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Changes in cranial base and craniocervical junction during growth in healthy individuals and in patients with osteogenesis imperfecta

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ACADEMIC DISSERTATION

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To my beloved

Abstract

Cranial base and craniocervical junction anatomy can be evaluated from MR and CT scans, and lateral skull radiographs. Cranial base anatomy changes during growth, as the form of the anatomic structures and their relative positions alter. In disorders of compromised bone quality, abnormal changes in the craniocervical junction can lead to pathological conditions with possibly life-threatening neurological complications. In this investigation these issues have first been addressed by making skull base measurements of healthy growing individuals to determine the age-specific normative values used to evaluate cranial base structures. These normative values were then employed in the following analysis of growing patients suffering from osteogenesis imperfecta (OI), a disorder of bone fragility due to abnormal collagen composition.

In healthy individuals' cranial base measurements, young children demonstrated significantly different results from those of older growing individuals and adults. Thus studies on patients younger than 9 years should include age-appropriate controls. Significant individual variation in skull base measurements during growth may appear, as revealed by longitudinal observation. The observed wide ranges, and the fact that the position of the odontoid process in relation to skull base structures may individually display alternating up- and downward movements, are important to consider when conducting follow-up examinations. Nevertheless, a notable deviation from the documented normal values is suggestive of pathological development in the craniocervical junction.

When comparing the age-specific normal values, skull base abnormalities were found to be present in approximately one fifth of OI patients; platybasia was the most frequent finding. Patients with haploinsufficiency mutations (which lead to a relatively mild phenotype) were less likely to have skull base abnormalities than patients with helical glycine substitutions. However, the strongest predictor of skull base abnormality was not the type of collagen mutation underlying OI but the clinical severity of the disorder, as expressed by the height Z-score. No evidence was found for a protective effect of

bisphosphonate treatment, nor for the progression of craniocervical junction pathology with age.

Based on this normative data and the observations made in patients with OI, age-appropriate screening limits for skull base pathology are proposed here, in addition to a recommendation for timing of radiological screening in patients with OI.

Contents

List of original publications

This thesis is based on the following publications, referred to in the text by their roman numerals. This thesis also contains some unpublished data.

I Arponen H, Elf H, Evälahti M, Waltimo-Sirén J 2008 Reliability of cranial base measurements on lateral skull radiographs. Orthod Craniofac Res 4:201-210.

II Arponen H, Evälahti M, Waltimo-Sirén J 2010 Dimensions of the craniocervical junction in longitudinal analysis of normal growth. Child's Nervous System 26:763-769.

III Cheung M, Arponen H, Roughley P, Azouz M, Glorieux FH, Waltimo-Sirén J, Rauch F 2011 Cranial Base Abnormalities in Osteogenesis Imperfecta: Phenotypic and Genotypic Determinants. J Bone Min Res 26:405-413.

IV Arponen H, Mäkitie O, Haukka J, Ranta H, Ekholm M, Mäyränpää MK, Kaitila I, Waltimo-Sirén J 2012 Prevalence and natural course of craniocervical junction anomalies during growth in patients with osteogenesis imperfecta. J Bone Min Res 27: 1142–1149.

Abbreviations

Ba	Cephalometric landmark basion at the midpoint on the anterior bony margin of the foramen magnum of the occipital bone
BMD	Bone mineral density
BMP	Bone morphogenetic protein
BMP1	Gene coding for bone morphogenetic protein 1
C1	First cervical vertebra also known as atlas
C2	Second cervical vertebra also known as axis
CRTAP	Cartilage associated protein
COL1A1	Gene coding for alpha 1 chain of type I collagen
COL1A2	Gene coding for alpha 2 chain of type I collagen
CT	Computed tomography
D	Cephalometric landmark dens at the cranial tip of the odontoid process
DXA	Dual-Energy X-ray Absorptiometry
ECM	Extracellular matrix
LEPRE1	Leucine proline-enriched proteoglycan. Gene coding for leprecan or prolyl-3 hydroxylase enzyme.
LS aBMD	Lumbar spine areal bone mineral density
M	Cephalometirc landmark M-point at the most caudal point of occipital bone
MRI	Magnetic resonance imaging
N	Cephalometric landmark nasion at the nasofrontal suture
OI	Osteogenesis imperfecta
Op	Cephalometirc landmark opisthion at the mid-point on the posterior bony margin of the foramen magnum
PNS	Cephalometirc landmark posterior nasal spine at the most dorsal point of the palatal bone
PPIB	Cyclophilin B. Gene coding for cyclosporine-binding protein.
S	Cephalometirc landmark sella turcica
SD	Standard deviation
TGF-β	Transforming growth factor beta
Wnt	Wingless homologue in vertebrates (transcription factor)

1. Introduction

Throughout life, bone mass is the net result of the processes of bone resorption and formation, which is regulated by genetic and environmental factors. Bone dysplasias, such as osteogenesis imperfeca (OI), disrupt the balance of bone remodelling.

OI is a heterogeneous group of connective tissue disorders characterized by bone fractures that arise from even low impact trauma. Progressive bone deformities develop frequently in severe OI types. OI is a disease of collagen I rich tissues; particularly bone, where disorganized matrix and reduced bone mass, result in the characteristic brittleness. The disorder is caused by a mutation in one of the two type I collagen genes; *COL1A1* or *COL1A2* in most patients with OI. Small numbers of patients, however, carry a mutation in genes involved in collagen protein assembly, leading to abnormal folding of type I collagen. (Marini *et al.* 2010)

A serious potential complication of OI is a deformity of the craniocervical junction. The craniocervical junction consists of the occiput, housing the foramen magnum, atlas and its axis, as well as ligaments and other associated structures. It forms a vital passage for the central nervous system. (Menezes 2008a) Craniocervical junction anomalies also affect a wider patient population; they are possible complications of several syndromes, such as Down or achondroplasia, or can arise from congenital malformations (Luyendijk *et al.* 1978, Menezes 2008e). The pathology of the craniocervical junction can be asymptomatic, or result in even life threatening symptoms caused by compression of the medulla, cerebellum, cervical nerve roots, lower cranial nerves or the vascular supply to these (Sawin and Menezes 1997, Smoker and Khanna 2008, Bishop 2010, Forlino *et al.* 2011).

Data on the normal dimensions of the craniocervical junction and the associated growth-related changes in healthy children has been scarce, despite being a prerequisite for the accurate diagnosis and better understanding of the development of basilar anomalies. Similarly, the natural course of craniocervical junction anomalies, in growing patients with OI, has for the most part been unexplored despite the importance of early intervention before serious symptoms arise.

2. Review of the literature

2.1. Bone metabolism and growth

The skeletal system, composed of cartilage and bone, aids locomotion, and supports body weight. The skeleton also protects the internal and sensory organs, the central nervous system, and the respiratory tract, whilst acting as storage for calcium and phosphate. The facial skeleton is embryologically derived from the cranial neural crest, whereas the rest of the skeleton, including cartilage, is derived from mesoderm (Leucht *et al.* 2008). Mature bone is actually specialised connective tissue composed of bone cells, proteins, and minerals. Histologically, mature bone can be divided into two types: 1. Cortical or compact bone that has a dense ordered structure and is mainly found in the diaphyses of long bones and the outer surfaces of flat bones. 2. Cancellous or trabecular bone, that is lighter, less compact, and has an irregular structure. Trabecular bone is less abundant and forms the metaphysis and epiphysis of long bones and the inner parts of flat bones. Trabecular bone is ideally suited to withstand compressive stress and hence is the predominant bone type in the vertebrae. (Datta *et al.* 2008)

Mature bone tissue hosts three types of cells: osteoblasts, osteocytes, and osteoclasts. Stromal marrow cell derived osteoblasts function in bone formation by synthesizing and secreting collagenous and non-collagenous matrix proteins (Shi *et al.* 1996). Approximately 10-15% of osteoblasts are transformed into osteocytes. Multifunctional osteocytes are the most abundant cell type in bone. They are believed to transmit signals for bone remodelling via dendritic processes and by apoptosis in response to mechanical strain. Hematopoietic stem cell derived osteoclasts are involved in the resorption of bone tissue by secreting matrix metalloproteinases and cathepsins. (Aarden *et al.* 1994) Osteoblasts are located on the surfaces of bone tissue, while osteoclasts are found both within the calcified matrix and on calcified bone surfaces. (Datta *et al.* 2008)

The extracellular matrix (ECM) consists of organic components (collagen fibers embedded in ground substance) and inorganic components (mainly calcium and phosphate

in the form of hydroxyapatite). These minerals contribute approximately 60% of the weight of the bone. About 90% of the matrix proteins are collagen, making it the most abundant group of proteins in the body. Collagen gives structural support to bone, and is an essential component of cartilage, skin, ligaments, and cornea. (Viguet-Carrin *et al.* 2006, Datta *et al.* 2008) Types I through V are the most abundant collagen types in the body. Type I collagen is the predominant constituent of bone, and type II the main component of cartilage. The tensile properties of collagen fibrils serve to increase the elasticity of these tissues. The banded collagen fibrils in connective tissues mostly consist of more than one type of collagen molecule similar in structure. (Burgeson 1988, reviewed by Lodish *et al.* 2000) Changes in the composition of the organic matrix affect bone mineralization and thus the mechanical properties of bone. (Bala *et al.* 2011)

Type I collagen accounts for approximately 90-95% of the collagen content of bone (Huber 2007), whilst approximately 5% comprises type V collagen, and the remainder is bone-specific phospho- and glycoproteins (Niyibizi and Eyre 1989). Type I collagen is mainly produced by fibroblasts and osteoblasts, and is composed of two alpha-1 chains and one alpha-2 chain encoded by *COL1A1* and *COL1A2* genes, respectively (Figure 1). These three polypeptide chains begin to fold into a triple-helical rod at the carboxyl-terminal (C-terminal) end, with the folding proceeding towards the amino-terminal (N-terminal) end. (Engel and Prockop 1991) Posttranslational modification in the endoplasmic reticulum includes hydroxylation of prolyl and lysyl amino acids. (Marini *et al.* 2010) Intracellular stages are required to process and assemble the procollagen molecule, and extracellular stages to convert it into collagen and incorporate the mature collagen molecules into stable, cross-linked fibrils. The resulting mature collagen fibrils form by lateral interactions of the triple helices. (reviewed by Lodish *et al.* 2000)

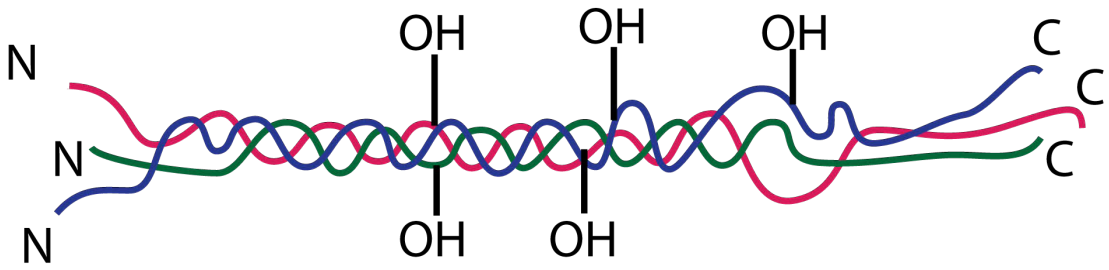


Figure 1 *Schematic illustration of a collagen trimer. Procollagen is composed of a twisted center and loose ends (C-terminal and N-terminal). Hydroxyproline aids crosslinking between the α chains and is therefore essential for stability of the collagen molecule.*

In order for the strength of the bone to be maintained, the process of bone turnover must be carefully regulated. Bone metabolism relies on complex Wnt/β-catenin and TGF-β/BMP mediated signaling pathways to achieve proper rates of growth and differentiation. These pathways modulate the key transcription factor Runx2 that is essential for osteoblast differentiation and embryonic bone formation. Signaling pathways include the action of several hormones, including parathyroid hormone, vitamin D, growth hormone, steroids, calcitonin, and insulin-like growth factor, as well as several other cytokines. (Canalis *et al.* 1988, Datta *et al.* 2008)

Bone undergoes constant remodeling as a response to functional demands transmitted via muscle attachments to reshape or replace bone during growth and following injury. Bone is a self-renewing tissue (Seeman and Delmas 2006). This inherent regenerative capacity is attributed to skeletal progenitor cells, located for example in the periosteum. Proliferating progenitor cells can differentiate into either chondrocytes or osteoblasts, which deposit new mineralized matrix. Matrix is continuously resorbed by osteoclasts, which in turn stimulates new osteoblastogenesis. (Leucht *et al.* 2008) During childhood, bone turnover is high and bone formation slightly exceeds resorption. In a healthy adult, the bone turnover rate is about 10% of the skeleton per year (Leucht *et al.* 2008). Peak bone mass is reached in the twenties, after which bone formation rate decelerates. The combined effect of rate of bone turnover, quality of the collagen-rich ECM, size, structure, geometry, and mineral density determines the overall mechanical properties of the skeletal

system (Seeman and Delmas 2006). These determinants are affected by diaseases such as osteoporosis, Paget's disease, osteopetrosis, and osteogenesis imperfecta.

2.2. Neurocranium and craniocervical junction

2.2.1. Anatomy

The cranial base, which is formed by the ethmoid, sphenoid, occipital, paired frontal, and paired parietal bones, can be divided into anterior, middle, and posterior cranial fossae. The cranial base houses a complex of foramina that form vital passages for neurovascular structures. The largest of these is foramen magnum in the occipital bone. The craniocervical junction is defined as the region comprising the occiput, atlas and its axis, as well as ligaments and other associated structures. The sagittal diameter of the foramen magnum is normally 35±4mm, and the transverse diameter is slightly less. (Menezes 2008a). Sagittal diameter less than 25mm has been suggested to be pathological (Hinck *et al.* 1961).

