Welcome address

It is with great pleasure that we welcome you to the 10th International Conference on Osteogenesis Imperfecta (OI) in Ghent. The programme committee has tried to create an attractive programme with broad coverage of the current progress in diagnosis, treatment and pathogenesis of OI. Besides reviewing important clinical and radiographic aspects, an update on the genetic and molecular aspects of OI will be presented and current view points and controversies on management and treatment of OI will be addressed. Experts on bone homeostasis and collagen biology will educate us on their knowledge and recent new insights of relevance to OI.

With this Symposium we hope to create a forum where a wide scope of medical specialities, including orthopaedic surgeons, rehabilitation physicians, rheumatologists, endocrinologists, ear-nose-throat specialists, paediatricians, molecular biologists and geneticists can interact and debate about various aspects of OI. We hope the meeting will be an enriching experience to all of you, trigger clinical and research interests in the condition and enhance consensus on nosology, diagnosis, management and treatment of patients with OI.

We would like to welcome you all to the welcome reception on Wednesday evening for a friendly and informal get-together. We also hope you will participate in the symposium dinner on Thursday evening, where we hope you will enjoy Belgian cuisine and musical entertainment. For those interested, a guided tour through the old town of Ghent will be organised on Friday evening.

We look forward to your presence at the symposium and invite you to enjoy this meeting in Ghent with us.

Anne De Paepe
Chair

Paul Coucke
Bart Loeys
Geert Mortier
Programme Coordinators

Scientific Committee

Bishop Nicholas
Byers Peter
De Paepe Anne
Devogelaer Jean-Pierre
Dhooge Ingeborg
Glorieux Francis
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Goemare Stefan
Loeys Bart
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Mestdagh Mieke
Mortier Geert
Renard Marjolijn
Symoens Sofie
Willaert Andy

PROGRAMME

Wednesday October 15th 2008

Location **PAC – W. Wilsonplein 2 – 9000 Ghent**

18:00 Welcome address
Anne De Paepe (Ghent University, Ghent, Belgium)
Paul Van Cauwenberge (Chancellor of Ghent University, Ghent, Belgium)

18:20 Lecture by Mrs Wallentin, President European Federation OI

18:40 Keynote lecture by Peter Byers (University of Washington, Seattle, USA)

19:30 Welcome reception in exhibition and poster hall

Thursday October 16th 2008

Location **PAC – W. Wilsonplein 2 – 9000 Ghent**

Session 1A: Clinical and radiographic aspects of OI

Chairs *Geert Mortier* and *David Sillence*

09:00 Introduction to the clinical aspects of OI **I.1**
Sillence D. (University of Sydney, Sydney, Australia)

09:20 OI: The classification revisited **I.2**
Glorieux F. (Shriner's hospital for Sick Children, Montreal, Canada)

09:45 The radiological differentiation of OI and non-accidental injury **I.3**
Hall C. (Great Ormond Street Hospital for Children, London, UK)

10:10 Overview of type I collagen mutations in OI (OI consortium) **I.4**
Marini J. (NIH, Bethesda, USA)

10:35 Coffee break in exhibition and poster hall

Session 1B: Molecular basis of OI

Chairs *Joan Marini* and *Paul Coucke*

11:00 Non-OI phenotypes in type I collagen **I.5**
Malfait F. (Ghent University, Ghent, Belgium)

11:25 Clinical and biochemical consequences of mutations in the C-propeptide coding domains of the COL1A1 and COL1A2 genes **I.6**
Pace J. (University of Washington, Seattle, USA)

11:50 The contrast of mechanisms of mRNA degradation vs. protein degradation phenotypes in COL1A2 nulls **I.7**
Byers P. (University of Washington, Seattle, USA)

12:15 A relational database for collagen mutations **I.8**
Dalgleish R. (University of Leicester, Leicester, UK)

12:40 Lunch and poster viewing in exhibition and poster hall

Session 2: Bone homeostasis and collagen biology

Chairs *Peter Byers* and *Matthew Warman*

13:30 Recent insights from genes involved in bone resorption and formation **I.9**
Van Hul W. (University of Antwerp, Antwerp, Belgium)

13:55 Structural biology and biosynthesis of collagens **I.10**
Bachinger HP. (Shriners Hospital for Children and Oregon Health & Science University, Portland, USA)

14:20 Folding, structure and interactions of mutant type I collagen in different forms of OI **I.11**
Leikin S. (NIH, Bethesda, USA)

14:45 Cell and matrix interaction domains on the type I collagen fibril **I.12**
San Antonio J. (Thomas Jefferson University, Philadelphia, USA)

15:10 Biochemical markers of bone turnover in children and adolescents with OI **O.1**
Åström E. (Karolinska Institutet, Stockholm, Sweden)

15:20 Coffee break in exhibition and poster hall

Session 3: Murine models for OI

Chairs *Brendan Lee* and *Paul Coucke*

16:00 The new aga mouse model for OI, with a mutation in the C-propeptide domain of Col1a1 **I.13**
Lisse T. (NIH, Bethesda, USA)

16:20 In utero stem cell therapy as a treatment for the OI knock-in murine model BrtlIV and evaluation of the differentiation capacity of mutant MSCs **I.14**
Forlino A. (University of Pavia, Pavia, Italy)

16:40 Alendronate effects on fracture healing in growing Brtl mouse model of OI **O.2**
Caird M. (University of Michigan, Ann Arbor, USA)

16:50 End of session

18:30 Symposium Dinner

Friday October 17th 2008

Location **PAC – W. Wilsonplein 2 – 9000 Ghent**

Poster available for viewing all day

Session 4: Recessive OI

Chairs *Joan Marini* and *Francis Glorieux*

09:00 Clinical and molecular features of OI due to CRTAP and P3H1 LEPRE1 mutations **I.15**
Lee B. (Howard Hughes Medical Institute, Houston, USA)

09:20 CRTAP and P3H1/LEPRE1 mutations: functional effects, complex interactions and founder mutations **I.16**
Barnes A., Chang W. (NIH, Bethesda, USA)

09:50 Other forms of recessive OI (non-collagen, non-P3H1, non-CRTAP) **I.17**
Pepin M. (University of Washington, Seattle, USA)

10:10 CRTAP mutations in lethal and severe OI: the importance of combining biochemical and molecular genetic analysis **O.3**
Van Dijk F.S. (VU University Medical Centre, Amsterdam, The Netherlands)

10:20 Recessive OI caused by LEPRE1 mutations: clinical documentation and identification of the splice form responsible for prolyl 3-hydroxylation **O.4**
Willaert A. and Malfait F. (Ghent University, Ghent, Belgium)

10:30 Coffee break in exhibition and poster hall

Session 5: Selected abstracts and poster session

Chairs *Bart Loeys* and *Matthew Warman*

11:00-11:30 Poster highlights

11:00 Increased dimensions of left ventricle and aorta in adult patients with OI **O.5**
Radunovic Z. (Aker University Hospital, Oslo, Norway)

11:10 How do adults with OI manage in daily life? A population based study **O.6**
Wekre L.L. (University of Oslo, Oslo, Norway)

11:20 Allele specific silencing of collagen I alpha 2 in primary human bone cells using RNAi – A step toward gene therapy in OI **O.7**
Lindahl K. (Uppsala University, Uppsala, Sweden)

11:30-12:30 Poster viewing

12:30 Lunch and poster viewing in exhibition and poster hall

Session 6: Orthopedic management and use of bisphosphonates in the treatment of OI

Chairs *Jean-Marc Kaufman* and *Frank Plasschaert*

13:30 Predictors of the response to pamidronate therapy **I.18**
Rauch F. (Shriner's hospital for Sick Children, Montreal, Canada)

13:50 Bisphosphonate therapy in OI: peroral vs intraveneously **I.19**
Bishop N. (University of Sheffield, Sheffield, UK)

14:10 Bisphosphonate therapy in OI: the Cologne experience **I.20**
Semler O. (University of Cologne, Cologne, Germany)

14:30 Study of the effect of growth hormone in combination with bisphosphonate treatment on bone metabolism in OI **O.8**
Antoniazzi F. (University of Verona, Verona, Italy)

14:40 Current evaluation of the response of OI adults treated with bisphosphonates **O.9**
Shapiro J. (Kennedy Krieger Institute, Baltimore, USA)

14:50 Coffee break and continued poster viewing in exhibition and poster hall

15:15 Radiographs, bone densitometry and biological parameters of bone remodeling **I.21**
Devogelaer JP. (Saint-Luc University Hospital, Brussels, Belgium)

15:40 Spine and limb surgery in OI pediatric patients **I.22**
Finidori G. (Hôpital Necker Enfants Malades, Paris, France)

16:05 (Re)habilitation in OI in childhood **I.23**
Engelbert R. (Amsterdam School of Health Professions, Amsterdam, The Netherlands)

16:30 Diagnosis and treatment of the functional muscle bone unit in OI **I.24**
Schönau E. (University of Cologne, Köln, Germany)

16:55 End of session

19:00 Guided tour in Ghent

Saturday October 18th 2008

Location **PAC – W. Wilsonplein 2 – 9000 Ghent**

Session 7: Hearing loss and Dentinogenesis Imperfecta

Chairs *Cor Cremers* and *Inge Dhooge*

09:00 Hearing loss in OI **I.25**
Kuurila-Svahn K. (University of Turku, Turku, Finland)

09:20 Management of hearing loss: Surgery (Incudostapedotomy and Malleostapedotomy) and outcome of the procedure **I.26**
Cremers C. (UMC St. Radboud, Nijmegen, The Netherlands)

09:40 Outcome of cochlear implantation in three patients with OI **I.27**
Mylanus E.I. (UMC St. Radboud, Nijmegen, The Netherlands)

10:00 The teeth in OI: diagnosis and prognosis **I.28**
Martens L. (Ghent University, Ghent, Belgium)

10:20 Factors influencing hearing loss in OI **O.10**
Chevrel G. (Edouard Herriot Hospital, Lyon, France)

10:30 Dental age of children with OI **O.11**
Waltimo-Sirén J. (University of Helsinki, Helsinki, Finland)

10:40 Coffee break in exhibition and poster hall

Session 8: New perspectives in treatment of OI and ethical aspects

Chairs *Nicolas Bishop* and *Anne De Paepe*

11:15 Stem cell therapy in children with OI **I.29**
Horwitz E. (Children's Hospital of Philadelphia, Philadelphia, USA)

11:55 Scientific, Medical and Ethical Prospects for Novel Therapeutics in OI **I.30**
Marini J. (NIH, Bethesda, USA)

12:30 Wrap-up and summary
Byers P. and De Paepe A.

12:45 Lunch in exhibition and poster hall

Free afternoon

INVITED SPEAKERS

I.1

Introduction To The Clinical Aspects Of Osteogenesis Imperfecta

David Sillence

Discipline of Genetic Medicine, University of Sydney

Osteogenesis Imperfecta encompasses over 11 disorders with 8 numbered types, two types of Bruck syndrome and Cole-Carpenter syndrome. Some clinical features in combination are highly suggestive but not pathognomic of OI. Blue sclerae (distinctly blue-grey hue), bone fragility (low impact), osteoporosis quantified by Dual Energy X-ray Absorbtiometry (DXA) or Peripheral Quantitative Computerized Tomography (pQCT) and Opalescent Dentine are indicative but all found in other Connective Tissue Dysplasias. Radiographic features including multiple Wormian bones, long bone fractures, long bone bowing and angulation and basilar impression again are highly suggestive of OI but not pathognomonic. However the finding of many of these clinical signs combined with any of the Mendelian patterns of inheritance may be diagnostic. At present five gene loci (COL1A1, COL1A2, P3H1-CRTAP, P3H1-LEPRE, and PLOD2) and a wide range of phenotypes arising from mutations in these loci are delineated.

Blue grey sclerae result not from thinning of the sclerae but as shown by Eicholtz from accumulation of an electron dense granular material found between the collagen lamellae. Is it this non-collagenous matrix which interferes with clotting and accounts for the easy bruising in OI type I. Osteoporosis is not found in some people with OI type I, IV or V at any age especially at a younger age. There is considerable inter- and intra-familial variability in fracture frequency. Progressive young adult onset hearing impairment, vertigo, basilar impression, joint hypermobility and skeletal deformity are all features frequently found in OI but also in other Connective Tissue Dysplasias.

I.2

Osteogenesis Imperfecta: the classification revisited

Francis H. Glorieux

Shriners Hospital for Children, McGill University, Montréal, QC, Canada

OI is characterized by bone fragility and reduced bone mass. The wide variability in the severity of the features among patients has generated several attempts at classifying the OI subjects according to clinical characteristics, well before the molecular abnormalities began to be discovered. First was the era of eponyms (Vrolik, Lobstein, etc…). Then the identity of the disease between adults and newborns was recognized, leading to the widely used denominations of OI tarda and congenita. Since 1979, the Sillence classification has been central to attempts at further delineating the various forms of OI. With the better understanding of the key role of collagen type I mutations, it was hoped that they could provide clues to explain the wide spectrum of severity in OI. Such efforts have been so far moderately successful. Recently the discovery that other genes (some of them yet to be identified) are critical for the development and organization of the bone matrix has broaden the spectrum to a point that it may be now timely to rethink the classification of OI taking into account simultaneously the clinical picture, the genetics, and the molecular events. A unifying picture may emerge that will be helpful both at bedside and in the laboratory.

I.3

The radiological differentiation of OI and non-accidental injury

Christine M Hall

Great Ormond Street Hospital for Children, London, UK

The paediatric radiologist is responsible for supervising the appropriate radiographic quality and the interpretation of a skeletal survey, undertaken either to confirm a suspected diagnosis of OI or where physical abuse is suspected in a child presenting with one or more unexplained fractures. The age of presentation in the majority of physically abused patients is in infancy, before fully mobile and whilst totally dependent on the regular carers.
The radiologist is only part of a team involved in the full evaluation of the child, which may include various medical and scientific personnel and social service departments.
The key radiographic features in OI in infancy at the milder end of the spectrum (types I and IV) will include a combination of two or more of the following findings:
- Wormian bones
- Decreased bone density with coarsening of the trabecular pattern
- Slender ribs and long bones
- Mild bowing (with shortening) of the femora

A pattern of fracturing usually presenting when independently mobile which excludes classic metaphyseal fractures (CML), skull fractures and unusual fractures.
Fractures most likely to involve the diaphyses of long bones including impacted fractures.
The features seen in physical abuse in infancy include two or more of:
- Classic metaphyseal fractures
- Skull fractures

Unusual fractures including fractures of the small bones of the hands and feet, acromion process, pubic rami, epiphyseal fracture separations and spinal fractures affecting the neural arches.
Absence of a generalised abnormality of the skeletal system.
Non-discriminatory fractures include:
- Vertebral body crush fractures
- Rib fractures (although posterior rib fractures are said to be more suggestive of physical abuse)
- Long bone fractures of the diaphyses. This is most commonly the presenting type of fracture in either group.

There remain a very small number of infants in whom differentiation from the radiographic and clinical findings is not possible and collagen testing is required. It should also be remembered that rarely OI and physical abuse may co-exist.

I.4

Overview of type I collagen mutations in OI (Representing the Bishop[1], Byers[2], de Paepe[3], Marini[4], Mottes[5], Roughley[6] and SanAntonio[7] Labs)

Joan Marini

1 Sheffield, UK
2 Department of Pathology, University of Washington, Seattle, USA
3 Center for Medical Genetics, University Hospital Ghent, Ghent, Belgium
4 Bone and Extracellular Matrix Branch, NICHD, NIH, USA
5 Department of Mother and Child, Verona, Italy
6 Genetics Unit, Shriners Hospital for Children, Montreal, Quebec, Canada
7 Department of Medicine,Thomas Jefferson University, Philadelphia, PA, USA

We have previously published the genotype-phenotype analysis of over 830 glycine substitutions and splice site mutations in the two alpha chains of type I collagen (Hum Mutat 28:209-221, 2007). Each chain had a distinct genotype-phenotype relationship. In the 2007 collection of mutations, mutations in the first position of glycine codons account for 78% α1(I) substitutions, with a substantial contribution from CpG dinucleotides, and about half of α2(I) substitutions. In α1(I), one-third of glycine substitutions were lethal, especially those with charged or branched residues. Substitutions in the first 200 residues were non-lethal, with variable phenotype thereafter. Two exclusively lethal regions aligned with Major Ligand Binding Regions, suggesting they disrupt crucial interactions of collagen with integrins, MMPs, fibronectin and COMP. Mutations in COL1A2 were predominantly non-lethal (80%). Lethal substitutions were located in 8 regularly spaced clusters along the chain, supporting a Regional Model. The lethal regions aligned with proteoglycan binding sites along the fibril, suggesting a role in fibril-matrix interactions. Recurrences of substitutions at the same α1(I) glycine frequently had different clinical outcomes, while those in α2(I) frequently resulted in concordant outcomes. Splice site mutations comprised 20% of the mutations assembled for the prior analysis. Splice site mutation were most often non-lethal because they led to frameshifts. We will update our prior analysis with the addition of mutations identified since the 2007 Consortium Report, correlating this analysis with information on structural domains along the helix and updated maps of collagen-ligand interactions.

