The Foundation of a New Paradigm of Disc Degeneration: *The Twin Spine Study*

Submitted for Consideration for the Kappa Delta Award

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Table of Contents

Abstract

Disc degeneration had been viewed for much of the last century as a result of aging and 'wear and tear' from mechanical insults and injuries. Thus, research and prevention strategies in lumbar degenerative changes and associated clinical conditions focused primarily on mechanical factors as primary causes using an 'injury model.' However, over the past decade a dramatic change in views of determinants of disc degeneration has been underway. The Twin Spine Study, a multidisciplinary and multinational research program on the etiology and pathogenesis of disc degeneration, has been at the forefront of recent related discoveries, and has been a substantial contributor to the dramatic paradigm shift over the past decade related to determinants of lumbar disc degeneration.

Among the most significant findings were a substantial influence of heredity on lumbar disc degeneration and the identification of the first gene forms associated with disc degeneration. Also, our studies on the effects of smoking and driving exposures using exposure-discordant identical twins have provided perhaps the most well controlled studies of the effects of these exposures on human disc degeneration to date. Furthermore, the results of one of our research group's most recent studies indicate that the effect of anthropometric factors, such as body weight, and muscle strength on disc degeneration, while modest, may be greater than that of occupational physical demands. This paper briefly summarizes the Twin Spine Study and some of the major discoveries that have resulted.

Knowledge gained through the Twin Spine Study and others' efforts over the past decade have substantially enhanced our understanding of disc degeneration and have provided a new paradigm. Disc degeneration is now considered a condition that is genetically determined in large part, with environmental factors, although elusive, also playing an important role. This advance in the understanding of disc degeneration provides a foundation from which to develop new hypotheses and more fruitful research that may help to elucidate the etiology of disc degeneration and associations with pain.

Background

Whether measured in terms of prevalence of symptom complaints in the general population[2,3], related medical visits[4], industrial injury claims[5], or disability pensions[6,6-8], back pain problems are among the most common and costly musculoskeletal conditions facing the developed countries of the world[6]. Unfortunately, prevention and treatment strategies have demonstrated only modest effects. The core challenge in developing rational, effective approaches to prevention and treatment is that the underlying pathology and risk factors are largely unknown. As John Frank et al concluded, "*current knowledge does not allow us to determine, by clinical examinations or any laboratory or imaging test, the exact medical cause of LBP in most patients. Indeed 97% of LBP is called 'nonspecific' or 'strain/sprain'…"* [9] Similarly, Deyo and Weinstein noted that a precise pathoanatomical diagnosis is not available in 85% of cases.[10] Yet, theories and models of underlying pathology and its etiology have been adopted over the past half century that have had profound effects on how the problem is viewed and approached by those afflicted, their health care providers and health and insurance policy-makers.[11]

Although the specific underlying pathology is unknown in most cases of back pain, lumbar disc degeneration is a primary suspect and is commonly believed to be responsible for back symptoms, as well as being a major culprit in sciatica and lumbar spinal stenosis (NIH-NIAMS workshops 1989, 1995 and 2005).[12-15] Consequently, the disc is a primary target for diagnostic and therapeutic interventions. Nachemson suggested that painful conditions may result from premature aging changes that render the disc mechanically incompetent, creating abnormal motion patterns that subject various spinal structures to undue stress.[16] Neuropathic changes, including abnormal firing in neurons innervating back tissues and nerve ingrowth into degenerated discs have been added to the list of suspected causal factors, as well .[17-19] In the case of symptomatic disc herniations, the findings of Olmarker and colleagues indicate that irritation of nerve roots may not only be caused by compression but also by biochemical effects of exposure to the nucleus pulposus.[20] There is also evidence that cytokines,

such as TNF-α, may be factors in nucleus pulposus induced neuropathy.[21,22] In addition, a possible role for bacterial infections in discs with patients with severe sciatica has been suggested.[23] Although the pain mechanisms are unclear and likely to be complex, evidence suggests that the disc plays a role in back symptoms, sciatica, and spinal stenosis,[24-26] but the extent of the role remains unknown.