The joint connecting the neurocranium and spine is comprised of the first cervical vertebra, atlas (C1), and its axis (C2). They are specialized to allow a wide range of head movement in flexion, extension, lateral bending, and lateral rotation. The ring-like atlas consists of an anterior and a posterior arch and two lateral masses encircling the vertebral foramen. The axis, in contrast, incorporates a large corticocancellous bony protuberance called the odontoid process or dens, which has a narrowed waist and thickened cortical tip. The joint surfaces of the skull base, atlas, and second cervical vertebra have little inherent stability. Thus, the stability of this region is mainly determined by ligamentous structures. (reviewed by VonTorklus and Gehle 1972, Menezes 1998)

The alar ligaments connect the medial surface of the occipital condyles to the lateral tip of the odontoid process. The apical ligament connects the caudal end of clivus to the superior tip of the odontoid (Figure 2). Tectorial membrane is a continuation of the posterior longitudinal ligament and is composed of a deep and a superficial layer. Between the

apical ligament and the deep layer of the tectorial membrane is the cruciate ligament bridging the clivus and the axis body, and a narrow transverse ligament, which extends between the upper ends of the lateral masses of the atlas. The transverse atlantal ligament and accessory atlantoaxial ligament are additional supportive structures. Posteriorly, the upper cervical spine has less ligamentous support; only the atlantoaxial and atlanto-occipital membranes. (Tassanawipas *et al.* 2005, Tubbs *et al.* 2011)

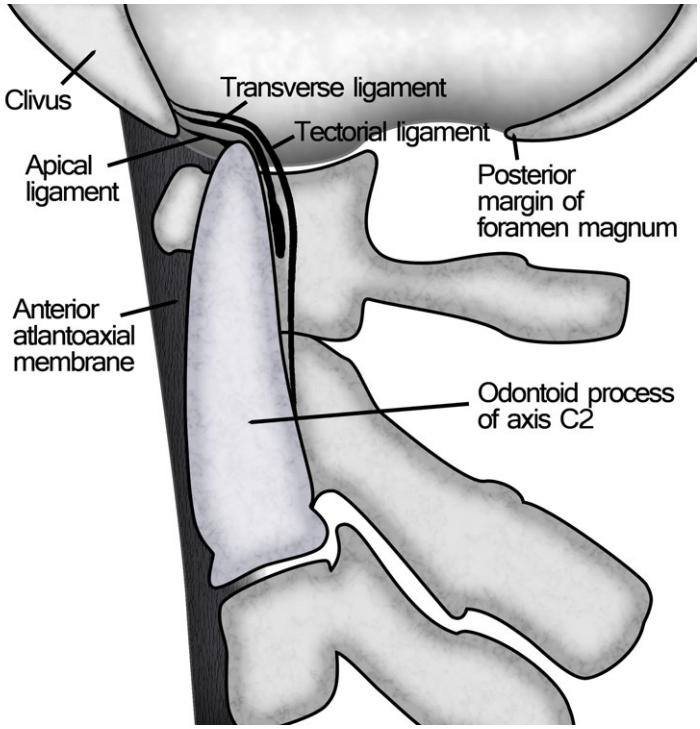


Figure 2 *Schematic illustration of the main ligaments of craniocervical junction connecting the clivus on the occipital bone to C1 and C2. Modified from Netter, Frank. Atlas of Human Anatomy 1989 Novartis, USA.*

2.2.2. Prenatal development

During human embryogenesis, 42 somites are formed by the end of the fourth week of gestation. Each somite differentiates into dermatome, sclerotome, and myotome. Of these, the sclerotomes go on to form the vertebral bodies, each of which receives contributions from two adjacent somites; whereas neural arch is derived from a single somite. The fourth occipital sclerotome, termed proatlas, divides to ultimately form the anterior

tubercle of the clivus, the anterior margin of foramen magnum, the occipital condyle, the lateral atlantal masses, the superior portion of the posterior arch of atlas, the apical tip of odontoid process, as well as the apical, cruciate, and alar ligaments. The most inferior part of the axis arises from the second cervical sclerotome (Figure 3). (Matsuoka *et al.* 2005, Menezes 2005, Pang and Thompson 2011)

Skull bones have two separate origins. Those of the face arise from the neural crest and are thus of ectomesenchymal origin, whereas those of the cranial vault and base arise from the paraxial mesoderm of the somitomeres and the first five somites (Cobourne 2000). Several studies and transgenic-mouse models demonstrate the role of gene families that act as regulators of gene transcription in determining the embryonic plan of the developing craniofacial complex. The Pax family of regulatory genes is involved in somatic differentiation and sclerotomal resegmentation (Pang and Thompson 2011). Combinatorial expression of the homeobox-containing Hox genes has also been shown to be important in patterning and positional identity of distinct regions of the cranium and cervical vertebrae (Cobourne 2000).

Sella turcica, the midpoint of which has been used as a reference point in our study, refers to the bony nest of the pituitary gland. The pituitary gland begins its development in the third gestational week and is a compound structure. The posterior lobe forms from a diverticulum of the diencephalic floor called the infundibulum, whereas the anterior lobe forms from an evagination of the ectodermal roof of the stomodeum called Rathke's pouch (Schoenwolf *et al.* 2009).

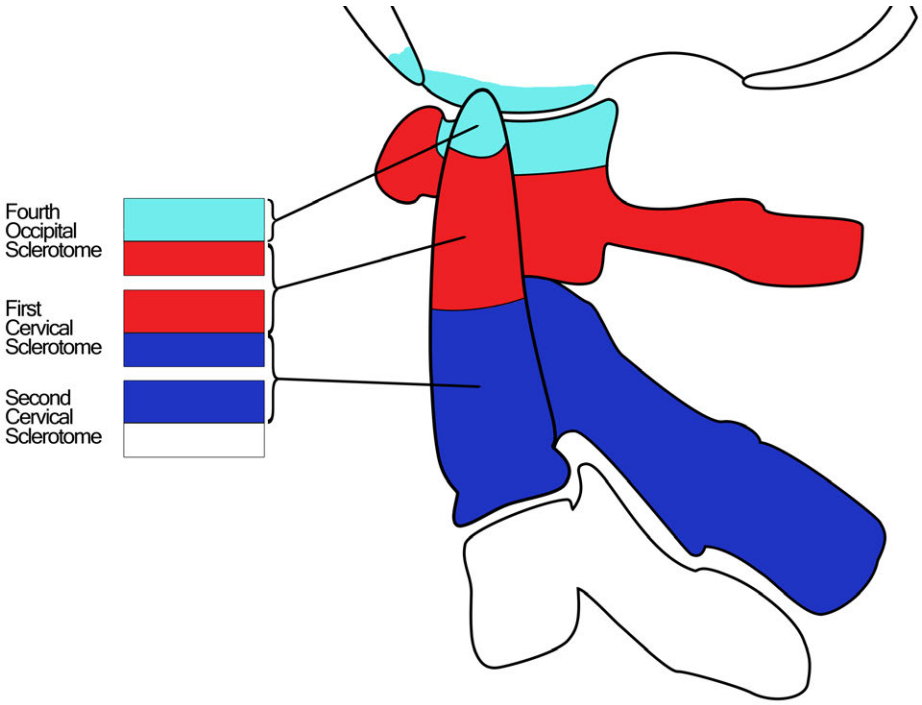


Figure 3 *Schematic illustration of the embryology of the craniocervical junction. The basiocciput, tip of the odontoid, and ligaments attached to it, develop from the fourth occipital sclerotome. The odontoid process is formed from the caudal part of the fourth occipital and the cranial part of the first cervical sclerotomes. The most inferior portion of the axis body is derived from the caudal part of the first cervical and the cranial part of the second spinal sclerotomes.*

Embryonic cartilage formation begins by the seventh week of gestation. The cranial base, that comprises a major part of the fetal chondrocranium, develops from three pairs of cartilaginous precursors: the prechordal, hypophyseal, and parachordal cartilages. These cartilages also contribute to protection of the brain and sensory organs together with cartilaginous capsules that develop around the otic and nasal pits. The caudalmost paired parachordal cartilages are derived from the occipital sclerotomes and the first cervical sclerotome. Thus they are modified vertebral elements. The parachordal cartilages form the base of the occipital bone. The hypophyseal cartilages fuse to form the body of the sphenoid bone. The prechordal cartilage gives rise to the ethmoid bone, which together with the nasal and turbinate bones encapsulates the nasal cavity. (Bosma 1975, Schoenwolf *et al.* 2009)

Bone formation takes place by two major mechanisms: endochondral and intramembranous ossification. During endochondral ossification mesodermally-derived osteochondral progenitors differentiate into chondrocytes to form a cartilage framework for the future bone (reviewed by Gilbert 2010). Ossification of the endochondrally formed cranial base begins during the second and third fetal months. Autonomously developing cranial base forms a platform for the growth of the rest of the cranium. Within the cranial base, synchondroses remain cartilaginous joints between the different ossification areas. Synchondroses are important growth centers that resemble histologically epiphyseal growth plates of long bones, but are bipolar in structure. (Björk 1955, reviewed by Bosma 1975) Long bones and vertebrae also develop via endochondral ossification (Mackie *et al.* 2011).

Facial bones and most of the cranial vault develop from neural crest-derived ectomesenchymal tissue through intramembranous ossification. During this process, osteochondral progenitors differentiate directly into osteoblasts to form membranous bone (reviewed by Gilbert 2010). Intramembranously formed flat bones grow to meet each other, and are then joined by sutures where appositional growth continues. Sutures are sites of growth (reviewed by Bosma 1975). Sutural growth in the cranial vault accompanies the enlargement of the brain, and similarly, growth of the respiratory tract, eye bulb, neck, and facial musculature are thought to contribute to the amount of growth in the number of sutures between facial bones. In addition to growth in sutures and synchondroses, the neurocranium also grows by surface apposition and resorption in response to forces from the enlarging brain. This occurs similarly in the face; the nasal cavity, for instance, vertically enlarges through lowering of the palate by resorption on the nasal surface along with apposition on the oral surface. Patency of the calvarial sutures provides malleability of the head during delivery. (Björk 1955, Shapiro and Robinson 1976, Moss 1997, Rice *et al.* 2003)

2.2.3. Postnatal development

The neurocranium is incomplete at birth. Similarly, the craniocervical junction of children is immature and differs from that of adults, both biomechanically and physiologically. Growth takes place sequentially, where the anterior fossa completes its growth first, followed by the posterior and middle fossae. Growth of the cranial base and face follow closely that of the calvaria. The calvaria, the cranial base, and the facial complex display an interrelationship, where forces from one complex influence the others. (Goodrich 2005)

Postnatal growth is regulated by genetic, epigenetic, and environmental factors (Bosma 1975, Enlow and Azuma 1975, Moss 1997). Environmental regulation is well displayed, for example in individuals with upper airway obstruction leading to altered craniocervical posture, which in turn affects the morphology of the first cervical vertebra. Growth changes include a smaller than average vertical dimension of the atlas dorsal arch. (Kylämarkkula and Huggare 1985, Solow 1992) Pathological conditions, such as craniosynostosis and hydrocephalus, leading to increased intracranial pressure, may in turn secondarily affect the cranial base morphology (reviewed by Bosma 1975, Perlyn *et al.* 2001, Goodrich 2005). The severity of the dysmorphology depends on the timing of the development of the pathology. Growing patients with untreated hydrocephalus exhibit a more obtuse anterior cranial base angle than healthy or treated children (Huggare *et al.* 1989).

The cranial base anterior to sella stabilises by 7 years of age through ossification of the spheno-ethmoidal and inter-sphenoidal synchondroses (Figure 4) (Björk 1955). Among cranial base synchondroses, the spheno-occipital synchondrosis is the most important site for sagittal and vertical growth. The spheno-occipital synchondrosis ossifies by 17 years of age until which time the clivus grows in length (Shirley and Jantz 2011). Simultaneously occipital bone drifts downwards and backwards as the brain is displaced by its growth resulting in a virtually parallel lowering of the foramen magnum in relation to the anterior cranial fossa. (Björk 1955)

During normal development, the anterior cranial base angle is obtuse at birth and decreases during the first five years, after which it remains fairly stable (Kerr and Hirst

1987). Most of the observed decrease in the anterior cranial base angle has been suggested to occur during the first two years after birth (George 2005).

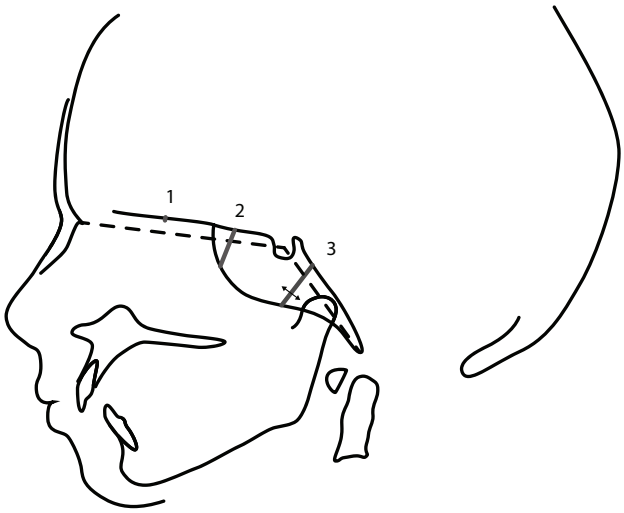


Figure 4 *Synchondroses of the anterior cranial base. 1.Spheno-ethmoidale 2.Inter-sphenoidale 3.Spheno-occipitale. The anterior cranial base angle is displayed with dashed lines.*

In early infancy the development of atlas proceeds from pre- and postnatally formed ossification centres that expand towards each other to form ventrally neurocentral synchondroses. Atlas should be a complete ring by 3 years of age as a result of fusion of the lateral atlantal masses. (Wang *et al.* 2001, Menezes 2008b)

At birth, the odontoid process is still separated from the body of the axis by a cartilagenous band, termed dentocentral synchondrosis (Figure 5). This synchondrosis lies below the superior level of the vertebral body (corpus of the axis C2) and is not visible on radiographs. The dentocentral synchondrosis gradually closes between 3 and 8 years of age. It contributes to the overall longitudinal growth of the odontoid process and vertebral body. Growth in height of the odontoid process also occurs inferiorly at the end plate physis and at the tip of the odontoid. (reviewed by VonTorklus and Gehle 1972, Menezes 1998, Piatt and Grissom 2011) The odontoid process is mineralized enough to be observed on lateral skull radiographs at birth, whereas the tip, called ossiculum terminale, is visible only after around 3 years of age when its ossification has proceeded. The tip of the dens fuses with the remainder of the odontoid process by 12 years of age. (Ogden 1984,

reviewed by Lovell *et al.* 2006) In healthy children, observed from the age of three months until 15 years, the growth rate of odontoid height has been found to be fastest during the first five years after birth, and become linear thereafter (Wang *et al.* 2001). The height of the odontoid process has been proposed to range from 14.4 mm to 21.3 mm in adults. In adult females, the height is on average 4.5 mm less than in adult males. (Schaffler *et al.* 1992, Çokluk C *et al.* 2006)

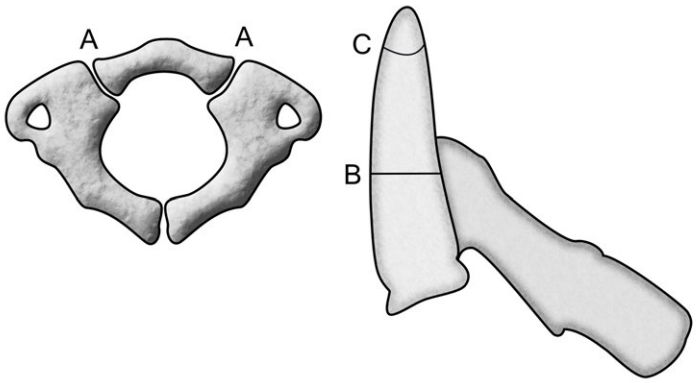


Figure 5 *Development of the cervical vertebrae. The paired neurocentral synchondroses (A) unite the neural arch to the anterior arch (body) of the atlas. Neural arches fuse posteriorly by 3 years of age, and neurocentral synchondroses by 8 years of age, at which time the vertebral canal has reached its adult size (Piatt and Grissom 2011). The odontoid process develops from two primary ossification centers that fuse by 3 months of age; the C2 corpus appears by the fifth fetal month and is separated from the dens by the dentocentral synchondrosis (B). Centrum has superior and inferior epiphyses and subsequent ossification centres. The dentocentral synchondrosis fuses at 3–8 years. The ossiculum terminale (C) at the tip of the dens appears after 3 years of age and coalesces with the odontoid process by 12 years of age. The inferior epiphyseal ring appears at puberty and fuses at about 25 years of age.*

Growth after birth involves rate variations at different ages in addition to changes in body proportion. The head makes up 25% of the standing height in infancy but only 13% at skeletal maturity. The height of the vertebral column will nearly triple from birth to adulthood. The measurement of sitting height provides an indirect reflection of spinal growth. During the first 5 years of life, sitting height and subischial length (i.e. the growth of the lower limbs) display similar growth velocity. From the age of 5 years to puberty,

sitting height accounts for one third of the gain, whereas subischial length accounts for two thirds. From puberty to maturity, the ratio is reversed. In an adult, the vertebral column makes up 60% of the sitting height, whereas the head represents 20% and the pelvis 20%. Strong correlation exists between armspan and standing height. (Dimeglio 2001)

Post-adolescent growth in the vertebral column progresses slowly, similar to most areas in the head and face. Vertebral bodies continue to grow between the ages of 20 and 30 years by apposition of bone to both ends. During this time, the total vertebral body height increases by 3-5 mm on average. After the age of 50 years, the vertebral height starts to decline. (reviewed by Harrison *et al.* 1977) On the other hand, intervertebral discs have been found to increase in height until 10 years of age and remain constant thereafter. (Stokes and Windisch L 2006).