I.5

Non-OI phenotypes in type I collagen

Fransiska Malfait, Sofie Symoens, Paul Coucke, Anne De Paepe

Center for Medical Genetics, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium

Type I collagen, a heterotrimer consisting of two α1(I) and one α2(I) chains, is the most abundant extracellular matrix protein in humans and the major structural protein of bone, tendon, skin and cornea. It is synthesized as a procollagen molecule containing a central helical domain, which is flanked by non-helical amino- and carboxyterminal propeptides. Cleavage of these propeptides is required to form mature collagen molecules, which assemble into fibrils. The majority of mutations in the genes encoding type I collagen (*COL1A1* and *COL1A2*) result in substitution of a glycine for a bulkier AA, leading to osteogenesis imperfecta (OI). A special class of mutations results in the rare Ehlers-Danlos syndrome (EDS) arthrochalasia type (EDS VIIA&B). Work by us and by others has recently provided evidence that defects in type I collagen may also be associated with a spectrum of phenotypes which differ from OI or EDS VIIA&B. Many of these phenotypes present during childhood with joint hypermobility and/or skin hyperextensibility, and some have increased risk for cardiac and/or vascular fragility later in life. In general, these defects can be classified into three major classes:
(1) Total absence of the pro-α2(I) chain, due to a homozygous *COL1A2*-mutation, may present as EDS hypermobility type during childhood, but is associated with severe cardiac valvular anomalies.
(2) Arginine-to-cysteine (R-to-C) substitutions at different positions of the α1(I) helical domain, are associated with a range of phenotypes, including Caffey disease, classic EDS, arterial rupture and mixed EDS/OI.
(3) Mutations in the most amino-terminal part of the collagen type I α-helices result in a distinct EDS/OI overlap phenotype with variable degrees of joint hypermobility and bone fragility and – in some patients - increased vascular fragility with bleeding diathesis. These mutations interfere with removal of the N-terminal propeptide, even though the N-proteinase cleavage site remains intact.
In conclusion, these findings broaden the phenotypic range of defects in type I collagen. Patients with these defects may come to medical attention because of a variety of problems, including musculoskeletal problems such as joint hyperlaxity, arthralgia and osteopenia, because of arterial rupture, or because of cardiac valvular problems. Early recognition and confirmation of diagnosis by means of accurate biochemical and molecular analyses may prove important for follow-up, including awareness for cardiovascular problems, and genetic counselling.

I.6

Clinical and biochemical consequences of mutation in the C-propeptide coding domains of the *COL1A1* and *COL1A2* genes

James M. Pace

University of Washington, Seattle, Washington, USA.

The C-propeptide domains of the type I procollagen alpha chains contain the sequences necessary to govern assembly of two proα1(I) chains and one proα2(I) chain to form a trimeric molecule. Thus, unlike helical defects, alterations of C-propeptides impede the initial oligomerization event and allow uniform overmodification of helical domains, and if assembly does occur, triple helix formation proceeds without interruption and without structural alteration. Therefore, phenotypic consequences of these defects appear to be due to decreased amounts of normal procollagen molecules and the presence of small populations of structurally normal but overmodified trimers. Most missense mutations of this type in *COL1A1* lead to death in the perinatal period. Comparable mutations in *COL1A2* usually result in mild forms of osteogenesis imperfecta (OI); this moderated severity may result from the unusual formation of proα1(I) homotrimers that are compatible with normal skeletal development. Characterized mutations also illustrate the significance of several C-propeptide elements including proteolytic conversion sites, cysteine residues involved in inter- and intra-chain disulfide cross-linking, and chain selectivity domains. In one instance, a patient with mild OI was found to harbor two mutations on the same *COL1A2* allele: the first, a glycine substitution which, when by itself, results in severe clinical and biochemical consequences; and the second, a missense mutation in the C-propeptide coding domain, which leads to a relatively mild phenotypic outcome. The second mutation was "dominant" to the first, whose severe consequences were muted. Thus, two mutations can be better than one, an observation that may have relevance for gene therapy strategies.

I.7

The contrast of mechanisms of mRNA degradation vs. protein degradation phenotypes in COL1A2 nulls

Byers P.

University of Washington, Seattle, USA

I.8

A relational database for collagen mutations

Raymond Dalgleish

Department of Genetics, University of Leicester, Leicester LE1 7RH, United Kingdom.

For nearly two decades the database of *COL1A1* and *COL1A2* mutations has existed first as printed documents and latterly as static web pages of data. However, earlier this year, the data were transferred into a MySQL relational database and given a feature-rich interface using the LOVD (Leiden Open Variation Database) system. In addition to *COL1A1* and *COL1A2*, data have also been compiled for the more recently discovered OI-causing mutations in the *CRTAP* and *LEPRE1* genes. The move to LOVD allows for more comprehensive information to be stored and easily searched. A custom data column has been added for mutation descriptions using legacy amino acid numbers and links are also now provided to difficult-to-locate meetings abstracts that provide the only published accounts of some mutations.
In parallel with the database changes, new reference DNA sequences from NCBI have been adopted. Based on the existing NCBI RefSeq mRNA sequences, these RefSeqGene genomic DNA sequences have been developed in consultation with NCBI. Derived from the current genome assembly, they match base-for-base with the corresponding RefSeq mRNA sequences. LOVD can use RefSeqGene sequences to validate mutation nomenclature at the cDNA (c.) or genomic (g.) level. The next stage will be the progression to using NCBI LRG (Locus Reference Genomic) sequences which will guarantee stability of the DNA sequence and provide cross-references to legacy numbering systems for exons, bases and amino acids. NCBI Genome Workbench will be developed to parse LRGs and generate mutation descriptions using HGVS-compliant nomenclature with cross-checking to legacy numbering systems.

I.9

Recent insights from genes involved in bone resorption and formation

Wim Van Hul

Department of medical genetics, University of Antwerp, Belgium

Bone mass is, throughout life, the net result of the processes of bone resorption and formation. Both of these are regulated by a number of genetic factors as well as environmental factors. In the last 10 years, a lot of new insights have been gained in these processes and their regulation, partially by studying monogenic conditions with an abnormal bone mineral density. These include the sclerosing bone dysplasias, a heterogeneous group of about 40 different clinical entities. These studies have resulted in some cases in the identification of previously unknown genes involved in bone homeostasis. In other cases, by illustrating the involvement of previously known genes in sclerosing phenotypes, further insights have been gained in the precise functioning of these genes and the pathways they participate in.

The heterogeneous group of the osteopetroses includes most of the conditions with impaired bone resorption. The clinical and radiological heterogeneity is reflected by the different genes shown to underlie one of these conditions with a function in either the differentiation of osteoclasts (RANK, RANKL), the acidification of the extracellular compartment between the osteoclast and the bone tissue (CAII, TCIRG1, CLCN7,OSTM1 and PLEKHM1) and finally the degradation of the bone matrix (CATHEPSIN K).

At the other end of the spectrum, some sclerosing bone dysplasias are due to an increased bone formation. The major finding of positional cloning of the genes involved, is definitely the essential, previously unknown, role of wnt signaling in the process of bone formation. Mutations in both the *SOST* gene, encoding the sclerostin protein, and the *LRP5* gene significantly influence bone formation rate and functional studies indicated the role of both proteins in wnt signaling.

In conclusion, positional cloning efforts on monogenic conditions with an abnormal bone density have contributed to the present understanding of bone homeostasis. Furthermore, natural variants within these genes might have an effect on bone mineral density in the general population and finally this can lead to new potential drug targets for the treatment of osteoporosis.

I.10

Structural biology and biosynthesis of collagens

Hans Peter Bächinger, Janice A. Vranka, Elena Pokidysheva, Kazunori Mizuno, Yoshihiro Ishikawa, Jackie Wirz, Nena Winand and Kazuhiro Nagata

Research Department, Shriners Hospital for Children, Portland, Oregon
Department of Molecular and Cellular Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan
Department of Molecular Medicine, College of Veterinary Medicine, Cornell University, Ithaca, New York

The biosynthesis of collagens involves numerous posttranslational modifications. The 3(*S*)-hydroxylation of proline residues is a post-translational modification that so far has only been observed in collagens. Studies with synthetic model peptides that incorporate 3(*S*)-hydroxyproline in the Xaa or Yaa position of the Gly-Xaa-Yaa triple helical sequence, indicate that 3(*S*)-hydroxyproline can only be incorporated in the Xaa position, the position were 3(*S*)-hydroxyproline is found in collagens. The crystallographic structure of such a peptide shows that the incorporation of 3(*S*)-hydroxyprolines does not change the triple helical parameters. However, peptides with 3(*S*)-hydroxyproline show a decrease in the transition enthalpy, which is compensated by a decrease in the transition entropy, resulting in an approximately equal stability of the triple helix, when compared with the corresponding proline peptides. The rate of folding of the triple helix in these peptides is not affected by the presence of 3(*S*)-hydroxyproline.
The interstitial collagens type I, II and III contain a single 3(*S*)-hydroxyproline per chain near the carboxy-terminal end of the triple helix. During the biosynthesis this modification requires a protein complex that consists of Prolyl 3-hydroxylase 1 (P3H1), CRTAP and cyclophilin B (CypB). Mutations in P3H1 and CRTAP in humans result in *Osteogenesis Imperfecta*. We show that the P3H1/CRTAP/CypB complex is a multi-functional complex that also acts as a molecular chaperone. P3H1 knock-out mice show a profound tendon phenotype, have a lower bone density and fragile skin and the pups are significantly smaller than their heterozygous or wild-type litter mates.

I.11

Folding, structure and interactions of mutant type I collagen in different forms of OI

Leikin S.

Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA

Abnormal folding of type I collagen appears to be responsible for the majority of severe Osteogenesis Imperfecta cases. However, the relationship between the abnormal folding and bone pathology remains largely unclear. In this presentation, I will review some recent advances and new approaches to this problem. I will focus on the emerging consensus that Endoplasmic Reticulum (ER) stress in osteoblasts plays an important role in the pathology. Our data suggest that the ER stress may be caused by two main factors. First, reduced stability of mutant procollagen prevents its folding without synthesis of additional chaperones required for stabilization of the triple helix. Second, even folded mutant procollagen is often recognized as abnormal, preventing its transport from ER to Golgi and resulting in its accumulation in ER. The severity of the ER stress may depend both on the identity and location of the mutation along the triple helix. For instance, the identity of a Gly substitution affects local helix disruption in the immediate vicinity and interactions of other molecules with the substitution site. However, it generally does not affect the overall triple helix stability. The latter is determined primarily by the mutation location. Thus, the substitution identity and its location affect the ER stress via different molecular mechanisms. These mechanisms are now beginning to come into the light. In conclusion, I will discuss how better understanding of these mechanisms may help us to solve the puzzle of OI phenotype variation and find new targets for treatment.

I.12

Cell and matrix interaction domains on the collagen type I fibril

[1]Sweeney, S.; [2]Orgel, J.; [3]Fertala, A.; [4]McAuliffe, J.; [5]Ala-Kokko, L.; [6]Forlino, A.; [7]Marini, J.; and [3]San Antonio, J. [1]

Cardiovasc Inst, U PA, Phil, USA; [2]Cntr Synchr. Rad Res, Dept Biol, Chem, Phys Sci, Ill Inst Tech, Chicago, USA; [3]Depts Med & Derm, Thom Jeff U, Phil, USA; [4]Dept Stat, Wharton, U PA, Phil, USA; [5]Dept Med Biochem & Molec Biol, U Oulu, Oulu, Finland; [6]Dept Biochem A Castellani, Univ Pavia, Pavia, Italy; [7]NICHD, NIH, Bethesda, MD.

Type I collagen, the predominant protein of vertebrates, polymerizes with type III and V collagens and non-collagenous molecules into large cable-like fibrils. Yet, how the fibril interacts with cells and other binding partners remains poorly understood. To help reveal insights into the collagen structure-function relationship, a database was assembled including hundreds of type I collagen ligand binding sites and mutations on a two-dimensional model of the fibril. Visual examination of the distribution of functional sites, and statistical analysis of mutation distributions on the fibril suggests it is organized into two domains. The "Cell Interaction Domain" is proposed to regulate dynamic aspects of collagen biology including integrin-mediated cell interactions and fibril remodeling. The "Matrix Interaction Domain" may assume a structural role, mediating collagen cross-linking, proteoglycan interactions, and tissue mineralization. Molecular modeling was used to superimpose the positions of functional sites and mutations from the two-dimensional fibril map onto a three-dimensional X-ray diffraction structure of the collagen microfibril *in situ*, indicating the existence of domains in the native fibril. Sequence searches revealed that major fibril domain elements are conserved in type I collagens through evolution, and in the type II/XI collagen fibril predominant in cartilage. Moreover, the fibril domain model provides potential insights into the genotype-phenotype relationship for several classes of human connective tissue diseases, mechanisms of integrin clustering by fibrils, the polarity of fibril assembly, heterotypic fibril function, and connective tissue pathology in diabetes and aging.

I.13

The new aga mouse model for OI, with a mutation in the C-propeptide domain of Col1a1

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Osteogenesis imperfecta (OI) is the most common heritable cause of skeletal fractures and deformities in humans associated mostly with mutations in type I collagen genes. We recently characterized the ENU-derived mouse mutant Aga2 (abnormal gait 2) which possesses an alternative splice site mutation affecting the C-terminal end of Col1a1. Heterozygous animals develop a mild to severe phenotype associated with a marked increase in bone turnover and disrupted native collagen network. In-depth molecular analysis revealed disturbances in type I collagen heteroduplexes, which lead to intracellular accumulation of aberrant procollagens in the ER. This is associated with the induction of an ER stress-specific unfolded protein response involving upregulation of BiP, Hsp47 and Gadd153 with caspase-12 and -3 activation causing apoptosis in osteoblasts both in vitro and in vivo. Beside the skeletal hallmarks, the primary screen of Aga2 in the German Mouse Clinic depicted alterations in the lung. We found a reduction in the respiratory frequency, an increase in expiratory time as well as a pronounced decrease in the mean expiratory flow rate, which seem not to be correlated with rib-cage fractures. Our studies identified a role for intracellular modulation of the ER stress associated unfolded protein response machinery in OI, and beside the bone, the lung might also be primarily affected in Aga2.

I.14

In utero stem cell therapy as a treatment for the OI Knock-in murine model BrtlIV and evaluation of the differentiation capacity of mutant MSCs

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We evaluated an in utero cell therapy treatment for Osteogenesis Imperfecta using the murine model BrtlIV. The bone marrow cells isolated from eGFP mice were injected into E14.5 embryos and mice were analyzed at 2 m. The engraftment, detected in various organs by microscopy, was quantified in bone by confocal microscopy and in different tissues by FACS and by Real Time PCR. Analysis of type I collagen extracted from bone revealed a reduction of the mutant collagen in treated mice. PQCT showed a statistical increase in trabecular and cortical density in long bones epiphysis of Brtl treated animals and micro CT analysis of mid-shaft femur detected improvement in Tissue Mineral Content, Tissue Mineral Density, Cortical Thickness and Cortical Area.

Ultimately to evaluate the cellular therapy effects is necessary to have a better knowledge of how the endogenous stem cells behave. We investigated in Brtl the MSCs proliferation and differentiation toward adipocytes and osteoblasts. MSCs proliferation was statistically higher in Brtl mice than in WT. No difference was detected in the CFU-F number, but the ability of mutant cells to differentiate to adipocytes was greater and their ability to differentiate toward mineralizing osteoblasts was less than in WT.

Based on our in utero treatment the stem cell transplantation seems promising to cure the disease. The alteration in the MSCs differentiation signal pathway identified in our OI model will be further analysed to optimize and better evaluate the cellular therapy approach.

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I.15

Clinical and molecular features of OI due to CRTAP and P3H1 LEPRE1 mutations

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Autosomal recessive OI is caused by dysregulation of prolyl 3-hydroxylation in part of fibrillar type I collagens and is due to mutations either in CRTAP or LEPRE1. Complete loss of function is associated with a severe OI phenotype with the corresponding biochemical signature in skin and bone collagen. The clinical features resulting from CRTAP or LEPRE1 loss of function mutations were difficult to distinguish at birth. Infants in both groups had multiple
fractures, decreased bone modeling (affecting especially the femurs), and extremely low bone mineral density. Hypomorphic mutations and residual functional activity appear to correlate with milder phenotypes and survival, as is the case in OI VII. From a pathophysiological perspective, the consequences of loss of prolyl-hydroxylation need to be distinguished from the loss of independent functions of CRTAP or LEPRE1. Together, they lead to altered collagen triple helical assembly, post-translational over modification, but qualitative increased rates of secretion. On a tissue level, there are thickened collagen fibers, decreased osteoid deposition, but accelerated rates of mineralization. Because of the evolutionary conservation of this biochemical modification and the potential for diverse collagen targets, dysregulation of prolyl 3-hydroxylation reflects a more general connective tissue disease and raises questions about co-morbidities for example in soft tissues and cartilage and potential adjunctive treatments.

I.16

CRTAP and P3H1/LEPRE1 mutations: functional effects, complex interactions and founder mutations

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We identified 15 cases of recessive OI which are caused by null mutations in CRTAP (Cartilage-Associated Protein) or P3H1/LEPRE1 (Prolyl 3-Hydroxylase 1), two of the three components of the collagen prolyl 3-hydroxylation complex in the endoplasmic reticulum. We have designated these cases as types VII (OMIM #610682) and VIII OI (OMIM #610915), respectively. The phenotype of recessive OI ranges from lethal to severe, clinically similar to types II/III OI but with distinctive histomorphometry and white sclerae. A founder mutation in LEPRE1, which was transported from West Africa to the Americas with the colonial slave trade, is lethal in homozygous infants. We demonstrated that this mutation has a carrier frequency of 1-2% in contemporary West-Africans and 1/200-300 in African-Americans. Biochemically, recessive OI is characterized by loss of CRTAP or P3H1 transcripts and protein, and lack of 3-hydroxylation of (1(I)Pro986. Similar absence of 3-hydroxylation occurs in skin, dermal fibroblasts, bone and osteoblasts, demonstrating lack of redundancy of this modification. Paradoxically, collagen from these probands is overmodified by prolyl 4-hydroxylase and lysyl hydroxylase. Also, collagen secretion is increased in LEPRE1-null cells. P3H1 and CRTAP protein is absent or minimally detectable in CRTAP-null or LEPRE1-null fibroblasts, respectively, despite normal levels of LEPRE1 or CRTAP transcripts in these cells. This suggests that CRTAP and P3H1 are mutually protected in the ER complex. Transfection of full length CRTAP expression constructs into CRTAP-null cells can rescue P3H1 protein and reduce overmodification of type I collagen. Examination of ER stress in proband fibroblasts showed increased expression of IRE1, BiP and EDEM1, while HSP47 protein levels were increased to help relieve the ER stress burden. Increased XBP1 splicing was not detected in proband fibroblasts by PCR and restriction digestion. Loss of the 3-hydroxylation complex and ER stress adaptation may contribute to the phenotype of recessive OI.