Prior to the past decade, the traditional injury or repetitive loading model of disc degeneration had dominated related prevention strategies and research for nearly half a century.[27] Such a model of disc degeneration implied that overloading from exposure to a single excessive force or repetitive loading results in structural damage (e.g. accelerated disc degeneration or herniation), which in turn leads to symptomatic conditions. Among the factors most commonly suspected of accelerating degenerative changes in the discs were various occupational physical loading conditions.[28] In particular, attention has been given to heavy materials handling, postural loading, and vehicular vibration.[29] Numerous studies of the relationship between heavy materials handling and postural loading resulted in mixed findings related to the presence and degree of association with disc degeneration.[3,30-38]

Vehicular driving had been associated with a higher incidence of back symptoms and degenerative changes, which were attributed to the effects of whole-body vibration on the intervertebral disc.[39] Yet, in an extensive review of the scientific literature, Kjellberg and colleagues from the Swedish National Institute for Working Life [40] cautioned that although the majority of studies revealed significantly higher frequencies of back symptoms and degenerative changes in the vertebrae and intervertebral discs of drivers compared to referents, "uncontrolled confounding factors may have affected the results in all studies, and the conclusions about the causal role of whole-body vibration for the observed injuries and/or disorders, therefore, becomes uncertain." Buckwalter cited several mechanisms of age-related deterioration of intervertebral discs, but acknowledged that activities and agents that accelerate degeneration remain speculative.[41]

Based on the studies available at the time, Frymoyer summarized the state of knowledge on determinants of "degenerative disc disease" 15 years ago. He wrote *"Among the factors associated with*

its occurrence are age, gender, occupation, cigarette smoking, and exposure to vehicular vibration. The contribution of other factors such as height, weight, and genetics is less certain".[42] A decade later Ala-Kokko conducted a literature review on the same topic, "degenerative disc disease," and concluded *"Even though several environmental and constitutional risk factors have been implicated in this disease, their effects are relatively minor, and recent family and twin studies have suggested that sciatica, disc herniation and disc degeneration may be explained to a large degree by genetic factors."[43]* A dramatic change in views of determinants of disc degeneration was underway.

Disc degeneration which was once viewed as a result of aging and 'wear and tear' from mechanical insults and injuries is now viewed as being determined in great part by genetic influences,[1,44,45] suggesting new models through which to conceptualize and study disc degeneration and associated pathology. We will summarize briefly some of our group's research, through the Twin Spine Study, that has been at the forefront of this major paradigm shift.

The Twin Spine Study

The Twin Spine Study, which started in 1991, is a multidisciplinary and multinational research project with collaborators primarily in Canada, Finland, the United States and the U.K. The resulting collaborative work has been at the forefront of recent discoveries related to determinants of disc degeneration, and has been a substantial contributor to a dramatic 'paradigm shift' over the past decade related to determinants of lumbar disc degeneration. Among the most significant findings were a substantial influence of heredity on lumbar disc degeneration and the identification of the first gene forms associated with disc degeneration.[45,46] Also, our studies on the effects of smoking and driving exposures using exposure-discordant identical twins have provided perhaps the most well controlled studies of the effects of these exposures on human disc degeneration to date.[47,48] Furthermore, the results of one of our research group's most recent studies indicate that the effect of individual physical factors, such as body weight, and muscle strength on disc degeneration, while modest, may be greater than that of oc-

cupational physical demands.[49] Following is a brief description of the subjects and data on which the studies summarized in this paper are based.

Subjects of the Twin Spine Study came from the population-based Finnish Twin Cohort (with 13,888 male pairs of known zygosity) based on relevant prior information available from surveys in 1975 and 1981, which had elicited response rates of 89% and 84%, respectively. The cohort has been found to be representative of the general Finnish population.[50] They include 147 male monozygotic (MZ) twin pairs and 153 dizygotic (DZ) male twin pairs later recruited for further genetic analyses. The initial selection of 117 pairs of MZ twins was based solely on discordance between twin siblings for a specific common behavioral or environmental factor (e.g. sedentary or heavy occupational physical demands, routine exercise participation, or occupational driving). The factors were selected because of their suspected importance in the etiology of spinal degeneration and back symptom complaints, as well as the availability of relevant information from the Finnish Twin Cohort database. In addition, a random sample of 30 MZ pairs, stratified by age, were added, as were 153 pairs of DZ twins selected using analogous criteria, yielding a total sample of 600 subjects. The volunteer rate was approximately 82%.

Study subjects were found to be quite representative of the Finnish Twin Cohort, which is representative of the Finnish population. No statistically significant differences were observed for level of education, social class, smoking, level of leisure-time physical activity, or history of work-incapacitating neck, shoulder or back pain, or sciatica. The study pairs differed from the entire Finnish Twin Cohort only for work status, they were somewhat more likely to be working, and physical loading at work (slightly higher among study subjects), due to the inclusion of related factors in the selection criteria.[51] DZ pairs were selected in an analogous fashion. The validity of zygosity was studied previously in a subsample of 104 twin pairs. The agreement in classification between the questionnaire data and 11 blood markers yielded an estimated probability of misclassification of less than 1.7%[52] and zygosity has since been confirmed by DNA analyses.

