# Genetics of osteoarticular disorders

**Florence (Italy), February 22-23, 2002**

**Organized by**
**DEPARTMENT OF INTERNAL MEDICINE**
**UNIVERSITY OF FLORENCE**
**FONDAZIONE INTERNAZIONALE MENARINI**

**Sala Verde**
**Villa Vittoria**
**Piazza Adua, 1 - Florence (Italy)**

## Friday, February 22, 2002 - Morning

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| 08.30 | **M.L. Brandi** (Florence, I)<br>Welcome address and introduction |
| | **Session I** |
| Chairmen: | **M.L. Brandi** (Florence, I)<br>**R. Nuti** (Siena, I) |
| 09.00 | **G. Novelli** (Rome, I)<br>Quantitative genetics: problems to be faced in the future |
| 09.30 | **T.D. Spector** (London, UK)<br>Genetics of osteoarthritis |
| 10.00 | **R. Nuti** (Siena, I)<br>Osteoporosis: a multifactorial disorder |
| 10.30 | Coffee break |
| 11.00 | **A.G. Uitterlinden** (Rotterdam, NL)<br>Vitamin D Receptor gene polymorphisms |
| 11.30 | **J.A. Eisman** (Sydney, AUS)<br>Functionality of Vitamin D Receptor gene polymorphisms |
| 12.00 | **S.H. Ralston** (Aberdeen, UK)<br>*COL1A1* Sp1 binding site polymorphism and the genetics of osteoporosis |
| 12.30 | General discussion |
| 13.00 | Lunch |

## Friday, February 22, 2002 - Afternoon

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| | **Session II** |
| Chairmen: | **J.A. Eisman** (Sydney, AUS)<br>**S.H. Ralston** (Aberdeen, UK) |
| 14.30 | **L. Masi** (Florence, I) |

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| | The estrogen response in the genetics of osteoporosis |
| 15.00 | **L. Gennari** (Florence, I)<br>Genetics of male osteoporosis |
| 15.30 | Coffee break |
| 16.00 | **A. Falchetti** (Florence, I)<br>Genetics of familial osteoporosis |
| 16.30 | **E.F. Wagner** (Vienna, A)<br>Genetic analysis of Fos proteins in normal and pathological bone development |
| 17.00 | General discussion |

## Saturday, February 23, 2002 - Morning

### Session III

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| Chairmen: | **M. Matucci-Cerinic** (Florence, I)<br>**A.G. Uitterlinden** (Rotterdam, NL) |
| 09.00 | **M. Matucci-Cerinic** (Florence, I)<br>Osteoarthritis: a multifactorial disorder |
| 09.30 | **O. Ethgen, J-Y. Reginster** (Liège, B)<br>Epidemiology of osteoporosis and osteoarthritis |
| 10.00 | **C.J. Williams, S.A. Jimenez** (Philadelphia, USA)<br>Genetics of primary generalized osteoarthritis |
| 10.30 | Coffee break |
| 11.00 | **J. Korkko** (New Orleans, USA)<br>The genetics of familiar osteochondrodysplasias |
| 11.30 | **M.L. Brandi** (Florence, I)<br>Pharmacogenomics inosteoarticular disorders |
| 12.00 | General discussion |
| 13.30 | Lunch |

Dear Colleagues,

on behalf of the Fondazione Internazionale Menarini, I would like to invite you to participate in the International Symposium on Genetics of Osteoarticular Disorders to be held in Florence, Italy, on February 22-23, 2002.
Osteoporosis and Osteoarthritis are the two most common age-related chronic disorders of articular joints and skeleton, representing a major public health problem in most developed countries. Apart from being influenced by environmental factors, both disorders have a strong genetic component, and there is now considerable evidence from large population studies that these two disorders are inversely related. Thus, an accurate analysis of the genetic component of one of these two multifactorial diseases may provide data of interest for the other disorder.

The focus of the Symposium is on recent work in genetic research of osteoarticular disorders. The discovery of risk and protective genes for Osteoporosis and Osteoarthritis promises to revolutionize diagnostic and therapeutical strategies of these diseases. The quick expansion and advances in animal and human genomics of quantitative disorders not only will lead to a better understanding of skeletal biology, but will certainly open new avenues in pharmacological opportunities and pharmacogenomics of specific drugs.
The programme comprises several invited lectures to summarize our knowledge in the field. Lecture blocks were patterned to build a foundation of knowledge in the two selected topics. The primary goal of this Symposium is to bring together scientists and clinicians working in Osteoporosis and Osteoarthritis in order to identify the most promising and collaborative approaches for the coming decade. The site of Symposium has unique facilities to host this type of Conference. The city of Florence itself offers the opportunity of cultural and sight-seeing activities. I look forward to seeing you in Florence for this exciting event that should attract scientists and physicians with an interest in Osteoporosis and Osteoarthritis.

Maria Luisa Brandi, MD, PhD
President of the Meeting

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**Under the Auspices of**
Società Italiana dell'Osteoporosi del Metabolismo Minerale
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Società Italiana di Reumatologia
Società Italiana di Endocrinologia
Società Italiana di Genetica Umana

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**Quantitative Genetics: problems to be faced in the future**

Giuseppe Novelli

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Understanding genetics of complex disorders, or better, common oligogenic diseases, is one of the current challenges in medical genetics. With large numbers of coding (cSNPs) gene variants as well as polymorphic genetic markers available (SNPs), it will be possible to increase our understanding on disease pathogenesis. Now that third map of the human genome (http://genomebiology.com/2001/2/7/research/0025) revealed that human genome contains an estimated 65.000 - 75.000 genes, twice as many as proposed by the first two maps, the search for disease genes will not end but will enter a new phase. Monogenic disorders with Mendelian transmission have been identified. However, in diseases caused by more than one gene is even more difficult. Common examples include atherosclerosis, cancer, Alzheimer's disease, asthma, diabetes, glaucoma, osteoporosis, schizophrenia and psoriasis. There have been conflicting reports on the roles of associated genes. Even with population-based case-control studies and new statistical methods such as the sib-ship disequilibrium test and the discordant alleles test, there is no agreement on whether a2-macroglobulin (A2M) is a gene for Alzheimer's disease. Another example is the HCR gene on chromosome 6 and psoriasis. Ethnic variation causes further complications. In our investigation of UFD1L and PCQAP variants in schizophrenia, we did find dissimilar results in Italians compared to other Caucasians. On the other end, Nod2 mutation associated to Crohn's disease is found with the same frequency in different populations. At least four areas are critical for future work. First, we should understand what to look for and what to expect to find. To this end, the genetic theory and modelling of populations and diseases need to be considered. Many arguments speak for a relatively simple genetic background for many common diseases. Second, streamlined genotyping methods are needed even in centres that deal with modest numbers of samples and polymorphisms. In almost all settings, considerable savings, higher speed and improved quality can be obtained by centralised, high-throughput genotyping. Third, computational tools and methods of data analysis need to be reconsidered and tailored to make optimal use of the data sets available. Fourth, tight interactions are needed between clinicians (expertise on phenotyping) and geneticists (understanding of genetic principles) to support sufficiently large-scale projects.

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**Genetics of osteoarthritis**

Tim D. Spector, Alex J. MacGregor
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Rare inherited diseases of cartilage have been recognised for many years and the familial aggregation of Heberden's nodes was noted by Stecher in the 1940's. However until recently the genetic influence on the common forms of osteoarthritis was unclear. A number of studies in the last few years have shown unequivocal evidence that at least 50% of the variance of OA in the hands, knees and hips is accounted for by genetic factors. These include classical twin studies of unselected populations as well as population based family studies and affected sib pair studies. Segregation analysis has suggested that a major gene may also be present in addition to polygenic effects. Recently a genetic effect on disk degeneration, and spinal osteophytes, has also been demonstrated in twins using MRI data.