I.17

Other forms of Osteogenesis Imperfecta (non-collagen, non-P3H1, non-CRTAP)

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The mechanism responsible for OI type II or III that express a type I collagen defect is now quite well understood. The proportion with recessive OI remains a question. By randomly selecting 63 individuals with OI type II or II/III confirmed by collagen electrophoresis and completing genomic studies of *COL1A1* and *COL1A2* followed by *CRTAP* and *LEPRE1* sequencing, the underlying molecular basis of OI was found in each instance. 95% had an alteration in either *COL1A1* or *COL1A2*, 3% with a *LEPRE1* recessive mutation and 2% with *CRTAP* defect, confirming that the recessive forms comprise less than 5% of the OI II/III cases. To understand the proportion of individuals with non-lethal forms of OI who may have a "non-traditional" causative mutation we reviewed samples of 260 individuals with simultaneous testing of proteins and COL1A1/2 gDNA sequencing. Between 2004 and 2008, 60 samples with **normal** collagen electrophoresis studies were followed up with gDNA sequencing of COL1A1/2. A disease-causing mutation was found in 8 individuals. In each instance, the clinical diagnosis of OI was likely. Of the remaining 52, the clinical findings were often equivocal. Evidence from study of several consanguinous families with normal collagen screening and the exclusion of CRTAP, LEPRE1 and SMPD3 as candidate genes suggests that there are additional genes to be identified for OI. The clinical examination including medical history, physical exam, family history and radiographs is key in determining the need for continued testing.

I.18

Predictors of the Response to Pamidronate Therapy

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Intravenous pamidronate is widely used in children and adolescents with osteogenesis imperfecta (OI). The drug reliably inhibits bone resorption by inactivating osteoclasts. In our studies on children and adolescents with OI, all 139 patients who received pamidronate for the first time experienced a decrease in urinary bone resorption markers. The treatment effect on the amount of mineralized material on the whole-bone level is determined by the interaction between the drug and growth. In our patient cohort, lumbar spine areal bone mineral density z-scores increased in 139 of 144 patients during the first treatment year. On the functional level, changes in pamidronate-treated patients depend on a multitude of factors, such as bone deformities, fracture rates, muscle force, motivation, attitudes toward risk (of patients and caregivers), access to physiotherapy and occupational therapy, quality of orthopedic care. In our group of pediatric OI patients, gross motor function increased in 53 of 59 patients (90%) during the first 3 years of treatment. Thus, the clinical course of patients receiving pamidronate depends on many factors other than the medication. Pamidronate therefore be seen as part of a treatment concept rather than as a stand-alone therapy.

I.19

Bisphosphonate therapy in OI: peroral vs intraveneously

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Over 30 published studies in adults and children have described the effects of a variety of bisphosphonate interventions in infants, children and adults. The characteristic changes reported in response to therapy include improved bone mass, size and shape, reduced pain and improved mobility.
Adverse effects appear to be few during childhood; acute phase reactions are typically limited to the first exposure to the drugs, there is some undertubulation of the ends of long bones without apparent adverse mechanical sequelae and there have been no reports of osteonecrosis of the jaw in OI patients. Hypocalcaemia is seen only occasionally. Concerns over accumulated microdamage appear unfounded thus far, but the effect of accumulated bisphosphonate within the skeleton in subsequent decades remains to be determined.
The tissue level effects of bisphosphonates in OI are well described and the efficacy of the intervention appears to relate primarily to increased bone size in tubular bones and improved retention of trabecular and cortical bone in the axial skeleton.
Questions that remain are over the choice of drug, route of administration and the indications for starting therapy – should all those with OI receive this therapy?

I.20

Bisphosphonate therapy in OI – the Cologne experiences

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Treatment of children and adolescents with i.v. pamidronate is an established treatment in Osteogenesis Imperfecta. According to the literature i.v. treatment with neridronate is also an effective therapy and much more convenient for the individual patients.
Method: We analyzed the results of our first patients treated with i.v. neridronate regarding safety and effectiveness. The patients were treated according to the experiences published by Gatti et al in 2005. For safety assessments we gave a questionnaire to 24 patients/parents [16 male, median age 6.6years (0.2 – 14.6 years)] after the first infusion of neridronate. Concerning the effect of treatment we assessed the reshaping of lumbar vertebrae in 12 of these patients who had taken 2 lateral spine x-rays over a period of 12 months. We used the concavity index (mh/ah)

Results:

Effects (n=24)	Frequency
Body temperature (38,0^0 C - 38,9^0 C)	25%
Body temperature (>39,0^0 C)	21%
Decrease in bone pain	43%
Increase in bone pain	7%

There were no clinical sings of hypocalcaemia and the serum calcium and creatinine levels before the first and second infusion were in normal range.
There was a reshaping of the lumbar vertebrae after 1.1 years of treatment.

Vertebra	Change of concavity index	p-value
L1	+ 7.2 %	0.132
L2	+ 12.4%	0.034
L3	- 1.4%	0.766
L4	- 4.3%	0.354

Conclusion: Neridronate i.v. is a safe and convenient therapy for children with severe Osteogenesis Imperfecta. After first infusion the frequency of side effects were comparable to a treatment with pamidronate. There was a reshaping of vertebral deformities after one year of therapy.

I.21

Radiographs, bone densitometry and biological parameters of bone remodeling

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Osteogenesis imperfecta is a genetic disorder characterized by an increased bone fragility and low bone mass. Therapy with intravenous cyclical intermittent bisphosphonates became the cornerstone of therapy after the papers published by the Glorieuxí team. It is not yet clear, however, how long should bisphosphonate therapy be maintained ? As long as the child is growing ? As long as BMD is still increasing ? Continued therapy could indeed lead to some osseous after effect in children, even if in postmenopausal women suffering from osteoporosis, such side effects have not been clearly demonstrated. Growing bone might not react as the skeleton in the elderly. The cyclical intermittent treatment with pamidronate is characterized by the appearance of dense zones (zebra zones) visible on all metaphyses, particularly the iliac crest, the wrist, the knee and the upper femur. The distance between the condensed rings is proportional to the growth rate of each cartilage, which can differ according to the muscular stresses and bone hyperemia after a fracture or an orthopedic operation for example. After stopping bisphosphonate therapy, they tend to disappear slowly, because they undergo turnover. There is no real freezing of bone. At the spine, however, they seem to persist longer and represent a bone in bone image.
Pamidronate therapy does not interfere with growth. There is indeed no slowing down of growth spurt either in boys or in girls. As far as BMD is concerned, the effect of therapy appears to be much more efficient during the 2 first years of therapy. The increase in lumbar BMD amounted to about 2 Z-scores (age-corrected SD) during the 2 to 3 first years of therapy, but tended to level off afterwards. This was true only for children aged less than 16 years. Older patients did not show such a dramatic increase of BMD, a behaviour a little similar to what was observed in therapy of postmenopausal osteoporosis. We suggest weaning from therapy when BMD levels off, i.e. about after 4 years. After stopping therapy, some patients, but not all show a decrease in BMD. Resuming therapy induces a re-increase in BMD. The parameters of bone remodeling (NTX and CTX) decreased rapidly and dramatically after each series of 3 monthly infusions, re-increasing before the next infusions. However, a slow and progressive decrease was observed with elapsing time, more marked during the first 2 – 3 years and tending to plateauying afterwards. This image constitutes a mirror image of the gain in lumbar BMD. After stopping therapy, there is a trend to re-increase in the parameters of bone remodeling, decreasing again after recurrent therapy. The age of the patient before starting therapy, his puberty stage, the follow-up of BMD and of the parameters of bone remodeling should help the clinician to modulate the therapy and its duration.

I.22

Spine and limb surgery in OI pediatric patients

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O.I. is a constitutional disease, which can be dramatically worsened by secondary accidents: bone deformities, fractures and immobilisations will increase the osteoporosis and the severity of the pathology. Medical treatment and physiotherapy are very effective. Surgery is very useful and central medullary osteosynthesis is largely used. According to our experience, Bailey and Dubow telescopic nails are a very efficient solution to protect the femurs. It is now possible to perform osteotomies, realignment and nailing with minimal surgical approach or percutaneously. The main problem is to avoid a varus position of the femoral neck during the nailing.
For the humerus, the forearm and the tibia, telescopic wiring - generally done percuteaneously - is a simple, non-invasive and effective procedure.
In very severe forms extra diaphyseal wiring can be an elegant solution.

The onset of an important scoliosis or kyphoscoliosis is very frequent in severe forms of O.I.. Bracing is poorly efficient. The lack of spine growth, the shortness and deformity of the trunk induce a major respiratory insufficiency, which is the main cause of premature death in O.I. adult patients.
Vertebral posterior arthrodesis is an effective treatment. Surgery must be performed on young patients before the onset of an important and rigid deviation, especially in kyphosis, without taking bone maturity into account and when there is no more growth of the trunk.
A progressive halo-cranial reduction during the pre-operative period can induce an important correction of the angulations and can enable an improvement of the size of the trunk and of the respiratory functions.
Posterior arthrodesis gives stable results if it is done on a spine with mild deformities. Therefore, surgery must not be performed too late.

I.23

(Re)habilitation in OI in childhood

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Introduction: "Active now, healthy later" is also important in children with chronic conditions. I will present studies on exercise tolerance in children with chronic diseases (eg. spina bifida, juvenile idiopathic arthritis, leukemia, congenital heart disease) and focus on Osteogenesis Imperfecta

Objective: To study the effects of a physical training program on exercise capacity, muscle force, and subjective fatigue levels in patients with mild to moderate forms of osteogenesis imperfecta (OI).
Study design Thirty-four children with OI type I or IV were randomly assigned to either a 12-week graded exercise program or care as usual for 3 months. Exercise capacity and muscle force were studied; subjective fatigue, perceived competence, and health-related quality of life were secondary outcomes. All outcomes were measured at baseline (T 0), after intervention (T 1), and after 6 and 9 months (T 2 and T 3, respectively).

Results: After intervention (T 1), peak oxygen consumption (VO2peak), relative VO2peak (VO2peak/kg), maximal working capacity (Wmax), and muscle force were significantly improved (17%, 18%, 10%, and 12%, respectively) compared with control values. Subjective fatigue decreased borderline statistically significantly. Follow-up at T 2 showed a significant decrease of the improvements measured at T 1 of VO2peak, but VO2peak/kg, Wmax, and subjective fatigue showed no significant difference. At T 3, we found a further decrease of the gained improvements.
Conclusion: A supervised training program can improve aerobic capacity and muscle force and reduces levels of subjective fatigue in children with OI type I and IV in a safe and effective manner. Implications for clinical practice are provided.
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I.24

Diagnosis and treatment of the functional muscle-bone-unit in Osteogenesis Imperfecta

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Muscle force is the most important factor for the growing skeleton. The training of this system is often difficult in children with Osteogenesis Imperfecta due to the increased risk of fractures.
The Cologne Concept of functional physiotherapy is a new approach to combine different therapeutic strategies to strengthen muscles and bones and to improve mobility and independency in children and adolescent with Osteogenesis Imperfecta.
Intervention: The patients participated in an intensive multimodular rehabilitation program for 2 weeks at the beginning and for one week after 3 months. One important part is a Whole Body Vibration training with the side alternating platform Galileo®, conducted by the patients over a period of 6 months at home.
Methods:
31 patients (f=11; m=20; OI-type III = 14; OI-type IV =10; OI-type I =7) participated in this programme. 11 patients are still training, 20 patients completed training and 6 droped out. For quantifying changes during training we used DXA scans, the Brief Assessment of Motor Function and a modified Gross Motor Function Measurement Test.

	Changes during 6 months of training	p-value
Bone mass (whole body DXA) [n=11]	+ 14.15 %	< 0.003
Muscle mass (whole body DXA) [n=11]	+ 4.26 %	< 0.001
Brief assessment of motor function [n=13]	+ 13.8 %	0.0151
GMFM	+ 14.19 %	0.004

Conclusion: The Cologne concept of a functional training of the musle-bone system in children and adolescents including a whole body vibration training was beneficial for the participants and led to an increase in muscle- and bone mass. Additional the patients showed an improved mobility and became more independent.

I.25

Hearing loss in OI

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In population studies on OI including audiometric studies, hearing loss has been reported in up to 58% of the patients. Sometimes it begins in childhood, but the bilateral, progressive hearing loss most often starts in early adulthood. It is strongly age-related, and often proceeds from conductive to a mixed and sensorineural type with increasing age. Hearing loss affects patients with all types of OI. The characteristics of hearing loss are not correlated with clinical features of OI or the mutation.

Although hearing loss in OI is otosclerosis-like, these are two histologically, enzymatically and etiologically distinct entities. Hearing loss in OI has a tendency to earlier onset, more severe middle ear involvement, and a higher incidence of sensorineural hearing loss. Functional ossicular discontinuity is the main etiology for the conductive hearing loss in OI. Cochlear hair cell loss, stria vascularis atrophy and calcification, tectorial membrane distortion and perilymph hemorrhage have been suspected of accounting for sensorineural hearing loss.

Hearing loss in OI may be treated with hearing aid or stapes surgery. In severe deafness, cochlear implantation may be indicated.

The hearing loss in OI apparently is a result of multifactorial, yet unknown genetic and environmental effects. Misleading subjective assessment of hearing ability in adults with OI emphasizes the importance of regular audiometric studies in OI-patients. While early detection and treatment of hearing loss are of utmost importance to avoid aggravation of physical handicap, audiometry should be performed if a hearing deficit is suspected, and in asymptomatic OI patients at the age of 10 years with repeated audiograms every third year thereafter.

I.26

Management of hearing loss: Surgery (Incudostapedotomy and Malleostapedotomy) and outcome of the procedure

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Objectives: To provide additional data on the effects of stapes surgery in patients with Osteogenesis Imperfecta (OI) we present the audiometric results of 13 ears with primary surgery (incudo-stapedotomy) and 2 ears with revision surgery (malleo-stapedotomy), performed between 2000 and 2008. These results will be compared with results from the literature.

Methods: Twelve patients underwent mutation analysis to confirm the clinical diagnosis of OI. Intra-operative findings were recorded. Audiometric evaluations took place at regular intervals.

Results: In all patients a disease causing mutation in the *COL 1A1* gene has been identified.
Surgical findings in OI patients may involve atrophic, mobile stapes crura, fixation of the stapes footplate, thickened footplate, and hypervascularized or thickened middle ear mucosa.
The outcomes for hearing in the incudo-stapedotomized ears were good since the air-bone gaps at short-term follow-up were reduced in all cases. These results were maintained in the long-term, with exception of one ear in which progression of the sensorineural component occurred shortly after the operation.
Although initial success was noted in both ears with revision surgery, the air-bone gap relapsed to preoperative values within 12 months.

Conclusions: Stapes surgery is a reasonable alternative to amplificate hearing in OI patients even in the long-term. Hearing loss in OI is mostly of the mixed type and the sensorineural component in OI has been reported to be progressive. Stapedotomy may only be a part of hearing rehabilitation in OI to improve the hearing level and to facilitate the rehabilitation with a hearing aid in the short- or in the long-term. Since in 12 candidates for stapes surgery mutation could be located in the *COL1A1* gene, conductive hearing loss in OI caused by stapes fixation is possibly linked to a mutation in this gene.

I.27

Outcome of cochlear implantation in three patients with OI

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Osteogenesis Imperfecta (OI) is a heterogeneous disease of the connective tissue caused by a defective gene that is responsible for the production of collagen type I, leading to defective bone matrix and connective tissue. Hearing loss affects 35-60% of the patients and will progress to deafness in 2-11% of OI patients for whom cochlear implantation may become the only remaining treatment option.

Objective: to evaluate the outcome of cochlear implantation in three patients with OI.

Methods: Three patients with OI were retrieved from the Nijmegen Cochlear Implant Centre's database. Data concerning imaging, surgical findings and rehabilitation were noted. Furthermore, electrophysical measurements and pitch perception measurements were performed.

Results: Most of the specific observations in ear surgery on patients with OI, such as brittle scutum, sclerotic thickening of the cochlea, hyperplastic mucosa in the middle ear and persistent bleeding, were encountered in these 3 patients. In Case 3, with severe deformities on the CT scan, misplacement of the electrode array into the horizontal semicircular canal occurred. In all 3 cases, programming was hindered by non-auditory stimulation. Even after reimplantation, non-auditory sensations lead to Case 3 becoming a non-user. Averaged electrode voltages (AEVs) in Case 3 were deviant in accordance with an abnormally conductive otic capsule. Spatial spread of neural excitation responses in Cases 1 and 2 suggested intracochlear channel interaction for several electrodes, often in combination with facial nerve stimulation (FNS). In Case 1, the estimated pitch of the electrodes that caused FNS varied consistently. Despite the electrophysiological changes, after 1 year follow up, open set phoneme scores of 81% and 78% were reached in Cases 1 and 2, respectively.

Conclusions: When aware and prepared for the specific changes of the temporal bone in OI, cochlear implantation can be a safe and feasible procedure. Preoperative imaging is recommended to be fully informed on the morphology of the petrosal bone. In case of severe deformities on the CT scan, during counselling the possibility of misplacement should be mentioned. Rehabilitation is often hindered by FNS requiring frequent refitting.

I.28

The teeth in OI: diagnosis and prognosis

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People with Osteogenesis Imperfecta (OI), well-known as a heterogeneous connective tissue disorder manifestating brittle bones and caused by genetic defects of type I collagen, commonly show oral manifestations consequently having oral implications. Paralleling the phenotypic heterogeneity of OI, the oral manifestations attributable to OI vary widely with malocclusions and Dentinogenesis Imperfecta (DI) representing the classical dental findings. Each of the 4 accepted subtypes of OI may further be subdivided on the presence of DI. DI in association with OI is termed DI type I. However, there are 2 non-OI associated types of DI recognized (type II and III).

All three forms of DI present an amber-brown to blueish-grey hue to the teeth with various degrees of transparence, cracking/loss of enamel and attrition. The radiographic features of DI types I and II are largely similar, exhibiting bulbous tooth crowns, distinct cervical constrictions, short roots, obliteration of the pulp chamber, and periradicular radiolucencies. DI type I affects both the primary dentition as well as the permanent dentition.

Receiving appropriate dental care is an important element contributing to the patient's overall health and sense of well-being. Therapeutic strategies to preserve oral function, vertical tooth dimension, and normal facial growth and esthetics should be instituted as early as possible – this is, already in the primary dentition. Providing full-coverage tooth restorations or overdentures may be necessary to achieve these goals. Selective orthodontic and orthognatic therapy may be beneficial in limiting malocclusion. Although the overall risk of maxillofacial/alveolar fracture appears to be low, all dental care should be provided in a very cautious and measured way.

