

Meeting report

Genetics of osteoarticular disorders, Florence, Italy, 22–23 February 2002

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Abstract

Osteoporosis (OP) and osteoarthritis (OA), the two most common age-related chronic disorders of articular joints and skeleton, represent a major public health problem in most developed countries. They are influenced by environmental factors and exhibit a strong genetic component. Large population studies clearly show their inverse relationship; therefore, an accurate analysis of the genetic bases of one of these two diseases may provide data of interest for the other disorder. The discovery of risk and protective genes for OP and OA promises to revolutionize strategies for diagnosing and treating these disorders. The primary goal of this symposium was to bring together scientists and clinicians working on OP and OA in order to identify the most promising and collaborative approaches for the coming decade. This meeting put into focus the importance of an adequate genetic approach to several areas of research: the search for the genetic determinants underlying new susceptibilities, the optimization of previously acquired data; the establishment of correlations between genetic polymorphism and functional variants, and gene–gene and gene–environment interactions (particularly those between genes and nutrients). An adequate genetic approach is also essential with regard to determining more selective criteria for phenotypic definition of familial OP, in order to obtain more homogeneous and statistically powerful family-based studies. The symposium concluded with an interesting overview of the future perspectives offered by DNA microarray technologies for identifying novel candidate genes, for developing proteomics and bioinformatics analyses and for designing low-cost clinical trials.

Keywords: estrogen, genetics, osteoarthritis, osteochondrodysplasia, osteoporosis

Introduction

Lectures were mainly on the genetics of complex osteoarticular disorders, and were grouped in such a way as to build a foundation of knowledge on the two selected topics. The meeting was attended by 120 Italian endocrinologists, rheumatologists, geriatricians, radiologists, general practitioners and postdoctoral researchers.

The first session dealt with both general and specific aspects of quantitative genetics of OP and OA. It also included presentations of polymorphisms of *VDR* and *COL1A1* genes and their corresponding functional variants. The second session introduced an interesting concept, the estrogen response in the genetics of OP,

and then focused on genetic aspects of male and familial OP, concluding with the role of Fos proteins in bone pathophysiology in mice. The third session was on the pathophysiology of OA, the genetics of primary generalized OA and familial osteochondrodysplasias. An interesting perspective on the future role of pharmacogenomics in osteoarticular disorders concluded the meeting.

This report is organized into eight distinct topics; it will focus on general genetic concepts and study models and new perspectives for approaching these complex diseases. The concepts and arguments presented in each topic area are common to all of the speakers in each section, except where the speakers are individually named.

Quantitative disorders

G Novelli (University of Rome at Tor Vergata, Rome, Italy), A Falchetti (University of Florence, Italy) and R Nuti (University of Siena, Italy) discussed quantitative disorders.

A Falchetti and G Novelli discussed how quantitative disorders are common multifactorial diseases; both genetic and environmental factors contribute to their pathogenesis. They exhibit a polygenic pattern of inheritance. OP and OA represent common quantitative disorders and difficulty exists in identifying responsible genes. They are extremely heterogeneous conditions, with contribution from low-penetrance, common alleles and environmental factors, often unknown or not measurable. Twin and sib-pair studies have clearly assessed the genetic background for both diseases. Incomplete penetrance, phenocopies, gene interactions and other transmission mechanisms complicate the genetic analysis.

A general approach to quantitative genetics may identify four areas as being critical for future work, as presented by G Novelli. The first area is what to look for and what to expect to find. Genetic theory and modeling of populations and diseases need to be considered. It is possible that a relatively simple genetic background exists for many common diseases. The second area consists of streamlined genotyping methods, which are needed even in centers that deal with modest numbers of samples and polymorphisms. Centralization of high-throughput genotyping is recommended. Thirdly, computational tools and methods of data analysis need to be considered and tailored to make optimal use of the data sets available. The fourth area is communication, as close interactions are needed between clinicians and geneticists to effectively support large-scale projects.

Genetics of osteoarthritis

The genetics of OA was discussed by T Spector (Twin Research and Genetic Epidemiology Unit, St Thomas' Hospital, London, UK), M Matucci-Cerinic (Department of Internal Medicine, University of Florence, Italy) and O Ehtgen (WHO Collaborating Center for Public Health Aspects of Rheumatic Disorders and Department of Epidemiology and Public Health, University of Liège, Belgium).