Further clues come from a large number of studies which have uncovered rare families with mutations in the collagen 2AI gene who express precocious OA and varying degrees of chondrodysplasia. Studies have also implicated collagen IX mutations. To date these mutations do not appear to influence common forms of OA but do offer exciting insights into disease mechanisms.

Candidate genes for common forms of OA, which have shown associations in studies of varying size, include the Vitamin D Receptor gene (which influences bone density and is near the Col 2AI

locus) as well as IGF-1 genes, TGFb, and ColA1 genes. Linkage studies using families and affected sib pairs have to date shown suggested loci in an area of chromosome 2q and other larger studies are ongoing to try to confirm and pinpoint this region.

OA is now recognised as a heterogeneous group of conditions with a wide variety of different pathological processes leading to a common outcome of joint destruction and disability. A more useful understanding of the physiology and genetic mechanisms of the complex disease may be obtained by studying intermediate phenotypes individually or in combination. These are obtained by dividing OA into its constituent parts. These intermediate phenotypes may operate independently or together in clusters determined by pleiotropic genes. The definition of 'generalised OA' however has no consensus by clinicians and epidemiologists and may not exist as a genetic entity. Bone is an overlooked phenotype in OA. There is increasing evidence for a major role for bone in the pathogenesis of OA. OA patients have 5-8% greater bone density than controls and this is seen up to five years before the first osteophyte is visible on x-rays. There is also some evidence for modest increases in bone turnover (itself genetically mediated) in early OA, which may precede x-ray change. A recent study of discordant twins for OA hip suggested the possibility of shared genes with those influencing bone density.

In conclusion, OA is a strongly genetic disease, which is likely to be a complex polygenic disorder. Understanding how the individual genes influence the many intermediate processes is likely to be a fruitful avenue to provide insight into disease pathways and potential new drug targets.

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**Osteoporosis: a multifactorial disorder**

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Bone loss of postmenopausal women must be considered as a result of many factors which modify the rate of bone remodeling inducing an imbalance between the activity of osteoclasts and osteoblasts (1). However, a reduction in bone mass below the average for age may be also a consequence of inadequate accumulation of bone in young adult life. Determinants of a low peak bone mass are considered genetic factors, low calcium intake during childhood, low body weight, sedentary lifestyle, and delayed puberty (2).

At maturity the two main causes promoting bone loss are estrogen deficiency and aging. The major mechanism of the phase of rapid bone loss that lasts for five years in women is estrogen deficiency. The slow phase of bone loss is attributed to age-related factors such as an increase in PTH levels, a reduction in intestinal calcium absorption, and to osteoblasts reduced activity. In men the slower phase of bone loss starts at about age 55 years (3-4). Indeed osteoporotic fractures increase with age: vertebral fractures in the 60 years, hip fractures in the 70 years. A twofold higher incidence amog women compared with men for all age-related fracture sites was calculated: because life expectancy is longer for women, it results in a greater fracture prevalence among women than would be predicted from the age-adjusted incidence ratio (5).
Moreover many environmental factors have been considered capable to increase the risk for osteoporosis. These are: nutritional deficiencies and particularly reduced calcium intake; reduced physical activity and mechanical loading; medications, such as corticosteroids; lifestyle factors as smoking, alcohol, and caffeine; increased susceptibility to falls. In a U.S. representative sample of postmenopausal women who have never been on estrogen therapy it was observed that only some modifiable (physical activity three to five times per week, body mass index) and nonmodifiable (age, race, mother's history) factors are significantly related to the prevalence of osteoporosis (6). In men weight loss from maximum of 10% or more was related to elevated hip fracture risk; subjects who reported low nonrecreational physical activity or who were current smokers were somewhat more likely to experience a hip fracture, but the risk was not statistically significant; a modest association between higher protein consumption or higher calcium intake and lower hip fracture risk was found (7).
A recent european survey examined a middle-age women population, and detected four independent predictors for ankle fracture: smoking, multipharmacy, fracture history, overweight status (8).
As regards physical activity, jogging was found to be associated with higher femoral neck bone mineral density in men and thus may provide some protection against osteoporosis and fracture (9). A study performed in elderly women and men indicate that risk factors consistently associated with bone loss include female sex, thinness, and weight loss, while weight gain appears to protect against bone loss for both men and women. Moreover, current estrogen use may help to maintain bone in women, whereas current smoking was associated with bone loss in men. Surprisingly, bone loss was not affected by caffeine, physical activity, serum 25OHD, or calcium intake (10). Other reports underline that caffeine's effect on bone loss may be associated with VDR genotype, and that a moderate caffeine intake is not associated with increased bone loss (11). Several studies suggest that a high animal protein and/or phosphate consumption may induce an increase in the risk of osteoporotic fractures promoting hypercalciuria: probably this pathogenetic factor must be limited to populations with unusual high intake of protein; more frequently low protein consumption is an aspect of a general malnutrition, and this is associated with low bone mass and high risk of fractures. As regards sodium intake and risk of bone loss, high sodium diets increase urinary calcium excretion and consequently PTH secretion; however, the clinical significance of this aspect is uncertain.

Other factors contribute to make osteoporosis a multifactorial disorder: uderlining chronic disease, as gastrectomy, hyperthyroidism, hypercortisolism; alterated physical characterisctcs of bone, as density, size and geometry, microarchitecture, and composition (12).

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**Vitamin D Receptor Gene Polymorphisms**

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The role in skeletal metabolism of the steroid hormone vitamin D and its nuclear receptor (VDR) is well known. While deleterious mutations in the VDR gene cause 1,25-diydroxyvitamin D resistant rickets, the influence of more subtle DNA sequence variations (polymorphisms) in the VDR gene remains unclear. Three adjacent RFLP's for *Bsm*I, *Apa*I, and *Taq*I, respectively, in intron 8/exon 9 at the 3' end of the VDR gene are the most frequently studied sofar.
Early on in the field of genetics of osteoporosis, Morrison et al., reported that the *Bsm*I RFLP in the last intron of the VDR gene was related to osteocalcin serum levels (as a marker of bone turnover) [1]. Subsequently, these authors also reported this polymorphism to be associated with differences in bone mineral density (BMD) in postmenopausal women [2]. In the following years dozens of papers were published analysing the same RFLP in relation to BMD but with varying results. Possibilities to explain the discrepancies between studies are small sample size resulting in statistical scatter of the data, unrecognized population admixture and variable linkage