Data acquisition involved transporting twins from all parts of Finland to a central location where a team of project investigators, technicians and other staff ensured that interviews, physical examinations and clinical testing were completed over a two-day period for each twin pair.

A structured interview - was used by trained interviewers to obtain data on lifetime exposures of interest from adolescence through the present. Interviewers were blind with respect to the specific discordance or selection criteria for the twins and project investigators avoided discussions with the interviewers regarding the study hypotheses. Demographic information and health history; occupational history; history of regularly performed leisure time activities/exercise; specific recalled incidents or trauma resulting in acute 'back injury'; general dietary history, particularly related to calcium intake; smoking and driving history were obtained from the interview. For example, for each job held during a subject's lifetime, the subject was asked to describe the job activities, including his most common lifting activity and estimate the weight lifted, the frequency of lifting, and the number of hours spent sitting during an average work day. This information along with the job title was used to appropriately categorize the job in terms of its general demands related to materials handling and postural stress. Exposure to cigarette smoking was calculated in pack years. Optimal means of acquiring adequate estimates of lifetime exposure data is an unresolved issue in research requiring such data. However, using standardized in-depth interviews noting common life 'milestones' to assist with recall are expected to assist in providing valid estimates of exposures of interest. Coded data were checked for congruence, outliers were identified and in some cases phone calls were used to verify unclear or unusual recorded responses.

One year following initial data collection, all subjects were asked to complete an additional questionnaire, which was provided by approximately 98% of subjects. The follow-up questionnaire afforded the opportunity to determine response reliability for several exposure history variables. Responses were compared with those at the time of the initial interview among those who said there had been no change in their jobs. The intra-class correlation coefficient (ICC) was 0.75 for estimates of time

spent sitting, 0.77 for driving and 0.60 for total lifting per day. Also, a five-year follow-up interview and examination was conducted on a subgroup of 150 MZ subjects that allowed for reliability estimates for lifetime exercise history data. Test-retest reliability of lifetime exercise history (using a five-year interval) yielded an ICC of 0.69 for lifetime years of exercise type and 0.73 for associated mean exercise hours per week.[53]

Clinical examinations - included anthropometric measurements (weight, height, % body fat using acoustic impedance) and evaluation of spinal range of motion, isokinetic lifting strength, back muscle static endurance, psychomotor reaction time, and blood and urine samples (for inflammatory mediators, connective tissue markers, and DNA analysis). The Twin Spine Study was provided extraordinary access to the 1.5 Tesla MRI scanner at Kuopio University Hospital and magnetic resonance images of the lumbar spine were obtained for all subjects using a set protocol. Collected blood samples were appropriately stored and transported to the Department of Human Molecular Genetics at the Finnish National Public Health Institute, where DNA was extracted.

Defining Disc Degeneration - The accuracy of phenotype measures is critical in genetic epidemiology when trying to identify gene forms for conditions with multifactorial etiologies, and in studies of gene-environment interactions. The strengths of the Twin Spine Study have been the acquisition of data on a broad spectrum of determinants and possible confounding factors, which can be controlled in analyses when appropriate, and the accuracy of the outcome measures, particularly with respect to degenerative and structural variations.

From the beginning of the Twin Spine Study, the research team has invested much time in methodological developments, such as in spine MRI protocols and image analysis programming. We recognized the importance of standardization and the development of quantitative measures to replace or augment the gross qualitative ratings of spine degeneration in common use, but with suboptimal reliability and precision.[54]

Then there is the deceptively simple issue of defining disc degeneration. The term disc degeneration is commonly used for an overall subjective impression of imaging findings, including signal loss, bulging, herniation, endplate irregularities, osteophytes, and narrowing of the disc space, but no universally accepted standard definition exists. One might expect degenerative findings to correlate with age, but such correlations have been modest within the 35-year period spanning 35 to 70 years of age using qualitative MRI findings (Figure 1).[55] The finding most highly associated with age to date has been disc signal based on MRI T2 sequence, a measure of tissue hydration. Still age explains only a minor portion of the variance in disc signal.