2.3. Osteogenesis imperfecta

Osteogenesis imperfecta (OI), also known as brittle bone disease, is a heterogeneous group of inherited connective tissue disorders. The term OI was established in 1895 (Buday 1895), prior to which various names of the condition were used. Some of the most common alternatives were Ekman-Lobstein syndrome, Vrolik syndrome, and the “glass-bone disease”. The earliest known studies on OI date back to 1788, performed by the Swede Olof Jakob Ekman. He described the condition in his doctoral thesis delineating a family in which persons in three generations had a condition that he termed "osteomalacia congenita". (King and Bobechko 1971)

2.3.1. Molecular basis

The etiology and genetic basis of OI remains partly unknown today. OI is caused by abnormal formation of type I collagen; either as a quantitative or a structural defect. Deposition of new osteoid by single osteoblasts is reduced in OI. Subsequent increase in cell number is not sufficient to compensate for the deficiency, resulting in low bone mass

(Rauch *et al.* 2000). In addition to defects in collagen production, mutations causing OI also affect the overall composition and organization of bone matrix (Forlino *et al.* 2011). Altered noncollagenous protein content in matrix is believed to disrupt the mineralization dynamics leading to matrix hypermineralization. Hypermineralized bone is a common feature of quantitative and qualitative collagen defects, as well as of *CRTAP* defects in humans and mice. Thus, in addition to passive deposition of minerals, an active process is believed to take place, where matrix abnormality leads to a differentiation defect of osteoblasts or osteocytes that alters expression of noncollagenous proteins involved in the control of mineralization. (Cabral *et al.* 2011, Forlino *et al.* 2011)

The phenotypic severity of OI depends on which amino acid chain in a collagen molecule is affected. A defect in α1(I) chain tends to result in a more severe clinical outcome than α2(I) defects, resulting in two helix compositions. Complete absence of the α1(I) chain leads to a lethal phenotype (Forlino *et al.* 2011). Missense mutation of glycine in the triple-helical region of type I collagen generally leads to OI. Phenotypic severity is affected by the position of the glycine substitution in the helix and which amino acid the glycine is substituted to. (Roughley *et al.* 2003, Forlino *et al.* 2011) The general tendency is towards a more severe clinical outcome when the glycine substitution occurs in the α1(I) chain towards the carboxyl-terminal end of the molecule, as compared to milder phenotypes occurring by a similar mutation towards the amino-terminal end (phenotype gradient model) (Roughley *et al.* 2003). Bodian *et al.* (2008) have presented a more detailed model for lethality prediction. Approximately 80% of glycine substitutions in α2(l) and the majority of splice sites are non-lethal. To date, more than 1000 independent mutations causing OI are recognized, of which a majority are a glycine substitution in the triple helical region (Dalgleish 1997 and 1998; www.le.ac.uk/genetics/collagen/index.html, visited on March XV, 2012).

The majority of patients with OI have an autosomal dominant form caused by primary defects in type I collagen, which in addition to affecting ECM structure and mineralization, cause intracellular stress, disrupt interactions between collagen and non-collagenous proteins, and cause abnormal cell-cell and cell-matrix interactions. (Forlino *et al.* 2011) Type I OI is caused by frameshift or point mutations in most cases, or rarely by a

splice site mutation, creating a premature termination codon in *COL1A1* transcript. This produces unstable transcription products that are destroyed by nonsense-mediated decay, leading to reduced formation of α1 chain and haploinsufficiency of type I collagen (Willing *et al.* 1996, Bishop 2010). In types II to IV OI, 80% of cases are due to a glycine substitution and 20% are due to a splice site in either *COL1A1* or *COL1A2* genes (located on chromosome 17 and 7 respectively) leading to structural alteration of type I procollagen (Table 1). Substituting residues may disrupt non-covalent bonds causing local unwinding of the triple helix and excess post-translational modification of the collagen helical region. Mixture of normal and mutant α-chains results in matrix heterogeneity and contributes to phenotypic severity. (Roughley *et al.* 2003, Forlino *et al.* 2011)

Autosomal recessive forms collectively account for approximately 5% of the OI cases detected in Europe and North America (Venturi *et al.* 2012). They are usually caused by a null mutation leading to deficiency of proteins involved in collagen posttranslational modification, folding, or secretion. A mutation affecting the enzyme complex responsible for posttranslational hydroxylation of the position 3-proline residue of *COL1A1* is often present. Such genes are *CRTAP* (cartilage-associated protein), *LEPRE1* [leucine proline-enriched proteoglycan, also called leprecan or prolyl-3-hydroxylase-1 (P3H1)], and *PPIB* (cyclophilin B). (Marini *et al.* 2010, Forlino *et al*. 2011) Gene products of these three form a complex that modifies proline into hydroxyproline, a process that appears to be critical for the normal folding and assembly of collagen (Barnes *et al.* 2006, Baldridge *et al.* 2008). Recessive OI can also be caused by a defect in collagen chaperone genes such as *SERPINH1*, *FKBP10*, and *OSX/SP7*, that are essential for correct helical structure. (Van Dijk *et al.* 2009, Christiansen *et al.* 2010, Lapunzina *et al.* 2010, Kelley *et al.* 2011) Mutation in *SERPINF1*, encoding pigment epithelium-derived factor, leads to a mineralization defect with a distinctive histology (Homan *et al.* 2011). Mutation in *BMP1* has also been linked with autosomal recessive OI (Asharani *et al.* 2012, Martínez-Glez *et al.* 2012).

Patients with dominant types of OI, and recessive types VII and VIII, have high bone turnover with elevated osteoblast and osteoclast activity. Their observed bone tissue abnormalities include lower bone mass than in age-matched controls, lower cortical bone

thickness, and osteopenia (Jepsen *et al.* 1997, Kocher and Shapiro 1998, Boyde *et al.* 1999, Edouard *et al.* 2011, Forlino *et al.* 2011). In children, OI can cause clinical osteoporosis that differs from idiopathic juvenile osteoporosis by its hereditary nature. No deficiency in serum calcium and vitamin D metabolite levels is present in OI as in osteomalacia. (Kocher and Shapiro 1998, Gloriex 2008, Edouard *et al.* 2011) The observed fragility of bones in OI, is caused by high stiffness combined with low bone mass.

2.3.2. Clinical features

A population incidence of 1:5000-1:10 000 inhabitants has been reported worldwide for OI (Stoll *et al.* 1989, Andersen and Hauge 1989, Byers *et al.* 1991, Glorieux 2008). Reports on rare recessive mutations are often of specific populations or isolated families, which thus display a high local disease frequency (Barnes *et al.* 2006, Baldridge *et al.* 2008). Official calculations on the number of OI patients have not been conducted in Finland. The Finnish Skeletal Dysplasia Registry at Helsinki University Central Hospital, maintained on a research basis, includes more than 300 known OI cases. The Finnish disease heritage includes a variety of rare hereditary diseases that are overrepresented in Finland (Norio 2003). Greater incidence than average of recessive OI types has not been documented in Finland, however.

Even though genetic testing has become more common in recent years, diagnosis of OI is still most often based on typical clinical and radiological findings. Medical and family histories are cornerstones in arriving at the correct diagnosis. Distinguishing OI from non-accidental injury, such as child abuse, can be complex in some cases. (Paterson *et al.* 1993)

A Dual-Energy X-ray Absorptiometry (DXA) assessment of bone mineral density (BMD) may help to establish diagnosis of OI, which usually associates with low BMD. Typical radiological findings include vertebral fractures, long bone fractures and bowing, generalized decrease in density and thinning of bones, coarsening of the trabecular pattern, excessive intersutural Wormian bones, and certain characteristic features associated with

the recessive OI types. (Cremin *et al.* 1982, Hayes *et al.* 1999, Glass *et al.* 2004) Diagnostic methods can also include molecular analysis of type I collagen on cultured fibroblasts from a skin biopsy, or genomic sequence studies on DNA obtained from blood or saliva specimens, for example.

2.3.2.1. General symptoms

OI is associated with bone fragility, bone pain, short stature, and joint hypermobility (Glorieux 2008, Basel and Steiner 2009). Joint hypermobility in OI is believed to be related to the collagen defect. This view is supported by the overlap in symptoms between OI and other connective tissue disorders, such as Marfan and Ehlers-Danlos syndrome (Grahame 1999). Lax joints tend to be less stable, to sublux or dislocate, and are generally more susceptible to trauma.

Truncal height is reduced more in the severe forms of the disorder than in the mild forms, and the same pattern applies for the ratio between armspan and height; an increase is seen in children with severe disorder types (Lund *et al.* 1999). The cause of the short stature seen in OI is unclear. Collagen type II predominates in the growth plate cartilage of long bones, and thus linear growth could be expected to be normal in patients with OI. Nevertheless, morphological changes in the growth plate have been observed in OI (Sanguinetti *et al.* 1990). Compression fractures contribute to the reduced vertebral height, but the main cause of short stature is likely to be associated with the function of osteoblasts. Unresponsiveness of osteoblasts to normal growth factors or defective osteoblast/bone matrix feedback on the growth hormone and somatomedin axis have been suggested in the dominant OI types (Marini *et al.* 1993). The greater impact on growth of qualitative collagen defect than of quantitative defect could reflect their different consequences on the osteoblast differentiation (Shi *et al.* 1996, Lund *et al.* 1999). In the severe forms of OI height is already reduced at birth. Platyspondyly is a common finding in older patients, corresponding to the reduction of truncal height. Whether platyspondyly is caused by biomechanical (reduced bone mineral content), or by biosynthetic abnormalities (reduced growth potential of the spine), is yet to be determined. (Lund *et al.*

1999) The recessive types, with extreme short stature, are caused by deficiency of proteins that notably function in both cartilage and bone (Marini *et al.* 2010).

In severe forms of OI progressive deformity, barrel shaped chest, and scoliosis are commonly found. Patients are often in a hypermetabolic state with increased respiratory and heart rates. Recurrent pneumonia, right-sided heart failure, valvular insufficiency, and aortic root dilatation also are typical. Cardiovascular and respiratory system manifestations are considered secondary to skeletal changes, such as scoliosis and rib fractures. (Hortop *et al.* 1986, Singer *et al.* 2001, Forlino *et al.* 2011) Vertebral deformities are generally common in OI, with prevalence ranging from 39% to 100%. (Falvo *et al.* 1974, Norimatsu *et al.* 1982, Ishikawa *et al.* 1996)

Blue sclerae is a typical characteristic of certain OI types. Patients often display progressive hearing loss that usually begins in the early twenties or thirties and is twice as common in women as in men. Conductive hearing loss is caused by otosclerosis-like damage to the ossicles in the middle ear. Its prevalence in the Finnish adult OI population is 58%. No correlation has been found between hearing loss and collagen mutation type or mutated collagen gene (types I, III and IV OI examined) (Kuurila *et al.* 2000, Kuurila *et al.* 2002).

Typical clinical characteristics also include certain dental and occlusal features. A total of 10-50% of OI patients display dentin dysplasia that clinically, histologically, and radiologically resembles dentinogenesis imperfecta (Lukinmaa *et al.* 1987, Waltimo and Lukinmaa 1997). The presence of dentin dysplasia, as well as normal color of sclera, implies qualitative collagen defect. OI patients may exhibit hypodontia, delayed tooth eruption, impaction of teeth, and a high incidence of malocclusion. (Lukinmaa *et al.* 1987, O'Connell and Marini 1999, Malmgren and Norgren 2002, Waltimo-Sirén 2011).

2.3.2.2. Classification

At the beginning of the 20th century, OI was divided into two forms; congenita and tarda. The latter was considered a milder form, in which fractures occurred only after birth. In

the following years, the vast clinical variation of symptoms and the inherited nature of the disorder was recognised. As a result, Sillence and co-workers (1979a) presented a more comprehensive classification. The original Sillence classification that gained general acceptance is based on clinical and radiological characteristics and divides the disorder into types I through IV (Sillence *et al.* 1979a and 1979b). Sillence classification was later extended to include histologically, radiologically, and genetically distinct OI types (Table 1) (Roughley *et al.* 2003, Rauch and Glorieux 2004). The classification of OI is a subject of ongoing debate among researchers.

OI continues to be an essentially clinical diagnosis; clinical severity is the central parameter of the classification (Van Dijk *et al.* 2010). Classification of an individual patient is not always straightforward, however, because the clinical symptoms present a continuum. Therefore, a genotype-based classification has also been proposed (Forlino *et al.* 2011). Mutation-based classification appears more individual and might lead to an assumption of individual mutation-targeted treatment, whereas phenotype-based classification corresponds more closely with the current phenotype-guided treatment protocols. In general, the phenotype-genotype correlation in OI is somewhat limited (Rauch *et al.* 2010, Van Dijk *et al.* 2010).

Type I is the most common form of OI. It is clinically the mildest type with normal or nearly normal height, increased risk of fracture, but usually no deformity. Blue sclerae is a common finding. Type II OI is considered lethal perinatally due to respiratory problems. Type III OI is characterized by progressive bone deformity and short height. Fracture incidence without medication remains high throughout life. Dentine dysplasia is a frequent finding. Type IV OI is clinically the most diverse type. Total body height is often decreased from mean values for age. (Sillence and Rimoin 1978)

Types V-VII share phenotypic characteristics with types I-IV, in addition to each having some distinguishing traits. These OI types are associated with normal teeth and sclerae. Diagnosis is made on the basis of clinical and bone histological findings, and mutation analysis (Roughley *et al.* 2003). Type V OI is of unknown etiology. The distinguishing histology is lamellar organization of bone into irregular mesh-like appearance. Type V OI

is associated with hyperplastic callus formation at fracture sites and calcification of intraosseous membrane between the bones of the forearm. A dense band is seen on a radiograph adjacent to the growth plate of the long bones, and radial head dislocation is also typically seen. (Glorieux *et al.* 2000, Fassier *et al.* 2007, Cheung *et al.* 2008)

The defining feature of type VI OI is an abnormal histologic lamellation pattern with fish-scale like appearance of the bone lamellae and the presence of excessive osteoid. This OI type is moderate in severity with vertebral body involvement. No Wormian bones or rhizomelia are usually present. (Roughley *et al.* 2003, Bishop 2010, Homan *et al.* 2011) Type VII OI patients exhibit varying phenotypes from moderately deforming to lethal. Typical clinical findings are short humeri and femur. Type VIII OI is associated with extremely low BMD, severe growth deficiency, and bulbous metaphyses. Typical radiological findings in childhood are popcorn-like bone structures. Type IX OI patients display a phenotype varying from moderate to lethal. (Bishop 2010, Forlino *et al.* 2011) Type XI OI is also called Bruck syndrome 1. It has been reported in single Turkish and Mexican families (Alanay *et al.* 2010, Forlino *et al.* 2011). Other syndromes and disorders with overlap in phenotypic features with OI include Cole-Carpenter syndrome and Ehlers-Danlos syndrome.

Table 1 *Classification of OI.*

Type	Inheritance	Gene defect	Phenotype
I	AD	*COL1A1* or *COL1A2*	Non-deforming
II	AD	*COL1A1* or *COL1A2*	Perinatally lethal
III	AD	*COL1A1* or *COL1A2*	Severe deformity, rhizomelic dwarfism
IV	AD	*COL1A1* or *COL1A2*	Moderate deformity, slightly shortened height
V	AD	?	Moderate to severe deformity, hyperplastic callus, mineralised intraosseous memebrane.
VI	AR	*SERPINF1*	Moderate deformity, abnormal bone architecture
VII	AR	*CRTAP*	Severe to lethal osteochondrodysplasia and rhizomelic dwarvism
VIII	AR	*LEPRE1*	Severe to lethal osteochondrodysplasia
IX	AR	*PPIB*	Moderate to lethal deformity
X	AR	*SERPINH1*	Severe to lethal deformity
XI	AR	*FKBP10*	Progressive deformity

2.3.3. Treatment

As no cure for OI currently exists, treatment is multidisciplinary and aimed at preventing and controlling symptoms, as well as maintaining good life quality. This can be achieved by non-surgical management, such as physical therapy, bracing and splinting, surgical

management, such as intramedullary rods and spinal surgery, and/or medico-pharmacological management.