disequilibrium between populations (because of the use of anonymous RFLPs). Also, gene-environment interactions, involving dietary calcium intake and circulating 1,25-dihydroxyvitamin D levels, may explain discrepancies in the literature. Several meta-analyses have indicated that the overall difference in BMD between the VDR gene variations studied was small (1-2%) and might also be influenced by other factors such as age-at-menopause [3,4].
In the largest study published sofar [5], analysing 1782 Dutch elderly men and women, actually no effect of single RFLP's was observed, whereas a small effect was detected employing haplotypes constructed of the three adjacent 3' RFLP's. This indicates that accurate recognition of allelic heterogeneity (by haplotypes rather than by single polymorphisms) is important to identify the risk alleles at this part of the VDR gene. This notion is corroborated by the observation of substantial sequence variation in the 3'UnTranslated Region (3'UTR) which spans 3.2 kb. Although the function of the 3'UTR is ill-defined, several experiments have suggested that polymorphisms in this region might alter VDR function [3,6].
The analysis of the genomic organisation has shown that the VDR gene is quite large spanning at least 80 kb [7], and has an extensive promotor region capable of generating multiple tissue-specific transcripts [8]. In view of the genome-wide observed frequency of Single Nucleotide Polymorphisms, one can expect over 100 polymorphisms present in the VDR region alone. These polymorphisms will be present in areas that are functionally relevant, such as the 5' promotor regions and in the coding sequence. For example, a substitution (T to C, detected by *Fok*I) at exon 2 eliminates the first ATG translation initiation site and allows a second one 9 bp downstream to be used. Thus, two variant forms of the VDR protein can be translated that differ by three amino acids resulting in proteins of 427 and 424 amino acids. Although this polymorphism was found to be associated with BMD, this finding was not universal.
The interpretation of the polymorphic variations used sofar in the VDR gene is severely hindered by the fact that many of the polymorphisms considered are anonymous. However, current data indicate that multiple polymorphic variations exist in the VDR gene that could each have different types of consequences. Thus, 5' promotor variations will affect mRNA expression patterns and levels, whereas 3' UTR sequence variations could affect the mRNA stability. In combination as haplotypes, these genotypic differences are likely to affect the VDR mRNA levels, and most likely also VDR protein levels. In combination with different protein variants of the VDR, the functional effect of polymorphisms might be depending on the cell type, developmental stage, and activation status.
To understand the functionality of polymorphisms it is therefore crucial to first identify all polymorphisms across the VDR gene. Therefore, our efforts are focussed on finding novel sequence variations and establishing the phase of alleles across the entire VDR gene by defining haplotype patterns. The ultimate goal of this enterprise is to identify the functional VDR sequence variants that matter. Once these polymorphisms and haplotypes have been identified, large scale association studies, preferably in different populations, will allow to estimate the effect size in relation to biological parameters (endpoints) most likely affected by the vitamin D endocrine system. Although BMD is a frequently used endpoint in association studies of the VDR gene polymorphisms, it might not be the one on which the vitamin D endocrine system has the largest influence. This notion is supported by the meta-analyses mentioned above but also by associations reported for VDR polymorphisms with fracture risk, independent of differences in BMD [9]. In line with this notion is also the observation that VDR gene polymorphisms are associated with different diseases such as osteoarthritis, prostate cancer, infection susceptibility and diabetes [reviewed in 10]. Together these associations indicate the pleiotropic nature of the vitamin D endocrine system, a characteristic not uncommon for steroid hormones. In depth analysis of VDR polymorphisms might therefore not only be important for the fields of osteoprosis and osteoarthritis but also in other medically relevant areas.

## References

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**Functionality of Vitamin D Receptor Gene Polymorphisms**

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Bone density, as a predictor of osteoporotic fractures, has been the focus of intense study in relation to hormonal, life style, medical and, more recently, genetic factors. In twin and family studies, 50 - 85% of the age-specific variance is attributable to genetic factors. Allelic variants of the vitamin D receptor (VDR) gene were the first to be linked to and associated with physiological variation in bone density [1-8]. This work was followed by reports of association (and some linkage) studies with bone density and even fracture outcomes of allelic genes of the oestrogen receptor, collagen 1µI, various cytokines and their receptors, the PTH receptor and, most recently, the LDL receptor-related protein 5 gene [9-33]. While the VDR polymorphism studies established the candidate gene approach in relation to bone density, turnover and fractures, they have been controversial with subsequent studies finding weaker (or no) associations [34-42]. This inconsistency, as reviewed in two meta-analyses for the VDR [43, 44], has been noted for most of the other linked or associated genes. The presence of multiple polymorphic sites and the lack of linkage disequilibrium, even between closely spaced polymorphisms, has stressed the requirement for formal haplotyping, by inference or by direct analysis, over extended regions in specific gene loci. Furthermore, when considering functionality of polymorphisms, statistical power [45-47] and differing ethnic and racial backgrounds, environmental and hormonal factors need to be assessed concurrently with gene haplotypes. In almost all association studies of gene alleles, the precise mechanisms for the polymorphisms have not been defined. In contrast with typical genetic disorders, virtually none of the allelic differences relate to coding region differences. The polymorphic region in a nuclear co-factor binding site of the collagen 1µI gene may alter collagen gene expression. Similarly covert differences in 5'- and intronic regulatory regions in linkage with the overt polymorphic sites in most other allelic genes may mediate the physiological outcomes. VDR polymorphisms, in addition to their associations with bone and calcium homoeostasis, have been associated with differences in susceptibility to and prognosis of immunological disorders and infectious diseases, diabetes and obesity as well as prostate, breast and colon malignancy [48-63]. Some studies also suggest an effect of the VDR alleles on osteoarthritis of spine and hip [64-66]. Thus the VDR gene can be a useful model to investigate some of the mechanisms of wide-ranging allelic polymorphism effects. These can be considered at several levels from molecular mechanisms associated with gene

regulation through to responses to physiological, hormonal and environmental factors, including regulation of gut calcium absorption and long-term bone density response to calcium intake, physical activity and vitamin D treatment.
Bone homoeostatic responses to dietary calcium intake that range from less than 500 to more than 1500 mg/day in different population groups may interact with genetic factors. Some but not all studies have noted differences in gut calcium absorption, parathyroid gland function and renal calcium excretion in relation to VDR alleles [31, 67-78]. Other studies have found similar effects over time in change in bone density under control conditions or in response to vitamin D or analogue treatment [79-81]. Again results have been inconsistent [77, 82, 83]. Possible associations with body weight may confound some of these analyses [82, 84-89]. At the molecular level, the polymorphic sites may be in linkage with further 3' polymorphisms and in some, but again not all, studies these may influence VDR mRNA stability [90-101]. It has also been suggested that a more 5' polymorphism that changes the length of the final hVDR protein may influence function [102]. This could also be related to the relative expression of the "standard" VDR or the recently described longer B1 isoform [103]. Moreover some of these "effects" may be influenced by gene-gene interactions, for example with the oestrogen receptor and collagen 1µI polymorphism [10, 38, 104]. The complexity of these potential mechanisms and interactions make it difficult to directly assess the functionality of individual polymorphic gene effects including those for the VDR. Further work is clearly required to evaluate potential mechanisms underlying these allelic differences. Understanding how they may act and interact with other genes and environments may help improve regimens and strategies for optimal individualised therapy.

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## *COL1A1* Sp1 binding site polymorphism and the genetics of osteoporosis

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Osteoporosis is a common disease with a strong genetic component. Data from twin and family studies suggest that up to 85% of the variance in bone mass is genetically determined and other factors which predispose to osteoporotic fracture risk such as bone turnover, femoral neck geometry and ultrasound properties of bone also have a strong genetic component. A family history of osteoporotic fracture predicts the occurrence of fractures independent of bone mass, indicating that genetic influences on osteoporosis are complex, involving variation in genes which not only affect bone density, but also those which also influence skeletal geometry, bone turnover and bone quality.