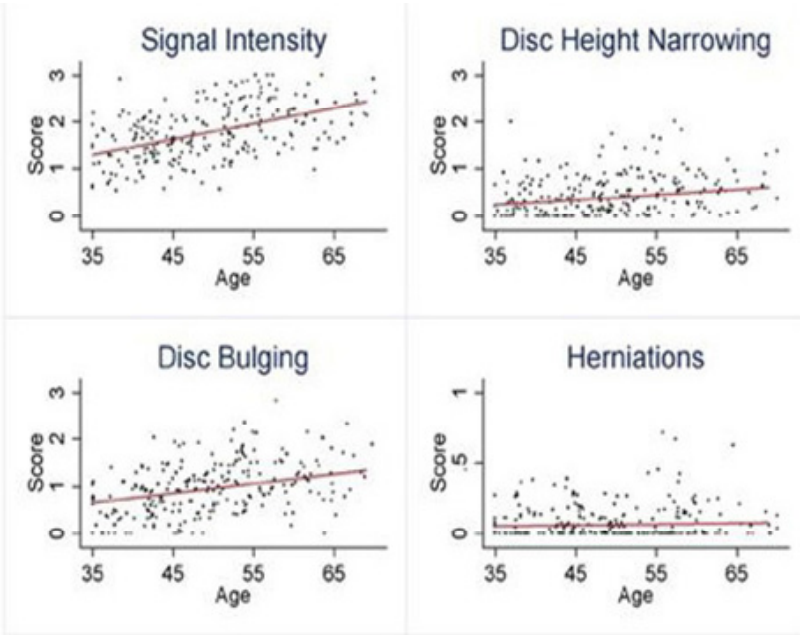

Figure 1. Associations between age and four common findings of disc degeneration based on Spine MRI. The overall associations are weak. (From *Spine*, Battié et al 2004[55])

The various MRI findings associated with disc degeneration represent both atrophic (i.e. annular tears) and proliferative (i.e. osteophytes) changes and may appear at different times in the overall sequence of events collectively termed disc degeneration. Findings may also differ with respect to effects on the occurrence or severity of symptoms. Furthermore, the influence of various risk factors may vary in dif-

ferent stages of the degenerative process. Thus, we made a decision early in the Twin Spine Study to examine the distinct findings associated with disc degeneration separately, as opposed to using summary scores that aggregate different findings, which has proved useful particularly for studies of genetic influences.[45,56]

In an effort to refine MRI assessments of disc degeneration and explore the development of quantitative measurements using the digital data, we initially developed a UNIX based image analysis program in 1994,[1] and the latest version has been programmed to run on Windows NT to provide outcome files simultaneously for all spine levels and regions of interest. It also allows new measures to be easily programmed, as such needs routinely arise as new questions are posed.[56-59,59] To obtain quantitative measurements, the contours of the anatomical boundaries of lumbar discs, vertebrae, and the spinal canal are manually segmented on sagittal PD images (Figure 2). The evaluator follows the contour of the vertebrae including the anterior and posterior longitudinal ligaments, and posterior wall of the spinal canal. To segment the disc from the vertebrae, the evaluator follows the boundary between the vertebral endplates and disc. Segmented areas are then adjusted using T2 and T1 weighted images, if necessary, taking advantage of different contrasts. The areas created by the intersections of those segmentation lines form the regions of interest corresponding to the disc and vertebra from which measures are derived by the software. Manual segmentation is also used in axial slices, for example, to evaluate mid-axial disc area and the central spinal canal.

Perhaps the most useful quantitative measure developed was of disc signal, adjusted for the intra-body reference of adjacent CSF. This measure has been more highly correlated with age than any of the other degenerative signs in the disc. We also found it to be the measure of disc degeneration exhibiting the greatest change over a five-year follow-up period, as compared to little mean progression in disc narrowing, bulging and other measures.[59] The importance of quantitative measures of greater reliability and precision was demonstrated in our earlier study of associations with Vitamin D Receptor