Medication treatment is either aimed at reducing bone resorption or enhancing bone formation. Currently bisphosphonates are virtually the drug of choice at least in the severe OI forms. They decrease bone turnover by inactivating osteoclasts and inducing their apoptosis thus increasing bone mass. Several observational studies have reported on the benefits of bisphosphonate treatment in patients with OI since 1998 (Åström and Söderhäll 1998, Glorieux *et al.* 1998, Phillipi *et al.* 2008, Vuorimies *et al.* 2011, Ward *et al.* 2011). Reported benefits include increased BMD, reduction in fracture rate and deformities of long bones, decreased bone pain, increase in the mean cortical width and vertebral size, and increased mobility (Plotkin *et al.* 2000, Roughly *et al.* 2003, Rauch *et al.* 2006, Bishop *et al.* 2010, Semler *et al. 2011*). More benefits are gained with severely affected patients than with the milder OI forms. The optimal dose and duration of bisphosphonate treatment remains to be determined. At present, maximum gain seems to be obtained after 2-4 years of treatment during growth (Rauch *et al.* 2006). Bisphosphonates are well tolerated. An acute phase reaction has been reported with first exposure. The long-term effects of accumulated bisphosphonates are, however, uncertain.

Attempts to aid bone formation with growth hormone and parathyroid hormone therapy have been unconvincing in patients with OI. Vitamin D and calcium intake needs to be monitored during growth and supplemented when necessary. Stem cell and enzyme replacement therapy might become an alternative treatment in the future. (Monti *et al.* 2010)

2.4. Basilar pathology

Basilar pathology refers to anomalies of the cranial base and/or craniocervical junction that cause neural compression and/or vascular compromise. In these conditions, brain stem compression can lead to neurological symptoms and other symptoms, such as hydrocephalus and macrocephaly, resulting from the prevention of cerebrospinal fluid

circulation (Charnas and Marini 1993, Sawin and Menezes 1997). Basilar pathology can be caused by dysplasia, malformation or deformation (Spranger *et al.* 1982).

In the literature, early reports on basilar pathology date back to 1790 (Ackermann 1790). Subsequently Chamberlain drew wider attention to these pathologic conditions with his work on Bantu Africans (1939) suggesting that in healthy individuals atlas and axis should lie caudal to the "base line" drawn from the dorsal margin of the hard palate to the posterior edge of the foramen magnum, also called opisthion. McGregor introduced a reference line modified from that of Chamberlain's in 1948, running from the upper surface of the posterior edge of the hard palate to the most caudal point of the occipital curve (McGregor 1948). His reasoning for the new reference line was that the lowest point on the occipital curve is easier to locate from a lateral skull radiograph than the opisthion point. Bull later confirmed the increased reliability, expressed as smaller variation in results, when McGregor's reference line was used instead of Chamberlain's. Bull also concluded that, when assessing basilar pathology in adults, age is an insignificant variable. Instead he found a gender difference in normal values (Bull *et al.* 1955).

Pathological conditions of the cranial base and craniocervical junction are divided into basilar invagination, basilar impression, and platybasia (Figure 6). Sillence proposed the nomenclature to describe these entities that can occur simultaneously or as isolated findings. (Sillence 1994, Kovero *et al.* 2006) In several studies the terms have been used synonymously making comparison of results difficult.

Diagnosis of basilar pathology can be made from a lateral skull radiograph, or from mid-line sagittal MR or CT image, because all but one reference line commonly used in the literature are situated at the midsagittal plane: Chamberlain's line (Chamberlain 1939), McRae's line (McRae 1953), McGregor's line (McGregor 1948), the anterior cranial base angle, DM distance (Kovero *et al.* 2006), Ranawat's line (Tassanawipas *et al.* 2005), Wackenheim's clivus baseline, the clivus-canal angle, Bull's angle, and Landzert's angle, (Smoker 1994). The atlanto-occipital joint axis angle is measured from an antero-posterior or coronal view of the skull (Smoker 1994). Cronin *et al.* (2009) demonstrated a similarity of results using the different imaging modalities with Chamberlain, McGregor, and

McRae's measures. MR image is ideal for the evaluation of soft tissues, neural structures, and ligaments. CT images, on the other hand, provide excellent images of the osseous structures. Traditional radiographs are recommended as an initial screening method of basilar pathology, and in epidemiologic studies (Janus *et al.* 2003, Kovero *et al.* 2006). In case of a pathological finding in a lateral skull radiograph, CT scan or MR imaging should be conducted (Menezes 2008b). The advantages of plain radiographs include low radiation dose compared to a CT scan, low cost, easy accessibility, and short exposure time, making it simple for the patient.

Basilar pathology can be symptomless or cause a spectrum of symptoms varying in impact and strength. Possible symptoms include motor myelopathy, sensory abnormalities, brain stem or lower cranial nerve dysfunction, vascular compromise, and basilar migraine. Motor myelopathy can be non-specific, manifesting as a lack of endurance or as paresis. Sensory abnormalities include posterior column dysfunction, hyperalgesia, and bladder dysfunction. Brain stem dysfunction can be expressed as nystagmus, apnea, ataxia, or dysmetria. Lower cranial nerve dysfunction presents often as dysphagia, soft palate paralysis, or trapezius muscle weakness. Symptoms of vascular compromise are syncope, vertigo, and intermittent paresis. Basilar migraine pain is documented in 25% of children with basilar invagination. (Hayes *et al.* 1999, Menezes 2008c, Smoker and Khanna 2008) The physical appearance of patients often includes a relatively short neck and restricted neck movement (Pearce 2007). Basilar pathology can also lead to respiratory arrest and sudden death (Sawin and Menezes 1997).

2.4.1. Pathology of craniocervical junction

Craniocervical junction anomalies are congenital osseous, developmental, or acquired abnormalities of the occipital bone, foramen magnum, or the first two cervical vertebrae. First, congenital structural abnormalities include anomalies of the atlas often involving the posterior arch, such as complete or partial aplasias, hypoplasia, and clefts, as well as anomalies of the axis. Odontoid hypoplasia is a rare condition ranging in severity from presence of rudimentary dens to complete aplasia (Menezes 2008b). Occipital bone anomalies include basiocciput hypoplasia, also called short clivus, occipital condyle

hypoplasia, and atlantooccipital segmentation failure, also termed occipitalization of the atlas. These anomalies are often associated with basilar impression and basilar invagination. Second, developmental anomalies can be secondary to systemic disorders that affect skeletal growth and development. Finally, primary acquired abnormality is the result of local bone destruction caused by, for example, a tumour or trauma. (Menezes 1998, Smoker and Khanna 2008)

2.4.1.1. Definitions and measurements

Pathology of the craniocervical junction can be divided into basilar invagination and basilar impression. Basilar invagination is defined as the protrusion of the uppermost vertebral structures into foramen magnum. Definition of basilar impression, in contrast, is a relative lowering of the cranial base with consequent positioning of the uppermost vertebral structures above the caudal border of the skull.

Basilar invagination is commonly evaluated by measuring the perpendicular distance of the tip of the odontoid process to McRae's reference line, also called the foramen magnum line, that extends from the anterior to the posterior edge of foramen magnum, that is from basion to opisthion (Figure 1, Study I) (McRae 1953). Basilar invagination is diagnosed when the tip of the odontoid extends above the McRae's line, thus the measure is positive (McRae 1953, Kovero *et al.* 2006).

Basilar impression can be evaluated by measuring the perpendicular distance of the tip of the odontoid process to Chamberlain's line or McGregor's line, or by measuring the D-M distance (Chamberlain 1939, McGregor 1948, Kovero *et al.* 2006). The reference line to measure D-M distance is a line parallel to the nasion-sella line passing through the lowest point of the occipital curve (Figure 1, Study I). This reference line was first introduced in Kovero et al. (2006) to obtain a reference level more stable in OI patients than the one involving the posterior nasal spine. Several values have been suggested as a threshold limit of pathology (McGregor 1948, Hayes *et al.* 1999). Kovero *et al.* (2006) revised the threshold limits of the Chamberlain measure by more than 10.5 mm, of the McGregor measure by more than 11.9 mm, and with a vertical D-M distance more than 9.4 mm as

diagnostic radiological criterion for basilar impression in the adult population. These values are 3 standard deviations above average of healthy controls. The group further suggested that patients, whose value exceeds that of healthy controls by 2 standard deviations, warrant further examination.

Other measurements, more rarely used in literature, to evaluate craniocervical junction pathology include the Ranawat measure, which is the perpendicular distance between the centre of the sclerotic ring of C2 and a line drawn along the antero-posterior axis of C1 (Tassanawipas *et al.* 2005), Bull's angle, which is the intersection of the hard palate and the plane of the atlas (Bull *et al.* 1955), and Wackenheim clival line, which is a line drawn along the superior surface of the clivus. Protrusion of the odontoid posterior to the line's projection is considered a pathological finding (Thiebaut *et al.* 1961).

2.4.2. Platybasia

2.4.2.1. Definitions and measurements

Platybasia is defined as the flattening of the skull base, and is often associated with craniocervical junction pathology (Pearce 2007). It is infrequently accompanied by a developmentally shortened clivus (Pang and Thompson 2011), however platybasia can be asymptomatic and therefore its clinical significance is disputed (McRae 1953).

Platybasia is commonly assessed by measuring the anterior cranial base angle, which is an angle between the nasion-sella and sella-basion lines. Nasion is defined as the most anterior ventral point of the suture between the frontal and nasal bones, whilst sella is the midpoint of the pituitary fossa. McGregor considered an angle above 148 degrees to be highly suggestive of platybasia in young adults (McGregor 1948), whilst Kovero *et al.* (2006) set the threshold limit at 146 degrees for adults.

Another, more rarely used, measure to evaluate platybasia is the NTB angle of Welcker that joins the nasion, tuberculum sellae, and basion (Pang and Thompson 2011).

2.4.3. Basilar pathology in OI

A wide range of craniofacial morphology is observed in OI, from nearly normal to severe dysmorphology (Waltimo-Sirén *et al.* 2005). Typical radiographic findings include a generalized decrease in density and thickness of calvarial bones. Delayed closure of fontanelles and sutures, and excessive Wormian bone formation might also be visible. (Hayes *et al.* 1999) Intersutural Wormian bones are most evident along the lambdoid suture, which connects the parietal and temporal bones with the occipital bone on the posterior aspect of the skull (Cremin *et al.* 1982, Glass *et al.* 2004). Extremely osteoporotic bone might in some cases make diagnosis of basilar pathology challenging from a plain radiograph (Janus *et al.* 2003).

The pathogenesis of craniocervical anomalies in OI remains obscure. Anomalies have been observed in OI types I, III, and IV, but not in the perinatally lethal type II (Sawin and Menezes 1997). A generally accepted hypothesis is that craniocervical junction anomalies are caused by skeletal dysplasia and force induced deformation. The weight of the brain compresses the cranial base, causing microfractures that result in relative upward migration of the craniocervical structures (Figure 6) (Frank *et al.* 1982, Sillence 1994). Findings reported by Sawin and Menezes (1997) support this hypothesis; they report a frequent intraoperative finding of thickened proliferative bone that resembles callus in the skull base.

In patients with OI, basilar impression has been noted to be a more frequent finding than basilar invagination (Hayes *et al.* 1999). Reported symptoms of craniocervical junction pathology in patients with OI include Chiari malformation (herniation of the cerebellum through the foramen magnum), hydrocephalus, and other consequences of compression of brain stem and related structures by bone or soft tissues. Symptoms can also result from indirect compromise of the blood supply. (Frank *et al.* 1982, Sawin and Menezes 1997, Charnas and Marini 1993, Smith *et al.* 2010) Sudden infant death has also been reported following respiratory or cardiac failure (McAllion and Paterson 1996, Menezes 1997). Basilar invagination, clinically exhibited as short neck, has been associated with the occurrence of sleep apnea in patients with OI (Li *et al.* 2002). In severe forms of the

disorder, macrocephalia has been described (Charnas and Marini 1993, Primorac *et al.* 2001).

The prevalence of basilar invagination or basilar impression has been studied in children and adults with types I, III, and IV OI (Table 2). The prevalence figures range from 0-100% in type I, 18-100% in type III, and 4-71% in type IV (Charnas and Marini 1993, Sillence 1994, Jensen and Lund 1997, Engelbert *et al.* 1998, Janus *et al.* 2003, Kuurila *et al.* 2003, Kovero *et al.* 2006). The medication status of the patients has not been reported in these articles. Studies on paediatric patients report the onset of craniocervical pathology from the age of 2 to 3 years onwards (Rush *et al.* 1989, Sillence 1993). In concordance with these studies, basilar pathology has been suspected to be progressive in patients with OI (Charnas and Marini 1993, Sillence 1994, Kovero *et al.* 2006).

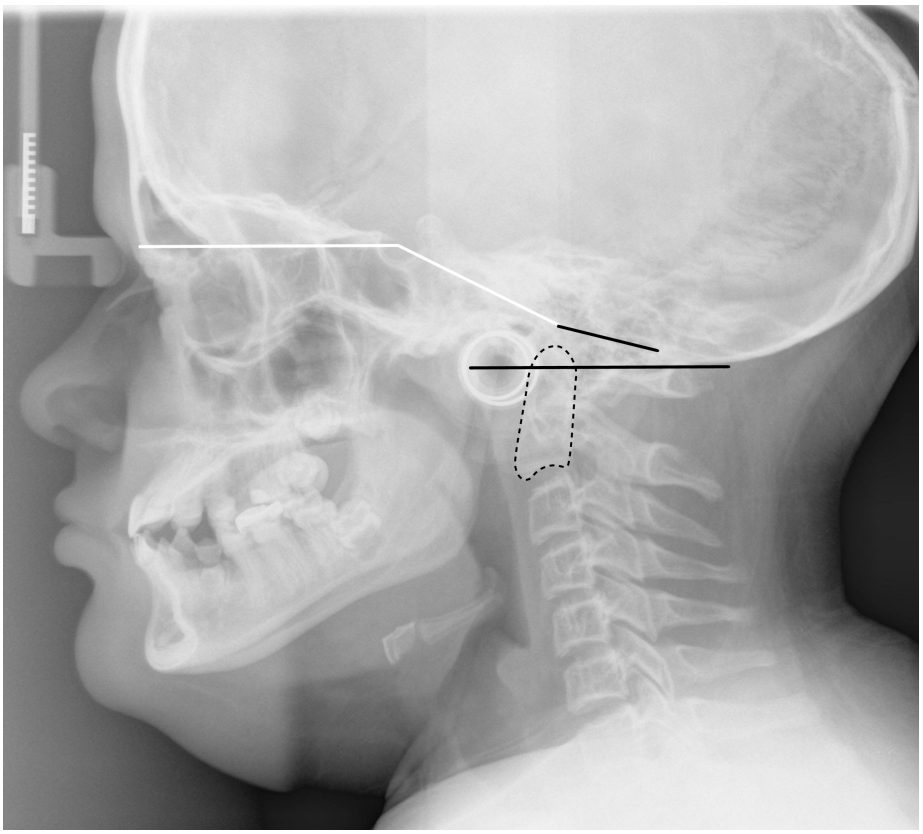


Figure 6 *Radiograph of a female aged 13.5 years with type III OI exhibiting basilar impression and platybasia in the absence of basilar invagination. McRae's line and D-M distance line are indicated in black, and the anterior cranial base angle in white.*

Table 2 *Prevalence of basilar pathology in different OI types*

Author	Age of patients (years)	Type I n/N(%)	Type III n/N(%)	Type IV n/N(%)	Criteria for abnormality
Charnas & Marini 1993	0.3-65	0/4(0)	6/33(18)	2/31(6)	McRae >0 McGregor >5mm
Sillence 1994	NA	(10-100)	(30)	(5-71)	Chamberlain >5mm McGregor >7mm
Jensen & Lund 1997	13-62	1/35(3)	4/4(100)	5/13(38)	Chamberlain >5mm McGregor >7mm
Engelbert *et al.* 1998	1-16	0/17(0)	7/16(44)	1/14(7)	McGregor (threshold value not reported)
Janus *et al.* 2003	0-18	0/85(0)	7/21(33)	1/24(4)	Chamberlain and McGregor (threshold values not reported)
Kuurila *et al.* 2003	19-69	3/21(14)	2/5*(40)	4/15(27)	McRae >0 Chamberlain >10mm
Kovero *et al.* 2006	16-69	4/29(14)	3/5(60)	7/19(37)	McRae >0 D-M distance >9.4mm

n = number of patients with pathological finding

N = total number of patients in the study

NA data not available

* one patient was either of type III or IV

2.4.4. Basilar pathology in other disorders

As stated earlier, primary basilar pathology can result from congenital structural anomalies. Atlantooccipital fusion, or occipitalization of the atlas, is the most common congenital abnormality of the upper cervical spine. The incidence is around 0.25% in the

general population (Menezes 2008c). Atlantooccipital fusion refers to partial or complete congenital fusion between the atlas and the base of the occiput resulting in an increased risk of atlantoaxial instability. The severity ranges from a complete bony fusion to a fibrous band. Atlantooccipital fusion is a result of segmentation failure between the fourth occipital sclerotome and the first spinal sclerotome, as in Klippel-Feil syndrome. (Menezes 1998, Gholve *et al.* 2007, Smoker and Khanna 2008)

The majority of axis anomalies are associated with os odontoideum, which is defined as a failed fusion between the tip of the odontoid and the body of C2. The base of the dens is often hypoplastic. (Smoker and Khanna 2008) While the exact etiology is unclear, os odontoideum is believed to result from a childhood trauma or a fusion failure of the base of odontoid. This anomaly is often asymptomatic, and thus only incidental radiographic findings of a separate ossicle moving with the clivus or anterior arch of the atlas are seen. The ossicle may, in fact, fuse with the clivus. The condition may also be revealed with the onset of symptoms following minor trauma. Os odontoideum can cause atlantoaxial instability and compression of the spinal cord. (Menezes 1999) Increased incidence of os odontoideum has been found in Down syndrome, Morquio syndrome, spondyloepiphyseal dysplasia, Klippel-Feil syndrome, and Larsen syndrome (Smoker and Khanna 2008).