The COLIA1 gene, which encodes the alpha 1 chain of type I collagen is an important candidate gene for regulation of BMD and bone fragility. Missense mutations and splice site mutations in COLIA1 which lead to null alleles have been reported to result in a phenotype which overlaps between mild osteogenesis imperfecta and severe osteoporosis. In view of this, we investigated the hypothesis that polymorphisms of the regulatory regions of COLIA1 may predispose to osteoporosis. We identified a polymorphic Sp1 binding site in the first intron of the COL1A1 gene that was associated with reduced BMD and an increased risk of osteoporotic fractures in women from the UK 1. Whilst subsequent studies in many populations confirmed these associations, not all investigators reported a positive association between COLIA1 genotypes and BMD or osteoporotic fractures. In view of this, we carried out a meta-analysis of these studies and this revealed that the risk of osteoporotic fracture in patients who carried the unfavourable allele was too great to be accounted for by allele-specific differences in BMD, implying that the polymorphism may act as a marker for reduced bone quality2. In keeping with this, functional studies showed that the polymorphism was associated with increased binding affinity for Sp1, and increased allele specific transcription of COL1A1 in vitro. Further studies were conducted to examine the functional consequences of these abnormalities in collagene gene regulation. Ex-vivo mechanical testing of bone cores from patients of different genotype showed reduced yield strength (adjusted for bone density) in G/T heterozygotes (n=7) when compared with G/G homozygotes (n=10) (mean + SD = 4.60 + 0.3 vs 3.55 + 0.2 Mpa; p=0.03). Composition analysis of these bone samples suggested that bone from G/T individuals had a reduced inorganic content when compared with G/G homozygotes. This was confirmed by quantitative backscatter electron imaging analysis which showed reduced mineralisation of bone in G/T heterozygotes (n=7) when compared with G/G homozygotes (n=6) (21.5 + 0.5 % vs 20.2 + 0.7%; p=0.04) and increased heterogeneity of

mineralisation as reflected by broadening of the bone mineral density distribution peak (4.5 + 0.5 % vs 3.5 + 0.5 % p=0.04). Osteoblasts from G/T heterozygotes also produced an abnormal ratio of collagen alpha I (1) chains, relative to alpha I (2) when compared with G/G homozygotes (2.3:1.0 vs 1.99:1.0; p=0.007), even though cell growth, total protein and alkaline phosphatase were similar in the two groups. Cultures from G/T heterozygotes also had an impaired ability to form mineralised bone in vitro when compared with G/G homozygotes, as reflected by Alizarin Red S staining of b-glycerol phosphate supplemented cultures (1.64 + 0.1 vs 0.6 + 0.1 mM Alizarin/105 cells; p<0.0001). Our data indicate that the COLIA1 "T" allele influences the ratio, but not total amount of collagen type I alpha chains produced by bone cells, leading to abnormal mineralisation of bone both in vivo and in vitro and reduced bone strength. Other factors may also contribute to the relationship between COLIA1 genotype and fracture risk however, since some investigators have reported positive associations between the COLIA1 Sp1 alleles and ultrasound properties of bone as well as associations with other pheotypes relevant to the pathogenesis of osteoporotic fractures including femoral neck geometry 3.

In conclusion, the assembled data suggest that COLIA1 alleles predispose to osteoporosis by several mechanisms including a reduction in BMD, an alteration in bone composition leading to reduced bone strength and an alteration in bone quality and femoral neck geometry.

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**The estrogen response in the genetics of osteoporosis**

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Sex steroids are important regulators of bone physiology, play an essential role in the maintenance of bone health throughout life, and adverse effects of hormone deficiency can be seen in the young and old, in men and women. The mechanisms by which these effects are mediated remain incompletely known. In recent years, our understanding of the role of estrogens in both females and males has expanded greatly. For example, considerable emphasis has been focused on the regulation of extragonadal estrogen biosynthesis, in particular that which occurs in adipose tissue and bone, and its importance in the well-being of the elderly (1). There is increasing evidence that both men and women extraglandular production of C(18) steroids from C(19) precursors is important in normal physiology as well as in pathophysiology states. The enzyme aromatase is found in a number of human tissues and cell including bone and it is important in the locally catalyzation of C(19) steroids to estrogens. The observation of a marked bone phenotype in men with mutation of either the estrogen receptor (ER) a or aromatase (2, 3) have led to the conclusion that local estrogen production in bone cells plays an important role in the maintenance of bone mineralization and the prevention of osteoporosis in men and in women (1). Of equal significance is the realization derived from studies of ER a-knock-out (ERKO) (4) and aromatase knock-out

(ArKO) (5) mice, which are characterized by a low bone, mass.
Extragondal sites of estrogens biosynthesis possess several fundamental features that differ from those of the ovaries. The first important point is that the estrogen synthesized within these compartments acts predominantly at the local tissue level in a paracrine or intracrine fashion (6). Thus, the total amount of estrogen synthesized by these sites may be small, but the local tissue concentrations achieved are probably high and exert biological influence locally (7). In addition, the regulation of estrogen biosynthesis in each tissue site of expression is unique and this leads to a complex physiology situation, which make, for example, interpretation of circulating estrogen levels very difficult (8). The key steroidogenic enzyme involved in the conversion of androgens to estrogens is the aromatase that derived from CYP19 gene (9, 10). In bone, aromatase is expressed primarily in osteoblasts and chondrocytes and aromatase activity in cultured osteoblasts is comparable to that present in adipose stromal cells (11). Thus it appears that in bone also, local aromatase expression is the major source of estradiol (E2) responsible for the maintenance of mineralization and it likely that circulating E levels merely reflect the sum of local formation in various sites. In addition, estrogen production in these extragonadal sites is dependent on an external source of C19 androgenic precursors because the extragonadal tissues are incapable of converting cholesterol to the C19 steroids. As a consequence, circulating levels of testosterone (T) and androstenedione as well as DHEA and DHEAS become extremely important in terms of providing adequate substrate for E biosynthesis in these sites. It should be pointed out that in the postmenopausal women, circulating T levels are in order of magnitude grater than circulating E2 levels. This by itself suggest that circulating androgens might be more important for maintaining local E levels in extragonadal sites than are circulating E2 levels (7). In this context, it is appropriate to consider why osteoporosis is more common in women than in men and affects women at a younger age in terms of fracture incidence. Simpson et al. (12) suggested that uninterrupted sufficiency of circulating T in men throughout life supports the local production of E2 by aromatization of T in E-dependent tissue such as bone protecting tissue against mineral loss. It is well known that multifactorial diseases such as osteoporosis involve multiple genes and environmental factors and result principally from genetic variations that are relatively common in the general population. Genes involved in estrogen metabolism (the aromatase gene) and in estrogenic response (the estrogen receptor a gene) are possible contributors to the abnormal pathophysiological processes associated with osteoporosis (13, 14). It is know that dinucleotide (TA)n repeat polymorphism at the human ERa is associated with low BMD in postmenopausal women indicating a role of this gene in the regulation of bone metabolism (15). In addition, genetic variants in the human aromatase gene, for example, could alter estrogen metabolism with several repercussion on bone metabolism. A polymorphic repeat in CYP19 has recently been associated with bone loss, risk of fractures (16) and with breast cancer risk (17). In order to evaluate a possible functional role of this polymorphism we studied the aromatase activity in fibroblast cells from patient with opposite genotypes. We found that patients with a genotype associated with a high bone mass and with a low fracture risk synthesized a higher amount of estradiol in comparison with the opposite genotype. These data not only throw light on the role of locally-produced estrogens in health and diseases processes, but may also lead to new modalities of therapy in the future.

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**Genetics of male osteoporosis**

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Recent epidemiological studies pointed out that male osteoporosis is an increasingly important health problem. It has been estimated that a 50-years-old man has about a 6% risk of hip fracture

and a 16% to 25% of any osteoporotic fracture in his remaining life (1). Because of the increasing in life expectancy, the number of elderly men with osteoporosis will increase dramatically in the next years, so that the number of fractures in men is expected to double by 2025. Despite the increasing importance of the problem, until recently, the focus of osteoporosis research has been made on women, and much less is known regarding those factors that may influence bone mass or loss in older men.