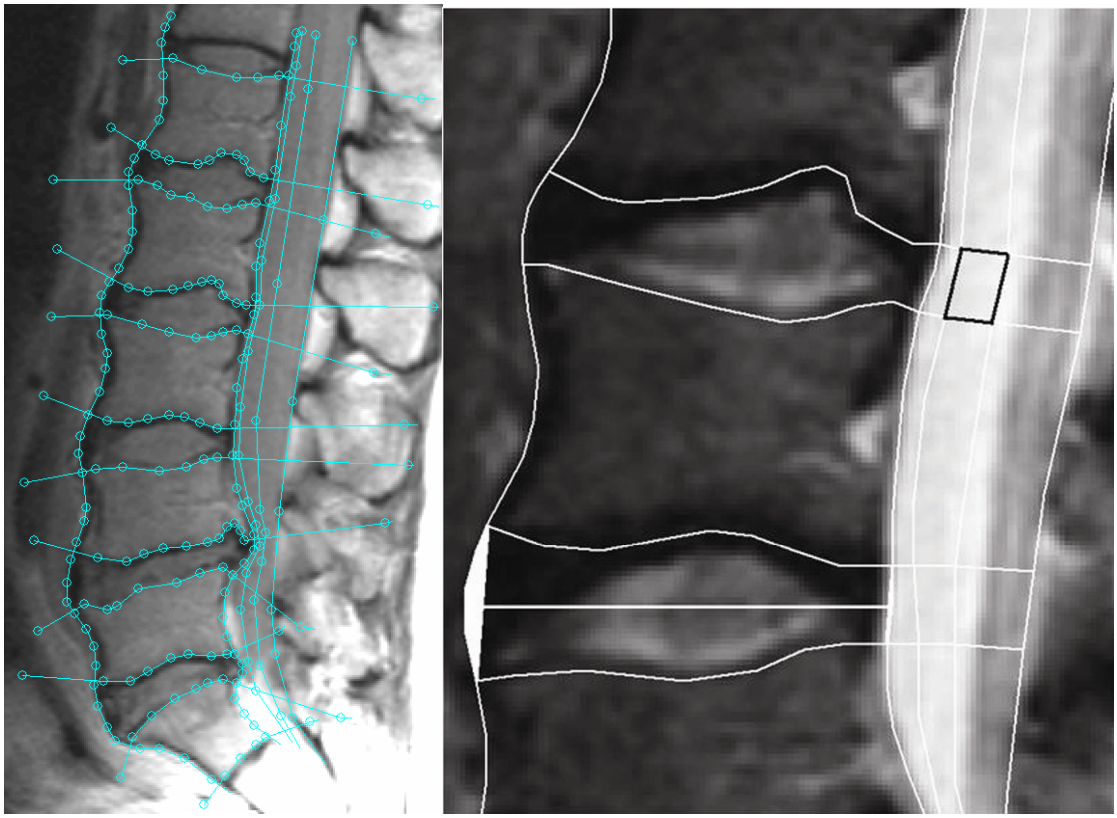

Figure 2. The left picture shows how the manual segmentation was performed using PD- weighted images: the 1st and 2nd vertical line follow the anterior and posterior longitudinal ligaments, the 5th vertical line follows ligamentum flavum, and the 3rd and 4th lines provide cerebrospinal fluid (CSF) samples adjacent to the disc (this is confirmed in T2 weighted images). The horizontal lines follow the disc-vertebra interface. The picture on the right shows the areas in interest created: in the upper disc level the mean disc signal and mean signal of adjacent CSF (black lines) is obtained. The lower disc level demonstrates the bulging areas and the remaining disc area (minus bulging areas) is divided by its diameter (the horizontal 'mid-disc' line). The disc area divided by its length = mean disc height. [60]

polymorphisms, which were identified when using the quantitative measure of signal intensity, but would have been missed using the gross ordinal scales of qualitative measurements.[56]

Quantitative degenerative measures are of particular interest for longitudinal studies where more precise measurements of change are needed than available through ordinal rating scales. Quantitative measures include: disc signal intensity adjusted by the signal intensity of adjacent CSF, disc volume, disc height, anthropometrics and adjusted signal intensity of vertebrae, disc bulging and osteophytes. Intra-rater reliability varies from 0.91-0.99.

Although quantitative measurements have many benefits in terms of reliability and precision, there are many findings that remain best evaluated by qualitative means. Thus, we have used a combination of qualitative and quantitative image analysis measures to depict various findings associated with degeneration. Each of the 600 subjects' films was assessed by one experienced spine specialist following a set protocol. The assessor was blinded to subject exposures and twinship. Among the specific findings assessed, either quantitatively or qualitatively, were:

from sagittal sections:
- disc signal (desiccation)
- disc height
- annular tears
- disc bulging and herniation
- endplate irregularities and sclerosis
- vertebral osteophytes

from transverse sections:
- disc signal (desiccation)
- dural sac compression
- annular tears
- disc bulging and herniation
- spinal canal size diameter/area

Exposure-Discordant Twin Studies of Suspected Environmental and Behavioral Risk Factors

We began our research on the etiology and pathogenesis of disc degeneration under the paradigm that disc degeneration was primarily the cumulative result of tissue injuries and degradation from trauma and repetitive loading. Yet, findings of studies of suspected physical loading risk factors were often contradictory or equivocal, possible confounding was a major concern, and dose-response relations were unclear. Also, at the time we began the Twin Spine Study, MRI was just becoming available and most prior epidemiological or clinical studies had been limited to evaluating disc degeneration through radiographs. Thus, in an effort to clarify the effects of a variety of suspected risk factors we elected to use MRI and a unique study design that we had seen used successfully in the examination of exposure effects on cardiovascular disease.[61] We used an exposure-discordant twin model. Studying MZ twin siblings grossly discordant for a suspected environmental exposure of interest, controlled not only for age and gender, but also genetic influences and many other known and unknown confounding factors due to the high degree of similarity in identical twins' home and social environments and exposures. We were fortunate to begin imaging subjects in 1992 following installation of the first 1.5 Tesla scanner in Finland.