Secondary developmental basilar anomalies, as in OI, have also been described in achondroplasia, spondyloepiphyseal dysplasia, Morquio syndrome, chondro-osteodystrophy, cleidocranial dysostosis, Goldenhar syndrome, Larsen syndrome, Weaver syndrome, Paget's disase, rheumatoid arthritis, rickets, hyperparathyroidism, and osteoporosis (Charnas and Marini 1993, Menezes 2005, Menezes 2008c). An increased incidence of atlantoaxial dislocation, possibly leading to basilar invagination, in occasional patients with Down syndrome, is due to ligamentous laxity (Uno *et al.* 1996). Similar occipitoatlantoaxial instability and increased incidence of basilar invagination are seen in Ehlers-Danlos syndrome and in some mucopolysaccharidoses, such as Morquio syndrome. In achondroplasia, on the other hand, the aetiology of craniocervical anomalies lies within underdevelopment of the skull base. (Milhorat *et al.* 2007, Smoker and Khanna 2008)

2.4.5. Treatment

Treatment of craniocervical junction pathology involves either non-operative approaches with skeletal halo traction, or operative approaches, with or without preoperative halo traction. The primary goal is to relieve compression at the craniocervical junction. When operative treatment is needed, generally either posterior decompression of the foramen magnum or anterior transoral-pharyngeal decompression is conducted, followed by occipitocervical fusion. Good long-term outcomes with sustained benefit in 60% of patients have been achieved with such decompression surgery and dorsal stabilization. (Kurimoto *et al.* 1991, Ibrahim and Crockard 2007, Menezes 2008d) Annual clinical examination, including neurological, and radiological follow-up, are recommended for surgically treated patients to monitor for possible recurrence of pathology (Sawin and Menezes 1997).

3. Aims of the study

The aim of this study was to analyse the morphology and developmental changes in the cranial base and craniocervical junction in an unselected control population and in patients with OI.

The following specific aims were set for the present study:

1 To analyze the reliability of identification of anatomic landmarks on lateral skull radiographs based on which craniocervical junction dimensions can be measured (I).

2 To explore whether any of the commonly used measurements of cranial base or craniocervical junction is less sensitive to errors than the others (I).

3 To study changes in the vertical dimensions of the craniocervical junction, and in the flexion of the anterior skull base in healthy individuals during growth (II).

4 To define diagnostic criteria for basilar anomalies in growing individuals (II).

5 To analyze the prevalence and clinical and genetic correlates of craniocervical junction anomalies in growing OI patients (III, IV).

6 To explore the natural course of development of basilar anomalies in growing OI patients (IV).

7 To establish guidelines for radiologic screening and follow-up of craniocervical junction area in patients with OI (IV).

4. Materials and methods

4.1. Diagnostic images

The study was based on lateral images of the skull. Lateral skull radiographs, MR, and CT images of the midsagittal plane were used. Radiographs had been taken with the central X-ray passing through the porion-porion axis and head position fixed with ear rods in cephalostat whenever possible.

4.1.1. Normal population cohorts

The subjects were a sub-sample of 248 individuals participating in a Longitudinal Growth Study, conducted on an unselected sample of healthy ethnic Finns (Caucasian Europeans) between 1967 and 1993 at the Institute of Dentistry, University of Helsinki, Finland. Subjects were randomly selected from those with a longitudinal series of at least five lateral skull radiographs obtained during growth (Table 3). Cut-off age was set at 25 years, up to which vertebral body growth has been reported to normally occur (reviewed by Harrison *et al.* 1977). The changes in craniocervical junction dimensions during growth were assessed for a mean observation period of 16.0 years (range 10.1 to 20.8 years).

For study IV, images of additional 15 healthy children, aged 0-3 years, were analysed (Table 3 and Figure 7). The children had been examined at the Children's Hospital, Helsinki University Central Hospital, for suspected accidental skull fractures possibly affecting calvarial or facial, but not skull base structures.

In study II the radiographs were grouped according to gender and age for analysis. In studies III and IV the healthy subjects were divided into two-year age groups that were age-matched with the patient cohort.

Table 3. *Number of subjects and images comprising the material of this thesis*

Publication	Controls		OI patients		Total number of images	Age range (years)
	A	B	C	D		
I	23				23	4.5-24.9
II	53				308	3.4-25.7
III	191		187		440	3.4-59
IV	53	15		76	474	0-39.1

A Helsinki Longitudinal Growth Study, Finland

B Young children, Helsinki University Central Hospital, Finland

C Shriners Hospital for Children, Canada

D Helsinki University Central Hospital, Finland

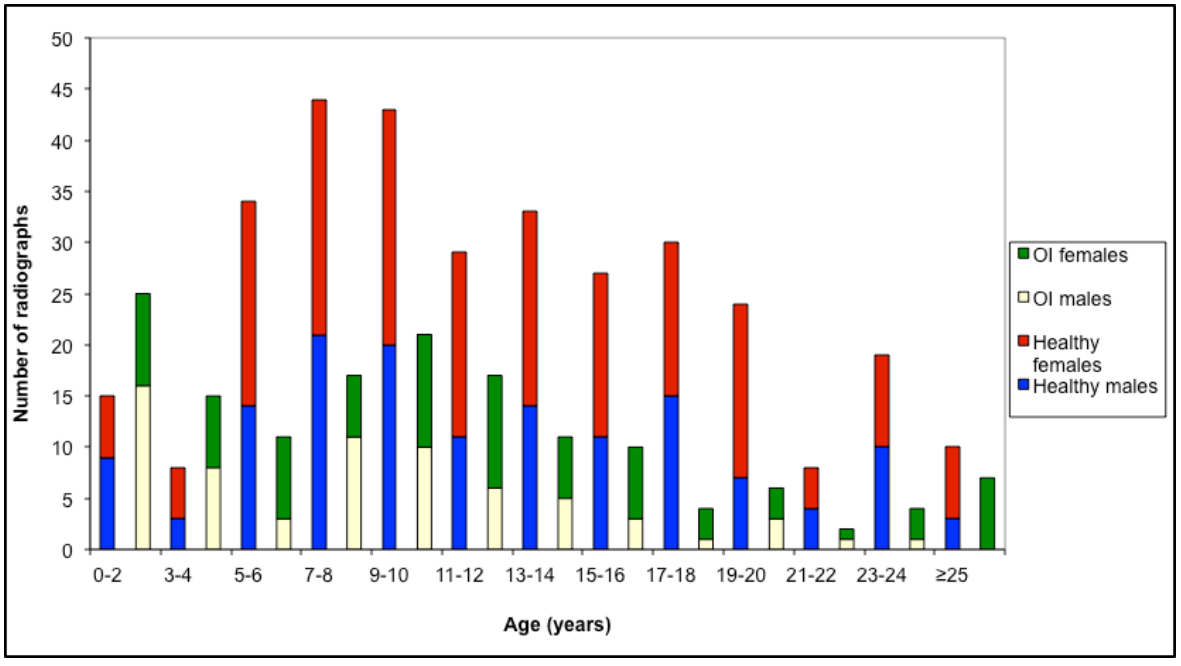


Figure 7 *Age and gender distribution of control and patient cohorts (IV).*

4.1.2. OI patient cohorts

Diagnosis of OI in all of the patients included in studies III and IV was based on clinical features, appropriate family history and/or identification of a mutation in the genes encoding type I collagen. In study III, the patient population comprised individuals with a clinical diagnosis of OI, who were examined at the Shriners Hospital for Children in Montreal, Canada. Of the 187 patients, 96 were female and 91 were male. When more than one skull radiograph of a single patient was available, the most recent one was used for the cross-sectional analysis. For the 62 patients (33%) with several available radiographs, the first and most recent ones were used for longitudinal analysis.

In study IV, all clinically diagnosed OI patients were included, examined at the Institute of Dentistry, University of Helsinki and/or Children's Hospital, Helsinki University Central Hospital, of whom an initial lateral skull radiograph or MR image had been obtained before the age of 25 years. Altogether 76 patients, 39 females and 37 males, from 69 families, met the inclusion criteria. Longitudinal data were available from 31 patients (41%). The first available image of each patient was used for cross-sectional analysis. In total, 143 radiographs and 7 MR images were analyzed. Table 4 displays the distribution of the OI types as well as selected clinical characteristics of the patients.

Table 4. *OI type and clinical characteristics of the patient cohorts in studies III and IV.*

OI type	I		III		IV		V*	VI*	VII*
Study	III	IV	III	IV	III	IV	III	III	III
N	88	47	30	13	51	16	11	4	3
Height (Z-score)	-1.2	-1.2	-6.7	-6.3	-3.5	-4.0	-2.6	-4.4	-2.1
aBMD (Z-score)	-2.3	-	-3.3	-	-2.6	-	-2.0	-1.9	-2.1
Dentin dysplasia (%)	17	-	87	-	51	-	0	0	0
Blue sclerae (%)	82	-	80	-	57	-	18	0	0
Wormian bones (%)	36	-	100	-	82	-	73	0	67
Bisph tx (%)	46	5.4	93	30.8	96	50	82	100	100
Scoliosis (%)	-	3.3	-	69.2	-	53.8	-	-	-
Head circumference (Z-score)	-	-0.2	-	-0.1	-	0.8	-	-	-

* No patients of this type in study IV

4.2. Radiographic measurements

Magnification at the midsagittal plane was calculated for each image according to the distance of the patient from the film and X-ray tube, then linear measurement values were corrected to natural size. When radiographic magnification was unknown, only angular measures were used, in addition to McRae's linear measurement as positive or negative.

Two examiners together identified 7 cephalometric landmark points on all of the images (Figure 8). Measurements from traditional radiographs were obtained with a hand ruler to

the closest 0.5mm distance or 0.5 degrees (°) angle (I, II, IV). Digital radiographs and MR images were analysed using a specially programmed algorithm in Viewbox software (version 3, dHal, Kifissia, Greece) (IV). In study III, the measurements were made to the closest 1mm or 1° from the average result of two independent observers.

In studies I-IV, the nasion (N), sella (S), and basion (Ba) points were located to measure the anterior cranial base angle. In studies I-III, the other points were traced to measure the perpendicular distance from the odontoid process (D) to four previously described reference lines;

1. McRae's line (Ba-Op)
2. Chamberlain's line (PNS-Op)
3. Modified McGregor's line (from posterior edge of the hard palate to the lowest point on occipital curve, PNS-M)
4. D-M distance

In study IV, the distance of the dens from the reference line of McRae, D-M distance, and the novel D-M angle were measured. D-M angle was defined as the angle between a line running through the lowest point of the occipital curve parallel to the sella-nasion line (as for D-M distance), and a line drawn from tip of the dens to the lowest point of occipital curve (Figure 8.). The linear measurements were considered positive when the dens point lay above the reference line, and the D-M angle when the line connecting dens and the lowest point on occipital curve was situated above the line running through the lowest point of occipital curve parallel to the sella-nasion line.

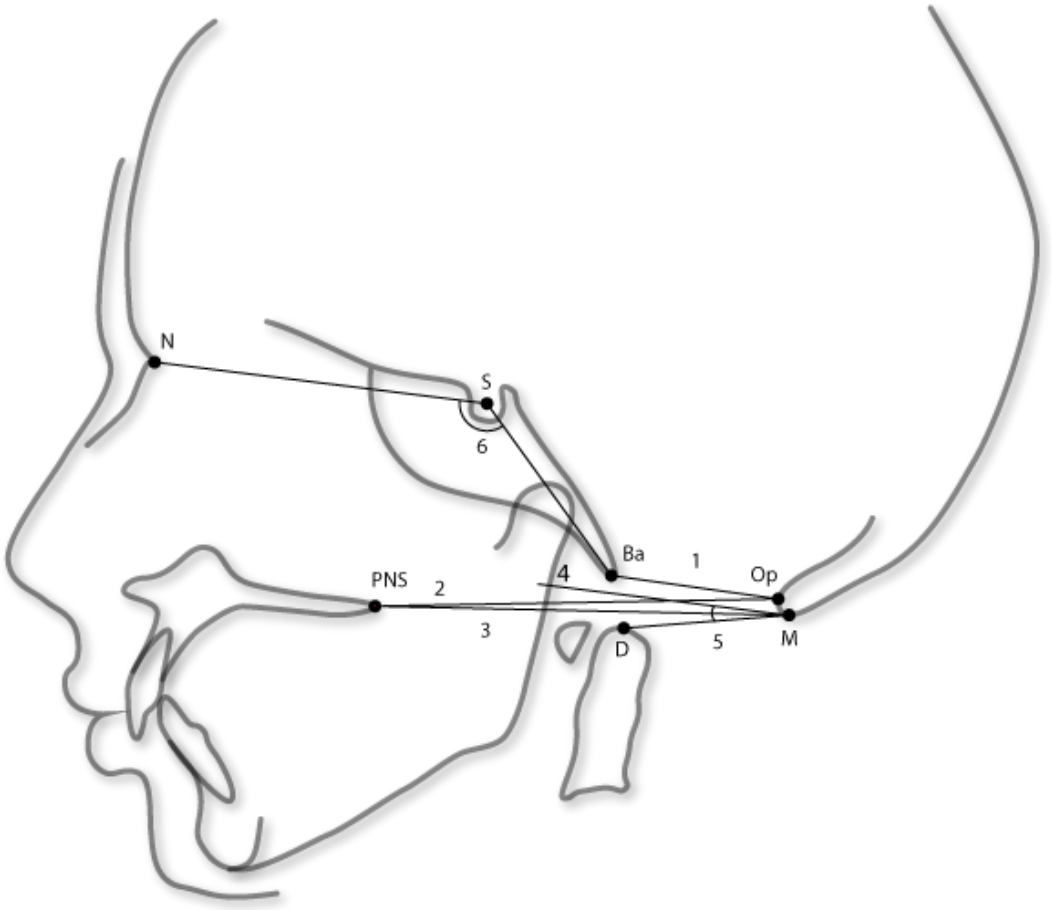


Figure 8 *Cephalometric landmarks, reference lines, and angular measurements used. Landmarks in alphabetical order were Ba (basion), D (dens), M-point, N (nasion), Op (opisthion), PNS (posterior nasal spine), S (sella turcica). Numbered reference lines and angular measurements were; 1.McRae's line 2. Chamberlain's line 3. McGregor's line 4. D-M distance 5. D-M angle 6. Anterior cranial base angle.*

The following definitions and diagnostic criteria were used:

- Basilar invagination was diagnosed when McRae measure was at or above 0.
- Basilar impression was diagnosed when Chamberlain measure, McGregor measure, or D-M distance were more than 3 SD above the average of age-matched healthy controls (III). In study IV, the criteria for screening patients for further evaluation with a MR or CT scan was lowered to a D-M distance or D-M angle more than 2.5 SD above the average of age-matched healthy controls.