In the past years, several epidemiological and clinical observations have underlined the importance of genetics in the pathogenesis of osteoporosis. It has been estimated that from 50% to 80% of the inter-individual variability in bone mass is genetically determined (2). Although most studies have been conducted in women, there is evidence to suggest that genetic factors and a positive family history of fracture are also important in determining osteoporosis in men (3, 4, 5). In rare instances, osteoporosis in men could be inherited in a simple Mendelian pattern. Examples of this include familial osteoporotic syndromes due to mutations in the aromatase and ERa genes (6, 7). Families have also been described in which high bone mass is inherited as an autosomal dominant trait, consistent with the effect of a single gene located on chromosome 11 (8). However, except these rare conditions, osteoporosis has to be considered a multifactorial disease in which genetic determinants are modulated by hormonal, environmental and nutritional factors. Possibly the same osteoporotic phenotype could be the result of different genetic and/or environmental interactions. It is also likely that some individuals genetically at risk for osteoporosis never become osteoporotics (*incomplete penetrance*) or, conversely, that individuals with no predisposing genes may develop osteoporosis with age, due to non genetic factors (*phenocopy*). The genetic effect on bone may also be site-specific, with different genes regulating bone density at different skeletal sites. Moreover, the possibility that a significant part of the heritability of bone mass is related to shared genetic contributions to skeletal size and body composition cannot be excluded. Indeed, it has been recently demonstrated that bone fragility leading to spine or hip fracture in men may be the result of site-specific deficits in bone size and volumetric BMD (vBMD). Men with vertebral fractures have reduced vertebral volume and reduced vBMD at the vertebral bodies but not at the femur, whereas men with femoral neck fractures have reduced femoral volume and reduced femoral neck vBMD, with more modest deficits at the vertebral bodies (9).

To date, most efforts toward understanding the genetic determinants of BMD and osteoporotic risk have largely relied on population-based case-control association studies of genes known to be involved in bone metabolism. By this approach positive and negative associations have been reported for several candidate genes but the individual contribution of these genes to the pathogenesis of osteoporosis is still controversial (2). Even though most of the work on the genetics of osteoporosis has focused upon women, there are now preliminary interesting data about some candidate genes with possible implications in male osteoporosis. These data were based on association studies and involve polymorphisms at the vitamin D receptor (*VDR*), collagen type I alpha 1 (*COLIA1*), insulin growth factor I (*IGF-I*), aromatase (*CYP19*), interleuchin 6 (*IL-6*), androgen receptor (*AR*) and estrogen receptor alpha (*ERa*) genes (10). It is important to remember that even though association studies are in some respects more powerful than linkage-based approaches to the study of complex diseases, they are also prone to give false positive results mainly due to population admixture and selection bias in recruitment of cases and controls. Thus, it is extremely important for a proper interpretation of results that a positive association is confirmed in large samples from different populations, and that the mechanism(s) by which the associated polymorphism influence osteoporotic risk is discovered not only at bone level but also at the molecular level. Moreover, the importance of genetic heterogeneity, including ethnicity, as well as environmental, hormonal and constitutional confounders (i.e. skeletal and body size) will need to be taken into serious account in the future genetic studies. Gene-gene and gene-environment as well as pharmacogenomic interactions in human and animal models will be critical targets for future research. At the same time, further developments in molecular genetics, such as microarray chips, as well as extended large-scale pedegree analyses will allow simultaneous identification of thousands gene polymorphisms segregating with osteoporosis. All these efforts will certainly improve our knowledges on the ethiopathogenesis of this invalidating disorder, allowing earlier preventive strategies and the development of more appropriate and effective treatment options.

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## Genetics of familial osteoporosis

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Understanding the genetic bases of multifactorial diseases represents the future task for scientists in order to explain new physiopathological aspects of complex traits. One of these diseases is represented by osteoporosis (OP). OP is a common disorder associated with reduced bone mineral density, affecting up to 50% of women (1) and 12% of men (2) at same point during life. Osteoporotic fractures are an increasing health care burden in all aging communities. Peak bone mass is the major determinant of bone mineral density for up to 10-20 years after menopause, until age-related factors become relatively more important in determining bone mass loss. Although OP is a multifactorial trait, genetic factors play an important effects on peak bone mass and in the pathogenesis of OP (3). The development in advanced techniques for measuring BMD made possible to have available a quantitative trait for segregation analysis. Up to 75% of variation in BMD has been suggested to be under genetic influences. However, the inclusion of OP in the list of genetic disorders is still debatable. Twin studies have shown a strong genetic effect of BMD at both peripheral and axial sites (4-8). The largest genetic influence was observed at sites of high trabecular bone content. Although twin studies have been powerful tools for studying genetic effects, they show some limitations and can only imply but not prove genetic influence. A variety

of experimental design appropriate models for establishing genetic background of OP have been proposed, such as linkage analysis, allele sharing methods, association studies and experimental crosses. In the last five years association studies gave origin to several novel information whose may quite controversial. These studies have been using the candidate gene approach and given the number of factors that are likely to be involved, there is a seemingly unlimited supply of candidate genes for OP. Discrepancy among studies can be explained on the basis of the quantitative polygenic nature of this disorder, where the effect of a given gene can easily be modified by epistatic and/or pleiotropic effects of other genes. It is likely that interactions between different genes could, at least in part, explain the discrepancy among the studies. Familial studies suggest a significant effect of genetic factors on peak bone mass (9). For example, using the early approach of metacarpal/cortical bone thickness, parent-offspring correlations indicated that bone mass was for a large portion genetically determined. In addition sib-pair studies, in premenopausal daughters of women with OP, have also shown modest but significant reductions in lumbar spine, femoral neck and femoral shaft BMD compared to premenopausal women without a family history of OP (10). Moreover, they demonstrated that mothers with osteoporotic fractures have daughters with lower bone density, but we do keep in mind that family-based studies are suffering because of the inevitable comparisons of subjects of widely different ages and year-of-birth cohorts and of familial similarities in lifestyle and choices. Unfortunately, only in rare known cases OP is inherited in a simple Mendelian manner (1) as it happens in Osteogenesis Imperfecta (11), aromatase gene (12) and estrogen receptor alpha (13) mutations. However, Familial OP (FOP) is still suffering of lacking of a clear clinical definition. While subsets of patients show a clear family history in other cases the separation of familial from environmental factors is difficult. Some families, with apparent transmissibility of OP, also exhibit clinical evidence of connective tissue dysplasia. We suggest a multistep approach designed to define and study FOP. In the first step we identify "interesting pedigrees" through the definition of phenotypic characteristics, such as a minimum of 4 subjects with OP or low BMD, the presence of also affected males, early onset, low physical activity and signs connective tissue dysplasia. We have to consider that the best approach should consist of recruiting large families from demographically restricted and rapidly growing populations, being ideal for genome-wide screens and for both parametric and non parametric analyses (14). Beyond narrowing the definition of disease and recognition of a rare subgroups of a common phenotype, we suggest other criteria to increase the *a priori* chance of success for linkage studies, such as the need for an ideal kindred to be multigenerational (up to 3 generations) exhibiting a pattern of inheritance with high penetrance. In fact, low penetrance and high expression variability of a considered quantitative trait represent two major problems when approaching to familial linkage analysis. In the second step genetic approaches, such as traditional linkage analysis to candidate genes (i. e. VDR, ERa, Aromatase, CTR, PTHR1, COLIA1) and/or wide genome scanning with high resolution linkage genetic maps (5 cM intervals) will be performed in the collected kindreds, making possible to distinguish between the genetic influences of a given candidate gene and nearby genes. This approach should make possible to map unknown OP-related genes to defined chromosomal regions containing Quantitative Trait Loci (QTL), to clone them and to identify their function. Indeed, the absence of a clear mendelian inheritance pattern makes extremely difficult, or impossible, to determine *a priori* the number of genes involved and the magnitude of their effects. We consider that a collection of a probably minor, but well clinically characterized, number of osteoporotic kindreds in as much as possible of the EC countries could represent an important tool not only for a national approach but also for a pooling of a discrete number of sufficiently clinically homogenous kindreds on which performing several genetic analyses due to the exchange of DNAs and/or immortalized lymphocytes.