As mentioned earlier, the primary suspected environmental risk factors for disc degeneration were various physical loading conditions, driving and associated whole-body vibration and smoking. Thus, we conducted a series of investigations with identical twins discordant for a common environmental factor suspected of influencing disc degeneration or risk of back symptoms. Our first 'pilot' study using the exposure-discordant twin design was of 20 pairs of smoking discordant twins (mean cigarette smoking discordance, 31.6 pack-years), which revealed a lumbar disc degeneration score 18% higher, in mean, for heavy smokers as compared to their 'non-smoking' siblings (Figure 3A). The total amount of variance in disc degeneration scores among all subjects explained by smoking, however, was less than 2%. The statistical power to detect this small effect size attested to the efficiency of the MZ twin study design.[47] Based on this experience we then established recruitment and data collection protocols for the Twin Spine Study and investigated the effects of various physical loading conditions at work and leisure,[1,62] including regular participation in various forms of exercise and occupational loading,[63] as well as driving and associated whole-body vibration (Figure 3B).

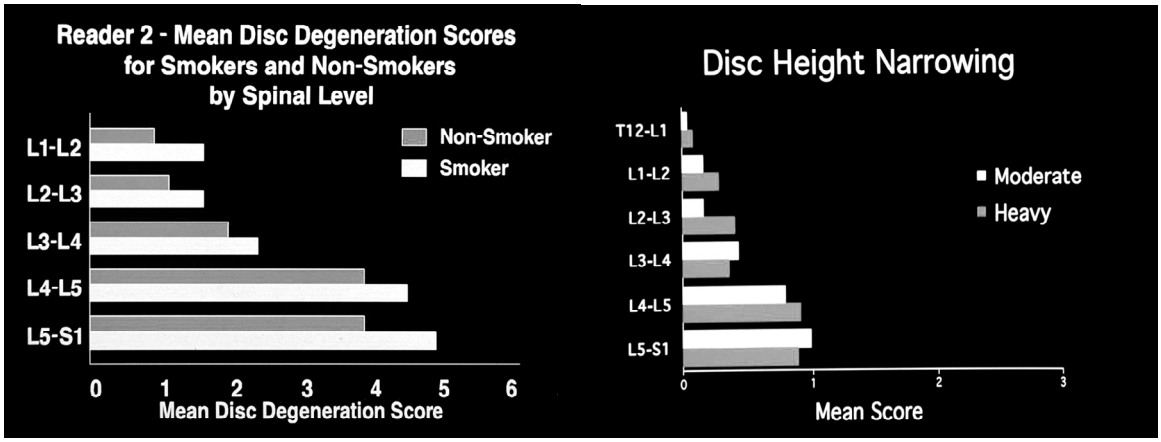

Figure 3. Figure A (left) shows the visual degeneration score for smoker and non-smoker monozygotic siblings by disc level. Smoking had a small harmful statistically significant effect across spinal levels. (Modified from *Spine*, Battié et al 1991[47]) Figure B (right) shows disc height narrowing score by disc level for monozygotic siblings with physically heavy vs. moderate lifetime work history. There was no consistent, statistically significant effect.

As mentioned earlier, a higher incidence of back symptom reports in driving occupations has been attributed to the effects of whole-body vibration on the intervertebral disc.[39] Our investigation of 45 pairs of MZ twin siblings highly discordant for occupational driving is arguably the most well controlled study of the effects of driving and associated whole body vibration on human discs to date, and did not demonstrate significant differences between siblings in MRI findings of the lumbar discs. As well as qualitative measures of disc degeneration, quantitative measurements of CSF-adjusted disc signal intensity were included, which should be highly sensitive for disc degeneration.[64,65] Yet, no tendency for greater disc degeneration was seen among drivers (Figure 4).