- Platybasia was diagnosed when the anterior cranial base angle was more than 3 SD above control average in study III, and more than 2.5 SD in study IV, above the average of healthy controls.
- Patients who received at least one of these diagnoses, were considered to have a cranial base anomaly.

4.2.1. Intra- and interexaminer error

Two examiners analysed 20 lateral skull radiographs, and 11 examiners 3 radiographs independently, to assess the intra- and interexaminer error in landmark identification (I). A sensitivity analysis was conducted, with the results of the most experienced examiner as a reference, to test the impact of the difference in the location of a single landmark on the measurements. This was done to simulate the clinical situation where an examiner, previously unfamiliar with the method, starts using it. Another purpose was to compare the sensitivity of the different measurements to errors.

4.3. Other measurements

Height, weight, and head circumference measurements were converted to age- and sex-specific Z-scores (III, IV), as well as lumbar spine (L_1-L_4) areal bone density (LS aBMD), as determined by DXA in the posteroanterior direction (III).

4.4. Statistical methods

Primary statistical tools were mean, standard deviation (SD), and range. Nonparametric statistics were applied when the data were not normally distributed. To test the reliability of cephalometric landmark identification, based on which the craniocervical junction dimensions are measured, the reproducibility of these landmark points was analysed using the Dahlberg's formula on repeated measurements (I). Dahlberg's formula combines systematic and random components of the error, and includes the assumption that the two determinations are made independently of each other (Dahlberg 1940).

Spearman's Rank Correlation test was used to calculate correlations between the measurements in the control cohort. One-way analysis of variance (ANOVA) was performed for each measurement to test whether the mean values of the age groups differed significantly. Mann-Whitney test was used to test differences between the measurements of males and females. P values less than 0.05 were considered statistically significant. (II)

Pearson's correlation coefficient was used to evaluate the correlation between D-M angle and D-M distance (IV).

Logistic regression analysis was used to evaluate the relationship between patient characteristics and the presence of cranial base abnormalities (III, IV). Potential predictor variables were age, gender, and height Z-score (III, IV), as well as OI type (type I, III or IV), intravenous bisphosphonate treatment history, weight, presence of dentin dysplasia (dentinogenesis imperfecta) or blue sclerae, and type of collagen type I gene mutation (III). Bisphosphonate treatment status at the time of imaging and craniocervical junction pathology were included as dichotomous variables (yes/no).

Calculations were performed using PASW Statistics software version 17.0 for Windows (SPSS, Inc., Chicago, IL, USA) and R language (version 2.13.0, R Development Core Team, R: A Language and Environment for Statistical Computing, Vienna, Austria, 2008).

4.5. Ethical considerations

Informed consent was obtained from all of the participants, or their legal guardians, for the Helsinki Longitudinal Growth Study. Images of patients with OI had been originally taken for diagnostic and/or treatment purposes and were thus justified. The ethics committee of the Institute of Dentistry, University of Helsinki, the Joint ethical committee of Helsinki University Central Hospital, and Shriners Hospital Institutional Review Board have approved these studies.

5. Results

5.1. Validation of methods

The distribution of landmarks in this study, determined independently by several examiners, was not random but systematic, following the anatomic form of the identified structure. The magnitude of difference in the landmark identification was dependent on the landmark in question. Inter- and intraexaminer errors were of a similar magnitude, although intraexaminer error declined in the repeated landmark identification. The extent to which variation in landmark identification affected the measurement results differed from landmark to landmark. Sensitivity analysis revealed that variation in the location of a landmark affected the linear measurement values on average less than the actual shift. In contrast, age of the subject did not affect the magnitude of random error. (I)

Of the linear measurements, the McGregor measure exhibited the smallest inter-examiner variation (SD on average 1.1 mm), and was least sensitive to errors in landmark positioning. McRae's measure, in contrast, displayed the largest interexaminer variation, and was the most sensitive to changes in landmark positioning. Notably, however, the basilar invagination diagnosis, based on McRae's measure, simply requires that dens vertically exceeds McRae's line, that is, the measure value is either positive or negative. Importantly, despite the inter-examiner variation, none of the consecutive results of the linear measurements or the anterior cranial base angle would have led to a false positive clinical diagnosis. (I)

In study III, D-M measure was confirmed to be a more sensitive indicator of basilar impression in patients with OI than Chamberlain or McGregor measures.

In study IV, a new angular measure, D-M angle, was constructed to overcome the need to correct the radiographic magnification. To obtain reference values for the novel D-M angle measure, the angle was calculated on 290 radiographs of a longitudinal series in a subgroup of 65 subjects (aged 0-26) from the control cohort. Correlation analysis

indicated a statistically significant positive relationship between D-M angle and D-M distance (r=0.980, 95% CI: 0.967-0.990; P<0.01, n=290), which suggests that D-M angle may be applicable as a measure of basilar impression.

5.2. Basilar structures in normal population

In young children, the tip of the odontoid process is situated relatively more caudally in relation to the skull base structures and reaches a level similar to that of adults by 7 years of age in both males and females. This age-related change was most evident with the McRae measure and least conspicuous in D-M distance. Therefore, it can be concluded that growth-related changes in the craniocervical junction have a tendency to bring the odontoid process and skull base closer to each other. Individual variation is notable, both in the distance of dens from the reference lines and in the direction of growth-related changes. In some subjects, the measures increased during growth, while in others they decreased, or periods of increase and decrease alternated. No specific age at which these changes occurred could be determined. This individual variation was camouflaged in the cross-sectional analysis. (II) The D-M distance increased in 43% of subjects, decreased in 55%, and remained constant in 2% of subjects with age (II). A decrease was evident in the anterior skull base angle during growth before the age of three years (II, III, IV). Table 5 displays the age specific measurement results for selected parameters.

Table 5. *Normal values for selected cranial base measures in different age groups. The mean, minimum, and maximum values are given for each parameter.*

Age group (years)		0-2	3-4	5-6	7-8	>9
McRae measure (mm)	Mean	NA	-5.1	-7.2	-5.1	-4.5
	Min	NA	-7.6	-13	-9.4	-10
	Max		-3.3	-2	-1	-1
D-M angle (degrees)	Mean	-6.9	0.7	-7	-2.4	-3.4
	Min	-23	-5	-23	-14	-24
	Max	3	8	7	9	11
Anterior cranial base angle (degrees)	Mean	136	130	131	130	129
	Min	131	116	116	123	121
	Max	147	136	139	140	139

NA data not available

The McRae measure was negative in all controls at all ages, indicating that the odontoid process was situated below foramen magnum level (II, III, IV). Similarly, none of the control subjects exceeded the clinical screening limit (+2.5 SD from the average) of platybasia or basilar impression (II). The age group means for linear measurements were similar in females and males, with only a few exceptions (II, III). In subsequent studies, the results of both sexes were pooled to ensure an adequate sample size for each age group. Based on these figures for normal development we constructed diagnostic age-dependent criteria for basilar impression and platybasia (Table 6).

Table 6. *Threshold values of anomaly. D-M distance, D-M angle, and anterior cranial base angle threshold values set at +2.5 SD from the normal average of each age group.*

Age-group (years)	0-2	3-4	5-6	7-8	≥9
D-M distance (mm)	NA	8.8	7.1	6.9	7.4
D-M angle (degrees)	10.8	14.3	13.0	11.7	12.4
Anterior cranial base angle (degrees)	146	146	143	141	143

NA data not available

5.3. Basilar structures in OI

5.3.1. Cross-sectional analysis

Craniocervical anomaly was found in all studied OI types. In study III, 41 patients (22%) had at least one cranial base anomaly (diagnostic level set at +3 SD). Isolated basilar invagination was found in 4 patients, isolated basilar impression in 7 patients, and isolated platybasia in 25. A combination of two of these conditions was evident in 4 patients (2.1%) and one patient with type III OI was diagnosed with all three.

In study IV, the incidence was 37% (28 patients) for an abnormal finding, exceeding the +2.5 SD limit, in one of the three cranial base measures. In the cohort, 12 patients were diagnosed only with platybasia, 1 with isolated basilar invagination and 1 with isolated basilar impression. Of those with an abnormal finding, 44% displayed more than one type

of anomaly. Six patients, of whom one had type I, one type IV, and four type III OI, displayed all three pathological conditions.

In study III, the analyses of the association between clinical characteristics and skull base anomaly were limited to a group of 169 patients with OI types I, III and IV, as the other OI types were too rare for detailed statistical evaluation. The presence of dentin dysplasia and Wormian bones were positively associated with occurrence of cranial base anomalies, whereas higher Z-scores of height, weight and LS aBMD as well as a diagnosis of OI type I were associated with lower odds for a cranial base abnormality. Age, gender, scleral hue, and a history of bisphosphonate treatment were not significantly related to the presence of anomalies (Table 4 in study III). The final regression model included only height Z-score as a significant independent determinant of cranial base anomalies (OR 0.53, 95% CI: 0.43 - 0.66; P<0.001). In the group of patients with a height Z-score below -3, the prevalence of anomalies was similar to patients who had received pamidronate in the first year of life (8 of 17 patients corresponding a prevalence of 47%) and to patients who had received bisphosphonates at a later age or not at all (21 of 44 patients, prevalence 48%; P=0.96 by chi square test), indicating lack of evidence for a suspected protective role of bisphosphonates in the development of basilar pathology (III). The regression analysis in study IV gave similar results, indicating a higher risk of having any of the pathological condition with a lower height Z-score (OR 0.64, 95% CI: 0.45-0.85; P<0.004, for one SD unit), independent of age or gender.

In study III, DNA sequence analysis was performed on 149 patients of whom 140 were positive for a mutation in *COL1A1* or *COL1A2*. Among them, cranial base anomalies were present in 6% of those with haploinsufficiency (frameshift or nonsense) mutations, in 43% of those with helical glycine substitutions in *COL1A1*, in 32% of those with helical glycine substitutions in *COL1A2*, in 17% of those with splice-site mutations affecting either *COL1A1* or *COL1A2*, in two of the five patients with C-propeptide mutations, and in one of the five patients with in-frame deletions. The type of collagen type I mutation was not independently associated with the prevalence of anomalies. (III)

The most prevalent diagnosis was platybasia (III, IV). In study III, platybasia was found in 16% of patients, whereas basilar impression and basilar invagination were diagnosed in 6% and 4% of patients, respectively. In study IV platybasia was present in 28.4% of patients, basilar impression in 14.9%, and basilar invagination in 13.2% of the patient cohort. Most of the Z-score values for cranial base measures were positive (above zero), indicating a relatively high position of the uppermost vertebral structures or flat cranial base (IV). Figures 4A,B, in study IV, display the cranial base measurement Z-scores of the cross-sectional patient analysis. Overall, in most measurements and age groups, the score was highest in type III OI, followed by type IV,and then type I, reflecting the general severity of the disorder.

5.3.2. Longitudinal analysis

In study III, cranial base anomaly was detected in nine patients (15%), as evaluated from the first available image. At the time of the last imaging, none of the 4 patients previously diagnosed with basilar impression fulfilled the criteria any longer. In contrast, platybasia persisted in all five patients originally diagnosed with it, and an additional seven patients had developed platybasia. Six patients had newly acquired basilar impression. Basilar invagination was not observed in the longitudinal patient group at either time point.

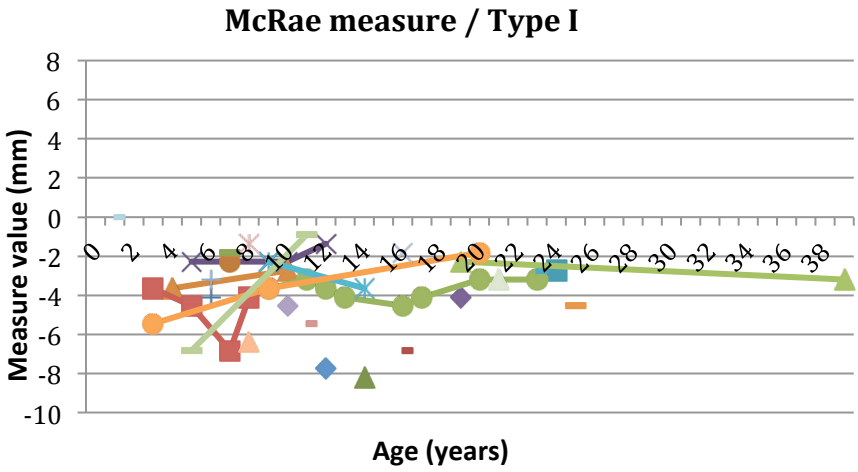
Likewise, in study IV we observed no statistically significant progression of craniocervical junction pathology with age. Longitudinal series were available for 31 patients of whom 25 had not been treated with bisphosphonates and were included in the final analysis. The beginning of series ranged from birth to 25 years. A normal McRae’s measure in the beginning of follow-up changed into a pathological one in two of the five type III OI patients and in all three of the type IV OI patients later fulfilling the criterion for basilar invagination. Similarly, basilar impression developed during the follow-up in two of the four type III OI patients and two of the three type IV patients. Platybasia developed during follow-up in one of the eight type III patients, and in the only type IV patient diagnosed with it.

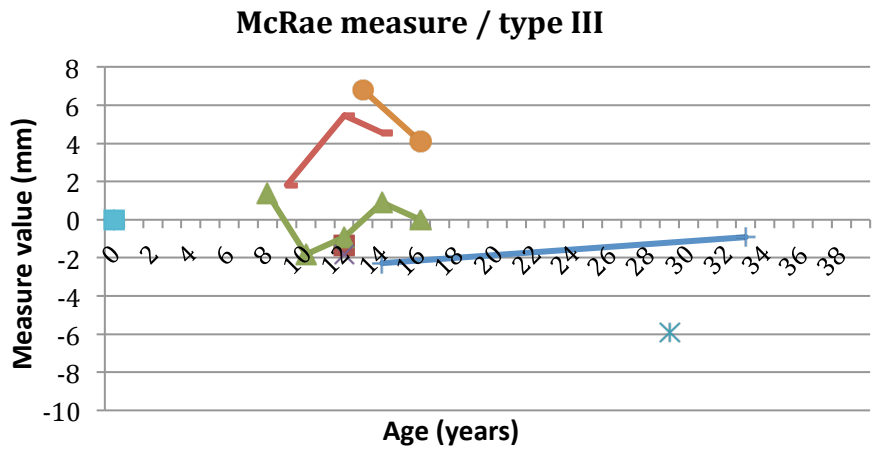
Once detected, basilar invagination persisted throughout the follow-up in all type IV patients, whereas in two type III patients, McRae's measure returned to normal at older age. Basilar impression persisted in the follow-up, with the exception of one type I patient, in whom the D-M angle measure returned to normal. Onset of basilar invagination and basilar impression was seen at all ages from 2 years onwards. Platybasia persisted in all the patients diagnosed with it (Figure 9). Platybasia was present from birth. Individual variation in measurement results was notable. (IV)

In study IV, the number of patients treated with bisphosphonates (n=14) was too small for detailed statistical evaluation. Visual exploration of the developmental curves of the cranial base measurements revealed, however, no difference between patients treated and not treated with bisphosphonates. Analysis of individual patients revealed that basilar pathology emerged even after treatment with bisphosphonates. One or more radiographs were available for eight patients after the onset of medication. For five of these patients, at least one image had been obtained before the bisphosphonate treatment. Platybasia was first detected in four patients (one type I, two type III, and one type IV), who had received intravenous pamidronic acid infusion. In two of these patients platybasia was not present before the treatment. Treatment time ranged from 1 to 3 years with a dose of 0.5-1.0 mg/kg per day, during 3 days, at 3 to 4 month intervals. One patient with type IV OI had initially normal craniocervical junction measure values, and developed basilar impression after 4 treatment episodes with zoledronic acid infusion. Platybasia improved in one type IV patient previously diagnosed with it, who had received intravenous pamidronic acid for 1.5 years at 3-month intervals, and later 3 treatment episodes with zoledronic acid infusion. Similarly, basilar impression could no longer be diagnosed, while platybasia persisted in one type IV patient, who had received 6 treatment episodes with zoledronic acid. Zoledronic acid was administred with a dose of 0.05 mg/kg as a single infusion every 6 months. One type IV patient acquired basilar invagination despite the treatment with pamidronic and zoledronic acid infusion for 3 years. The craniocervical status of this patient prior to medication was unknown. Despite the small sample of patients, similar tendencies in response to bisphosphonate treatment can be expected to appear universally in patients with OI.