General aspects of osteoarthritis

As discussed by T Spector, no universal consensus has been reached in defining a generalized OA phenotype. Bone density has been found to be greater in OA patients than in controls, years before the radiological appearance of osteophytosis; in addition, there is a slight increase in bone turnover during the early phase of disease [1]. A recent study of hip OA in discordant twins [2] has suggested that some of the same genes are involved in influencing hip OA and bone mass. Recently, magnetic resonance imaging data obtained from twins [3] showed

there is a genetic contribution to disk degeneration and spinal osteophytosis. At least 50% of the variants of OA in the hands, knees and hips are determined by genetic factors. Association studies [4–7] exhibited positive associations with polymorphisms of *VDR*, *IGF-1*, *TGFβ* and *COL1A1* genes, and linkage analyses strongly suggest involvement of loci on chromosome 2q. Spector (St Thomas's Hospital, London, UK) suggested that a research focus should be on intermediate phenotypes, individually or in combination, obtained by dividing OA into its constituent parts. They might occur independently or in clusters determined by pleiotropic genes.

M Matucci-Cerinic spoke of how it is important to take into account the role that environment and, specifically, nutrients such as vitamins C and D may play in the reduction of risk for disease progression and the ability of bone to respond to injurious processes in OA. Future efforts should be made to unravel gene–nutrient interactions, in order to apply effective preventive measures.

Genetics of primary generalized osteoarthritis

C William's (Division of Rheumatology, Department of Medicine, Thomas Jefferson University, Philadelphia, PA, USA) presentation focused on the genetics of generalized OA.

Subsets of OA, particularly primary generalized OA, exhibit a pattern of inheritance. Patterns of genetic contribution have been clearly demonstrated and both heritability and relative risk have been evaluated. Whole genome screens [7–9] pinpoint quantitative trait loci on 2q, 4q35 and 16p. Sib-pair analysis, by candidate-gene and genome-wide screening, has been extensively applied to the genetic dissection of primary generalized OA to overcome incomplete penetrance of traits, phenocopies, and environmental influences. A significant linkage was found at the *COL9A1* locus in a cohort of female pairs affected by hip OA, but screens also implicated 2q, 11q, 4q, 6p, 16p and 7p quantitative trait loci. Association studies failed to provide unequivocal results in differently selected populations.

Polymorphisms of *VDR* and *COL1A1* genes

Discussions of *VDR* and *COL1A1* genes were presented by AG Uitterlinden (Departments of Internal Medicine, Epidemiology and Biostatistics, and Clinical Chemistry Erasmus Medical Center, Rotterdam, The Netherlands), JA Eisman (Bone and Mineral Research Program, Garvan Institute of Medical Research, St. Vincent's Hospital, Sydney, Australia) and SH Ralston (Bone Research Group, University of Aberdeen, UK).

Presentations given by AG Uitterlinden and JA Eisman highlighted how polymorphisms of *COL1A1* and *VDR* genes, whose protein products are collagen type 1 and the vitamin D receptor, respectively, have been extensively

studied in OP, but there have been conflicting results on their association with bone fragility, intestinal calcium absorption and bone mineral density (BMD) at various skeletal sites [10]. The largest study published so far (1782 Dutch elderly men and women) has obtained consistent results using haplotype construction [11] of the three 3'-end restriction fragment length polymorphisms of *VDR*. Accurate recognition of allelic heterogeneity, by haplotyping, is important to identify the risk alleles at this part of the gene. Observation of substantial sequence variation in the 3' untranslated region suggests its influence on *VDR* function [12,13]. A known single nucleotide polymorphism at exon 2 of *VDR* alters the translation start site, thus determining two variant forms of receptor that differ by three amino acids; however, its universal association with BMD has not been reported. Recent findings [11,13] not only indicate that there are multiple polymorphic variations of *VDR*, but also that they could have different types of consequences. In fact, 5' promoter variations are able to alter mRNA expression patterns and levels, and 3' untranslated region polymorphisms could affect mRNA stability and most likely also *VDR* protein levels.

