Our findings suggest inhibition of entry into the chondrogenic pathway by MSC derived from patients carrying the Arg^{519}-Cys mutation in COL2A1. Our expectation was that these cultures would produce a mixture of wild-type and mutated type II collagen. Failure of these cultures to produce any detectable type II collagen and failure of the mutated pellet cultures to accumulate cartilage-like proteoglycans and to attain dimensions equivalent to the control cultures suggest interruption of the pathway(s) leading to the chondrocyte phenotype. It seems clear that the point mutation in COL2A1 produces an effect on these cultures that is developmentally more profound than might be expected merely from production of mutated type II collagen. It is known that patients with this mutation synthesize, produce, and accumulate mutated type II collagen in their cartilage[1]. While the ratio of wild-type to mutated α-chains found in cartilage from these patients is greater than 1:1, clearly, the mutated collagen is expressed, suggesting that the MSC are under a different set of regulatory mechanisms than the chondrocytes in cartilage of mutated patients.

Studies on regulatory mechanisms investigating the expression of genes associated with the chondrogenic phenotype suggest several avenues of further investigation:
1. Is COL2A1 the only chondrocyte-lineage gene affected? Are genes for chondrocyte-associated matrix proteins and proteoglycan core proteins similarly affected?
2. When chondrogenic effectors are added to the MSC cultures, is the expression of central regulatory elements in mutated cultures altered, in comparison with that in control cultures?
3. Are regulatory growth factors and cytokines expressed in an altered pattern by mutated cultures?

In summary, data from our laboratory support a potential role for impairment of reparative mechanisms resulting from altered MSC function in the development of OA.

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HOW IMPORTANT ARE GENETIC FACTORS IN OSTEOARTHRITIS? CONTRIBUTIONS FROM FAMILY STUDIES

Weiya Zhang and Michael Doherty

A familial tendency to OA was first suggested over 120 years ago when it was observed that Heberden's nodes may cluster within families[1]. However, it was Stecher in the 1940s who provided convincing evidence for a strong genetic predisposition to Heberden's nodes by observing that nodes were twice as common in mothers, and 3 times as common in siblings, of affected probands as in the general population[1]. This finding was subsequently confirmed in family studies and was extended to include radiographic OA of joints other than the hand[1]. Within affected families the risk of OA of the hands, knees, and hips is significantly higher than that in the general population, with heritability estimates ranging from 40% to 70%, depending on the joint assessed[2]. Our report presents an overview of the epidemiological studies of familial clustering and/or heritability of OA and updates our presentation in the 2002 Indianapolis Workshop on Osteoarthritis Outcomes.

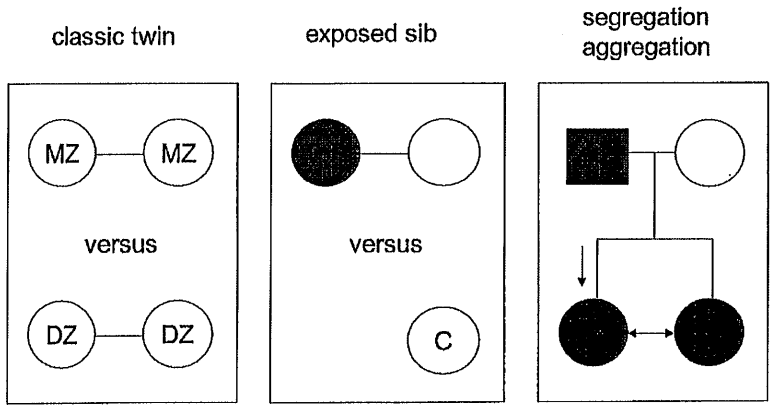

Figure 3. Familial risk and heritability of OA: different design strategies. MZ: monozygotic; DZ: dizygotic; TJR: total joint replacement; C: control; affected subjects are shaded.

Types of Family Studies

Family studies may be categorized into: (1) studies with no external comparator, such as classic twin studies and correlation and segregation analyses; and (2) studies with an external comparator, such as sibling or first-degree relative studies. While the former design calculates heritability or correlation coefficients among relatives, the latter estimates relative risk or odds ratio (OR), comparing risk (prevalence or incidence) of OA in relatives of probands with risk (prevalence or incidence) in the general population (Figure 3).

Classic twin studies. This strategy compares occurrence of disease in monozygotic (MZ) and dizygotic (DZ) twins. Assuming MZ and DZ twins share environmental factors to a similar degree but differ in their genetic similarity (MZ share 100% whereas DZ twins share, on average, only 50% of their genes), the difference in concordance for disease between MZ and DZ twins can be attributed to the genetic contribution. Thus "heritability," defined as the relative contribution of genetic variance to the liability to disease, can be calculated. This design is thought to afford a low level of confounding due to shared family environmental factors, such as diet, which may be risk factors for OA, although there is some evidence that MZ twins may have greater environmental sharing than DZ twins.