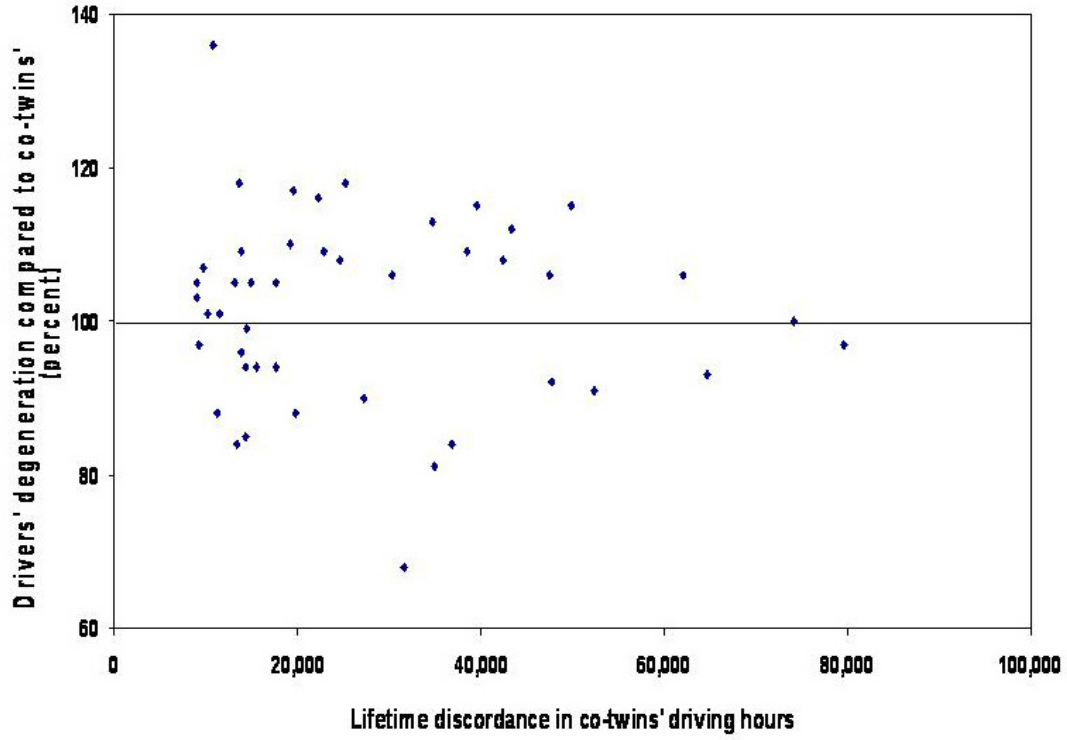

Figure 4. No differences in disc signal intensity between monozygotic twin siblings. There was either no trend of dose-response effect. (From the Lancet, Battié et al 2002[48])

Despite extraordinary discordance between MZ twin siblings in occupational and leisure time physical loading conditions throughout adulthood, surprisingly little effect on disc degeneration was observed. The findings indicated that while physical loading, that is handling heavy loads, bending, twist-

ing and static work in awkward postures, appears to influence disc degeneration, the effect size is very modest,[49,59,64] which would help explain the inconsistent results of previous studies of the effects of occupational physical loading. In a later analysis that aggregated the subjects from all the twin exposure-discordant studies, occupational and leisure time activities explained no more than 7 percent of the variance in disc degeneration.[1] Perhaps not surprisingly, we did not detect smoking effects in a larger, independent group of twins with substantially less discordance. As mentioned earlier, no evidence was found to suggest that exposure to whole body-vibration through motorized vehicles leads to accelerated disc degeneration, which was one of the primary hypotheses of possible mechanisms behind the association between driving occupations and back problems.[66]

Our findings of modest or negligible effects of the primary suspected environmental risk factors despite high exposures and gross discordance would explain the failure to demonstrate uniform, clear effects in earlier studies. We concluded that the particular extrinsic factors studied, which had been among those most widely suspected of influencing disc degeneration, had modest effects, if any. In fact, we found indications that routine loading may actually have some benefits to the disc. In a recent study, associations of anthropometric variables, including lifting strength and routine occupational and leisure time physical loading with disc signal intensity and narrowing were examined in multiple regression modeling.[49] Lower disc signal (representing disc desiccation) was associated with older age and various measures of less routine physical loading of the spine. In addition to greater age, lower body mass and lifting strength and larger disc area were associated with lower signal in multivariable analyses. While associations were more modest, greater age and occupational loading exposures entered the multivariable model explaining disc height narrowing. We concluded, "body weight, lifting strength, and axial disc area were more highly associated with disc degeneration than occupational and leisure physical activity histories, although all had modest influences. Furthermore, higher body mass, greater lifting strength, and heavier work were all associated with more disc height narrowing, but less disc desiccation contrary to current views."[49] These findings were the focus of the lead story of the BackLetter

the month after they were presented at the annual meeting of The International Society of the Study of the Lumbar Spine meeting in an article that asked the question, “Does physical loading strengthen the intervertebral disc and retard disc degeneration?”(BackLetter, July 2006) The discovery may represent an important finding in better understanding the relation between various loading conditions and disc degeneration and suggests that responses of the disc may be more in keeping with other musculoskeletal structures that benefit from adaptation to routine physical loading (Figure 5). The findings also suggest that determinants of disc degeneration and their effect sizes differ between specific degenerative findings. Thus, aggregating findings associated with disc degeneration into summary scores may mask relations.