As presented by SH Ralston, functional studies of the intronic polymorphism at the Sp1 binding site of the *COLIA1* gene, originally associated with reduction of BMD and increased risk of osteoporotic fractures, demonstrated that it correlates with increased binding affinity for the transcription factor Sp1 and increased allele-specific transcription of *COLIA1* *in vitro* [14]. Osteoblasts from heterozygotes produce an abnormal ratio of collagen alpha I (1) chains relative to alpha I (2) when compared with homozygotes. *Ex vivo* mechanical testing demonstrated different bone yield strength; composition analysis demonstrated a different inorganic content and different heterogeneity of mineralization according to this polymorphism [15,16].

Thus, in an apparently single metabolic pathway, many proteins interact and consequently the combination of polymorphisms can be pathogenic (AG Uitterlinden and JA Eisman). Future efforts will focus on the identification of all polymorphisms across the *VDR* gene by defining the haplotype patterns in order to better understand the pathophysiological role that the polymorphisms themselves exhibit in relation to the biological parameters influenced by the vitamin-D-related system. Not only does this important pleiotropic endocrine pathway influence BMD and BMD-independent fracture risk, but *VDR* polymorphisms have also been associated with OA, myocardial infarction, breast cancer, prostate cancer, diabetes and susceptibility to infectious diseases. Thus, the *VDR* gene can be a useful model for investigating some of the mechanisms resulting in wide-ranging allelic polymorphism effects. It should always be taken into consideration, however, that some of the effects may be influenced by

gene-gene interactions, for example estrogen receptor α (*ER α*) gene and *COLIA1* polymorphisms. Moreover, adequate daily calcium and/or vitamin D intake can mask or unmask a 'physiological' reduction in activity of the receptor associated with specific *VDR* gene polymorphisms. Understanding gene action, gene-gene and gene-environment interactions could improve both regimens and strategies for optimal individualized therapy.

Genetics of osteoporosis

Presentations on the genetics of OA were given by L Masi, L Gennari and A Falchetti (Department of Internal Medicine, University of Florence, Florence, Italy).

The estrogen response model and male osteoporosis

L Masi discussed the estrogen response model and male OP was discussed by L Gennari. Estrogen response represents the endpoint of an intricate network constituted by several genes, with multiple polymorphisms, encoding receptors and enzymes, with the intervention of co-activating and co-repressing factors. Furthermore, this network is complicated by all the possible interactions that all these components might have in various tissues. Thus, the final response could be dependent on a sort of physiological mosaicism.

Extragenadal estrogen biosynthesis has different features than ovarian. Locally synthesized estrogen predominantly acts at a local level in a paracrine or intracrine fashion. The aromatase gene (*CYP19*) encodes a specific enzyme that, at peripheral levels (particularly bone and adipose tissue), converts androgenic precursors into estrogen molecules. The observation [17,18] of marked bone phenotype in men with mutation of either the *ER α* or *CYP19* genes leads to the conclusion that local estrogen production in bone cells plays an important role in the maintenance of bone mineralization and the prevention of OP in both women and men. In fact, dinucleotide polymorphism of *ER α* gene, genetic variants of *CYP19* gene may generally alter the estrogen response at several sites. A polymorphic repeat of *CYP19* has been recently associated with bone loss, risk of fractures and risk of breast cancer [19,20]. Interestingly, fibroblasts from subjects with the *CYP19* genotype associated with high BMD and low fracture risk synthesize a higher amount of estradiol than fibroblasts with the opposite genotype. Such findings might also lead to new modalities of therapy in the future.

Little is known about the pathogenesis of male OP. The estrogen response uses mechanisms that might account for bone loss in males. Although common genetic variants of *ER α* , *CYP19* and other genes might act along with environmental factors to determine OP in men, different genetic determinants might explain the site-specific skeletal diversity of size and bone-loss rate in males with respect to females. Candidate genes for male OP are

mostly shared with women: *VDR*, *COLIA1*, *CYP19*, *IGF-1* (insulin-like growth factor 1), *IL-6*, *ER α* and *AR* (androgen receptor). Larger studies are needed to confirm preliminary findings.