A delay in the occurrence of symptoms following a radiological finding of cranial base pathology was observed in the study IV cohort. In the Finnish patient cohort, those with radiologically defined pathology were mostly free of clinical neurologic symptoms. One 18 –year-old female with type III OI and both basilar invagination and impression suffered from numbness in the upper extremities. One 20 –year-old male with type III OI, and all three types of cranial base anomalies, reported basilar headache induced by coughing. He died shortly after of pneumonia. Another male, with type III OI, and all three types of anomalies, underwent decompression surgery and posterior stabilisation at the age of 8 due to Chiari I malformation with a sole clinical symptom of muscle weakness in lower limbs first detected a year prior to surgery. He was the only one receiving treatment for the cranial base pathology (Figure 10). On detection of cranial base pathology, a MR scan and neurological examination were recommended for all patients.

Figure 9 *Cranial base measures (in millimetres or degrees) of patients not treated with bisphosphonates, according to OI type. Results include longitudinal follow-up and isolated findings with known radiographic magnification (IV).*




McRae measure / type III
Measure value (mm)
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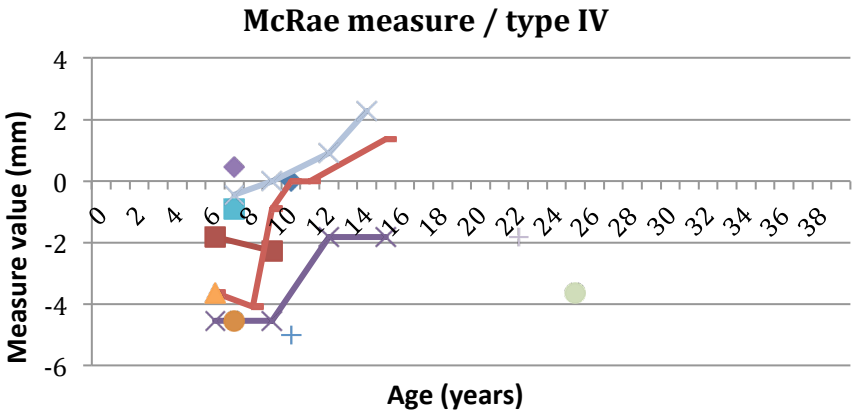
6
4
2
0
-2
-4
-6
-8
-10
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Age (years)


McRae measure / type IV
Measure value (mm)
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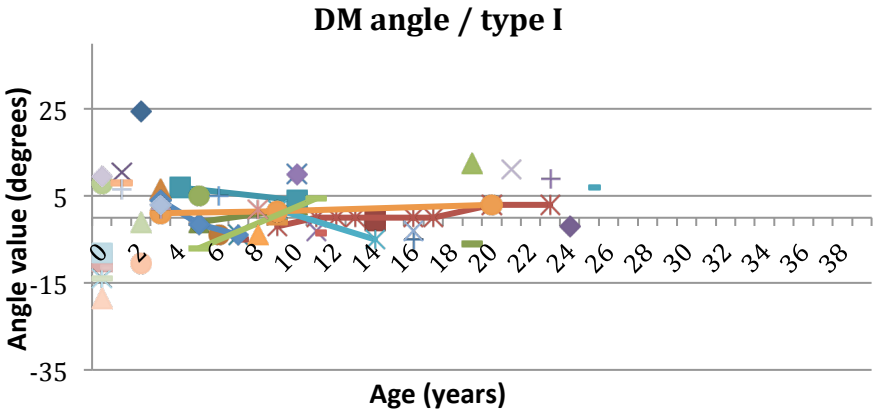
2
0
-2
-4
-6
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Age (years)


DM angle / type I
Angle value (degrees)
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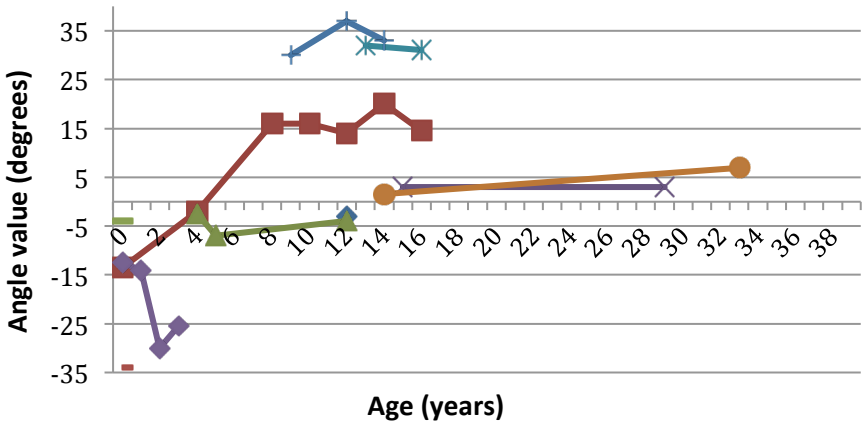
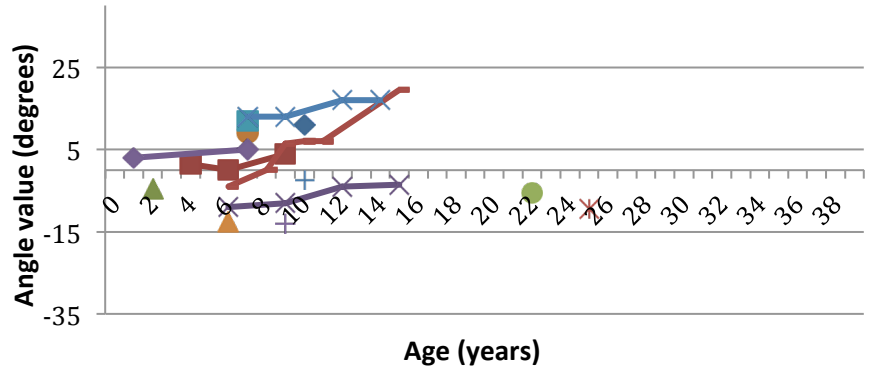
5
-15
-35
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Age (years)

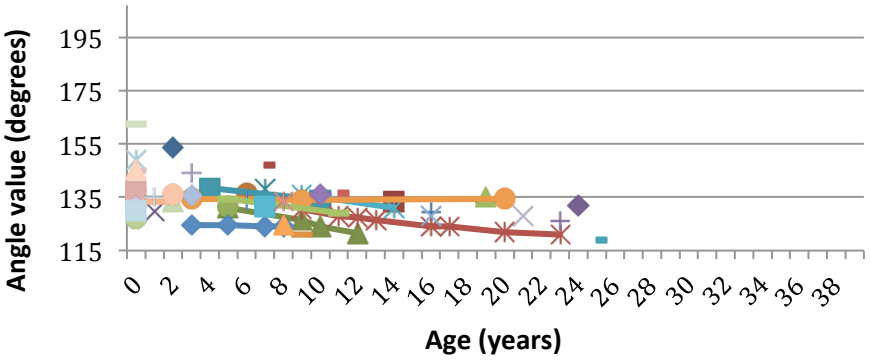
DM angle / type III

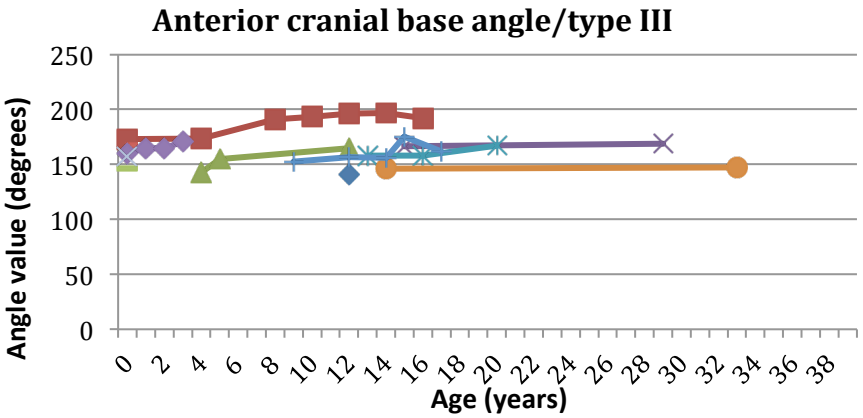


DM angle / type IV



Anterior cranial base angle /type I




Anterior cranial base angle/type III
Angle value (degrees)
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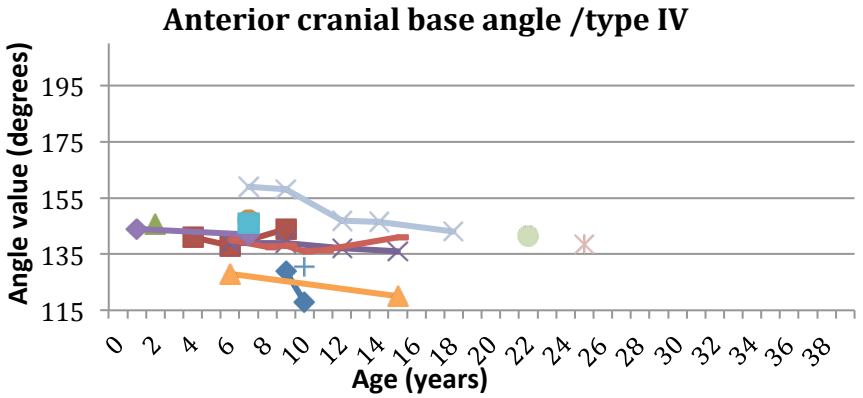
200
150
100
50
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Age (years)


Anterior cranial base angle /type IV
Angle value (degrees)
195
175
155
135
115
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Age (years)

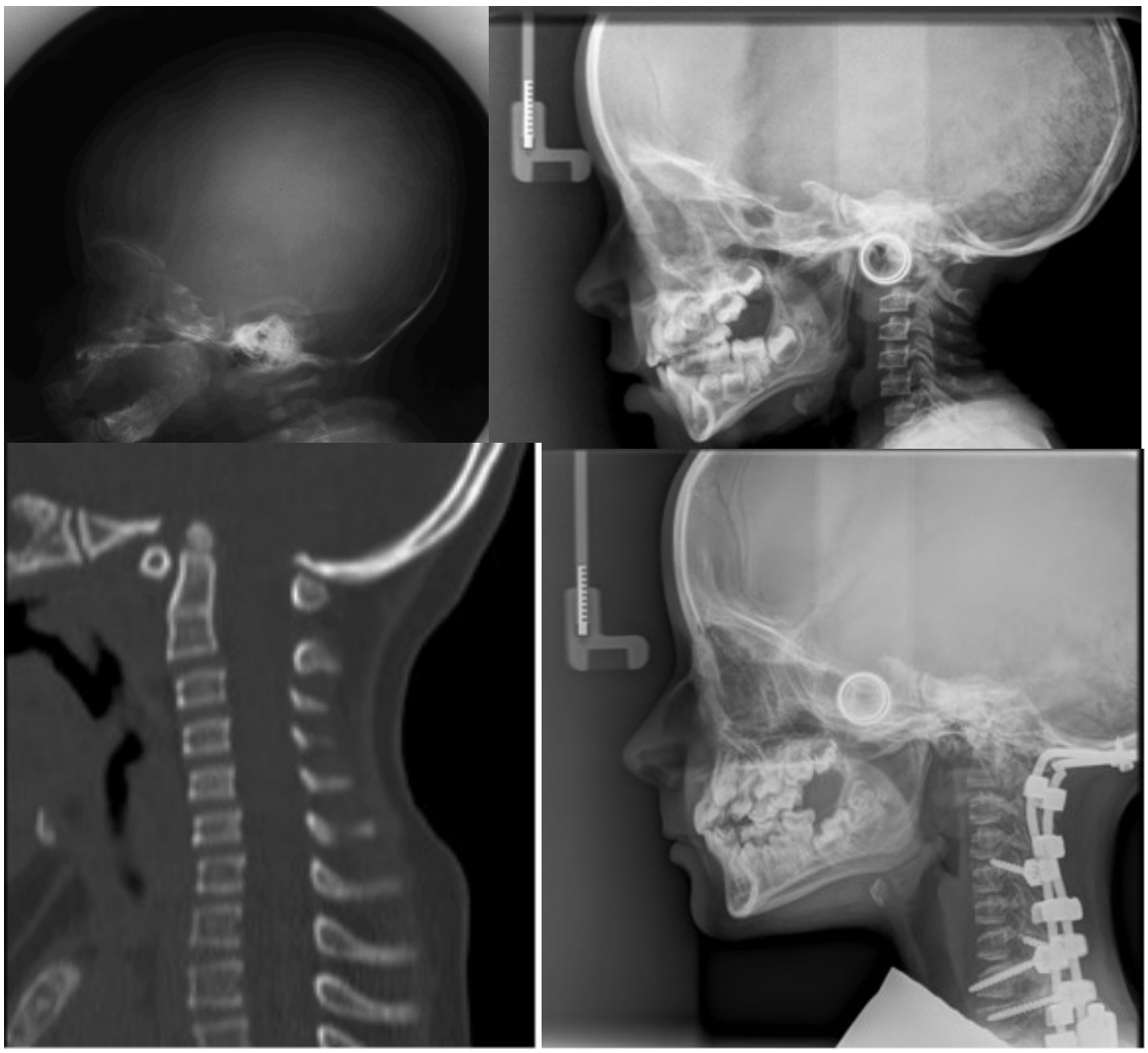


Figure 10 *Radiographs of a male with OI type III A) at birth and B-D) at ages 7.5, 8.5, and at 11.7 years. The patient presented with platybasia from birth and developed basilar invagination and impression by 4 years of age. Surgical decompression and posterior fixation was conducted at the age of 8 years due to evolution of Chiari I malformation following the craniocervical anomalies. The patient had been treated with bisphosphonates from the age of 4.5 years. Image D obtained after the surgery was not used in the longitudinal analysis.*

6. Discussion

6.1. Reliability of observations

The interpretation of radiographs, MR, and CT images invariably comprises some systematic and random errors, which can only be dealt with by repetitive analysis to a certain extent. Evaluation of the effect of the errors is vital for the reliability of observations. The observations made in this investigation suggest that, on average, variation in the location of a single landmark affects little the cranial base measurement values, whilst linear measurements and angles have been shown to be affected by the rotation of the head (Ahlqvist *et al.* 1986, Malkoc *et al.* 2005). For this reason, at the Institute of Dentistry, University of Helsinki, where the majority of the radiographs were obtained, images had been taken with ear rods stabilizing the positioning of the head and minimizing its rotation. Healthy individuals, old enough to stand independently in the cephalostat, and patients with OI, whenever possible, were positioned with the clinical Frankfurt horizontal plane parallel to the floor. Extension and flexion of the head have been shown, in contrast, to leave unaffected the distance of the tip of the odontoid process from the foramen magnum line (McGregor 1948). The cephalometric landmarks used in this study are located on the midsagittal plane, and thus the method is applicable, in addition to radiographs, to CT, and MR images of the same plane. An exception is the lowest point of the occipital curve, which might be visualised differently on a lateral skull radiograph and on MRI (Cronin *et al.* 2007). The lowest point is sometimes situated lateral to the midsagittal plane and thus is not visible on midline MRI. This can lead to the D-M measure reference line being located more caudally on a plain radiograph, and result in a more positive distance of the odontoid process to the reference line. The general reliability of the observations from a conventional radiograph were reported by Janus and co-workers (2003), who found that in all the studied cases, MRI findings confirmed the suspicion of pathology from a traditional radiograph in growing patients with OI.