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**Genetic analysis of Fos proteins in normal and pathological bone development**

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Fos proteins are transcription factors belonging to the AP-1 complex, which acts like a biosensor for many cells and is causally involved in many developmental processes, but also in cell differentiation and disease. One of the four members of the Fos proteins is c-Fos, which is a key regulator of bone development (1). Transgenic mice expressing exogenous c-Fos develop bone tumors, whereas mice lacking c-Fos are osteopetrotic due to a differentiation block in bone resorbing osteoclasts (2). We were also interested to study how c-Fos and its related protein Fra-1, which is c-Fos inducible, control osteoblast proliferation and osteoclast differentiation (3). Gene deletion experiments in mice demonstrated that Fra-1 is an essential gene for development (4) and genetic rescue experiments suggest that Fra-1 is not essential for osteoblast and osteoclast

differentiation. However, transgenic mice overexpressing Fra-1 develop the bone disease osteosclerosis, which is due to increased bone formation (5). The primary cause of the disease in Fra-1 transgenic mice is an osteoblast differentiation defect, although the transgenic osteoclasts are hyperactive in vitro. To test whether Fra-1 can substitute for c-Fos, we generated knock-in mice that express Fra-1 in place of c-Fos. Fra-1 rescues c-Fos dependent functions in bone development, which appeared to be gene-dosage dependent (6). However, Fra-1 failed to substitute for c-Fos in inducing expression of target genes in vitro. We are using these systems to identify novel Fos target genes by microarrays and with the help of bone-specific conditional alleles of c-Fos and Fra-1, we are studying the molecular mechanisms how Fos proteins govern bone cell development and differentiation.

Since Fos proteins need Jun proteins as partners to activate transcription, we are also investigating the function of c-Jun in bone cells using the conditional gene inactivation system called cre/loxP. Chondrocyte-specific inactivation using col2A1-cre transgenic mice results in severe scoliosis caused by failure of intervertebral disk formation and abnormal vertebral arch development, suggesting that c-Jun is a novel regulator of sklerotomal differentiation.On the other hand, deletion of c-Jun in the osteoclast lineage results in inefficient differentiation indicating that Jun proteins are also important regulators in skeletal development and differentiation. Recent experiments further supporting the relevance of the Fos and Jun transcription factors in bone disease will be discussed.

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**Osteoarthritis: a multifactorial disorder**

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Osteoarthritis (OA) is one of the most common diseases among mammals and its presence has been reported in Egyptian mummies and in dinosaurs. The impact of OA on disability is substantial, greater than any other medical condition in elderly persons. The process affect not only the cartilage, but also the entire joint structure, including synovial membrane, subchondral bone and all the other soft tissue structures. For this reason, OA is considered as an "organ disease". Many advances have been made in understanding the pathophisiology and epidemiology of OA, but untill

now it is unclear whether OA is a single disease or many disorders with a final common phenotype- "joint failure".
In fact, many joints with different risk factors may be targeted by symptomatic OA including small finger joints and large joints such as knees and hips. Indipendently from causes, osteoarthritic pathology occurs as a result of an inbalance between synthesis and repair (catabolic) processes. In this pathologic pathway the chondrocyte is well recognized not as passive bystander but as a metabolic active cell (1) playing a pivotal role in determining the cascade of events leading to the full expression of the disease. Moreover, many reports indicate OA as an inflammatory disease (2) considering the synovial inflammation not an innocent event pathologic process but a relevant aspect that contributes to the progression of the disease. Even if the prevalence of OA increases with age, the disease is not necessarily an inevitable consequence of aging but it is a complex disease due to a combination of risk factors (ranging from the mechanical to the biochemical). Risk factors for development of structural OA may be divided into systemic and local biomechanic factors playing a different pathogenetic impact in relation to the characteristic of the joint involved. For systemic risk factors, hormonal status (3) has been correlated with the disease suggesting that estrogen deficiency might play a role in causing OA. Moreover, high mineral bone density is associated with an increasd prevalence of hip, hand and knee OA. Also nutritional factors such as high dietary intake of antioxidant substance (4) (vitamin C) and of vitamin D (5) have been demonstrated to provide defence against both incidence and progression of hip OA. Ethnicity (6) is an additional systemic risk factor in particular for specific localization of OA (high prevalence of hip OA in Caucasian). The contribution of biological, lifestyle, socioeconomic and genetic factors to ethnic difference in OA are still unclear. Genetic studies give on important insight into the pathogenesis of OA. Genetic factors may have a strong role in the genesis of OA in hands and hips, which have been studied using strategies such as classic twins studies, estimation of the relative risk in sibling of OA patients and segregation analysis of clustering of OA within families. Genetic associations are now being identified on several cromosomes (7, 8) (2q, 11q and 16) and inheritance is likely to be poligenic and to involve common polymorphism, each with a relatively low attribution of OA. For what concerns local biomechanical factors, the effect of obesity on joints integrity has been well documented.
In persons who are overweight weight loss can reduce the risk of OA. Alterations of mechanical environment of the joint (knee laxity, proprioception abnormalities, alterated knee aligment) may contribute to the occurence of OA, in particular in weight baring joints. Acute injury and joint deformity, occupational factors, competitions and muscle weakness may predispose to disease occurence and progression.
The understanding of the risk factors for OA and of the disease pathogenesis, will give, in the next future, many opportunities to design new therapeutic strategies.

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**Epidemiology of osteoporosis and osteoarthritis**

