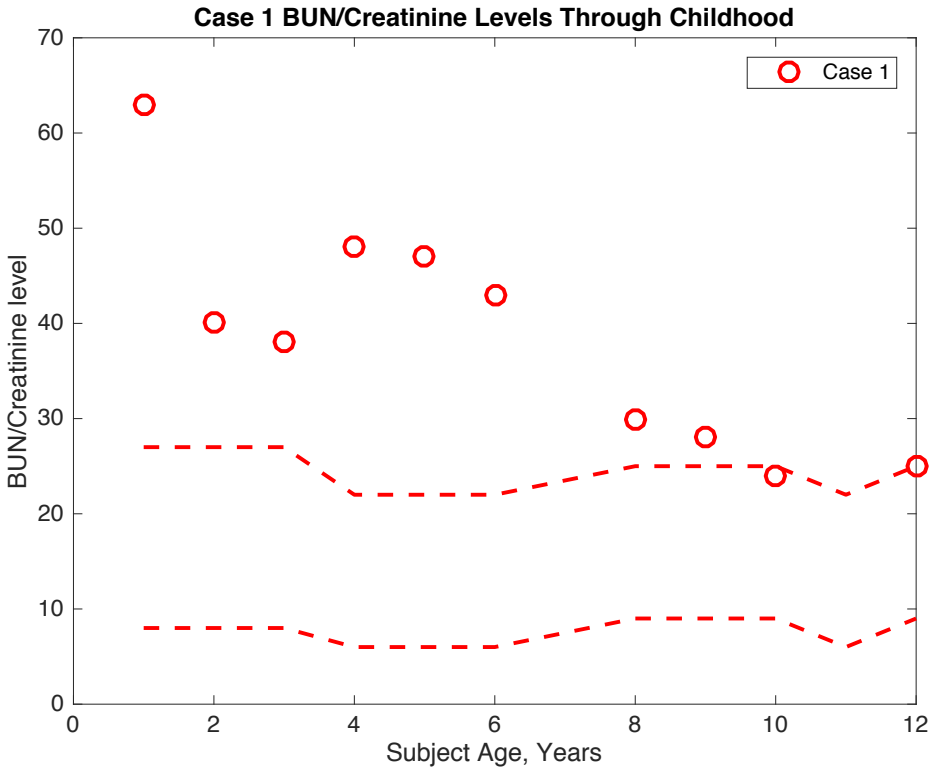

Figure 6: BUN/Creatinine measurements in Case 1 during childhood years. Shown above are the recorded BUN/Creatinine calculated values for the female patient, throughout her childhood years. In all but two measurements, the BUN/Creatinine level is higher than the reference range values, with two measurements falling in the extreme high end of the reference values.

Using the completion of analyzing the literature, the CMD database patient case files at the Reichenberger Lab at UCH, and lastly a comparison of two specific case files from the Reichenberger Lab CMD database, a list of clinical parameters relative to CMD worth further investigating was generated (Table 7).

Table 7: Clinical parameters suggested for investigation in future natural history of disease studies on CMD.
These variables for investigation were selected due to their considered importance in CMD diagnosis and disease progression.

Factors for Investigation	
1	Mutation
2	Head circumference
3	Bone thickness
4	Bone density
5	Calcium
6	Magnesium
7	Phosphorus
8	Alkaline phosphatase (ALP)
9	Tartrate-resistant acid phosphatase
10	Parathyroid hormone (PTH)
11	Calcitonin
12	Calcitriol (1,25-dihydroxyvitamin D3)
13	Vitamin D
14	Osteocalcin
15	Osteoprogeterin (OPG)
16	TNAP
17	Gap junction protein alpha-1 (GJA-1) = connexin 43 (*Cx43*)
18	BUN / Creatinine
19	Caclium/creatine (urinary)
20	Hydroxyproline (urinary)
21	Tubular reabsorption of phosphate
22	Auditory acuity
23	Visual acuity
24	Delayed primary dentition

DISCUSSION

Comparing two CMD patient cases

In both subjects' second years, clinical documentation indicates bone hyperplasia into the skull sinuses – mastoid sinuses in Case 1, and mastoid and paranasal sinuses in Case 2. Sclerosis of the base of the skull is also indicated to be severe enough in both cases to result in Chiari I malformations. Apparent reduction in bone hypertrophy of the calvarium also is documented to occur in both cases, however later in Case 2 (age 10) opposed to Case 1 (age 4).