In evaluating the reliability of this analysis, the limited size of the control cohort must be considered. Herein, this fact is displayed as a large SD of the age-group results, caused by

interindividual variation. The area under the normal variation curve below +2.5 SD covers 99.4% of all data values. Assuming that the control cohort follows the normal distribution, and the threshold limit of anomaly is set at +2.5 SD from the average, then theoretically 1 in 167 unselected patients without an anomaly would exceed the upper limit and thus receive a false positive diagnosis. A lower threshold limit was used to ensure the sensitivity of the screening, whereas higher limit yields better specificity of the diagnosis. In an adult population, the emphasis may be on limitation of false positive findings, whereas in children it is beneficial to identify all subjects that need closer follow-up.

6.2. Radiographic evaluation of cranial base dimensions

In healthy children, the more caudal relative position of the odontoid process (as measured from the tip of the body of the dens) detected below the age of 9 years, is likely to be due to the tip not being yet fully mineralized and fused to the body of the dens, which usually occurs by 12 years (III) (Menezes 2008b).

In assessing basilar impression, the use of Chamberlain's line as a reference has been criticised because of the difficulty in locating the posterior reference point; the opisthion (McGregor 1948). In addition, as pointed out by Kovero and collegues (2006), the hard palate in growing OI patients is likely to be an unstable reference structure. Our study confirms the recommendation of Kovero *et al.* (1996) of the suitability of the D-M measure as a reference line in growing individuals. No statistically significant age-related change in the measure was observed, as is expected from the early stabilisation of the nasion-sella level during growth. Contrary to the findings of Bull and colleagues (1955), age was shown to be a relevant factor in assessing basilar pathology, whereas gender is not. This view is shared by Cronin and co-workers (2007 and 2009), who demonstrated the lack of statistically significant differences, in cranial base measures, between the sexes in healthy adult population.

Diagnosis of basilar pathology is reliably done on an MR or CT scan. Lateral skull radiographs have been recommended as a screening method at individually adjusted intervals. A baseline cephalometric study has been recommended for all patients with OI before school age. (OI-consensus conference, 17-18 November 1999, Sørmarka, Norway)

6.3. Natural course of basilar pathology

The findings of this study are in agreement with earlier observations in that the anterior cranial base angle steepens with growth (Kerr and Hirst 1987, George 2005). Similar to the work by George (2005), a statistically significant decrease occurred only before the age of 3 years in healthy subjects. In the cohort of Kerr and Hirst (1987) the anterior cranial base angle was on average 142° at birth and reduced to 130° by 5 years of age. Here, the angle was 137° on average at birth and decreased to 130° by 5 years in healthy subjects. A growth-related steepening was seen, similar to that of healthy individuals, in OI patients. In growing OI population (aged 0-18 years) the anterior cranial base angle has been reported to have a range between 160-175° (Janus *et al.* 2003); however in this study cohort of the same age, the angle was 144° on average, and ranged between 118-197° (IV). In study III, the mean angle was 134° with a range of 94-170° for the whole cohort. McGregor (1948) considered an angle above 148° to be highly suggestive of platybasia in young adults, but the threshold limit was lower in all age groups in this study based on the values of healthy controls.

In the adult OI population, the prevalence figures of basilar pathology differ somewhat from those of growing patients. Basilar invagination has been documented in 22.2% of adults with OI (types I, III, and IV studied) (Kovero *et al.* 2006), however the corresponding figure for growing patients in study III was 4% and in study IV was 13%, when the same threshold value of +3 SD from the average of healthy age-matched controls was applied for both cohorts. In adult OI patients, the prevalence of basilar impression has been reported to be 17% (Kovero *et al.* 2006), whereas here, in growing patients, the prevalence was 6% (study III) and 9.5% (study IV) with the +3 SD threshold

limit. Platybasia was present in 11.1% of adults with OI. Platybasia was seen in 16% of patients in study III and 25.7% in study IV with the +3 SD threshold limit. The difference in the prevalence of cranial base anomaly between studies III and IV might be partly due to different examiners conducting the analyses. Moreover, in study III, the most recent radiograph was examined, whereas in study IV the first available one was selected. The percentage of patients with mild OI type I was similar in both study cohorts, however.

Menezes (2008e) postulated that adolescence is a critical stage for development of craniocervical junction pathology. In most disorders, where craniocervical junction anomaly is a possible complication, it emerges typically during the growth spurt, between 11 and 15 years of age. In patients with OI, the occurrence of cranial base anomalies has been previously observed at all ages from two years onwards (Sillence *et al.* 1993 as cited in Primorac), as was confirmed in this study. Likewise, no association has been found between age and development of scoliosis in growing OI patients (Engelbert *et al.* 1998).

In OI, the thinner cortices cause bone to bow and break easily. This bending of bone and/or microfractures, and/or possibly adaptive changes in the cartilaginous synchondroses, could explain the observed occurence of platybasia from birth. The spheno-occipital synchondrosis appeared exceptionally wide in our material, age considered, in several patients. This finding remains to be analysed in a forthcoming study. Repeated microfractures combined with bending of bone and ligament laxity would explain the reason for craniocervical pathology becoming evident after a child attains an upright posture. A delay in adoption of the upright position has been documented in children with OI. The delay is most evident in the severe forms of the disorder. The mean age of sitting by OI type is 11 months for type I, 19 months for type III, and 10 months for type IV. Similarly standing has been reported to occur on average by 11 months in type I, 40 months in type III, and 16 months in type IV. (Sutton *et al.* 2011)

Chamberlain (1939) suspected a progressive worsening of the craniocervical pathology or a delay in the appearance of symptoms to take place. Progressivity of anomalies was also suspected by Charnas and Marini (1993) and Sillence (1994). We found no statistical evidence of progression nor an age related factor in incidence of abnormality. The only

factor associated with occurrence of cranial base anomaly was the clinical severity of OI. Our findings were similar to those of Janus and co-workers (2003), who followed seven patients for 2-6 years with MR scans after an abnormal finding on lateral skull radiograph. They observed no signs of progression in six patients and progression in one. In the same seven patients, anterior cranial base angle increased in follow-up. Despite the anatomical abnormality, neurologic symptoms were not detected. Janus recommends obtaining an image of the cranium when symptoms are present, and suggests that children that have undergone occipitocervical stabilization or decompression surgery should be monitored carefully because growth of the posterior fossa and clivus continue past adolescence and might further alter the relations of the structures of craniocervical junction.

Platybasia did not improve spontaneously in the longitudinal follow-up (III, IV). In contrast, basilar invagination and impression interestingly passed off in some patients. The improvement in McRae measure is likely to occur as a result of growing number of microfractures lowering the posterior fossa leading to upward infolding of the occipital condyles and posterior edge of foramen magnum (Sillence 1994), thus raising McRae's line. The reason for the observed change into a negative value of the D-M measure remains unknown and this development is somewhat contradictory to the previously observed progressive change in skull shape towards overhanging of the occiput (Charnas and Marini 1993).

Severe growth failure was found here to suggest the presence of a craniocervical junction anomaly in OI patients. On the other hand, shortened distance between the skull base and shoulders, as caused by craniocervical abnormality, can in turn partly result in shortened overall height. Careful follow-up of subjects with a low height Z-score, combined with clinically short neck, is thus warranted.

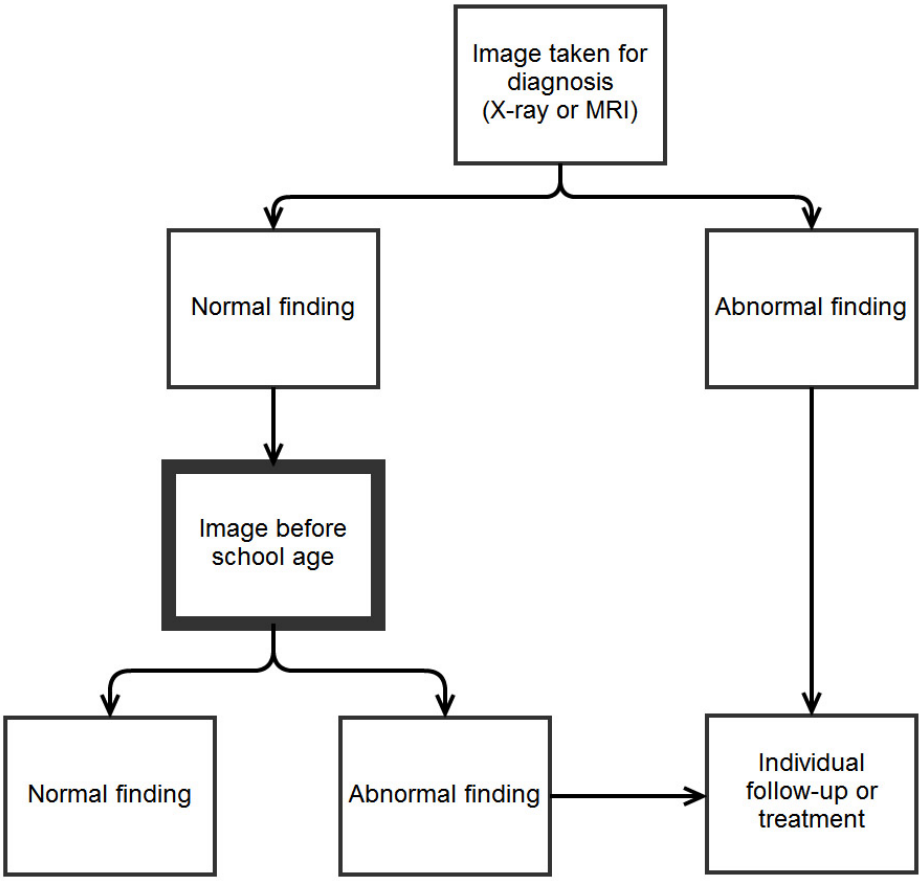
In patients with OI, the mean head circumference is within normal limits but has been reported to increase relative to body height (Lund *et al.* 1999). Charnas and Marini (1993) found that an increase in head circumference followed the normal curve for most patients. In 13 out of 46 patients, however, macrocephaly progressively advanced to reach an average head circumference Z-score of 2.1 ± 1.3. In 5 out of 76 patients, basilar

impression was discovered without related symptoms. Charnas and Marini (1993) documented basilar impression in all patients with large head circumference. In contrast, Engelbert and co-workers (1998) found no significant relationship between the head circumference Z-score and the presence of basilar impression in 47 paediatric patients. They also found basilar impression to be more commonly associated with kyphosis than with scoliosis. Children with generalized joint hypermobility had a lower incidence of scoliosis. Those children were also less prone to develop basilar impression. Engelbert speculated that microfractures of the vertebrae are the cause of spinal complications in patients with OI. Contrary to this view, laxity of spinal ligaments, thus vertebral instability, has been proposed to underlie progressive spinal curvature (Benson and Newman 1981, Hanscom *et al*. 1992). Scoliosis is a rare finding in patients with OI under the age of five, and is more common in the more severe forms of OI (Benson and Newman 1981). In evaluating scoliosis, change in sitting height is compared with angular spinal changes (Dimeglio 2001). Scoliosis has been previously noted to naturally progress at an individual rate in growing patients with OI without treatment (Hanscom *et al.* 1992). Hanscom and co-workers (1992) observed a partial loss of straightening, achieved in an arthrodesis operation, during a follow-up period. Of the 17 out of 56 patients with scoliosis, 11 also had craniocervical pathology, but association between these two could not be statistically shown (Hanscom *et al.* 1992).

Sillence (1994) recommends radiological screening of the cranial base in type I OI, at 3 to 5 year intervals, until skeletal maturity, after which no follow-up is needed if the findings are normal. In types III and IV, he suggests screening at 2 to 3 year intervals. Initial screening at the age of 5 years is recommended in severe forms. If no pathology is detected, a follow-up based on clinical symptoms is enough. Sillence also recommends delaying the upright position of a child. Notably his study reports a smaller frequency of neurologic symptoms than pathologic findings in radiographs or CT images. Based on our findings, we confirm that radiological analysis of cranial base dimensions with measurements described should be carried out for all patients before school age. In severely affected children, a radiograph or MR image is often taken already in infancy to facilitate diagnosis and classification of OI, and should be subjected to analysis of craniocervical junction. In case of normal findings from the image(s) taken before school

age, further imaging is unnecessary in symptomless patients to retain the patient radiation dose at a minimum. In the cases of abnormal findings in a radiograph and MR image, an individually adjusted plan for follow-up and treatment is warranted (Figure 11).

Figure 11 *The outline of suggested clinical management with imaging*



7. Conclusions

The radiographic analysis methods used here are applicable for screening of basilar pathology. By analysing lateral radiographs of the midsagittal plane, the variation in anatomic landmark location was found to lead to differences in numeric evaluation of the anatomic relationships of the cranial base and craniocervical junction. These differences were, however, shown to have little clinical significance. (I)

Of the commonly used measurements in evaluating basilar pathology, the McGregor measure was the least sensitive to errors in landmark identification. The McGregor measure is, however, significantly age dependent and thus not as suited to paediatric subjects as the least age sensitive measure, D-M distance (II). In patients with OI, D-M distance was confirmed to be a more sensitive indicator of basilar impression than the Chamberlain or McGregor measures (III). The novel D-M angle measure is unaffected by radiographic magnification and has high correlation with D-M distance, which further increases the superiority of D-M angle in evaluating basilar impression (IV).

The vertical dimensions of the craniocervical junction and the flexion of the anterior skull base change in healthy individuals during growth. Consequently the normal values for the cranial base measures are age dependent. Notable individual variation in measures during growth may appear. Our findings indicate that in 5–6-year-old children, the cranial base measurements are significantly different from older age groups, and studies on patients younger than 9 should be provided with age-appropriate controls. (II) We provide reference values for growing individuals (II, IV).

A notable deviation from the normal values is suggestive of pathological development and indicates a need for further examination. Cranial base anomalies were present in 22% of OI patients in the cohort of study III and 32% of the patient cohort in study IV, as assessed by the limit of pathology set at 3 SD above the values of healthy age-matched controls. Platybasia was the most frequent finding. (III, IV) Detection of platybasia from birth suggests intrauterine development, whereas basilar impression and invagination were

detected from the age of 2 years onwards (IV). Platybasia had a tendency to persist, whereas the natural course of basilar invagination and impression showed more variation.

Severely affected OI patients with very short stature are at the highest risk of developing cranial base anomalies (III, IV). We did not find evidence for progression of the pathology or for a protective effect of bisphosphonate treatment (III, IV). These findings favour the hypothesis that other than bony structures –skull base cartilage and craniocervical junction ligaments– might be involved in the development of these pathological conditions, in addition to changes in bones.

Early recognition of cranial base anomalies, and implementation of treatment is important before irreversible neurological defects arise. Based on the findings of this thesis, radiological analysis of cranial base dimensions with measurements described should be carried out for all patients before school age. In the case of normal findings from the initial image(s), further imaging is unnecessary in symptomless patients. Where abnormal findings in a radiograph or MR image are evident, an individually adjusted plan for follow-up and treatment is warranted.

8. Clinical recommendations and future considerations

The following clinical recommendations are proposed based on this study:

- Basilar pathology should be suspected in patients with OI and a low height Z-score.
- When assessing basilar anomalies, age-matched controls should be used with patients under 9 years of age.
- Basilar invagination is feasibly diagnosed with the McRae measure, basilar impression with D-M angle, and platybasia by measuring the anterior cranial base angle.
- The outline of suggested clinical management with imaging is presented (Figure 11).

With bisphosphonates becoming the treatment of choice from a young age, the natural course of development of cranial base anomalies in patients with OI may be lost, assuming that changes in bony structures bear a considerable responsibility for the development of the pathology. Future prospects certainly include studies on the effects of bisphosphonates on cranial base anomalies. Bisphosphonates are unlikely to reduce the prevalence of early onset platybasia, and do not directly affect the ligaments constituting an essential part of the junction. The increased stiffness of bone caused by bisphosphonates might even increase the prevalence of platybasia due to microfractures as the brain grows in volume. The favourable effects of bisphosphonate treatment on infants with severe forms of OI might lead to an adoption of an upright position earlier and as a result the weight of the cranium compressing the cervical spine earlier in growth.

The natural course of cranial base anomalies in other connective tissue disorders besides OI should also be examined. This study can serve as a benchmark for future studies.

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Espoo, May 9th 2012

Heidi Arponen

Try not to become a man of success but rather to become a man of value.
Albert Einstein (1879 - 1955)

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11. Original publications