In classic twin studies of OA of hands, knees, hips and spine, reported heritability estimates range from 40% to 70%, depending on the joint examined and the method used to define the OA phenotype[2]. Three further studies have been undertaken since 2003[3-5], reporting heritability [95% confidence interval (CI)] for radiographic knee OA of 50% (34%, 62%)[4] and for radiographic hip OA of 61% (18%, 86%)[5], in accord with previous observations. Heritability for knee cartilage volume, assessed by magnetic resonance imaging, has also been examined and found to be high — 61% (36%, 77%) for femoral cartilage, 66% (47%, 79%) for patellar cartilage, and 76% (56%, 87%) for tibial cartilage[3].

Twins have also been studied for gene–environmental interaction using a cross-trait cross-twin approach[4]. In this design, the OR between OA in one twin and an exposure [e.g., body mass index (BMI)] in the other is calculated. Stratified analyses for the association, based on MZ twins and DZ twins, are then undertaken, and interaction is identified if the OR between MZ and DZ are different. Corresponding logistic regression models may be used to adjust other confounding factors. Using this strategy it was shown that although high BMI has heritability of 55.7% (35%, 72%), the strong association between high BMI and knee OA is unlikely to be mediated through shared genetic factors, implying that environmental modification of BMI can influence knee OA. However, care must be taken, insofar as twins share similar family environmental factors, and the influence of environmental factors may thus be underestimated.

Correlation/segregation studies. This approach examines the distribution of OA within families. Correlations of the phenotype, such as radiographic scores, between relatives within families (e.g., parent-offspring and sib-sib) are calculated. The pattern of the correlations between different types of relative pairs is analyzed by variance components or path analysis to estimate the proportion of variance due to shared environmental and genetic influences. Heritability or the best-fit model of inheritance (e.g., segregation analysis) can be determined.

In addition to the 3 major studies described[1], one other recent study has used this approach[6]. Fifty-one probands who had undergone knee replacement surgery for OA and 128 siblings of these probands were studied. Heritability was determined for muscle strength, knee pain, cartilage volume, bone size, and radiographic OA. Estimates ranged from 42% to 85%, depending on the phenotype and the knee compartment assessed, with the lowest values for muscle strength (42%, 95% CI 1%, 83%) and the highest for medial tibial bone area (85%, 95% CI 46%, 124%). Interestingly, heritability for knee pain was 44% (95% CI –15%, 103%).

This study suggests that the individual characteristics that are commonly used to define the OA phenotype all demonstrate a strong genetic component.

Sibling or other first-degree relative studies. Previous studies have reported that the relative risk of OA in first-degree relatives of probands is 2- to 5-fold higher than that in the general population, depending on the joint assessed[1,2]. Several new studies have used a sibling or first-degree relative design[7-12] and, comparing siblings with the general population, have found odds ratios (OR) of 1.7 (95% CI 1.4, 2.2) for tibiofemoral OA, 2.9 (2.3, 3.7) for patellofemoral OA[10], and 2.0 (1.7, 2.3) for hand OA[8]. Comparisons have been extended to other relatives, such as parents, offspring, and cousins[8]. Further, in contrast to previous studies, phenotypes other than radiographic OA, such as knee pain (OR 2.85, 95% CI 1.70, 4.78), muscle strength (OR 0.61, 95% CI 0.40, 0.93)[7], gait pattern — such as degree of external foot rotation (continuous variable, 4.5° versus 13.5°, $p < 0.01$)[11], and chondrocalcinosis (OR 1.2, 95% CI 0.6, 2.3)[12] have also now been determined.

Major Issues and Future Work

Phenotype. Chiefly, Heberden's nodes and radiographic osteophytosis and joint space narrowing have been studied, but in recent years additional characteristics that can comprise phenotypes of OA have been extensively investigated, including symptoms (pain)[6,7], other structural changes (cartilage volume, bone size)[3,6], and risk factors or comorbidities (e.g., muscle strength, BMI)[4,6,7], that associate with OA. OA can no longer be viewed as a single disease entity with a uniformity of structural or functional damage. It is a complexity of disorders related to defects or dysfunction in bone, cartilage, surrounding muscles, and ligaments. International agreement on the way phenotypic information is collected would increase the possibility of combining cohorts for analysis and thus increasing statistical power — a key issue for the genetic study of common complex disorders.

Polygenic disorder. Current data suggest that common sporadic OA with late onset phenotypic expression is a polygenic disorder. Linkage and association studies have identified a number of candidate genes related to bone and cartilage[2], but these are polymorphisms present in a significant proportion of the population and each contributes only a small increased risk, either individually or by interaction with other genes or with environmental factors. This contrasts with early onset familial OA, where a single, but rare, major gene defect is largely sufficient on its own to cause OA (Figure 4).

Gene-environment interaction. Although family studies show a genetic component for common OA, up to half the variance remains to be explained. It may result from environmental risk factors and involve gene-environment and gene-gene interactions. Therefore, once specific genes and polymorphisms with risk of OA are identified, the next studies involve investigation of the interaction of such genetic markers with constitutional and environmental factors to elucidate how such genes actually cause the increase in risk.