Genetics of familial osteoporosis

The presentation by A Falchetti discussed how, although many diseases run in families (i.e. they cluster), it is not enough to conclude genetic factors are involved. In fact, it is entirely possible that a disease having no genetic etiology could also show evidence of familial aggregation or clustering due to a shared exposure or culturally transmitted risk factor. The genetic contribution accounts for peak of bone mass, the positive relationship between maternal history of fracture and recurrence risk, the higher risk of children having low BMD if their parents have low BMD, and variation of bone geometric measures, such as hip axis length.

Findings on a shared genetic contribution to BMD in males and females have been controversial. Many confounding factors exist for family-based studies, such as environmental factors acting differently over the course of the lifespan, the comparison of individuals of widely different ages and year-of-birth cohorts and familiarity in lifestyle choices. The classic linkage approach is less suitable for identifying all the OP genes, because of both the multigenic nature of disease and the difficulty in collecting multigenerational pedigrees. Most data, from sib-pair analyses, conflict and there are 'arbitrary' differences both in study design and in statistical approaches. Ideal prerequisites for investigating the genetics of familial OP would be the collection of extremely large numbers of families, the creation of adequate nonparametric linkage analysis packages, and the consideration of particular environmental factors not commonly experienced in many studies.

Research on familial OP still suffers at least four major problems: first, the lack of a clear definition of phenotype (e.g. BMD, Z-score, fractures); second, the difficulty in obtaining multigenerational kindreds (late onset disease); third, the lack of adequate statistical approaches to multifactorial diseases; and fourth, the genetic effect on bone may be gender-, age- and site-specific.

Phenotype assessment is a crucial issue in gene mapping of complex traits and its misclassification can lead to spurious results of genetic analysis. The lack of a standardized definition of a complex trait phenotype may hamper the comparison of genetic studies. An algorithm should be proposed for classifying an 'osteoporotic' phenotype in family members of probands with low BMD, similar to the one used for differentiating asthma from chronic obstructive pulmonary disease. Particularly, restriction of selection criteria, narrowing the recruitment of families, can provide a more homogenous population for family-based studies.

Genetics of bone development in animal models

EF Wagner (Research Institute of Molecular Pathology (IMP), Wien, Austria) discussed the genetics of bone development in animal models.

Fos proteins are transcription factors belonging to the AP-1 complex and are involved in many important physiological cell processes. In particular, c-Fos is a key regulator of bone development. Mice lacking c-Fos are osteopetrotic because of a block in osteoclast differentiation that results in changes in osteoclastic bone resorption activity. In contrast, transgenic mice overexpressing Fra-1 (a c-Fos-related protein) develop bone osteosclerosis as a result of an increase in bone formation due to a differentiation defect in osteoblasts, even if the Fra-1-transgenic osteoclasts are hyperactive *in vitro*. Moreover, gain of function of c-Fos, in an *in vivo* analysis, determines the transformation of osteoblasts and the occurrence of osteosarcomas. Interestingly, experiments on knock-in mice expressing Fra-1 in place of c-Fos demonstrated that Fra-1 is able to rescue the c-Fos-dependent functions in bone development in a gene-dosage dependent manner, but not the *in vitro* target gene expression. Such systems will be useful for detecting new c-Fos target genes using the microarray approach.

Molecular mechanisms underlying the role of Fos proteins are necessary for regulating bone cell development and differentiation. In osteoclasts, inactivation of c-Jun (the molecular partner of c-Fos in activating transcription) causes inefficient cell differentiation, suggesting an important role for Jun proteins in skeletal development and differentiation. Moreover, chondrocyte-specific c-Jun inactivation in *col2A1-cre*-transgenic mice results in severe scoliosis due to failure of intervertebral disk formation and abnormal vertebral arch development. This indicates a role for c-Jun in regulating sclerotomal differentiation.

Genetics of familial osteochondrodysplasias

The genetics of familial osteochondrodysplasias were discussed by J Korkko (Tulane University Health Sciences Center, Center for Gene Therapy and Department of Medicine, New Orleans, LA, USA).