An additional similarity between cases is bony overgrowth within the nasal cavity and nasal choanae with additional Class 2 occlusion and an open bite. These manifestations were both found to be congenital problems, which progressed with age. The hypovolemia and resultant hypoxia and need for bag resuscitation observed in Case 1 shortly after birth, could be explained by reduced nasal breathing complicated by bony overgrowth within the nasal cavity. Mild nasal flaring is listed in the respiration section of the subject's birth reports possibly indicating problematic nasal breathing directly since birth. Bone hyperplasia in both the nasal cavity and nasal choanae are prominent enough in both cases to cause both subjects to rely predominantly on mouth breathing.

Both cases are seen by craniofacial teams to monitor and progress the changing bone structure in each patient as they progress with age. Careful monitoring helps to detect changes in bone hypertrophy over the years, however most of the clinical summaries reporting the changes are qualitative and made by clinical observation. It is

difficult to compare bone hypertrophy between the cases, due to the inherent bias in having different specialists recording the observations for each case.

In the clinical reports, there has been significant variance of the clinical manifestations of CMD with age progression, between the two cases. While Case 2 presented sclerotic ribs and unaffected vertebrae, Case 1 differed with bony overgrowth within C1 severe enough to need correction by lamenectomy and minimal sclerosis of the ribs.

Auditory & visual acuity variation

Arguably, the most significant lifestyle differences between the cases are the secondary effects of CMD on the auditory and visual networks within the two subjects. In both cases, auditory deficits are significant enough to require hearing aids. Deficits appear to be most severe in the higher frequency spectrum in Case 1; however, it is unclear if this is replicated in Case 2. Though bilateral hearing loss is exhibited in both cases, the severity of the hearing loss is indicated to vary between right and left sides in the clinical work of both cases. Within individual patient cases, yearly auditory tests were documented as taken by different operators on different instruments between years, thus possibly adding a confounding variable when determining individual auditory changes over time.

With a population size of only two cases for this study, it is unclear exactly as to the causation of variance between the clinical manifestations of the two cases. One possible explanation could be that different mutations (Ser375del in Case 1, and

Phe377del in Case 2) play a role in the change of bone remodeling attributed to CMD. Variances may also be attributed to other variables such as sex, and lifestyle differences.

Importance of serum alkaline phosphatase

An important commonality found in this study, is the prevalence of high serum alkaline phosphatase levels within CMD patients. Serum alkaline phosphatase is a key hormone in the bone remodeling process. Serum alkaline phosphatase levels were determined to be abnormally high in both CMD subjects in early childhood. While still remaining above or within the high spectrum of the standardized range, serum alkaline phosphatase levels dropped off after birth. As this study is only a small representation of the CMD population necessary for a natural history of disease study for CMD, from these results, future studies may want to investigate serum alkaline phosphatase, calcium, phosphate, PPi and other blood parameter progression over time, in addition to the parameters mentioned in Table 7.

Collection of data on serum alkaline phosphatase must be carefully considered, as there are no verified reliable reference values below 4 years of age provided by the Mayo Clinic (see Appendix). Additionally, due to variances in buffers and testing protocols used, each laboratory measuring samples may produce values reproducibly within a range of their own independent reference values, however, different from the range supplied by the Mayo Clinic. In examining this enzyme for use in a natural history of disease study for CMD, a relatively large and heterogeneous sample size is necessary for comparison, due to the variances in enzyme concentration between sexes in healthy patients.

BUN/Creatinine measurements as a clinical blood parameter of interest.

A significant discovery within the clinical blood chemistry of Case 1, which was not available for Case 2, is the constantly high levels of BUN/Creatinine measurements. Case 1 had BUN/Creatinine measurements provided for all but two of the twelve years we studied, the case file for the patient in Case 2 contained no BUN/Creatinine measurements.

BUN/Creatinine is a clinical measurement indicating the kidney's ability to filter and successfully remove nitrates in the blood generated from the waste product urea, with respect to the relatively constant filtration of a separate muscular protein (creatinine). Both elevated and low measures of BUN/Creatinine are indicative of physiological disorders, where high BUN may be created by excess catabolism, and low BUN may occur as a result of excess anabolism.