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Osteoporosis (OP) and osteoarthritis (OA) are the two most common forms of musculoskeletal disorders in the elderly. Their high prevalence and the frequency of OP and OA related physical limitations make these disorders two of the leading causes of disability in aging people, especially with respect to weight-bearing functional tasks (1). Data on epidemiological aspects of osteoarthritis in various European countries became increasingly available of the recent years. Nevertheless, problems still arise in providing an overview of the situation, particularly at an European level for two reasons: first because of a lack of published data in some countries and, secondly, because very few of the epidemiological studies carried out today are strictly comparable, in terms of methodology. In Britain, reported prevalence rate of hip OA individuals over 55 years was in the range of 8.5% in women and 3.1% in men, for OA grades III or IV on the radiological Kellegren and Lawrence scale. Figures for Denmark in persons over 60 were 5.6% in women and 3.7% for men, while retrospective Swedish studies indicated that prevalence of coxarthrosis rose from less than 1% in population aged under 55 to 10% in those over 85. Prevalence figure for hip OA vary substantially across these, with figures of 40.7% for women and 29.8% for men in subjects aged 55-64. Data on prevalence of lumbar and cervical OA are relatively scarce, but at least one study found that it was one of the most prevalent sides of OA, with peak rates as high as 84% and 70% for cervical and lumbar spine OA, in older age groups and based on radiological OA. Relatively few studies of the incidence of OA have been reported in the literature in Europe. A 12-year follow-up study of 258 individuals aged over 45 from the general population showed that approximatively 25% of women and 10% of men developed radiographic knee OA during the study period, while in individuals between 75 and 79, the OA incidence in small joints of the hands was 13.6% and of knee OA 4.5% over a 5-year period (2). Overall, in the United States, about 1/3 of adults aged 25 to 74 years have radiographic evidence of OA involving at least one site. Specifically, 33% had changes compatible with definite OA of the hands, 22% of the feet and 4% of the knee. Among persons aged 55 to 74, corresponding prevalence ratios were 70% for the hands, 40% for the feet, 10% for the knees and 3% for the hips. A clinical diagnosis of OA, based on symptoms and physical findings, was made by the examining physician in 12% of the examinees from the NHANES-1 study (aged 25 to 74 years) (3). Using the World Health Organization criteria, based on the T-score of densitometry and/or the presence of at least one non traumatic fracture, for the definition of osteoporosis, it has been estimated that most American women under the age of 50 have normal BMD and osteoporosis is rare. With advancing age, an increasing number of women have osteoporosis so that, by the age of 80 years, 27% are osteopenic and 70% are osteoporotic at the hip, lumbar spine or forearm. 60% of the osteoporotic group will already have experienced one or more osteoporotic fracture. The prevalence of vertebral fractures varies, depending on the definition of fracture used, but it is estimated at between 10% and 25% in women aged 50 and over. There were an estimated 1.66 million hip fractures occuring worldwide in the over-35s in 1990, with about 50%, occuring in Europe and North America (4). In Belgium, we compared the

incidence of hip fractures in men and women and investigated the respective role of demographic and secular aspects in hip-fracture incidence changes during the period 1984 to 1996.
The mean annual incidence of fractures of the proximal femur increased from 107.8 per 100.000 inhabitants in 1984-1986 to 140.5% per 100.000 inhabitants in 1994-1996, whereas the incidence of femoral diaphysis used as control, remained stable from 14.5% per 100.000 inhabitants to 14.2% per 100 000 inhabitants. Hip fracture occured with a 2.3 to 1 female to male sex ratio. However, the incidence by age group was identical in men and women with fracture occurring earlier in women by about 7 years. The demographic changes only accounted for a 3.2% increase in the number of hip fractures, during this period, whereas the recorded increase was 30% (5). Hip fractures are currently a major health issue in most developed countries. If no comprehensive preventive policies are set up promptly, there will be a seven-fold increase in these fractures between now and the year 2050. Hip fractures in males, even if there are less common than in women, should not be underestimated and are likely to become a major health problem in the coming years.

In conclusion, osteoarthritis and osteoporosis are currently major issues for public health systems from developed and developing countries. If no decision is promptly taken regarding the appropriate preventive and therapeutic management of these disorders, the burden related to OA and OP in the future will become unbearable (6).

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**Genetics of primary generalized osteoarthritis**

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Human osteoarthritis (OA) is a heterogeneous and multi-factorial disease with multiple pathogenetic mechanisms implicated in its development and progression. Despite its complex pathogenesis, it is clear that certain subsets of OA exhibit a hereditary pattern. The most common form of hereditary OA is known as Primary Generalized OA (PGOA), a disease first recognized as a discrete clinical entity by Kellgren and Moore (1). PGOA is characterized by the familial

development of Heberden's and Bouchard's nodes in the dorsal aspect of the distal interphalangeal joints of the hands and the premature degeneration of the articular cartilage of multiple joints (2,3). The familial occurrence of Heberden's nodes was first documented by Stecher and Hersh, who concluded that these lesions were inherited as a single autosomal dominant gene with strong female preponderance (4). Subsequent studies provided additional evidence for the familial occurrence of Heberden's nodes, and of degenerative arthritis involving multiple joints (1-8), although later studies suggested a polygenic inheritance rather than a single gene defect (9,10). In addition to nodal involvement, the phenotype of PGOA includes clinical and radiographic features of precocious onset with loss of articular cartilage that is usually concentric or uniform, particularly in the knees and hips (5,11,12). Degenerative changes of the hip typically develop early in adult life and advance rapidly. Sclerosis, pseudocysts, and femoral head deformity are seen with late stage disease.

In the 1980's, the use of markers such as the HLA A1 B8 haplotype (10,13) and the MZ isoform of a1 antitrypsin (13) helped to establish patterns of genetic predisposition in PGOA patients. However, with the availability of genetic resources from the Human Genome Project in the 1990's, many more, and larger scale, epidemiological studies of OA "heritability" were undertaken. In the first large-scale study of OA twins, Spector et al. compared radiographs of monozygotic (MZ) and dizygotic (DZ) twins with generalized OA. Their analyses confirmed the conclusions of prior smaller studies that had suggested the importance of genetic contributions to the development of hand and knee disease: the overall heritability of OA was 39% - 65% with a concordance rate in MZ twins of 0.64 compared with 0.38 in DZ twins (14). The study also clearly suggested that there were environmental confounders that contributed to heritability, and that heritability in another frequently-affected OA joint, the hip, was lower than observed for the hands and knees. In an even larger study of Finnish OA twins, similar results were obtained with respect to heritability (15); interestingly, however, concordancy in MZ twins was remarkable only for females, leading to the suggestion that females bear a greater genetic burden for the development of OA than do males. Additional epidemiological studies have included clustering studies of sibs in order to determine relative risk. A sibling relative risk (ls) for probands that have undergone a total hip or total knee replacement was calculated to be 2.3, translating to a heritability factor of 27% for severe OA (16). Other cohorts of patients were similarly analyzed and familial aggregation of OA, particularly in families with severe and polyarticular disease, was observed (17). It must be noted, however, that not all epidemiological studies of heritability yield positive results. In some analyses, evidence of genetic contributors to OA development and progression has not been observed. Such results clearly demonstrate that PGOA is a heterogeneous disorder where the ethnicity, OA site, and gender of the cohorts used for analysis may significantly influence estimates of heritability.

Although segregation analysis of an OA cohort with hand and knee involvement has suggested that a major recessive locus may be involved in the inheritance of the disorder (18), OA is considered a complex genetic disorder with no recognizably Mendelian pattern of inheritance. Several rare exceptions have been reported for large families whose OA phenotype appears to be inherited as an autosomal dominant trait; furthermore, these families do not display any evidence of chondrodysplasia. Using parametric linkage analyses in genome-wide screens, loci on chromosomes 2q (as reported in ref. 19), 4q35 (20) and 16p (21) have been implicated in these cases of familial OA (only the locus at 2q has been observed in generalized OA). However, since primary OA that segregates as a familial disorder is rare, parametric linkage analyses have not been very useful for identifying putative disease loci. Rather, model-free methods, such as sib-pair analysis, have been used extensively to search for OA loci. These types of analyses have many advantages, including the ability to partially compensate for uncertainties due to incomplete penetrance, the effect of phenocopies, or the potential for environmental effects on disease development and progression. Sib pair studies have taken two paths in their evolution: firstly, candidate loci have been evaluated, and secondly, genome-wide screens for linkage have been performed. With regard to candidate locus analysis, one large study of sib pairs with severe OA, obtained suggestive linkage to the COL9A1 gene (the alpha 1 chain of type IX collagen) in a cohort of female affected pairs with hip OA (22). Most other studies of candidate loci have been under-powered, or have not displayed linkage to the level of significance (lod>3). In genome-wide scans,

however, at least 6 loci have been implicated; these include loci on chromosomes 2q, 11q, 4q, 6p, 16p, and 7p (23-27). In various studies, not all loci detected on a particular chromosome overlap with each other, suggesting that some chromosomes may harbor more than one susceptibility locus. Also, some loci, especially those on chromosome 2q, have been implicated in more than one study, strengthening the argument that loci on chromosome 2q may be important in the development and/or progression of OA. Taken together, the sib pair studies have confirmed the fact that PGOA is a polygenic disorder and, especially when sib pairs are stratified on the basis of gender or OA site, have provided numerous loci for further investigation.