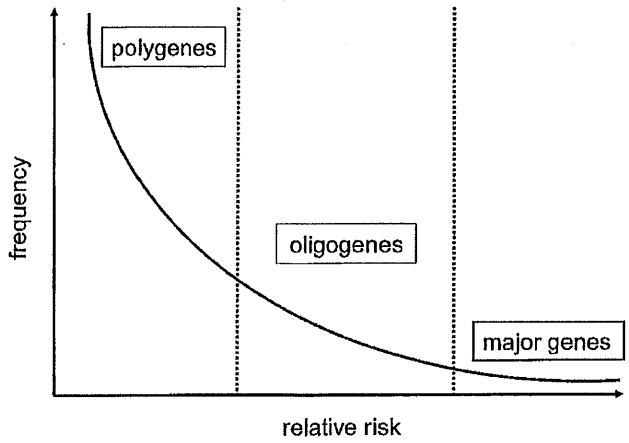

Figure 4. Genetic predisposition to disease. Relationship between frequency of genes associated with disease and their relative attribution to causing disease. Common polymorphisms have low attribution, but rare single mutations often, on their own, are sufficient to cause disease.

Power of studies. As the number of predisposing genes or polymorphisms increases, the sample size of study cohorts becomes critical in order to retain statistical power and reduce type I errors due to multiple comparisons. Most of the studies reported thus far appear underpowered. This, in part, explains the inability to replicate reported associations in different cohorts. Dissecting out the genetic details of polygenic disorders and adjusting for environmental and constitutional interaction and confounding present major challenges and require careful patient characterization of the subjects and large cohorts to study.

In conclusion, family studies show clear evidence of a strong genetic component to OA, with a relative risk 2- to 5-fold higher than that in the general population and a heritability of 40%–85%, depending on the phenotype examined. In addition to radiographic osteophytosis and joint space narrowing, other components of the OA phenotype also show strong genetic contributions. Following the identification of specific genetic markers, large-scale studies of gene-gene and gene-environment interaction should increase our understanding of this common, disabling, complex disorder.

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FROM BIOMARKER TO SURROGATE OUTCOME IN OSTEOARTHRITIS — WHAT ARE THE CHALLENGES?

L. Stefan Lohmander and David R. Eyre

There is continued interest in the identification and validation of biomarkers in OA. Such biomarkers have multiple potential uses, including the following: exploration of disease mechanisms and dynamics, identification of molecular targets for treatment, identification of patients at risk for rapid disease progression, monitoring effects of disease-modifying therapy, prediction of clinical responses, and tailoring treatment to biomarker levels. The need for biomarkers is particularly acute in the proof-of-concept stages in the development of disease-modifying OA therapy.

It may be speculated that access to useful biomarkers in OA could also eventually have public health benefits, by improving public awareness of risk and decreasing the number of patients required to be exposed to a new drug during the development stage. Availability of biomarkers could thereby also help speed drug development, allow testing of more alternatives in a shorter time, and help shape the design of future drug trials in this complex disease area.

The interest in OA biomarkers is fueled by the increasing prevalence of OA, due at least in part to aging of the population and the seemingly unstoppable increase in frequency of overweight and obesity in many countries. Further rationale for continued OA biomarker research is increasing awareness of the limitations of plain radiography as a method of monitoring OA outcome.

Despite much research in this area, biomarkers validated as surrogate outcome indicators in OA remain elusive. Where do the major difficulties lie? The following comments summarize some useful definitions and criteria for biomarkers and surrogate markers in OA, and highlight specific difficulties in identifying and validating markers for OA.

A clear definition of terms is important when discussing biomarkers:

- A biomarker is a structural or physical measure or cellular, molecular, or genetic change in a biologic process that can be identified and monitored, with resulting diagnostic or prognostic utility. Biomarkers must be reliably and reproducibly measurable by standardized, published methods, be used in several laboratories, and have undergone validation that they measure the intended process with sufficient specificity.
- A surrogate marker or endpoint, on the other hand, is a measurement or biomarker that serves to substitute for a clinically meaningful outcome or endpoint, as well as to predict the effect of a clinical intervention.
- A clinical endpoint, in contrast, is a characteristic or variable that measures how a patient feels, functions, or survives.

It follows from these definitions that, even in the best of cases, only some OA biomarkers can serve as surrogate endpoints for OA. To be validated as a surrogate endpoint a biomarker must be shown to be a reliable substitute measure for, or be able to predict, a clinically meaningful endpoint[1,2].

A significant challenge in the validation of a surrogate marker is that its measurement may not take into account adverse events, since the metabolic processes associated with an adverse event may not be monitored by the marker. Such adverse events may cancel all or some of the treatment benefit. Further, a surrogate marker may not register all beneficial effects of treatment if they are not in the marker pathway. Although a biomarker may have good face validity as a surrogate outcome, changes in concentration may not reflect the molecular or cellular process in the tissue that it is believed to monitor, leading to erroneous conclusions.

As mentioned, biomarkers may have several different potential uses. A general classification has been proposed on this basis[3]. According to this framework, a natural history marker is defined as a marker of disease severity that reflects underlying pathogenetic mechanisms and predicts clinical outcome independent of treatment. Such biomarkers