Osteochondrodysplasias, an extremely heterogeneous group of disorders, exhibit abnormalities both in cartilage and bone growth and development. More than 230 different osteochondrodysplasias have been classified. As a group of disorders they are quite common, with a significant socio-medical impact. A wide phenotypic spectrum characterizes familial forms. Genetic bases of these disorders encompass recurrent mutations of *FGFR3* (the gene encoding fibroblast growth factor receptor 3) in achondroplasia, and essentially private mutation in a large gene such as *COL2A1*. The search for *COL2A1* mutations is

time-consuming and expensive. Mutation detection never reaches 100%, even in patients with a classic phenotype. Linkage studies of large families are very useful for these disorders and recent advances in DNA microarray technology represent a promising methodological approach to reveal affected pathways or defective genes.

Pharmacogenomics in osteoarticular disorders

The presentation on pharmacogenomics in osteoarticular disorders was given by ML Brandi (Department of Internal Medicine, University of Florence, Italy).

Pharmacogenomics could drastically change the face and the outcome of drug development, reducing the number of people that must be included in clinical trials (in other words, only people known to be responders would be included in the study). Polymorphisms could be used as predictors of drug response, facilitating the drug design process. In the case of complex disorders, the 'one drug fits all' attitude allocates patients to empirical trial-and-error periods before acceptable regimens can be decided. Few examples concerning pharmacogenomics and osteoarticular disorders are actually available. It is known that healthy premenopausal women exhibit a different response to calcitriol according to the specific genotype at the 3' end of *VDR*, and the magnitude of the estrogen response differs according to which polymorphisms of *ER α* and *CYP19* genes they have. Genetic variants might be present in a drug's target receptor or might produce adverse reactions to the drug or altered drug metabolism. In fact, pharmacotherapy can be influenced by three general pharmacogenetic mechanisms: first, polymorphisms of genes associated with altered drug metabolism; second, genetic variants that produce an unexpected and undesirable drug reaction; third, genetic variation in a drug target that alters the clinical response and frequency of side effects. Data are available for asthma, cancer and psychiatric disorders, while little exists concerning drug effects in osteoarticular disorders. Pharmacogenomics represents the promise of treating people effectively and of creating 'tailored' individual therapy.

Conclusion

Current problems

To date, most of the reports on population-based genetic analysis of OP consider single candidate gene polymorphisms, sometimes limited to a single polymorphism. The candidate gene polymorphism approach suffers from confounding factors such as selection bias, differences among ethnic groups, inadequate sample size, environmental factors and linkage disequilibrium; prudence is mandatory in interpreting results from such studies. Moreover, correlation between DNA sequence variants and functional variants does not exist or has not been investigated or reported, importantly decreasing the statistical and biological power of such observations.

Potential solutions

Future efforts will identify all polymorphisms across the *VDR* and *COL1A1* genes, as well as in other/new candidate genes for OP and OA. Definition of the haplotype patterns will help us understand the pathophysiological role that the polymorphisms themselves might exhibit in the acquisition of specific bone/articular joint phenotypes. Thus, both multiple intragenic polymorphisms and multi-genic complex haplotypes have to be analyzed in defining any genetic susceptibility to these disorders. Larger population studies have to be performed, taking these approaches into account. Environmental factors, particularly nutrients, have to be accurately evaluated together with complex genotyping, in order to weight their importance in masking or unmasking functional variants with respect to specific genetic background in order to create more effective preventive strategies for OP and OA. Accurate standardized phenotype definitions are needed to add a more powerful statistical value to family-based studies. Probably, this will focus on subsets of kindreds more homogeneously defined, but adequate nonparametric linkage analysis packages must be developed. To date no general consensus exists on the parameters that ought to be considered for phenotyping generalized OA and familial OP.

Hopes for the future

Comparative genetics will add information on potentially interesting genes in humans once quantitative trait loci have been identified in animal models. Great results are expected from development of new DNA microarray and bioinformatic technologies, not only for gene variant detection, but also for proteomic and metabolomic aspects of the pathogenesis of OP and OA. These results will provide new opportunities for identifying people at risk of developing osteoarticular disorders. Data generated will enable us to develop new, tailored therapies for testing with newly designed clinical trials that involve more carefully selected individuals, creating the opportunity of avoiding or reducing severe side effects.

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