Another measure of progress in the genetics of PGOA is the wealth of association analyses that have been performed on candidate loci using case-control cohorts. Most of the candidate loci that have been studied include those coding for cartilage extracellular matrix (ECM) proteins, as well as genes that may be important in bone density. Since type II collagen is the most abundant of the collagens in the ECM, it has been the subject of several studies of association with PGOA and association with a high level of significance was reported in two studies (28, 29), but excluded in another (30), and of the candidate loci tested, only 2 genes (the COL9A1 and COL11A2 genes) that have shown evidence for linkage to OA, also demonstrated positive association (31). The diversity of observations of association between OA and candidate loci likely demonstrates population and phenotypic differences among cases and controls. These studies will clearly benefit from much larger sample sizes, the use of common markers in candidate genes and loci, stratification of cases according to gender and OA site, and, perhaps most importantly, proven replication of observed associations in order to identify OA-relevant alleles.

In conclusion, although there has been substantial progress in defining the genetics of PGOA and identifying regions of the genome that may harbor susceptibility genes, it is likely that additional OA loci are still to be discovered. The construction of a comprehensive list of susceptibility genes for PGOA is within the foreseeable future.

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**The Genetics of Familial Osteochondrodysplasias**

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The osteochondrodysplasias are a heterogeneous group of disorders characterized by abnormalities in cartilage and bone growth and development. They represent a difficult category of hereditary disease since the large number of similar but still distinct disorders affecting the osseous skeleton (1,2). Current classification includes more than 230 different osteochondrodysplasias (1). While individually rare, the osteochondrodysplasias as a group are common and have a significant social and medical impact (3).

Location of the radiographic changes, age of manifestation, severity of the disorder, body proportions and presence of associated clinical abnormalities have been used to establish the classification. In the other hand, this approach has also been used to combine some of these disorders into chondrodysplasia groups that share common features (1, 2). Recent developments in biochemical and molecular biology have helped towards understanding the underlying basic defects, and many of these dysplasia families have been confirmed to be allelic disorders caused be a defect in a single gene (such as diastrophic dysplasia group; 4) or in a gene group (multiple epiphyseal dysplasia; 5). However, a majority of the osteochondrodysplasias still have unknown etiology.

Many of these chondrodysplasia families are characterized by a phenotypic spectrum, ranging from barely detectable phenotype to perinatally lethal forms of a disease. Mild forms of certain osteochondrodysplasias can be difficult to distinguish from common bone and cartilage disorders such as osteoporosis and osteoarthrosis. Additionally, even though lethal ostechondrodysplasias as a group are typically readily detectable by antenatal ultrasound, establishing a specific diagnosis can be problematic (6).

Genetic basis of these disorders vary from the presence of recurrent mutations (such as FGFR3 c.1138G>A mutation in >95% of patients with achondroplasia, 7) to essentially each patient/family having a unique mutation in a large gene (such as COL2A1 mutations in type II collagenopathies; 8). Genetic testing is relatively straightforward for the former type but the latter presents a testing problem. This is especially true in diseases affecting cartilage and bone because the tissues are

rarely obtainable, and thus, the mutation analysis has to be done at the genomic DNA level instead of studying less complex mRNA. Screening a large gene for mutation is very challenging, labor intensive and costly.
A good example of this complexity is type I collagenopathies caused by defects in the COL1A1 and COL1A2 genes. The COL1A1 and COL1A2 genes encode for the alpha1(I) and alpha2(I) chain of collagen I, respectively. Collagen I is a major protein component of bone, tendon and ligment. It is a heterotrimer of two alpha1(I) and one alpha2(I) chains. Each chain consists of a large 1014 aa collagenous domain characterized by -Gly-X-Y- repeats, flanked by N-and C-terminal domain. Glycine as a smallest amino acid is obligatory in every third position to allow folding of the three chains. Thus, there are 338 obligatory glycines in both chains that, when substituted by a bulkier amino acid, result in a structural defect in the molecule. Exact structural defect is further dependant on the nature of the substituting amino acid (size, charge etc.). In addition to glycine substitutions, there can be various other mutations such as insertions, deletions and null allele mutations. This creates an almost endless possibilities for novel mutations. To date, glycine substitutions have been shown to associate with mild (osteoporosis, osteogenesis imperfecta [OI] type I), moderate (OI type IV), severe (OI type IV) and lethal (OI type II) osteochondrodysplasias (9).
Further complication in assessing the genetics of osteochondrodysplasias is that even in patients with classical phenotypes of a given osteochondrodysplasia, mutations are rarely detectable in 100% of the patients. This can be due to methodological limitations and/or genetic heterogeneity. There is still a vast amount of work to be done to determine the etiology of the remaining chondrodysplasias. Linkage studies are very useful for disorders where large families are available. For the others, candidate gene analysis has proven effective and recent advances in DNA microarray technology have a promise to reveal affected pathways or defective genes.

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**Pharmacogenomics in osteoarticular disorders**

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The Human Genome project and the possibility to survey for many genes introduced the concept of "omic" research, coined by various researchers as proteomics, metabolomics, immunomics, toxicomics, structural genomics, functional genomics and also pharmacogenomics. Indeed, many companies have for some time been looking beyond the sequencing of he genome to tap into its potential in human therapeutics. Inclusion of pharmacogenomics in regulations for clinical trials could drastically change the face and outcome of drug development. The number of people in the trial will decrease - speeding up the process - without any decline in statistical power because the success rate will be greater if only those likely to respond are included.
Of particular value are the technological advances that pinpoint individual variations in the genome (polymorphisms), making then routinely detectable and applicable in the clinical setting. Polymorphisms can be used as predictors of drug response, and, in some cases, can be integral components of the drug design process. Pharmacogenomics is likely to have a significant impact on medicine, analogous to the integration of imaging techniques. Most drugs show significant interindividual variation in therapeutic efficacy. Judgment of the effectiveness and safety of new drugs is still based on the average response of a study group. Inspection of the data from individual subjects, however, usually reveals significant numbers of patients with little or no response, as well as those who have dramatic responses. In cases of complex diseases, this "one-drug-fits-all" attitude subsets patients to empirical trial-and-error periods before acceptable regimens are found. This is often further complicated in disorders where severity of the phenotype waxes and wanes, making it difficult to predict the effects of changing a patient's medication.
In principle, three pharmacogenetic mechanism can influence pharmacotherapy. First and best studied to date are genetic polymorphisms of genes that are associated with altered metabolism of drugs. Second, genetic variants can produce on unexpected drug effect, often undesirable. Third, genetic variation in a drug target can alter the clinical response and frequency of side effects. Good examples are available for asthma, cancer, psychiatric disorders and infection diseases, while little has been done to identify genetic polymorphisms that underlie drug effects in osteoarticular disorders. The available information regarding this latter topic will be presented in detail.
Pharmacogenomics is not gene therapy or genetically modified foods or genetic engineering. Our language needs to be more precise and clear to prevent inaccurate vernacular usage creating a confused public perception of "genetic testing". Pharmacogenetic applications must be considered separately, acknowledging a distinct set of ethical, legal, social, and regulatory variables. This is not just a semantic argument; the promise to treat more people effectively must be preserved.