The subject in Case 1 reports high BUN/Creatinine levels in 8 out of 11 years in which her case has blood chemistry measurements for this parameter. With BUN/Creatinine measurements gradually decreasing towards the normal reference range over time. Though Case 1 repeatedly exhibits high BUN/Creatinine levels compared to the laboratory provided normal range, the variance between patient sample data and the range, is greater during early childhood, and decreases over time. This indicates a return of the BUN/Creatinine level in the patient toward normal physiological levels over age. One possible explanation of this could be an indication of constantly recurring bone formation and resorption in CMD patients during the early childhood ages. Increased bone resorption would produce large amounts of hydroxyproline resulting from the large

levels of broken down collagen. Hydroxyproline, when further broken down by ubiquitination and proteolysis, would result in high levels of blood nitrogen as one of the end products of the catalyzed bone.

Efficacy of comparing clinical data between CMD patients

Qualitative clinical measurements, such as changes in the location and severity of bone hypertrophy have not been entirely reliable during this study. In the two cases presented, most of the data was collected through observations collected in summaries from meetings with clinicians. Clinical measurements such as the degree of hypertrophy of bone mass between years are qualitative measurements, made at the physician's discretion. This leads to variance in judgment between different physicians responsible for separate cases, as they are not abiding by a unified guide of reporting clinical observations.

The ramifications of a lack of a unified procedure for reporting clinical observations are shown most evidently by the variation in available clinical data in the Reichenberger Lab CMD patient database case files (Table 4). Available clinical data, as reported by Table 4, is shown to vary drastically between patient cases. This could be due to physician's judgment for which clinical data to record, hospital's efficacy in maintaining patient data over time, and variances in tests conducted between years.

Though most of the important clinical parameters determined from the literature were surveyed when going through the Reichenberger Lab CMD database case files, one important clinical characteristic overlooked, was patient bone density measurements

taken with age progression. To quantitatively compare these changes in bone growth between patients, one suggestion would be to use radiographic measurement tools during CT and MR imaging to obtain precise diameters of bone thickness. Additionally, quantitative measurements of MR intensity can help measure bone density. This would be useful in comparing changes of bone composition over the years. Comparison would then be possible between these readings and previous studies indicating soft bone in CMD patients [23].

Future directions for a full scale natural history of disease study of CMD

From this pilot study, it is concluded that a sufficient number of patients for a future natural history of disease study can be recruited for a retrospective study. There is already a large sample size of CMD patients documented within the Reichenberger Lab, as well as other labs publishing CMD research within the literature. Of the 76 cases at the Reichenberger Lab, despite not having complete sets of clinical information, each case has contact information for the subject, the referring doctor, or both. Continued contact will prove to be quite important for acquiring the remaining uncollected data within these cases. Acquiring retrospective clinical data both descriptive and accurate enough for valuable comparison between cases may generate complications in continuity, and consistency within cases.

Opposed to the retrospective study, continuity and consistency of data collection may be easier found in a prospective study. In a future prospective natural history of disease study for CMD, all measurements should ideally be taken either at one clinical site, or at

multiple sites following one unified standard of observation, measurement, and documentation. Considering many CMD patients described in the literature live abroad, recruitment potential to one clinical site may not be the most feasible. Having a unified protocol for multiple sites in different countries may is one way to collect measurements from CMD patients in a consistent manner. This can allow for more quantitative conclusions to be drawn from the data collected. A prospective study is recommended over a retrospective study, for higher accuracy of collecting specific measurements.

With different *ANKH* and *Cx43* mutations associated with a wide range of CMD clinical manifestations, it may be useful to use data collected from a wider population of CMD patients to determine subclassifications of CMD. This could possibly help preemptively predict development and progression of clinical manifestations by characteristics unique to each subclassification.

Suggested clinical parameters for investigation

Clinical parameters of suggestion for investigation include many of the clinical parameters reported in the literature. This includes levels of blood chemistry markers, such as: calcium, phosphate, serum alkaline phosphatase, vitamin D, parathyroid hormone (PTH), calcitonin and calcitriol. For these quantitative measurements, it is important to ensure testing for all blood chemistry markers and blood isoenzymes to be measured during routine blood work examinations, taken yearly as routinely suggested by general practitioners. Consistency between measurements between patients temporally will help to identify any trends and commonalities. Other clinical parameters should be

measured to determine CMD's effect on bone growth. Bone density, bone thickness, skull circumference, and homogeneity of the long bones are worth further investigation, as it has been displayed to vary over age and location in the body, within both subjects in this study. A third set of clinical measurements to be made in future studies include those of the secondary effects of CMD. Measurements of auditory and visual acuity should be taken consistently, to measure any trends in degradation of perception of either of the special senses, in relation to CMD. A more detailed list of clinical parameters we suggest further investigation of CMD are summarized in Table 7:

Often unmeasured aspects of CMD patients' lives for consideration in future studies include the subject lifestyle choices. Arguably the most interesting to observe would be diet and exercise of CMD subjects, and how both relate to the development of bone growth. Of all of the CMD patients within the patient database at the Reichenberger Lab, only one subject case out of 76 included information on diet and exercise – a case which was not documented descriptively for either parameter. Exercise is an important factor in bone stress, nutrient demand, and remodeling of the mesenchyme and can be an overlooked confounding variable in the disease progression within these subjects' lives. Dietary choices also is key in nutrient availability, which may effect the manifestation of CMD - depending on dietary intake of associated components of CMD metabolic pathways, including but not limited to: calcium, inorganic phosphate, vitamin D, iron, magnesium. Collection of data on diet and exercise may be hard to obtain specifically; however surveying subjects may be a viable alternative. An example of some potential survey questions and their rationale is provided in the appendix.

Along with diet and exercise, other differences in lifestyle can be documented via a patient survey. Seen in these two cases are the subjects' differences in use of speech and sign language as forms of communication. One potential lifestyle difference thus, may include choice of schooling, learning with print or braille.

One interesting area of investigation, not nearly researched enough, is the impact of CMD development on mental health across all ages. Being a disease that has so many secondary effects on the body, CMD has a strong psychosocial impact on the lives of those with the disease, especially on children. Surveying can be a great way to investigate the uncharted waters of the psychosocial and psychological effects of CMD. Some considerations of psychosocial effects, that studies may choose to focus on are: dietary preferences, social anxiety, teasing, fear, headache, pain, reduced physical activity caused by pain, loss of sight, hearing, weak stamina and bone strength during exercise.

APPENDIX

Suggested questions for survey investigation:

1) Was CMD initially diagnosed or was another diagnosis suggested first? What was that diagnosis?

Rationale: Ask for distribution of misdiagnosis, to show CMD differential diagnosis is an important issue.

1a) Age when bone disorder was first diagnosed.

1b) Age at CMD diagnosis.

1c) Complications faced as a result of this misdiagnosis.

1d) Clinical treatments based on misdiagnosis.

2) What information were you given about CMD when you received your diagnosis?

Rationale: Show need for a natural history of disease study

2b) Physician awareness of CMD during the time of diagnosis.

2c) Number of referrals given before a correct diagnosis was made

3) What was the treatment plan for CMD?

Rationale: Show wide range of treatment options used for varying CMD patients, to show treatment needs to be patient specific

3b) Patient-specific tailored treatment plans for your individually distinct CMD symptoms.

3c) Differences in treatment plans between patients

4) What were your initial symptoms of CMD?

Rationale: Determine incidence and distribution of CMD disease manifestation.

4a) Changes in disease progression over time.

5) Reflect on your dietary lifestyle. List below what you would eat in an average breakfast, lunch, and dinner for one day.

Rationale: Determine dietary effects on blood chemistry levels.

5a) Analyze dietary decisions that may effect calcium, phosphorus, vitamin D, essential amino acid, protein and carbohydrate intake.

6) Have you ever had neurological problems since you've been born.?

Rationale: Determine effect CMD has on visual, auditory, and proprioception acuity.

6a) How have they developed over time

6a) Determine the epigenetic details of the spread of visual, auditory, and motor function deficits between CMD patients.

7) Has CMD ever been a problem in your physical activity and exercise?

Rationale: Determine if weakness in bone structure, muscle tone, or possible anemia secondary to CMD has exacerbated CMD's destructive effect on sensorimotor function.

7a) Analyze whether regular exercise increases bone density, bone mass, and bone & muscle strength, compared to subjects who do not exercise regularly.

7B) Determine how the biochemical pathways associated with osteogenesis and bone removal are compensated for, in CMD patients.

Mayo Clinic pediatric alkaline phosphatase reference values

Mayo Clinic Pediatric Test Reference Values. (2016, January 25). Retrieved from: http://www.mayomedicallaboratories.com/test-info/pediatric/refvalues/reference.php