I.29

Stem cell therapy in children with OI

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I.30

Scientific, Medical and Ethical Prospects for Novel Therapeutics in OI

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Parental mosaicism and type I OI provide lessons from nature on approaches to decrease the severity of OI. First, cell transplantation of normal osteoblast precursors mimics parental mosaics who have both normal and mutant osteoblast populations.
A second approach is suppression of mutant transcripts coding for alpha chains harboring a structural abnormality, by hammerhead ribozymes or siRNA; this mimics type I OI, in which a null or partial null collagen allele results in mild OI. Ribozyme specificity and efficiency is excellent *in vitro* and in stably transfected cells. We recently piloted hammerhead ribozymes *in vivo* in the Brtl OI mouse. We delivered ribozyme targeted to the mutant allele by mating Brtl with a transgenic mouse expressing RZ using the tet-off system. We found that female Brtl/Ribo/Tet vs Brtl/wt/wt mice have significantly greater body and femur length, femur and spine BMD at 2 month of age. Biomechanical and uCT studies are pending.
A third approach focuses on decreasing the ER stress in OI osteoblasts, as evidenced by increased CHOP/GADD153 in Brtl vs wt bone tissue. To decrease ER stress, we provided a chemical chaperone, sodium pheylbutyrate (SPB). Brtl and control mice were fed a diet containing 7 mg/gm SPB (PCI Synthesis, UK) *ad lib* from conception to age 2 months. Perinatal lethality was inconsistently decreased in SPB-fed Brtl mice, with some severely runted Brtl pups surviving several days after birth. Brtl mice fed SPB had fewer rib and upper extremity fractures at 14 days of age. Two-month-old Brtl males and females fed SPB-chow did not have increased body or femur length, femur or spine BMD vs. Brtl fed regular chow. Biochemical and uCT studies of femora are pending.
We explore the ethical considerations raised by application of these potential therapeutic approaches to children with OI.

ABSTRACTS OF ORAL PRESENTATIONS

O.1

Biochemical markers of bone trunover in children and adolescents with osteogenesis imperfecta

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Objectives: This prospective study was designed to investigate if biochemical markers of bone turnover could predict vertebral compression fractures in children with milder forms of OI, and assess their role in monitoring bisphosphonate treatment.

Methods: Bone mineral density (BMD) of the lumbar spine was measured by Dual-energy X-ray absorptiometry (DXA). Serum total alkaline phosphatase (ALP), bone ALP isoforms (in a subgroup), osteocalcin, type I procollagen carboxy-terminal propeptide, carboxy-terminal telopeptide of type I collagen and urine deoxypyridinoline were measured in 130 untreated children and adolescents, 0.25 to 20.9 years (median 6.7), with different types of OI. Sixty-nine of those were then at the median age of 8.3 years given monthly intravenous infusions of pamidronate. Effects on mobility and pain were assessed.

Results: In the larger untreated group some significant differences in bone markers were found between the OI types, but the variability within each type was substantial. No differences were observed between age-, type-, and mobility-matched subgroups of untreated children with mild form with and without vertebral compression fractures. All markers indicated decreased bone turnover during 1.0-12.5 years (median 4.3) treatment. ALP and deoxypyridoline changed most. Changes were not correlated to the improvement in BMD, mobility or pain.

Conclusions: Markers of bone turnover cannot at the individual level predict vertebral compression fractures, response in BMD, mobility or pain after treatment. Despite significant differences observed between OI types, bone markers are not clinically useful for classification. Serum ALP and urinary deoxypyridinoline appears to be sensitive in monitoring bisphosphonate treatment.

O.2

Alendronate effects on fracture healing in growing BRTL mouse model of osteogenesis imperfecta

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Objectives: This study was designed to test effects on fracture repair of subcutaneous alendronate injections either before fracture or through fracture healing in a growing Brtl IV mouse model of OI.

Methods: Two week-old male Brtl/+ and wild-type (WT) mice were assigned to groups which received no alendronate, weekly alendronate injections before fracture, or weekly alendronate injections before and after fracture. All treated mice received 6 weeks of alendronate at 0.219 microg/g prior to fracture. At 8 wks of age, one tibia underwent closed guillotine fracture and was allowed to heal for 1, 2, 3, or 5 wks. Tibiae were extracted, analyzed by microCT, and either mechanically tested in torsion or submitted for histology. Contralateral intact tibiae were mechanically tested.

Results: At 10-13 weeks of age, intact tibiae from Brtl mice have decreased energy to failure in torsion testing, compared to WT. Tibiae from both Brtl and WT mice treated before after fracture required more energy to break than did tibiae from untreated mice or mice treated only before fracture. Mice treated before and after fracture also had more bone in callus than untreated or treated-before-fracture groups. The torque at failure was higher in the group treated before and after fracture than in untreated mice. In the residual cortical bone, the tissue mineral density was highest when treatment was halted at the time of fracture. No statistically significant differences were detectable in fracture calluses of Brtl mice compared to WT counterparts.

Conclusions: Weekly subcutaneous injections of alendronate in growing Brtl mice led to increased bone in the fracture callus, which increased energy to failure of the callus in torsion testing.

O.3

CRTAP mutations in lethal and severe osteogenesis imperfecta: the importance of combining biochemical and molecular genetic analysis

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Autosomal recessive lethal or severe Osteogenesis Imperfecta (OI) is currently known to be caused by deficiency of Cartilage Associated Protein (CRTAP) and prolyl-3-hydroxylase due to respectively CRTAP and LEPRE1 mutations.
We describe five families in which 10 probands were diagnosed with lethal or severe OI. In three families consanguinity and/or recurrence was evident. All probands showed posttranslational overmodification on biochemical testing and mutation analysis of the COL1A1 and COL1A2 genes responsible for autosomal dominant OI was negative. However, molecular analysis of the CRTAP and LEPRE1 genes identified CRTAP mutations not previously described.
In literature certain differences have been described between autosomal dominant OI caused by mutations in the collagen type 1 genes and autosomal recessive OI caused by CRTAP and LEPRE1 mutations on physical and radiological examination. In the families described here these differences proved to be too subtle to diffentiate between autosomal dominant and autosomal recessive OI. In addition, autosomal dominant and autosomal recessive OI show similar posttranslational overmodification of collagen type 1 on electrophoresis.
In conclusion, in our families differentiation between autosomal dominant and autosomal recessive OI based on clinical, radiological and biochemical investigations proves difficult. Biochemical testing should therefore always be combined with molecular genetic analysis of the collagen type I genes. If no mutations in the collagen type 1 genes are found, mutation analysis of CRTAP and LEPRE1 genes should follow. This approach will facilitate identification of the genetic cause of lethal or severe OI, which is important in estimating recurrence risk in affected families.

O.4

Recessive Osteogenesis Imperfecta caused by *LEPRE1* mutations: clinical documentation and identification of the splice form responsible for prolyl 3-hydroxylation

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Recessive forms of Osteogenesis Imperfecta (OI) may be caused by mutations in *LEPRE1*, encoding prolyl 3-hydroxylase-1 (P3H1) or in *CRTAP*, encoding cartilage-associated protein. These proteins constitute together with cyclophilin B (CyPB) the prolyl 3-hydroxylation complex that hydroxylates the Pro986 residue in both the type I and type II collagen α1-chains. We screened *LEPRE1*, *CRTAP* and *PPIB* (encoding CyPB) in a European/Middle Eastern cohort of 20 lethal/severe OI patients without a type I collagen mutation. Four novel homozygous and compound heterozygous mutations were identified in *LEPRE1* in 4 probands. Two probands survived the neonatal period, including one patient, who is the eldest reported patient ($17^{7/12}$ y) so far with P3H1 deficiency. At birth, clinical and radiologic features were hardly distinguishable from those in patients with autosomal dominant (AD) severe/lethal OI. Follow-up data reveal that the longer-lived patients develop a severe osteochondrodysplasia that overlaps with, but has some distinctive features from AD OI. A new splice site mutation was identified in two of the four probands, affecting only one of three *LEPRE1* mRNA splice forms, detected in this study. The affected splice form encodes a 736 amino acid (AA) protein with a "KDEL" endoplasmic reticulum retention signal. While western blotting and immunocytochemical analysis of fibroblast cultures revealed absence of this P3H1 protein, mass spectrometry and SDS-urea-PAGE data showed severe reduction of α1(I)Pro986 3-hydroxylation and overmodification of type I (pro)collagen chains in skin fibroblasts of the patients. These findings suggest that the 3-hydroxylation function of P3H1 is restricted to the 736AA splice form.

O.5

Increased dimensions of left ventricle and aorta in adult patients with osteogenesis imperfecta

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Introduction: Since connective tissue is an important part of the heart, we wanted to investigate left ventricle (LV) dimensions and aorta diameter in patients with osteogenesis imperfecta (OI).

Methods: 99 patients, recruited into "The Norwegian Survey of Adults with Osteogenesis Imperfecta" were included and compared to 82 healthy individuals. LV end-diastolic dimensions and walls were measured by standard echocardiography. Sinus Vasalva (2D) was chosen as measure for aorta diameter. All these cardiac measurements were standardized by dividing with body surface area (BSA).

Results: LV end-diastolic cavity, LV septum and posterior walls were significantly larger in the OI patients than in the control group 2.98 ± 0.64 vs. 2.60 ± 0.29 cm/m^2 ($p<0.001$), 0.65 ± 0.23 vs. 0.48 ± 0.08 cm/m^2 ($p<0.001$) and 0.57 ± 0.18 vs. 0.44 ± 0.07 cm/m^2 ($p<0.001$), respectively. Similarly, aorta was increased in the OI group, 1.86 ± 0.51 cm/m^2 vs. 1.56 ± 0.19cm/m^2 ($p<0.001$). BSA in the OI patients was significantly smaller, 1.65 ± 0.3 vs. 1.91 ± 0.2 cm/m^2 (<0.001), while systolic and diastolic blood pressures were increased in the OI group as compared to the controls, 129.7 ± 19.4 vs.122.4 ± 12.4 mmHg ($p< 0.05$) and 82.2 ± 8.5 vs. 77.4 ± 8.1 mmHg ($p<0.001$), respectively.

Conclusions: Our study showed for the first time increased LV dimensions in adult patients with OI as compared to controls. These findings are probably due to the underlying connective tissue disease. The increased LV thickness is probably associated with higher blood pressures in the OI group.

O.6

How do adults with osteogenesis imperfecta manage in daily life? A population based study

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Objectives: One of the goals for patients with OI is to achieve relative independence in activities of daily living.The aim of this study was in adults with OI to assess the ability to perform activities in daily life (ADL) and to explore how ADL might vary as a consequence of the severity of the disease.

Methods: All adult OI patients, above 25 years of age (n=154) registered in Norway were invited, and 94 (61 %) (age range 25-83) attended the ADL-test. Classified according to Sillence 75 persons presented OI type I, 8 type III and 11 type IV.

To assess the ability to perform ADL, we used the Sunnaas ADL Index which contains ratings of 12 daily activities (including personal and instrumental activities).

Results: The ADL scores were found to vary across groups, both in terms of OI type and of the presence of deformities. Significant differences were found between type III and both type I and IV, in all ADL scales. Between type I and type IV, no significant differences were found. ADL scores for OI patients with deformities in the legs differed significantly from those without. Only 5 participants had a total ADL-score of ≤ 20 / 36. We did not identify special activity problems in the different ADL sub – indexes.

Conclusions: The results showed that most persons with OI are able to live their lives independently without organized assistance in the activities tested for.

O.7

Allele specific silencing of collagen I Alpha 2 in primary human bone cells using RNAi – a step toward gene therapy in OI

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Introduction: Recent studies have reported successful allele specific gene silencing by using small interfering RNA (siRNA) that discriminate between polymorphisms in mRNAs. Subsequently, siRNAs are interesting to explore as therapeutics in dominant monogenic disorders, such as Osteogenesis Imperfecta (OI).

Methods: Primary human bone cells heterozygous for a C/T polymorphism (SNP) in exon 6 of COL1A2 (rs1800222) were transfected with seven separate tiled siRNAs perfectly complementary to the T-allele, or with negative control siRNAs, using Magnet Assisted Transfection. Allele specific expression of COL1A2 mRNA was determined by quantitative sequencing of cDNA for the silenced C/T SNP and overall COL1A2 abundance was quantified by real-time PCR.

Results: Cells transfected with negative control siRNAs exhibited equal expression of the two alleles of rs1800222. siRNA3 induced significant allelic imbalance of COL1A2 and this siRNA was therefore further analyzed in three concentrations. The most optimal concentration rendered a T to C allele ratio of 34%. These results were verified by analysis of another heterozygous SNP in exon 25. COL1A2 expression was decreased by 72% of which 75% could be attributed to silencing of the targeted allele.

Conclusion: We show successful allele specific silencing of COL1A2 in primary human bone cells using siRNAs. The future aim is to design siRNAs that target mutated collagen alleles in fibroblasts cultured from patients suffering from OI, with the ultimate aim being siRNAs as gene therapy in patients suffering from OI.

O.8

Study on the Effect of Growth Hormone in Combination with Bisphosphonate Treatment on Bone Metabolism in Osteogenesis Imperfecta

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Introduction: In a randomized controlled 1-year clinical trial, we studied if the association of Growth Hormone (GH) with the ongoing bisphosphonate therapy can improve bone metabolism in patients suffering from Osteogenesis Imperfecta (OI).

Materials and Methods: after a 12 months observational period of Neridronate treatment, thirty prepuberal children suffering form OI (type I, IV and III) were studied. The patients, molecularly completely characterized in both type I collagen genes, were randomized into two groups comparable regarding sex, age, height, and clinical OI severity; fifteen patients were treated for 12 months with GH and Neridronate (Group A) and fifteen continued Neridronate alone (Group B). Auxological ad clinical parameters, number of fractures, calcium and other nutrients intake, physical activity and clinical symptoms related to the disease were evaluated every 3 months. Bone metabolic parameters and bone mass measurements were evaluated 12 months.

Results: Growth velocity was significantly higher during GH treatment in group A. BA did not advance faster than chronologic age in group A and B. The number of fractures did not increase in both groups. Bone DXA at the lumbar spine (L2-L4) and at Distal Radius showed a significant increase in Group A. Patients with quantitative defects, on the basis of mutations effect on type I collagen syntesis, had the best response to GH treatment in terms of growth velocity, bone DXA at the lumbar spine and at the radius.

Conclusions: The combined GH - bisphosphonate treatment, coupling the GH stimulation of bone apposition with the bisphosphonates inhibition of bone resorption, may give better long term results than bisphosphonate treatment alone in OI patients, particularly in patients with quantitative collagen synthesis defects, but also in patients with qualitative defects.

O.9

Current Evaluation of the Response of OI Adults Treated with Bisphosphonates

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This report summarizes the DXA response of 90 OI adults (60 males and 30 females, 39.5 ± 11 yrs) treated with IV and oral bisphosphonates: 63 OI type I, 15 type III, 12 type IV patients. Treatments included pamidronate, 1.5 mg/kg per infusion (max. 60 mg) q 3 months and oral agents weekly. There were 35 non-treatment controls, 28 patients received pamidronate, 17 alendronate and 10 residronate. A minimum of 18 months observation is reported.
Linear rates of change per year adjusted for age and sex across OI types are as follows. For L1-L4, the annual rate of change for controls was – 0.00036 gm/cm2 (p=0.92), for pamidronate, 0.0106 gm/cm2 (p< 0.001) and for oral bisphosphonates, 0.0038 gm/cm2 (p=0.046). For total hip BMD, the annual rate of change for controls was -0.005 gm/cm2 (p= 0.19), pamidronate, 0.0066 gm/cm2 (p=0.44), for oral bisphosphonates 0.0059 gm/cm2 (p=0.007). At femur neck controls rate of change was -0.0087 gm/cm2 (p=0.06), pamidronate -0.0033 gm/cm2 (p=0.44), for oral bisphosphonates (0.000043 gm/cm2 (p=0.99).
Conclusion: Considering rates of change by OI type, pamidronate showed statistically significant rates of change in Type I for L1-L4: 0.006 gm/cm2 (p=0.03) and OI Type III/IV for L1-L4: 0.016 gm/cm2 (p<0.001) and total hip: 0.011 gm/cm2 (p=0.046). The oral biphosphonates showed statistically significant rates of change in OI Type I subjects for L1-L4: 0.004 gm/cm2 (p=0.47) and total hip, 0.006 gm/cm2 (p=0.003). Femur neck BMD was unresponsive to any treatment. Biomarker response, duration of response and effect on fracture incidence will be presented.

O.10

Factors influencing hearing loss in OI

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Hearing impairment is a common symptom in OI, but its prevalence is highly variable, depending on criteria for referring patients and on still-unknown factors. This study was designed to determine prevalence of various types of HL (conductive, sensorineural and combined) in a random sample of patients with OI, not selected for hearing loss, and to find out factors that might influence hearing loss type and progression in OI.
Our study involved 61 patients affected by OI, 36 men and 25 women, aged 20 to 66. They underwent air and bone conduction pure-tone audiometry and speech audiometry. Tympanometry with acoustic reflex thresholds was performed to detect stapes footplate ankylosis. Age at stapes surgery was also considered in operated patients. Hearing parameters were compared to other clinical features (age, gender, sclera colour, OI type, family history, fractures rate, bone density, collagen gene mutations).
Prevalence of hearing impairment was 59% (44% being bilateral). 13% of patients had an asymptomatic acoustic reflex abnormalities. Only 28% of our population had normal results.
All patients with ascertained type I had at least one hearing anomaly. Sensorineural and combined hearing loss were the most frequent; only 6 ears were affected by pure conductive hearing loss. No link between gender, sclera colour, family history, fractures rate, or collagen gene mutations, and hearing was found. But there was a significant correlation between hearing thresholds and calcaneum ultrasound attenuation, and patients with stapes ankylosis had a significantly lower lumbar vertebral density.
As a conclusion, hearing impairment can be detected in OI much before patients are aware of it. OI type and bone quality explain a significant part of hearing loss variability.

O.11

Dental age of children with Osteogenesis imperfecta

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Introduction: Assessment of dental age is one method to estimate the biological developmental status of a young individual. Our purpose was to compare the dental age with the chronological age in children with different Osteogenesis imperfecta (OI) types.

Materials and methods: Dental ages of 21 Finnish children with OI were assessed from panoramic dental radiographs by so-called Demirjian's method using scores calculated for Finns. The OI type was IA in nine, type IB in four, type III in three, and type IVB in four patients, and in one it remained unclassified. The patients' decimal ages ranged from 2.99 to 13.75 years.

Results: Within type I OI, 77% displayed advanced dental age, but the proportion was smaller in type IB than in type IA subgroup. In type III, dental age was always delayed compared with chronological age. In type IVB, dental age was closest to the chronological age. Thus, the order of group means was: type IA, type IB, type IVB, and type III from highest to lowest dental age. The maximum differences between dental age and chronological age were +3.2 years in a boy with type IA, and -0.7 years in a boy with type III OI.

Conclusion: Dental age appears to be associated with general severity of the disease, type IA OI leading to the largest increase and type III OI to the largest decrease in the rate of dental development. It is of notable interest if these findings in OI children would correlate with other parameters of their biological maturity.

ABSTRACTS OF POSTER PRESENTATIONS

P.1

The sagittal balance of the spine in children and adolescents with Osteogenesis Imperfecta

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In osteogenesis imperfecta, compression fractures of the spine could be responsible for an increased thoracic kyphosis or a diminished lumbar lordosis. Theses changes in sagittal shapes of the trunk could be responsible for a global sagittal trunk imbalance. We compare the parameters of sagittal spinopelvic balance in young patients with OI to those parameters in a control group of healthy volunteers.

Methods: Eighteen patients with osteogenesis imperfecta were compared to a cohort of 300 healthy volunteers. A standing lateral radiograph of the spine was obtained in a standardized fashion.
The sacral slope, pelvic tilt, pelvic incidence, lumbar lordosis, thoracic kyphosis, T1 and T9 sagittal offset were measured using a computer assisted method.

Results: Comparison between OI patients and control group showed an increased T1T12 kyphosis in OI patients. T1 and T9 sagittal offset was positive in OI patients and negative in control group. This difference indicated that OI patients had a sagittal balance of the trunk displaced anteriorly compared to the normal population. Reciprocal correlations between angular parameters in OI patients showed a strong correlation between lumbar lordosis (L1L5 and L1S1) and sacral slope.

Conclusions: In OI patients, the T1T12 thoracic kyphosis was statistically higher than in control group and was not correlated with other shape (LL) or pelvic (SS, PT or PI) parameters. Because isolated T1T12 kyphosis increase without T4T12 significant modification, we suggest that vertebral deformations worsen in OI patients at the upper part of thoracic spine. Further studies are needed to precise the exact location of most frequent vertebral deformities.

P.2

Reliability of basilar pathology diagnosis on lateral skull radiographs

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Objectives: To explore how reliably one can identify in lateral skull radiographs of unaffected individuals anatomical landmarks that are used to diagnose pathological relationships in the craniovertebral junction for instance in patients with Osteogenesis imperfecta.

Methods: Randomly selected 20 lateral radiographs from Helsinki longitudinal growth study were separately analyzed and re-analyzed by two examiners. Both located 7 cephalometric points based on which 5 measurements were implemented. Similarly, three radiographs were analysed by 11 examiners. The differences of results were compared to assess the inter- and intra-examiner errors.

Results: Of the points defining the cranial base angle (to diagnose platybasia), nasion and sella were easier to locate than basion, which also marks anteriorly the foramen magnum line. Of the other points used to draw horizontal reference lines for detection of basilar impression and invagination, posterior nasal spine was easily found, whereas the lowermost point on the occiput, and opisthion, the posterior limit of foramen magnum, were less reliably located. Because they were distributed along horizontal anatomical structures, the variation in landmark location had little clinical significance on McGregor, Chamberlain and McRae values as well as on D-M distance, as long as dens point was vertically stable. Location of dens, the uppermost point of processus odontoideus, showed, indeed, little variation in the vertical direction.

Conclusions: Cephalometric analysis from traditional lateral skull radiographs is applicable in evaluating relationships between cranial base structures and cervical vertebrae as an initial screening method of pathology in the craniovertebral junction area.

P.3

Craniofacial and malocclusion traits in patients with different types of osteogenesis imperfecta

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Craniofacial and occlusion abnormalities have mainly been reported in patients with osteogenesis imperfecta (OI) type III. We have, however, observed severe malocclusions and a great variation in the craniofacial development in all types of OI. The aims were therefore to analyze these factors.

Methods: The degree of handicapping malocclusions were evaluated clinically in 51 patients with OI type I, 12 with type III and 24 with type IV (age 5-20, mean 11.8). The handicap was ranged from none (0) to extremely severe (4). A total of 76 cephalometric radiographs were analyzed. Twelve cephalometric landmarks and 11 variables were measured. Controls for comparison were a longitudinal population study of 136 healthy individuals.

Result: Severe malocclusions were found in 40% of the patients with OI type I, even in those with a mild form and normal thorax configuration, and in type III and IV in 92% and 58% respectively. The cephalometric analyses revealed that the maxilla (SNA), and the mandible (SNB), were significantly smaller, the mandible more posterior rotated (SN-ML), and the cranial base angle larger (SN-Ba) in the OI group than in the controls. The most significant differences were found between OI type I and III. Significant differences were also found between OI type IV and III.

Conclusion: OI patients type III had the most severe malocclusions and most deviating craniofacial development, but severe deviations were found also in the other types of OI. It is therefore recommended that well-trained dentists are included in an OI team.

P.4

No osteonecrosis in jaws of young patients with osteogenesis imperfecta treated with bisphosphonates

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Objectives: Several alarming reports have been published of unusual cases of distressingly painful exposure of bone in the jaws in patients using bisphosphonates. The clinical picture resembles the occupational disorder "fossy jaws" caused by exposure to white phosphorus during the manufacture of matches. This form of bone exposure has been termed osteonecrosis of the jaw (ONJ), because it has thus far only been reported in the jaws. As treatment with bisphosphonates has become an important symptomatic therapy in patients with osteogenesis imperfecta (OI) we found it important to investigate whether healing after surgical exposure of jaw bone was influenced by this treatment in our group of patients with OI.

Methods: Patients treated with intravenous infusions with pamidronate for 6 months or more were included (n=64). All data concerning the bisphosphonate administration were analysed. In 22 of these patients, 38 dental surgery procedures were performed after 0.03-7.9 years of treatment (mean 3.6). All patients had been examined clinically every 6 months by a dentist and/or a physician. The oral radiographs, which had been taken in all but two children who were younger than 3 years of age, were revised.

Results: Despite long-term intravenous monthly pamidronate treatment, none of the 64 patients had any clinical signs of ONJ.

Conclusions: The risk of ONJ in patients with OI must be considered so low that the patients with indications for treatment should be treated and get the chance to experience the well-documented beneficial effect for children with severe OI.

P.5

Randomized dose comparison of pamidronate in children with types III and IV osteogenesis imperfecta: 3 vs 6 month cycles

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AIM To determine whether the vertebral benefits of q3m infusion cycles can be attained on q6m cycles, with a lower cumulative dose.

Methods: Twenty-seven children with types III and IV OI were randomly assigned to receive 1mg/kg/3d IV pamidronate in q3 or q6 month cycles. All patients had spine radiographs, L1-L4 DXA, and musculoskeletal and function testing.

Results: L1-L4 DEXA increased significantly after 1 year of q3m cycles, with average change in z-score =+1.41 SD, but did not improve significantly with further treatment. In the q6m group, the average change in DEXA was not significant. Repeated measures analysis of DEXA z-scores yielded a z-score rate change of 0.064 SD/m for q3 vs 0.036 SD/m for q6 group (p=0.13). T12-L4 vertebral area and central height were determined from radiographs. Repeated measures analysis revealed significant improvement of q6m group average LI-L4 and T12-L2 vertebral height (p=0.05, 0.01) and area (p=0.002, 0.006). The rate of improvement of the q3m and q6m groups did not differ for L1-L4 area or height (p=0.52, 0.86) or T12-L2 area or height (p=0.28, 0.77). The OI children had no significant improvement in fracture incidence, manual muscle testing or BAMF motor scores in either group. Noteworthy, response to treatment was highly variable in each treatment group; improvement in vertebral area did not correlate with change in DEXA z-score.

Conclusions: Equivalent gains in vertebral height and area are obtained with q6m and q3m pamidronate cycles. For individual OI children, gain in DEXA does not correlate with extent of vertebral response.

P.6

Screening for molecular testing of the col1a1 and col1a2 genes in patients affected by osteogenesis imperfecta with an optimized dhplc protocol

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Objectives: Osteogenesis Imperfecta (OI) is a genetic disorder of connective tissue characterized by increased bone fragility and low bone mass.
Seven types of OI were distinguished by clinical and molecular findings, generating a wide range in severity; in particular, types I-IV are caused by a mutation in one of the two genes coding for the type I collagen protein.
We perform a pilot study by analysing Col1 genes in patients OI or suspected OI with clinical features ranging from types I-IV.

Methods: We propose an optimized and validated denaturing high-performance liquid chromatography (DHPLC)-based protocol for screening of all Col1A1 and Col1A2 coding exons in patients affected by OI.
The 51 exons of Col1A1 and the 52 exons of Col1A2, along with exon-intron junctions, were PCR amplified using specific primer pairs and analysed with DHPLC method.

Results: Any amplification product showing an abnormal elution profile were analysed with direct sequencing and under the optimised DHPLC conditions, all mutations were detected.
In some cases mutation analyses confirmed a clinical suspect (25%), while in cases with defined phenotype we found a mutation in 75% of patients.
The large number of mutation hits Col1A1 (75%) and the most frequent types are missense and frameshift mutations.
We will analyse negative cases also for big insertions and deletions and, if the phenotype is consistent, for recessive forms.

Conclusions: This protocol is a sensitive and expensive alternative for direct sequencing analysis of the Col1 genes.

P.7

Percieved activity level in a group of children with osteogenesis imperfecta type I and IV

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Objectives: Swedish children with Osteogenesis Imperfecta (OI) are generally referred to the national OI-team at the Astrid Lindgrens Childrens Hospital, Stockholm. The children will undergo medical examinations but are also assessed by a physio therapist and occupational therapist. We have noticed that children with mild OI often report activity level problems despite normal Paediatric Evaluation of Disability Inventory (PEDI) ratings. We aimed to find a more sensitive instrument.

Methods: In order to find out how school aged children with milder forms of OI perceive their activity level the occupational therapist (1) chose to use the Activity Scale for Kids (ASK), a self reporting questionnaire developed for children with muscular skeletal impairments and focuses on motor activities and ambulation. ASK has not jet been properly translated to Swedish so the purpose was also to find out if ASK could be an effective and client centred instrument for evaluating self perceived activity performance. Results: The great majority had some problems in daily activities. Most common difficulties were with: Running speed, keeping up with peers and being able to participate in sports, especially team related sports. Only a few of the children with OI typ I reported no limitations at all.

Conclusions: The major part of children with the milder forms of OI experiences some level of dysfunction in activity and participation. ASK seems to be sensitive for minor hindrance in the activity level and are therefore a feasible instrument for children with milder forms of Osteogenesis Imperfecta.

P.11

Long-term safety and efficacy of i.v. zoledronic acid (ZOL) in children with severe osteogenesis imperfecta (OI): 1-yr, open-label extension study

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Objectives: This 1-yr extension study was designed to assess the continued long-term safety and efficacy of ZOL in severe OI patients following quarterly ZOL or PAM (pamidronate) infusions for 12 months.

Methods: Severe OI patients who had completed 1-yr treatment in a core study (n=103) were randomized to either once-yearly or twice-yearly 0.05 mg/kg i.v. ZOL infusions (0.025 mg/kg if ≤3 yrs). Patients were analyzed based on their core treatment assignment (ZOL or PAM strata).

Results: The median percentage increase from core baseline in lumbar spine bone mineral density for once yearly and twice yearly infusions was 56.7% and 50.7% respectively in ZOL stratum vs. 43.4% and 44.3% respectively in PAM stratum. The incidence of clinical fractures in the extension period was 30.8% (16/52) in ZOL and 41.2% (21/51) in PAM strata. The incidence of femur and tibia fractures in the extension study (14.6% [15/103] and 13.6% [14/103] respectively), was not higher than in the core study (17.8% [27/152] and 9.2% [14/152] respectively). Majority of the adverse events (AEs) were mild or moderate in severity and not related to study drug. The incidence of serious AEs in the extension study was lower compared to the core study (18.4% vs. 25.7%). No long-term negative effect of ZOL was observed on renal function.

Conclusion: The efficacy of both once-yearly and twice-yearly i.v. ZOL infusions in the extension study was similar and consistent with the core study. No excess risk of fractures was observed during the extension period compared to the core study.

P.12

The spectrum of collagen I mutations causing osteogenesis imperfecta in Sweden

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Objective: Over 800 mutations causing Osteogenesis Imperfecta (OI) have been described in the genes encoding collagen type I. As COL1A1 and COL1A2 are large genes, there are still many codon positions where no mutations have been reported and only 10% of theoretically possible glycine substitutions have been described. The spectrum of mutations causing OI in Sweden has not previously been investigated.

Method: Exons and flanking intron sequences of COL1A1 and COL1A2 were sequenced in 28 unrelated OI-patients.

Results: In 23 of the 28 families a mutation was identified: nine of these were not previously reported, 14 were known to cause OI and in five patients no mutation was found. Fifteen mutations were located in COL1A1 and eight in COL1A2. Thirteen patients had a glycine substitution, while eight mutations were insertions, deletions or mutations leading to a premature stop codon. There was one non-glycine amino acid substitution and one mutation expected to cause a splicing defect. Three families were carriers of two separate mutations. However, only one of the mutations was of a typical OI-type. Two of the mutations were present in two separate families, not known to be relatives.

Conclusion: The spectrum of mutations causing OI described in this Swedish cohort is of the expected type, with the exception the non-glycine substitution. It is notable that in three patients two separate mutations were identified. It is unclear if both mutations influence the patient's phenotype. This study further illuminates the spectrum of OI-causing mutations and possible phenotypical outcomes.

P.13

Function and Deformities of Upper Extremity in Patients with Osteogenesis Imperfecta

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Objective: The aim of this study was to evaluate upper extremities' function and possible deformities of the patients with OI treated in our Department.

Methods: We retrospectively analyzed data of all patients that were surgically treated between 1981 and 2007. In addition, we have acquired physical examination (ROM, muscle strength, clinical signs of bone and joint deformities) and analyzed radiographs from 8 out of 19 operative treated patients called (response rate 42%). We used 'Capabilities of Upper Extremity' scale for the evaluation of the upper extremity function.

Results: All together, we performed 82 operations (37 primary operations and 45 reoperations) in patients with OI, of which 7 were procedures on upper extremity (8.5%, 3 primary operations and 4 reoperations). In the group of patients that underwent clinical examination, 5 out of 8 patients had a history of upper extremity fracture and only one of them underwent surgical procedure. Four patients had clinical and radiological sings of bone deformity of upper extremity. Limitation of shoulder and elbow ROM did not impair its function. 'Capabilities of Upper Extremity' questionnaire showed that our patients have very good function of upper extremities (between 94% and 100%).

Conclusion: The function of upper extremities remained very good and showed almost no variation in comparison to standard population. Patients with affection of upper extremities usually had one bone or joint affected, and those patients who had more than one bone or joint affected had mild deformities and no significant reduction of upper extremity function.

P.14

Pamidronate treatment of osteogenesis imperfecta in Croatia

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Objectives: Severe osteogenesis imperfecta (OI) is a hereditary disorder characterised by increased bone fragility and progressive bone deformity. Secondary osteoporosis is an important feature of OI. So far, no effective medical treatment is available. Antiresorptive activity of the aminobisphosphonates may improve clinical outcome in children.
We assessed the clinical impact of the administration of bisphosphonates in Croatian OI patients.

Methods: we report results of 1- 6 years treatment with intravenous pamidronate (APD) in 20 children (10 girls) with OI, age 3 months –14 years at entry. Pamidronate was administered in cycles as monthly infusions at a daily dose of 1-1,5 mg/kg during 6 months following pause for three months, or the same dose for three days every four months (mean [±SD] 6.8±2.5 mg/kg/year).

Results: During treatment dexa measurements showed a gradual increase in bone mineral density in all patients and Z score improved – 4.1±2.4 to –1.6±1.5. Number of confirmed fractures decreased in all (3.5±5.2/year to 0.8±1.1/year). The reduction in pain and improvement in well-being and mobility were observed. Well-known acute phase reactions were noted during first infusion cycle in two children and asymptomatic hypocalcemia in three children. During the treatment three children gained disproportionate weight compared to their height.

Conclusion: Although bisphosphonates do not correct basic abnormalities in OI, they significantly alter the natural course of the disease, increased bone mineral density and improve patients' quality of life.

P.15

Inhibition of adrenergic signaling in Brtl OI mouse by pharmacologic beta-blockade or genetic disruption (Adrb2-/-) improves bone and growth parameters

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Background: The sympathetic nervous system plays a key role in regulating endochondral bone formation and bone remodeling through beta2-adrenergic receptors (beta2-AR) expressed on osteoblasts and chondrocytes. Beta2-AR-deficient mice exhibit a high bone mass phenotype, as do normal and ovariectomized female mice treated with beta-blockers.

Objective: We examined the consequences of both genetic ablation and pharmacological inhibition of the beta2-AR on bone and growth parameters in the Brtl OI mouse.

Methods: Mice homozygous for a mutation that eliminates the expression of beta2-AR (Adrb2-/-) were crossed with Brtl, which is heterozygous for a col1a1 G349C substitution; 2-month G4 (F0 X Brtl) mice were analyzed. Propranolol, a non-selective beta-AR antagonist, was administered daily i.p. (20ug/g/day) for 5 weeks (age 3-8 weeks). Bone and tissue parameters were monitored using the Lunar PIXImus DXA scanner.