LIST OF JOURNAL ABBREVIATIONS

Acta Otolaryngol	Acta Oto-Laryngological
Am J Hum Genet	American Journal of Human Genetics
Am J Med Genet A	American Journal of Medical Genetics Part A
AMA Arch Intern Med	American Medical Association's Journal of Internal Medicine
Br J Ophthalmol	British Journal of Opthalmology
Br J Plast Surg	British Journal of Plastic Surgery
Cell Reprogram	Cellular Reprogramming
Clin Genet	Clinical Genetics
Dentomaxillofac Radiol	Dento-maxilofacial Radiology
Dis Nerv Sys	Diseases of the Nervous System
Eur J Pediatr Surg	European Journal of Pediatric Surgery
Hum Mol Gen	Human Molecular Genetics
Indian J Pediatr	Indian Journal of Pediatrics
J Bone Joint Surg Am	The Journal of Bone and Joint Surgery
J Bone Miner Res	Journal of Bone and Mineral Research
J Clin Invest	Journal of Clinical Investigation
J Clin Psychology	Journal of Clinical Psychology
J Laryngol Otol	The Journal of Laryngology & Otology
J Med Gen	Journal of Medical Genetics

J Psychopharmacol	Journal of Psychopharmacology
Neurol India	Neurology India
Otol Neurotol	Otology & Neurotology
PostGrad Med J	PostGraduate Medical Journal
Paediatr Child Health	Journal of Paediatrics and Child Health
Pediatr Intl	Pediatrics International

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CURRICULUM VITAE

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Permanent Address: 124-05 133rd Ave, South Ozone Park, NY 11420
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Education

Boston University School of Medicine, Boston, MA
M.S. Medical Sciences, (*School of Medicine, May 2016*)

Boston University College of Engineering, Boston, MA
B.S. Biomedical Engineering *(College of Engineering, May 2014*)
Minor: Chemistry (*College of Arts & Sciences, May 2014*)

Experience

Lab Assistant at the Center for Regenerative Medicine (CReM)
Boston University School of Medicine, Boston, MA
August 2012-August 2014

- Adapted spinner flask suspension culture techniques developed in prior independent experiments, to begin the development of a new standardized directed differentiation protocol to bioreactor batch and fed batch culture.
- Independently designed and executed stem cell experiments in the scaling up of mouse embryonic stem cells in suspension culture. Determined optimal LIF concentration needed to maintain pluripotency and viability.
- Given autonomy to train an undergraduate student in both static and suspension cell culture techniques.

Research Trainee at The Focused Ultrasound (FUS) Laboratory
Brigham and Women's Hospital / Harvard Medical School, Longwood, MA
August 2013 – June 2014

- Lead developer of a research team designing and prototyping a novel real-time ultrasound imaging feedback device to be used in ventriculostomy procedures.
- Arranged meetings with clinicians for the incorporation of marketable designs in the precision and usability of an ultrasound guided tool for ventriculostomy procedures.

Patient Advocate at Health Leads
Codman Square Health Center, Dorchester, MA

May 2011 – August 2012

- Researched patient specific resources addressing the social determinants of health (Housing, Food, Utilities.)
- Filled the role of liaison between patients and both private and government agencies.
- Provided continued support with 20 patient cases simultaneously while maintaining individual attention to each.

Projects

Directed Differentiation of Embryonic Stem Cells in Suspension Culture

January 2013-August 2014

- Derived & optimized an unprecedented scaling up protocol for the adaptation of static culture directed differentiation of mESCs into suspension culture, increasing experiment cost efficacy.

Forest Fire Detection System

January 2014 - May 2014

- Drafted a business plan indicating the profitability of selling fire detection systems to state and federal governments.
- Designed a technically feasible new fire detection system using innovative LiDAR technology.

Oculomotor System Calibration

September 2013 - December 2013

- Developed a software algorithm to analyze and predict eye saccade movement, determining perception bias through use of electrooculography.

Skills

Laboratory: IR & NMR Spectroscopy, UV/VIS, AAC, TLC, ELISA, Protein Purification, Tissue Pathology, Gel Electrophoresis, PCR, Flow Cytometry, Assaying, ES & iPS Cell Culture, Immunohistochemistry.

Computer: C++, MATLAB, Mathematica, Microsoft Office, MestReNova, ImageJ, FlowJo, InDesign, Photoshop, Social Media.

Organizations *Society of Asian Scientists and Engineers: Boston University Chapter*

April 2013 – April 2015

- Corporate Relations Chair & Social Chair. Expanded club membership by over 300%.

- Directed and organized panel lectures and networking workshops for undergraduate students.
- Led my university chapter to successfully hosting the organization's Northeast Regional Conference in February 2015.

Publications Faik C. Meral, Michael A. Persaud, Aaron E. Silva, Abhishek Mundra, Greg T. Clement, Kirby G. Vosburgh, Phillip J. White. A novel device for guiding ventriculostomy with transcranial ultrasound. *The Journal of the Acoustical Society of America.* 04/2014; 135(5):2211.