Results: Propranolol treatment resulted in a 6.1% increase ($p=0.025$; n=11 males) in femoral BMD compared to 2-month untreated Brtl mice, but without a significant change in body weight or femur length. Beta2-AR-deficient Brtl mice (Brtl/Adrb2-/-) had a 14.4% ($p=0.004$) and 16.9% ($p=0.005$) increase in femoral and lumbar spine BMD, respectively, compared to Brtl/Adrb2+/+ animals (n=7-15 females). Brtl/Adrb2-/- mice also had a comparative 28.3% increase ($p=0.0002$) in femoral BMC. Both femur and body lengths of Brtl/Adrb2-/- mice were increased compared to Brtl/Adrb2+/+ by 5.3% ($p=0.006$) and 2.8% ($p=0.05$), respectively. The weight of Brtl/Adrb2-/- mice was increased by 11.8% ($p=0.005$). There was no change in body fat percentage.

Conclusion: Selective inhibition of the beta2-AR improves bone quality and growth in Brtl mice and holds promise as an OI therapy.

P.16

Far-red fluorescent pamidronate: A new tool for assessing bisphosphonate deposition and retention in vivo

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Objectives: Bisphosphonate use in osteogenesis imperfecta (OI) has been expanding, outpacing understanding of local drug deposition and retention in a growing skeleton. This study demonstrates an imaging strategy to use fluorescent bisphosphonates as in vivo biomarkers for drug deposition and retention in models of skeletal disease such as OI.

Methods: A far-red fluorescent pamidronate (FRFP) was used to investigate bisphosphonate binding. In vitro competitive inhibition was performed with mineralized dentin with increasing concentrations of non-labeled pamidronate or alendronate. In vivo, competition inhibition was performed by injecting mice with constant FRFP and increasing pamidronate dose, followed by fluorescent imaging. The Brtl mouse model for type IV OI was used to assess in vivo time course of FRFP binding and co-localization of FRFP deposition with alendronate treatment.

Results: FRFP binding was inhibited with increasing concentrations of non-labeled bisphosphonate in vitro, demonstrating consistent binding sites between drugs. In vivo, competitive inhibition is not encountered, indicating excess availability of pamidronate binding sites in the proximal tibia, distal femur, and mandible. In vivo, FRFP tibial localization is rapid, with 87% of maximum signal reached within 2 hours. FRFP co-localizes to osteosclerotic metaphyseal lines formed in vivo from alendronate injection in Brtl and WT mice, demonstrating co-localization of treatment dose with FRFP deposition. FRFP is retained in the bone over the course of a seven week protocol.

Conclusions: FRFP is an effective in vivo biomarker for bisphosphonate deposition and will be useful to investigate local deposition and long-term retention of bisphosphonates in mouse models of OI.

P.17

Surgical Management Of Basilar Impression In OI Type IV By Transnasal Clivectomy

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Basilar impression with upward displacement of the clivus occurs in 25% of subjects with moderate to severe OI. Once clinical or neuroradiologic signs of progression and incipient neurologic compromise become apparent, surgical decompression is indicated. Transpalatal ventral decompression has been the standard approach until recently.

Objective: To perform ventral decompression clivectomy via the transnasal route in a 26 year old with OI type IV with opalescent dentine. Basilar impression had been diagnosed at screening 16 years previously but was progressive over a 2-3 year period, despite concurrent bisphosphonate therapy, with progressively severe cough headache and abnormal neurologic examination.

Methods: Access to the postnasal space and central skull base was gained via a transnasal exposure. This was achieved using a novel enlarged “mono-nostril”, swinging septal and anterior sphenoidotomy approach. Endoscopic transnasal resection of the clivus, anterior arch of C1 plus the odontoid process of C2 was then undertaken followed by occipital-C6 posterior fusion.

Results: Total operative time was 15 hours. Extubation was achieved on post-operative day 3. Immediate post-operative relief of cough and laughter headache was achieved. Although headache returned after 3 months, duroplasty and ventriculo-peritoneal shunting for Chiari anomaly provided relief of symptoms.

Conclusion: Transnasal clivectomy, a minimally invasive endoscopic approach to brainstem decompression, is especially applicable to OI patients because of their platybasia and the high position of odontoid relative to the hard palate which makes the traditional transoral/transpalatal route more difficult. With this approach there is rapid recovery of feeding and swallowing.

P.18

The International Nosology of Osteogenesis Imperfecta – Standardization of Severity Evaluation and Where To Now

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The International Nosology of Constitutional disorders of the skeleton (amended) includes 8 numbered types of OI with 3 named syndromes. Taking into account the heterogeneity in OI type II and III, the nomenclature encompasses over 19 OI syndromes. Five gene loci are known to contribute to the pathogenesis of these syndromes. These loci are COL1A1, COL1A2, CRTAP, PH31/LEPRE1 (coding for Leprecan) and PLOD2. However there are a number of patients whose clinical phenotype or biochemical molecular findings fall outside the classified syndromes.

We have investigated a late onset form of OI characterized by low bone turnover and recurrent stress fractures. This female proband experienced stress fractures without trauma at 11 years and during adolescence had over 50 stress fractures including jaw, ribs, spine, metatarsal bones and hands. She has marked cutaneous striae, telangiectatic rash on arms and legs and spontaneous aseptic necrosis of jaw. There are a number of well characterized mouse mutants for which human homology has not been established. These include the Sphingomyelinase Phosphodiesterase-3 deficient mouse (SMPD3). It is likely that further OI syndromes remain to be delineated. Furthermore in our experience there are several OI phenotypes with ocular involvement.

To clarify confusion between Nosology of OI syndromes and grading of severity, we have developed grading criteria for mild, moderate, severe and extremely severe OI. These were used to assess children for inclusion in the POISE (Pediatric OI Safety and Efficacy) study of Risedronate in mild OI.

P.19

A Multidisciplinary Care Program for Children with Osteogenesis Imperfecta

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Children with Osteogenesis Imperfecta attending the Children's Hospital at Westmead are cared for by a multidisciplinary OI management team composed of specialists in rehabilitation, genetics, bone and mineral medicine, dentistry, adolescent medicine and orthopaedics, as well as physiotherapy, occupational therapy, social work, orthotics, nursing and genetic counselling.

Fractures are painful at all ages and children gain considerable relief from effective analgesia in conjunction with appropriate splinting techniques to support the fracture during movement. Parents are taught first aid and splinting techniques to reduce pain and shock. Fractures are managed by orthopaedic specialists with team involvement in planning for rodding or other procedures. All children are considered for treatment with Cyclic Intravenous Bisphosphonates.

To minimise Basilar Impression and scoliosis, we advise reclined supported seating until the child has sufficient trunk control to maintain upright posture without slumping into a kyphotic deformity. Developmental therapy, hydrotherapy, judicious splinting, physical activity programmes and musculoskeletal management, especially of joint hypermobility, improve long term outcomes. All children are referred to paediatric dentistry for initial assessment and annual review particularly if they are treated with bisphosphonates due to the very low risk of Osteonecrosis of the Jaw. Bone density and hearing assessments are performed at regular intervals during childhood.

Skilled genetic counselling and education helps families, children and schools understand this condition. All families are referred to an OI support organisation. Emotional support, medical and orthopaedic management and practical assistance, provided by an experienced multidisciplinary team, are essential to achieve optimal outcomes for a child with OI.

P.20

Recombinant Human Growth Hormone in Children with Osteogenesis Imperfecta Types I and IV

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Objective: To evaluate the growth and bone effects of recombinant human growth hormone (rhGH) treatment in children with Osteogenesis Imperfecta (OI).

Methods: Open labelled cohort study of rhGH, 17iu/m^2/week, administered to 15 children with Osteogenesis Imperfecta Types I (n=6) and IV (n=9). Major assessments occurred at baseline, 12 and 24 months. Bone mineral density (BMD) in subjects was compared with extensive normal data.

Results: Height and weight z-scores had increased at 12 months (Delta z=0.37 and 0.30, respectively; $p<0.05$) and at 24 months (Delta z=0.50 and 0.46; $p<0.05$). Insulin-like Growth Factor-1 and urine hydroxyproline increased from the baseline ($p<0.05$). There was a trend towards an increase in alkaline phosphatase ($p=0.17$).
Bone age increased appropriately throughout the study period. Second metacarpal cortical thickness ($p<0.005$), external diameter ($p<0.005$) and percent cortical area increased from baseline ($p<0.005$ at 12, $p<0.05$ at 24 months). Vertebral size improved at 24 months ($p<0.005$), but concavity index remained unchanged. One child had a rapid progression of kyphoscoliosis.
Both total BMD age z-score and bone mineral content (BMC) for lean tissue mass (LTM) z-score decreased at 12 ($p<0.005$) and 24 months ($p<0.005$). No significant change was noted in lumbar spine BMD.

Conclusion: 24 months of rhGH in children with OI Types I and IV improved height, cortical thickness, percent cortical area and vertebral size while increasing bone turnover. This study failed to show a benefit of rhGH on total BMD, lumbar spine BMD, BMC for LTM and vertebral shape and it may worsen spinal deformity.

P.21

Mutations in the Type I collagen C-propeptide cleavage site cause a distinct phenotype based on slower procollagen processing

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Introduction: Mutations in type I procollagen C-propeptide cleavage site are of particular interest because they disrupt a unique processing step. We identified two children with mild OI who had cleavage site mutations in COL1A1 (P1: a1(I)Asp1041Asn) or COL1A2 (P2: a2(I)Ala1029Thr). P1 had a normal LRP5 sequence.

Methods: Clinical features, including BMD, histomorphometry and radiographs, were compared to collagen modification, chain incorporation and procollagen processing in fibroblasts.

Results: P1 DEXA Z-score and pQCT vBMD were +3, contrasting with radiographs demonstrating osteopenia and os-in-os vertebrae, and histomorphometry revealing increased bone remodeling, without mineralization defect or signs of osteosclerosis. P2 had a DEXA z-score of 0, gracile long bones with radiographic osteopenia, and decreased BV/TV and increased BFR without a mineralization defect on histomorphometry. Steady-state collagen electrophoresis showed slight backstreaking of a1(I) and a2(I) in cell layers of both probands. The baseline of P1 chains was delayed, while those of P2 migrated normally. Chain incorporation was normal in P1 and slightly delayed in P2. Pericellular processing of P1 was delayed, with increases in both pCa1 and proa2, while P2 had increased pCa2 and proa2 and normal processing kinetics.

Conclusions: These mutations define a novel phenotype within type I collagen defects. In combination with a recently reported adult with proa1(I)Ala1040Thr substitution (Int Conn Tis 82S1: CC01), our cases suggest that defects in proa1(I) processing lead to high BMD in childhood, with signs of osteopetrosis occurring subsequently. Pro-a1(I) cleavage appears crucial to C-propeptide processing, while defective pro-a2(I) specific or non-specific cleavage occurs after a1(I) processing.

P.22

The o.i. italian reference centre in verona: clinical and molecular diagnosis

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Introduction: University of Verona offers a wide and complete service for the clinical and molecular diagnosis and for the therapeutic follow-up of italian patients with Osteogenesis Imperfecta.
Monthly, inside the G. B. Rossi Hospital, specialistic physical examinations are organized in presence of specialists (pediatrician, orthopaedic sp., physiatric sp.) with the aim to identify or to exclude potential O.I. patients.
In the case of high pathological suspicion, subject is signaled to the molecular laboratory for the genetic investigations.

Material and Methods: Patients' gDNA is isolated from peripheral blood sample and the entire coding regions of COL1A1 and COL1A2 genes, with relative splicings boundaries, are amplified and sequenced by capillary electrophoresis.
In a small number of subjects with clinical features of quantitative defect but with negative genetic screening, a cell culture was established from skin biopsy to obtain RNA for RT-PCR investigation and null-allele test to confirm causative effect of collagene I genes.

Results and Conclusion: During 4 years of activity, our molecular laboratory produced more than 100 molecular responses: about 80% of them concerns positive diagnoses of O.I. while the remaining 20% can be justified expecially by uncertain clinical phenotypes but also by O.I. forms not involving COL1A1 and COL1A2 genes and in a minor extent by intrinsecal limits of the methodic.
Besides classical variations, of particular interest are those out of the triple elix region. As concern, our case history produced: 4 frameshift / stop-codon mutations in N-term. propeptide, 1 aa substitution and 1 large deletion in C-term. propeptide of COL1A1 gene.

P.23

High carrier frequency of founder mutation causing severe/lethal recessive type VIII osteogenesis imperfecta in west africans and african-americans

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Objectives: We identified a recurring LEPRE1 mutation (IVS5+1G>T) causing Type VIII OI (OMIM #610915) in 6 infants born to carrier parents of West African or African-American descent, suggesting the existence of an African founder mutation which had been transported to the Americas. We investigated carrier frequency of the mutation in contemporary West African and African-American populations and the molecular anthropology of the mutation.

Methods: We screened gDNA from several populations using PCR and RE digestion, or a custom SNP assay, followed by PCR confirmation of positive samples. For mutation age, we have used microsatellites and short tandem repeats covering a 4.2 MB region surrounding the LEPRE1 gene on chromosome 1p to determine the conserved haplotype.

Results: The recurring mutation was identified in 5 of 995 Washington DC, 5 of 1429 Pennsylvania and 2 of 631 Maryland samples (~1/200-300 carriers). Fifteen of 1097 unrelated individuals (1.37%) from Nigeria and Ghana were heterozygous for LEPRE1 IVS5+1G>T, all but one from Kwa-speaking tribes. Haplotype analysis of the type VIII OI African-American and West African pedigrees has revealed a conserved region of less than 450Kb, which would be consistent with a single mutation that arose over 300 years ago.

Conclusions: West Africans have a high (>1%) carrier frequency for a founder mutation causing recessive type VIII OI. This mutation alone would cause an incidence of recessive OI in West Africa (1/21,000) equivalent to the incidence of de novo dominant OI, compared to 5-7% recessive OI in North America. The age of the founder mutation is consistent with transportation via the Atlantic slave trade. Contemporary Mid-Atlantic African-Americans have a predicted incidence of homozygosity for this mutation of 1/160,000-380,000 births.

P.24

Cellular mechanism of decreased bone in BRTL mouse model of osteogenesis imperfecta: imbalance of decreased osteoblast function and increased osteoclasts and their precursors

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Objectives: We investigated the cellular contribution to bone properties in the Brtl mouse, a knock-in model for moderate OI which is heterozygous for a col1al G349C substitution.
Methods: We examined the Brtl bone phenotype in 2 and 6 month old mice using static and dynamic histomorphometry, quantitative immunohistochemistry and real-time RT-PCR.

Results: Brtl cortical and trabecular bone are reduced before and after puberty, with BV/TV decreased 40-45%. Brtl ObS/BS is comparable to wild-type, and Brtl and wild-type marrow generate equivalent number of CFU at both ages. However, OcS/BS is increased in Brtl at both ages (36-45%), as are TRAP+ cell numbers (57-47%). After puberty, Brtl ObS/BS decreases comparably to wild-type mice, but osteoblast matrix production (MAR) decreases to half of wild-type values. In contrast, Brtl OcS falls only moderately (~ 16%) and Brtl TRAP staining remains significantly elevated compared to wild-type. Consequently, Brtl BFR declines from normal at 2 months to half of wild-type values at 6 months. Immunohistochemistry and real-time RT-PCR reveal increased RANK, RANKL and OPG levels in Brtl, although a normal RANKL/OPG ratio is maintained. TRAP+ precursors are markedly elevated in Brtl marrow cultures and form more osteoclasts, suggesting that osteoclast increases arise from more RANK-expressing precursors.

Conclusions: Osteoblasts and osteoclasts are unsynchronized in Brtl bone, which contributes to the tissue-level mechanism of decreased bone volume in Brtl. The disparity in cellular number and function results from poorly functioning osteoblasts in addition to increased RANK-expressing precursors that respond to normal RANKL/OPG ratios to generate more bone-resorbing osteoclasts. Interruption of the stimulus which increases osteoclast precursors may lead to novel OI therapies.

P.25

Audiologic and genetic determination of hearing loss in patients with Osteogenesis Imperfecta

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Introduction: Osteogenesis Imperfecta (OI) is a hereditary connective tissue disorder, clinically characterized by a triad of symptoms including bone fragility, blue sclerae and hearing impairment. In general, the disease is inherited in an autosomal dominant pattern. Causal mutations for OI are located in the COL1A1 or the COL1A2 gene.
A protocol is developed to determine the characteristics of the hearing loss which affects about half of the patients with OI. To elucidate the known inter-individual variability in OI-associated hearing loss and to get an insight in the underlying pathogenetic mechanisms of the hearing impairment, these characteristics will be correlated with the patients' genotypes.

Methods: 25 families in which 2 to 8 family members were affected by – genetically confirmed – OI, will be invited for an ENT-examination and an extensive audiological evaluation. CT and MR imaging techniques will be used to gather information about deficient ossification at the level of the temporal bones.

Expectations: Previously no correlation could be found between the mutated gene or mutation type and the hearing pattern in OI (1). It was suggested that the hearing loss was the result of multifactorial and possibly still unknown genetic effects. In our study, environmental factors will therefore be taken into account. Additionally, attention will be given to Single Nucleotide Polymorfisms located in genes associated with hereditary deafness and otosclerosis.

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P.26

Bone growth in children with Osteogenesis Imperfecta treated with Telescopic Fassier-Duval and Bailey-Dubow Nails

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The telescopic nails are used in children to allow them to grow after the correction of a deformity in a long bone, and usually in Osteogenesis Imperfecta (O.I.).
In this study we present the resulting growth in two types of telescopic nails used to treat Osteogenesis Imperfecta (generally type II of Sillence), 13 nails were Fassier-Duval and 4 nails were Bailey-Dubow.
18 patients were treated with a total of 74 surgeries, 15 on the right femur, 17 on the left femur, 3 on the right tibia and 4 on the left tibia. We did not treat upper extremities with telescopic nails. None of the nails failed in the elongation, which was satisfactory until July 31, 2007, with no asymmetry nor physeal detention and the ones that ceased to grow was caused by the basic pathology, not by the nail.
The most relevant inconveniences which occurred during the study period were extrusion of the telescopic nail, curving in same and following the direction of deviation of the bone and a vascular necrosis of the intermediate fragment.
The total growth was between 0,3 cm. and 12,2 cm. in the right femur (an average of 7,6 cm.) and from 0,2 cm. to 12,8 cm. in the left femur (an average of 6,5 cm.), from 0,3 cm. to 3,7 cm. in the right tibia, (an average of 2 cm.) and from 0,4 cm. to 2,7 cm. in the left tibia (an average of 1,5cm.), in proportion with their age, with a maximum age of 23 and a minimum age of 3.
In our experience, the nail could be elongated adequately in 98% of the cases and despite the typical complications of the implant and the type of patient, the results indicate that they are useful in paediatric pathology (OI) which require a telescopic nail, maintaining the alignment while it grows and the complications in evolution are fundamentally due to the alterations in the bone where the nail is placed.

P.27

Bone mass in adults with osteogenesis imperfecta, a population based study

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Objectives: By measuring bone mineral density (BMD) in an adult population with osteogenesis imperfecta (OI), we wanted to answer the following questions: 1. Is the rate of fractures related to BMD? 2. How common is osteoporosis (T<-2.5) in patients with OI? 3. Are there differences in BMD related to Sillence type?

Methods: All adult OI patients, above 25 years of age (n=154), registered in Norway were invited, and 88 (57%) (Age range 25-83) had a DXA measurement. Classified according to Sillence 68 persons had OI type I, 9 type III and 11 type IV.
Rate of fractures was reported by the patients. BMD g/cm^2 of the spine (L2-L4), femoral neck and the total body skeleton were determined by Lunar DPX-1.As BMD is sex- and age-specific, the individual BMD values were converted into Z-scores. To diagnose osteoporosis we used the criteria of the World Health Organization (WHO).

Results:
1. Higher rate of fractures was related to with lower BMD.
2. In all measured regions, Z-score was significantly lower than zero in all OI types. Only 10% had osteoporosis defined as T<-2.5 in at least one skeletal region (3 patients type I and 6 type III).
3. Type III had significantly lower BMD compared to type I and IV

Conclusions: The fracture rate was highest in patients with low BMD. Although there was a high fracture rate, only 10% were classified as osteoporotic. Differences in BMD were found between types of OI.

P.28

Gait analysis and energy consumption in walking patients affected by osteogenesis imperfecta

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Objectives: Osteogenesis Imperfecta (OI) is associated to musculoskeletal abnormalities. The quantification of the gait pattern in these patients may be useful for rehabilitations an surgery decisions.

Methods: In this study, 8 patients affected by OI (range: 4-27 years) and 22 healthy children (range 5-27 years) were evaluated. gait analysis was conducted using a 8-camera optoelectronic system to measure the kinematic of movement, two force platforms , to obtain the kinetic of movement and a synchronized video system. A breath-by-breath system was used to assess energy expenditure during walking. Parameters were extracted to quantify kinematic and kinetic data and energy consumption data.

Results: OI patients were characterised by common limitations during deambulation: reduced velocity and anterior step length, anterior pelvic tilt, excessive hip flexion during gait cycle, reduced knee flexion in swing and abnormal external foot rotation, abnormal knee extension in midstance, anomalous ankle dorsiflexion in pre-swing and in swing, and reduced ankle propulsion at push-off. The results related to energy consumption confirmed the severity of their condition, too: the patients present, in fact, higher heart rate and oxygen cost during deambulation than control group.

Conclusion: The quantitative evaluation of functional limitation of OI patients, using GA and K4b2, gives additional information to those provided by clinical evaluations: these results may be clinically useful to define the more effective surgical and rehabilitative treatment in OI subjects.

P.29

The use of TEN nails with "sliding nail" technique in paediatric patients with Osteogenesis Imperfecta (OI)

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Background: Intramedullary fixation with use of a telescopic rod is one of the standard methods for long-bone stabilization in growing children with oi, however, various authors are trying to remedy their limitations and complications by changing telescopic rod or using new synthesis techniques with non telescopic devices.
We present our experience related to the treatment of 16 diaphyseal fractures of long bones of the lower limbs with application of TEN nails in children with OI.

Methods: We operated on 39 children (age 0-13y) suffering from oi type I, III, IV, according to Sillence classification, who have Nerixia by 12 months and we have run clinical and X-ray evaluation for 1y before and after surgical treatment of 16 segments (3 tibiae and 13 femora) with sliding central medullary nailing with 2 TEN nails.

Results: All nails were inserted without an arthrotomy of the distal and proximal joint, and the 69% telescoped successfully.
Nails had to be changed in 29% of the bone segments, in case of migration of nail (12%), nail deformation (14%) and nail overlap (3%) as a result of trauma following the surgical treatment.
Morever, for the patients who had suffered a second trauma after the first surgical treatment, for the 83% of cases, central medullary TEN nails avoid the breakdown of the fracture fragments, but in 43% was a needs to change nails (improving bone synthesis, improvement of stabilisation).
The cumulative survival rate of the nails at 1y postoperatively was 71%.
Moreover, the clinical and X-ray evaluation performed before surgery and at the end of follow-up, showed no appearance or worsening of axial deviations or heterometry of treated segments.

Conclusions: Both insertion and removal of a TEN nails are much less invasive than insertion and removal of a conventional telescopic rod.
In addition, TEN be inserted without an arthrotomy and obliquely through the growth plates so they don't cause epiphysiodesis and damage to cartilage.
In addition TEN nails have a lower cost compared with telescopic rod and are available in 5 different sizes offering the advantage of better adapt to the diameter often very small of the Osteogenetic bone that in some cases needs to be recanalizate.
Use of telescopic rod before the age of five years increases the risk of complications while most of the complications observed with TEN occur after the age of five years.

P.30

Application of geometric measurements at the hip region (HSA) by DEXA in patients with Osteogenesis Imperfecta

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Osteogenesis Imperfecta (OI) is an inherited disorder characterized by increased bone fragility with recurrent fractures that leads to skeletal deformities in severe cases. The phenotypic expression is heterogenous with the most severe forms being fatal in the perinatal period to mild forms diagnosed only in adulthood. OI is characterized by a low bone mass, a reduced trabecular thickness and number and a decreased bone formation in adults. Animal and human studies suggest that skeletal fragility in OI is due to the defect in collagen synthesis, whereas the abnormalities in bone turnover and mineral are inconsistent. Since reliance on BMD alone do not provide the best predictive ability for peripheral fracture risk in patients with OI the aim of our study was to compare DXA BMD measurements and femoral geometric dimension measurements (Hip Structure Analysis) between different patient groups and the occurrence of peripheral fractures. We performed DXA measurements (Lunar iDXA, software version 11.2, GE Healthcare) in 21 patients with OI (mean age 45 +/-5 yrs) and a multiple fracture history and consequently abnormality of the stature and analyzed the different features of DXA like BMD at different measuring sites and geometric measurements. Hip structure analysis (HAS) including cross-sectional moment of inertia (CSMI), and cross-sectional area (CSA) and femoral srenght index (FSI) is known to correlate to bone mass distribution and fracture. These structural variables can be derived from cross-sectional absorption curves generated by dual-energy X-ray absorptiometry (DXA). BMD accounts for only about half of the variation in strength estimated by CSMI, indicating that CSMI,CSA and FSI contribute additional information regarding femoral strength not contained in BMD. The same tools were applied to a control group (CO) of age matched healthy females (25) and an age matched patient group(30) with osteoporosis with multiple fractures (OPO). Apart from differences in BMD measurements between the different groups our results show significant decreases of the different geometric parameters (HAS, CSMI and CSA) when compared to an age matched control group and a moderate difference to patients with premenopausal osteoporosis.

group	age	HSA(FSI)	CSM(mm4)	CSA(mm2)	BMD neckg/cm2)
OI	45±5	0.8± 0.5	8.3± 3.0	119.3± 24	7 0.81± 0.1
OPO	51±7	1.0± 0.4	7.3± 2.2	98.6±16.2	0.66± 0.1
CO	46±6	1.5± 0.7	14.9± 6.2	177.0± 53	1.01± 0.3

We conclude that geometric structural measurements made at the femoral neck by DXA are of more clinical relevance and may be more reliable for fracture prediction than BMD measurements in patients with Osteogenesis Imperfecta.

P.31

Operative treatment of the long bones in Osteogenesis imperfecta – preliminary results about the influence of bisphosphonates and selection of osteosynthetic devices

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Objectives: Intramedullary stabilization after multiple cor-rective osteotomies in OI in the past was often associated with extensive surgery and a number of complications. With the availability of biphosphonates (BP) one could expect better bone stability. The aim of our investigation was to analyze the influence on our surgical management.

Patients and methods: Retrospective analysis of 47 OI-Patients. 40 where operated on long bones for the following indications: fracture, clinical relevant deformities and non-union, 25 patients had no additional BP-treatment (Group A), type of OI I: 6, III: 9, IV: 9 and V: 1. Op. /patient: 6 [1...13]. The total number of operations was 131. In the majority of group A osteosynthesis was done with telescopic nails, rushpins, locking nails and K-wires. Since 2002 15 Patients with BP-therapy where operated. Type of OI I: 2 , III: 6, IV: 7. Age at first treatment initiation with BP´s: 2 years [1,5…21]. Age at first intervention: 2,5 years [1,5...30]. Op./patient: 2 [1...6]. We performed 31 operations. Osteosynthesis was done with ESIN, rushpins, cerclage rather than telescopic nails. In 2 patients we used screwlocking plates. The postoperative treatment consists of initial short time immobilisation and early functional treatment. Results: We found good healing of fractures and corrective osteotomies under BP´s 13 of 15 Group B-patients. Overall mobility seems to be better in Group B. We found no significant differences in fracture rates between both groups after intervention. Complications where seen in 46/128 interventions (36%) of group A and 7/31 (22%) in group B.

Conclusions: With introduction of BP´s there is a trend toward less invasive procedures. Better bone stability allows the reduction of necessary corrective osteotomies per bone and additional use of skrewlocking plates for short distances. With operative fracture-treatment one can achieve early restoration of function. We recommend BP´s and prophylactic intramedullary stabilization at walking age. The risk of nonunions after surgery seems to depend rather on age, type and thermic damage of the osteotomized bone than on BP-influence. Further studies and longer follow up periods are necessary to elucidate the influence of BP´s on operative management.

P.32

Multi disciplinairy outpatients' clinic. (para) medical care of the adult patient with osteogenesis imperfecta

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Introduction: In the Netherlands (para-)medical care of children with OI is centred in the Wilhelmina Children's Hospital Utrecht (UMCU). However, expertise and specialised care for the adult patient was lacking. In close collaboration with a Nurse Practioner, the Dutch organization for individuals with OI initiated the development of a regional outpatients' clinic in Zwolle (situated in the eastern part of The Netherlands).

Methods: The Nurse Practioner supervises the patients during their one day outpatient visit at the clinic. At the end of the day the multidisciplinary team discusses the patient's health status. Our intention is to examine the patients on a yearly basis.

Participating disciplines are:
- Advanced Nurse Practitioner
- Orthopaedic surgeon
- Endocrinologist
- Rehabilitation consultant M.D.
- Occupational therapist

Specialised care is focused on:
- Advising and determining prescription policy on:
  - Orthopaedic interventions
  - Fracture reduction and treatment of osteoporosis.
  - Rehabilitation medical screening
  - The use of ortheses and suggestions to improve activities of the daily living
  - Vitamin substitution and antiresorptive therapy.

Results after 6 months:

- High number of requests for the outpatients' department
- Calcium intake is lower than expected
- Bone densitometry
- Vitamin D deficiency occurs in one-third of the patients

P.33

Quantitative magnetic resonance imaging of the calcaneus in patients with osteogenesis imperfecta

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The aim of this study was to evaluate the ability of quantitative MRI to assess bone quality in patients with osteogenesis imperfecta (OI). The apparent transverse relaxation time (T2*) was measured in 12 subjects (7 men and 5 women; mean age 32.1 +/- 17.1 years) affected by mild OI, types I and IV, with varying degrees of spinal bone mineral density (BMD). Five subjects had osteoporotic vertebral fractures as assessed by radiographs of the spine. MRI of both feet was performed on a 3T scanner using a fast gradient-echo technique. Fifteen sagittal images with echo times between 3.1 ms and 14 ms were acquired for each calcaneus and T2* values were estimated in the superior, anterior and posterior regions. BMD was determined at the lumbar spine and total femoral neck by DXA. HR-MAS NMR spectroscopy of urine samples was performed to analyze the metabolic profiles of all subjects investigated. Calcaneal T2* values showed regional variations being typically shortest in the superior region of the calcaneus. A significant increase ($p<0.05$) in T2* was found in 4 OI patients, especially in the anterior and posterior regions of both calcanea. For these subjects, R2* (1/T2*) showed a significant positive correlation with BMD at the lumbar spine ($r=0.62$; $p<0.05$). A higher correlation with BMD was observed for two of these patients characterized by an atypical metabolic profile. This study shows that T2* of the calcaneus is sensitive to alterations in bone quality and appears potentially useful for a better radiological typing of this disease.

P.34

Structural Heterogeneity of Type I Collagen Triple Helix and Its Role in Osteogenesis Imperfecta

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Gly substitutions in type I collagen cause over 80% of severe OI cases, suggesting that structural defects in the triple helix underlie the disease. However, no simple correlations of OI severity with the predicted or measured triple helix stability have been reported. To test whether location of mutation within different structural regions in the triple helix may modulate the OI phenotype, we mapped these regions by systematic measurements of mutant collagen stability. Using differential scanning calorimetry and circular dichroism, we measured variation in the collagen melting temperature (Tm) for 50 different Gly substitutions from OI patients. Using infrared spectroscopy and known stabilities of collagen-like peptides, we determined variation in the activation energy for local unfolding along the triple helix. Combining the resulting Tm and local stability maps, we delineated three flexible regions and two stable regions in type I collagen triple helix. The flexible regions overlapped with regions important for collagen fibril assembly and ligand binding. One of them also coincided with the largest known region of lethal alpha1(I) Gly substitutions. The stable region close to the C-terminal end of the triple helix coincided with the other region of predominantly lethal alpha1(I) Gly substitutions. We found that the mutations within the stable N-terminal region cooperatively unfold this region and prevent normal N-propeptide cleavage. The uncleaved pN-collagen incorporates into fibrils and leads to the distinctive OI/EDS phenotype.

P.35

Could the island of Sardinia be considered a genetic model for the study of Type 1 Osteogenesis Imperfecta?

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The island of Sardinia, located in the middle of the Mediterranean Sea, is considered a genetically homogeneous, isolated area having high disease incidence and prevalence. Sardinian population is an ancient genetic isolate with unique distribution of common genetic disorders such as type 1 diabetes mellitus, thalassemia and multiple sclerosis.
Purpose of this study is to comprehend if the prevalence of Osteogenesis Imperfecta (OI) in the island could be significantly higher than the rest of Italian population, in order to present a model for further studies of the genetics of Type 1 OI.
As long as Italian health system does not keep a proper record yet, the only codified, epidemiological data are related to the number of hospitalizations with diagnosis of OI.
According to our observations, Sardinia was the Italian place with the higher rate of hospitalization for OI in the past five years, after the two Italian regions with dedicated centres for OI.
We have found at least four families presenting familiar histories of type 1 OI, with at least six or more cases each. In one family a new mutation in COL1A1 gene, not previously reported, was isolated (COL1A1 IVS39-2/-1 AG>CC g.12100-1). The overall number of patients with Type 1 OI counted so far in the northern part of the island was fifty.
Further studies will be necessary to ascertain whether or not the high prevalence of this disease in Sardinia represents a coincidental occurrence.

P.36

Linking Collagen Genotypes to Clinical Phenotypes

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Osteogenesis imperfecta (OI) manifests a wide range of severities, from mild to lethal, and the relationship between genotypes, molecular phenotypes, and clinical phenotypes is poorly understood. For OI forms resulting from missense mutations in a type I collagen gene, it is known that the phenotype depends on the nature of the mutation and its location with the gene; however, it is not currently possible to reliably predict the disease severity from the mutation. We are developing a computational method to predict the lethality of OI-associated collagen mutations using a divide-and-conquer approach. Mutations likely to share a mechanism of lethality were identified and each set was modeled separately. Models were validated both computationally, using independent test sets of mutations, and experimentally. The computational and experimental results together lend insight into the effect of mutations on the structure and function of collagen and the etiology of OI.

P.37

Importance of Anthropometric Measurement in Diagnostics of Osteogenesis Imperfecta Types

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Introduction. Osteogenesis imperfecta (OI) is the most common inborn disease of fibrous connective tissue characterized by increased fragility of skeleton. Congenital bone fragility is the cause of multiple fractures and biomechanical serious deformities of the long bones and the axial skeleton.
The aim of our study was specifying of the diagnostics of individual types of OI with taking advantage of clinical, precise anthropological, radiological, biochemical, biomechanical and genetic methods.
Patients and methods. Group of 55 patients (27 M/ 28 F) aged from 1.5 to 56 years is followed and treated in the Ambulant Centre for Defects of Locomotor Apparatus in Prague. Patients were classified into 7 types of the OI according to both Sillence (1979) and the new Glorieaux (2003) classification. The cohorts of patients were characterized with accent on the body height, proportionality of trunk and extremities, shape of thorax, head, and nutritional status. Anthropometric parameters were expressed in z-score. All the collected data of patients were gathered into the complex database and statistically evaluated trying to find some relations among anthropometric, clinical and biochemical parameters.
Results. The most important parameters distinguishing the types are body height and length of lower extremities. Meanwhile the type I is characterized by shortening of the trunk, the type IV shows even more severe involvement of length of lower extremities.
The most important factor which affected the severity of disease was the type of OI, determined by the severity of collagen affection. Patients with more severe type of OI have more fractures and severe deformities. Parameters explaining the variability in body height among patients within the same type of OI are: platyspondyly, deformity of ribs (in coincidence with trunk deformity) and dentinogenesis imperfecta. We also demonstrated lower body height in patients with high urine deoxypyridinoline level and decreasing of its level in patients on antiresorptive therapy.
Conclusion. We affirm that the dividing of OI types according to the new classification gives more consistent pictures of the OI types. The type of OI determines the severity and progress of the disease. It reflects the rate of bone structural involvement and tells us how often the fractures and microfractures which cause the deformities will occur. Anthropometric parameters can be useful in differential diagnostics among OI types (especially between type I and IV). One of the most important parameters which can be used to distinguish the types is body height. The length of lower extremities and whole body proportionality are on the second place.

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P.38

A new form of hereditary osteoporosis in a 3-generation Finnish family

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Juvenile osteoporosis has been considered a sporadic and self-limiting disease, unless diagnosed as OI. This view is challenged by new genetic findings, including osteoporosis-causing mutations in LRP 5 and LRP6. The pathogenesis of juvenile osteoporosis still remains largely unknown. We describe clinical, genetic, radiological and histological findings in a three-generation pedigree with a new form of autosomal dominant primary osteoporosis.

The proband presented with painful vertebral compression fractures at the age of 35 years. Secondary causes of osteoporosis were excluded. She had no features of OI and no history of peripheral fractures. The bone mineral density (BMD) lumbar Z-score was -2.7 and she had compression fractures in Th VI-IX. Further assessment revealed osteoporosis in eight additional family members, many suffering from asymptomatic compression fractures. The youngest affected patient is a boy of 12 years with asymptomatic compression fractures in his thoracic spine, no history of peripheral fractures and a lumbar BMD Z-score of -1.7.

Transilial bone biopsies from two treatment-naïve adults revealed severe low turnover osteoporosis with low trabecular bone volume, decreased osteoid and low numbers of osteoblasts. The mineralization and resorption rates were normal.

Genetic testing for mutations in LRP5 and LRP6 was negative. A genome-wide micro-satellite analysis revealed no linkage in areas encoding type 1 collagen. Further analyses are under way to identify the disease-causing genetic defect.

We describe a new form of autosomal dominant early onset primary osteoporosis. The discovery of the underlying genetic defect may provide important new information about biological and pathogenetic mechanisms in osteoporosis.

P.39

Bone turnover and type I collagen C-telopeptide isomerization in adult osteogenesis imperfecta: associations with collagen gene mutations

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Newly synthesized Col I comprises non isomerized C-telopeptide (aCTX), but with bone matrix maturation aCTX is converted to its isomerized b form (b CTX). Urinary a/b CTX ratio has been proposed to reflect collagen maturation. We investigated changes in bone turnover and Col I isomerization in adult patients with OI and their relationship with Col I gene mutations.
Sixty four adult patients [25 women, 39 men mean age (SD): 36.2 (11.6) year] with OI participating in a randomized study and 64 healthy controls of similar age and gender distribution were investigated. In patients with OI and controls, we measured the following biochemical markers of bone metabolism: serum type I collagen N-propeptide (PINP) an index of Col I synthesis, osteocalcin a marker of osteoblastic activity, urinary Col I helical peptide, a marker reflecting the degradation of the helical portion of Col I, urinary aCTX and urinary and serum bCTX. Based on the putative functional effects of Col I gene mutations which were identified in 56 OI subjects, patients were divided in those with haploinsufficiency (n=29), patients presenting with helical domain alterations (n=17) and others (n=10).
Compared to healthy controls, patients with OI had decreased levels of PINP (-22.7%, $p<0.0001$), increased osteocalcin (+73%, $p<0.0001$) and increased Col I helical peptide (+58%, $p=0.0007$). Urinary aCTX was increased (+31%, $p=0.03$) whereas urinary (-15%, $p=0.022$) and serum (-9.9%, $p=0.0056$) bCTX were significantly decreased, resulting in a 49% ($p<0.001$) higher urinary a/bCTX ratio. Patients with Col I gene mutations resulting in haploinsufficiency had lower PINP levels than patients with helical domain alterations (26.4±15.3 vs 41.6 ± 27.4 ng/ml, $p=0.0043$) and controls ($P <0.01$).
Adults with OI are characterized by decreased Col I synthesis -especially those with haploinsufficiency mutations- increased Col I degradation and decreased Col I C-telopeptide isomerization.

P.40

Adult OI patients: peripheral fracture rate. An observational study

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In adult OI patients, the incidence of fractures decreases after puberty and remains low until menopause in women and the age of 70 in men. Longitudinal studies evaluating fracture rate are not available. Our study attempts to determine the rate of peripheral fracture in adult OI patients.
We send a questionnaire about the peripheral fractures to the 64 adult patients having participated in a 3-year, randomised, double-blind, placebo-controlled trial of oral alendronate between 1999 and 2003. In this study, alendronate had no significant influence on the fracture rate.
We have received 53 responses representing a follow-up of 51 +/- 12 months. 41 patients have received alendronate after the randomised study during in mean of 39 +/- 15 months and 12 no. 15 patients treated by alendronate have presented at list an event of fracture and 8 in the non-treated group. In this observational study, no difference was found between the group of patients having received alendronate during the randomised study and the others. 22 patients (41 %) presented at list 1 event of peripheral fracture: 17 patients 1 event, 3 patients 2 events, 1 patient 3 events and 1 patient 9.
As a conclusion, peripheral fractures are common in adult OI patients. Researches in new therapeutic approaches capable avoid peripheral fractures are necessary.
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Correlation between temporal bone CT-scan and hearing in OI

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This study attempts to determine tomodensitometry interest finding out asymptomatic ear pathology in OI.
44 patients affected by OI underwent pure-tone audiometry by air and bone conduction, acoustic reflex measurements, and temporal bone tomodensitometry (TDM) less than 3 months before or after hearing tests. CT-scans were interpreted by a radiologist and an otology surgeon, who didn't know the hearing features of patients. They assessed stapes footplate and pericochlear TDM aspects by the same scale for each ear. Correlation between hearing and CT-scan parameters was investigated by regression analyses.
Better correlations were obtained between peri-cochlear CT-aspect and sensorineural hearing loss (r2 from 0.32 to 0.56) than between stapes footplate CT-aspect and conductive hearing loss (r2 from 0.12 to 0.38). Among 16 ears with significant peri-cochlear demineralization, 5 just had a limited hearing loss on 4 and 8 kHz, and one had all hearing thresholds below 20 dB. All ears with an average pure-tone hearing loss above 50 dB had some degree of perilabyrintic demineralization. Some cases with clear discrepancies between CT and audiometric data will be shown, as well as some unusual ossicular and periotic bone anomalies.
The statistical correlation between temporal bone CT and hearing mainly reflects presence of perilabyrintic demineralization in significant sensorineural hearing loss. When hearing is normal or mild, CT can show demineralization, which might be an early sign of future hearing deterioration. A longitudinal study of hearing is needed to confirm this hypothesis.

P.43

Growth Trends for OI Types III & IV

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Objectives: To determine the natural history of growth in OI types III and IV using the longitudinal NIH pediatric OI population and the predictors of adult height.

Methods: Retrospective review of 33 growth curves (17 type IV OI, 14 type III OI and 2 OI type III/IV) from birth to18 years for correlation of stature with unaffected same-gender children and stratification by OI type and final height age (HA).

Results: Final stature for type III and IV OI young adults is HA=3.0 + 1.5 years (range 1- 6 yrs) and 7.2 + 2.8 years (range 3.5-10 years), respectively ($p<0.001$). At 36 months of age, OI type III and IV children have the lengths of average 8.3 + 4.0 month (range 3-14 months) and 15.4 + 6.5 month old infants (range 4-29 mos), respectively ($p<0.001$). We stratified the children into 4 non-overlapping groups by final HA. Groups 1 (n=8), 2 (n=12), 3 (n=9) and 4 (n=4) children have final HA of 9-12, 4-7, 2.5-3.5 and less than 2 years, respectively. Type IV children are in groups 1 and 2, while type III children are in groups 2, 3 and 4. At CA=36 mos, HA>15 months, only for Group 1. In Group 1, only 2 children had scoliosis >15°, while most children in Groups 2 and 3 have scoliosis >20° and/or spinal stabilization, and all group 4 children require spinal stabilization. Group 2 children lack a pubertal growth spurt, while Group 3 children grow slowly throughout childhood and teen years and Group 4 children fall progressively behind during childhood.

Conclusions: Mid-parental height is not a useful tool in estimating final height in OI children. Children with types III and IV OI have significantly different final HA. Further predictive discrimination is possible using HA at 36 months and scoliosis

P.45

Identification of Skin Abnormalities in Osteogenesis Imperfecta Patients by Magnetic Resonance Imaging--A Pilot Study

CL Raggio, KW Fishbein, EM Carter, M Kim, N Pleshko, RG Spencer

Osteogenesis imperfecta (OI) is a genetic disorder characterized by bone fragility and frequent fractures. Diagnosis is based on clinical and radiological criteria, which can be nonspecific in mild-to-moderate cases, and increasingly by genetic testing, which is time-consuming. We tested the hypothesis that magnetic resonance imaging (MRI) can detect skin abnormalities that correlate with OI genotype and phenotype. Our primary research objectives were to determine: 1. whether MRI can differentiate between skin from OI and control subjects; 2. whether nondestructive MRI analysis is supported by invasive but highly specific Fourier transform infrared spectroscopic imaging (FT-IRIS); and 3. whether there is a relationship between type I collagen genotype, skeletal phenotype, and skin phenotype across patients of all ages. MRI analysis of 3-mm full-thickness forearm skin biopsies from OI (n=6) and non-OI control (n=2) subjects was performed, followed by FT-IRIS. MRI parameters, including T1, T2, and magnetization transfer (MT), were compared to FT-IRIS parameters characterizing dermal collagen. Initial findings showed clear differences in both MRI and FT-IRIS parameters between patients with OI and controls. Findings within the OI group correlated with the severity of clinical phenotype; epidermal and dermal layers were thinner in OI patients compared to controls with the degree of thinning correlating with the severity of OI phenotype. MRI revealed fat deposits within the dermis of OI skin only. FT-IRIS revealed differences in collagen orientation in the dermis of OI skin compared to controls. We conclude that MRI is sensitive to presence and severity of OI in human skin, as confirmed by FT-IRIS analysis. This supports the potential for developing an MRI approach for rapid non-invasive diagnosis of children with OI.

P.47

Transient Osteoporosis in Osteogenesis Imperfecta: Potential for Mis-Diagnosis

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Transient osteoporosis (TO) is a clinical syndrome affecting men more than women characterized by the occurrence of migratory joint pain. Pain occurs in hip, knees or ankles, and is found in conjunction with radiographic evidence of bone edema. TO may be confused with avascular necrosis (AVN) particularly at the proximal femur. TO is self-limited whereas (AVN) is a progressive lesion. Treatments differ.
Case Report: A 54 year old woman with type IV OI presented with persistent left hip pain of 4-5 months duration. 6 months earlier she had developed right hip pain aggravated by weight bearing. MRI revealed intense marrow edema of the anterior one-half of the right femoral head suggesting either avascular necrosis (AVN) or bone edema. No fracture was seen. Surgical intervention for AVN was recommended but deferred when the lesion was defined radiologically as TO. Pain resolved over 5 months on conservative treatment, but was followed in 1 month by severe left hip pain. MRI of the left hip again showed intense marrow edema. Residronate therapy was initiated with gradual resolution of the hip pain.
TO is a recognized but extremely uncommon cause of hip or knee pain in OI. In 1997, Noorda summarized 17 cases in male and female OI patients involving their hips, knees and ankles. Micro-trauma may evoke intense bone edema.
Conclusion: TO should be included in the differential diagnosis of hip or knee pain in the OI patient. Mis-diagnosis places the patient at risk for inappropriate surgery. Treatment with bisphosphonates may be useful.

P.48

Dentinogenesis imperfecta and related diseases to osteogenesis imperfecta

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Introduction: The bone and teeth dysplasias represent a numerous series of growth defects of hereditary origin. Their symptomatology exhibit a large scale of abnormalities which are the aim of this presentation.
From the broad scale of the diseases we demonstrate following conditions: dentinogenesis and or osteogenesis imperfecta, some syndromes with manifestation in orofacial area.

Material and methods: In all observed cases the clinical observation was acompanied by genelogical and laboratory methods as well as stomatological methods as well as stomatological examination.

Results: The analysis of our data shoiwed some interesting relationships between bone, ard dental tissues and mesenchymal orofacial structures.

Point of special interest: The most promising direction of our further research seems to be correlation between osteoblasts and odontoblasts in selected cases.

Conclusions and recommendations: The precise ethiopathogenetic knowledge promotes the correct genetic diagnostics and further medical preventive tactics.

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Clinical, Radiographic and Histological Characterization of Non-Lethal Type VIII OI

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Objectives: Type VIII osteogenesis imperfecta is a newly defined recessive form of lethal or severe OI caused by null mutations in LEPRE1, encoding collagen prolyl 3-hydroxylase1. We present here the first complete description of two non-lethal cases of type VIII OI.

Methods: Mutations were identified by DNA sequencing. We examined 3-hydroxylation of α1(I) Pro986 by mass spectroscopy and collagen fibrils by electron micrography. Clinical tests included serial radiographs, detailed analysis of blood, urine and bone histomorphometry.

Results: We identified null mutations in both LEPRE1 alleles of two children, ages 17 and 10 years old, with severe bone dysplasia, rhizomelia, white sclerae and extreme short stature. There is no P3H redundancy in bone vs. skin with ≤6% 3-hydroxylation of alpha1(I)Pro986 in proband bone and skin tissue, and cultured osteoblasts and fibroblasts. Collagen fibrils have normal diameters but irregular borders. Although Lepre1 was reported expressed in renal glomeruli and tubules, renal ultrasound was normal and detailed examination of random and 24-hour urine samples revealed only subtle abnormalities. Serum testing was distinctive from other types of OI, with elevated acid phosphatase, an osteoclast product, consistent with elevated bone turnover. Bone histomorphometry confirmed extremely high bone turnover, along with elevated Mineral Apposition Rate and faster matrix mineralization (lower MLT) than type VII OI. Stained sections demonstrate abnormal osteoblast morphology, with irregularly shaped cells piled up on the newly deposited matrix, rather than normal cuboidal osteoblasts in a monolayer. An additional distinction is a broad osteoid seam on all trabecular surfaces.
Conclusions: Non-lethal Type VIII has distinctive characteristics which distinguish it from severe AD type III OI. These traits provide a diagnostic guide for clinicians and a basis for further investigations of the mechanism of recessive OI.

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10 years study of Osteogenesis Imperfecta in children.Experience of the Pediatric and Orthopaedic Departaments of Central Children Hospital "Grigore Alexandrescu", Bucharest-Romania

Carasava L.

Daniela Ciotlos,orthopaedic surgeon,Central Children's Hospital "Grigore Alexandrescu", Bucharest

1. We studied 17 patients with osteogenesis imperfecta, aged between 6 days -13 years. The objective has been to evaluate the effects of pamidronate therapy on the evolution of the clinical course in these patients.

2. The DEXA method has been used for the first time in Romanian children during this study.

3. Audiometric measurements have been used ,also for the first time to study the pathology in Romanian children.

4. We describe a case with osteognesis imperfecta type I associated with hypothyroidism.

5. This is the first Romanian study on pamidronate effects in children with osteogenesis imperfecta.

6. Best treatment results occurred in patients who started pamidronate in very early infancy

7. Our youngest patient was 6 days old at the start of therapy. This might be the youngest baby who ever received pamidronate.

8. Our study recommends pamidronate as part of the complex therapy prescribed for osteogenesis imperfecta infantile patients.

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Osteogenesis imperfecta (OI): noninvasive ventilation (NIV) for respiratory insufficiency

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Introduction: OI patients often develop scoliosis and deformities of the chest wall. Chronic hypoventilation (breathless, tiredness, sleep disturbance, headache) may occur and viral or bacterial infections then lead to severe pneumonia. NIV is effective to treat hypoventilating neuromuscular patients - but there are few reports concerning OI patients.

Method: 3 patients with severe OI, all wheelchair-bound, were admitted after pneumonia having respiratory problems. Data before NIV, spontaneous breathing:

P 1: 19 yr, 2000: FVC:0,4 L, pCO2 52 mmHg, pO2 57 mmHg
P 2: 21 yr, 2001: FVC:1,8 L, pCO2 52 mmHg, pO2 57 mmHg
P 3: 29 yr, 2008: FVC:0,3 L, pCO2 65 mmHg, pO2 33 mmHg
(FVC = Forced Vital Capacity)

Noninvasive ventilation (overnight 6-9 hrs) was initiated with nasal masks (Respironics ®: P 1- 3), mobile respirators (PV 403 BREAS ®: P 1 & 2, Clevair plus Versamed ®: P 3) and pressure controlled modes (13-18 mbar).

Results: All patients accepted NIV, the pulmonary situation stabilized, no severe adverse reactions (rip fracture, pneumothorax) occurred. Essential was the change to tight fitted individual masks for long-term ventilation. Follow-up, spontaneous breathing 2001-2008 (except P 3):

P 1: 27 yr, median 2000-8: FVC: 0,76 L, pCO2 37 mmHg, pO2 79 mmHg
P 2: 28 yr, median 2001-8: FVC: 2,2 L, pCO2 38 mmHg, pO2 82 mmHg
P 3: 29 yr, median 2008-8: FVC: 0,49 L, pCO2 54 mmHg, pO2 49 mm Hg

Conclusion: To detect respiratory problems early regular pulmonary tests are recommended for severely affected OI patients. NIV is suitable for chronic respiratory insufficiency over years and enabled the patients to do a qualified job and to continue participating in social and daily activities.

General Information

Date

October 15-18, 2008

Venue

Auditorium 'De Schelde', Floor 1
'Provinciaal Administratief Centrum (PAC) – Het Zuid', W. Wilsonplein 2,
9000 Gent
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Registration Desk – Opening hours

Wednesday	15/10/2008	15.00 – 20.00 hrs
Thursday	16/10/2008	08.00 – 17.00 hrs
Friday	17/10/2008	08.00 – 17.00 hrs
Saturday	18/10/2008	08.00 – 13.00 hrs

Public Transport

Ghent St. Pieters train station > Brussels International Airport (Zaventem)
There is 1 direct train per hour between Ghent and Brussels International Airport.
For detailed train information, go to: www.b-rail.be

Parking Facilities

P3 (=Parking Zuid) is located opposite the 'Provinciaal Administratief Centrum'.
When coming from the E40 motorway signs 'Centrum' and 'P3'.

Language

The official language of the symposium is English.

Accreditation

An application for accreditation units has been submitted. Participants will receive a certificate of attendance at the registration desk of the Symposium.

Weather

The weather is usually mild on October, with average temperatures around 15-18°C. An occasional shower is possible.

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**Proof of official PhD status and age needs to be supplied with your registration*

The registration fee includes access to the scientific sessions and the exhibition, the coffee breaks and lunches as indicated in the programme. The welcome reception is also included in the registration fee.
Semico will send a confirmation of registration by email after receipts of both the registration form and the payment.