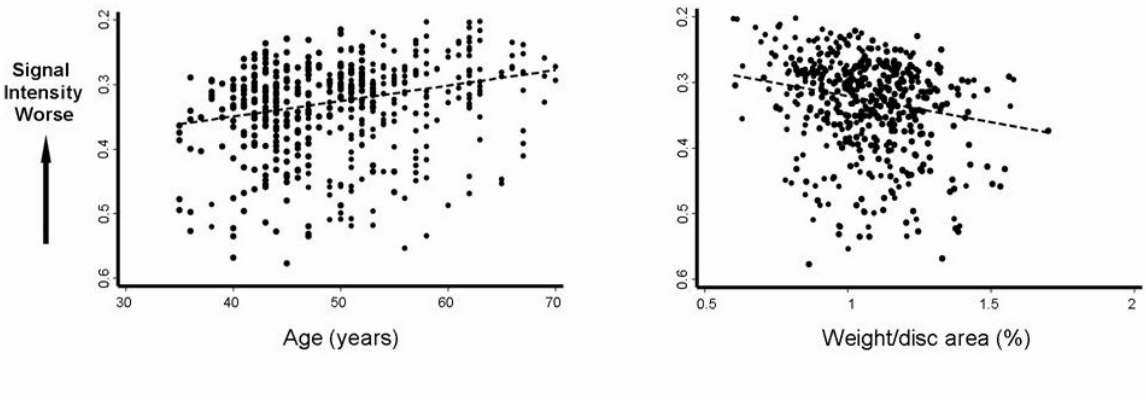

Figure 5. Scatter plots of quantitative CSF-adjusted disc signal versus age and body weight/axial disc area. Higher body weight per disc area (and other indicators of greater routine loading on the spine) was associated with better disc signal. (From *Spine*, Videman et al 2007 [49])

In summary, the findings of the exposure-discordant twin studies raised questions about the adequacy of an injury model or ‘wear and tear’ view of disc degeneration. Moreover, more recent findings suggest that greater routine physical loading may actually have some beneficial effects on the disc. During the course of the exposure-discordant twin studies, the striking observation of anyone who had the opportunity to view twin sibling images side-by-side was the strong resemblance in disc degeneration, not just in the degree of degeneration, but also in the types of findings and spinal levels involved. These observations led us to pursue studies of genetic influences.

Heredity as a Major Determinant of Disc Degeneration

The observations of co-twin similarities led to two studies of independent samples of MZ twins to systematically evaluate familial aggregation of disc degeneration. Familial aggregation in MZ twins can be viewed as representing the upper limit of genetic influences, as similarities can reflect both shared genes and shared early environments. Because there are very few traits that exhibit shared environmental (i.e. non-genetic familial) effects in adulthood, familial aggregation is generally viewed as a proxy of total genetic effects. The resulting two papers were published in 1995 and were the beginning of a major shift in the way disc degeneration and its determinants are viewed.

Although occupational physical loading and other environmental exposures had received much attention as possible risk factors[28], detailed studies focusing on hereditary aspects of disc degeneration were lacking[67]. Before our work, there were only case series reports of similarities between twin siblings and relatives in the extent and location of degenerative changes in the spine and other joints.[68,69] We first conducted a systematic evaluation of lumbar degenerative changes blinded to twinship using the 20 twin pairs of MZ twins enrolled in the 'pilot' study of twins discordant for smoking. We found a striking degree of similarities (matching by type of finding and spinal level) within identical twin pairs, well beyond that expected by chance or because of similarities in age (Figure 6).[46] This was followed by a larger, more comprehensive investigation of the role of familial aggregation and environmental influences in disc degeneration, which has been among our team's most important contributions to date. [1] Spine MRIs from 115 pairs of MZ twins were used to estimate the effects of commonly suspected risk factors on disc degeneration relative to the effects of age and familial aggregation, representing both genetic and early shared environmental influences. In the multivariable analysis of the T12-L4 region, 61% of the variability in disc degeneration was explained by familial aggregation, beyond that of age and occupational physical loading that together explained 16%. In the L4-S1 discs,

11% of disc degeneration was explained by physical loading and age, which rose to 43% once familial aggregation was added to the model (Figure 7). In contrast to the upper lumbar

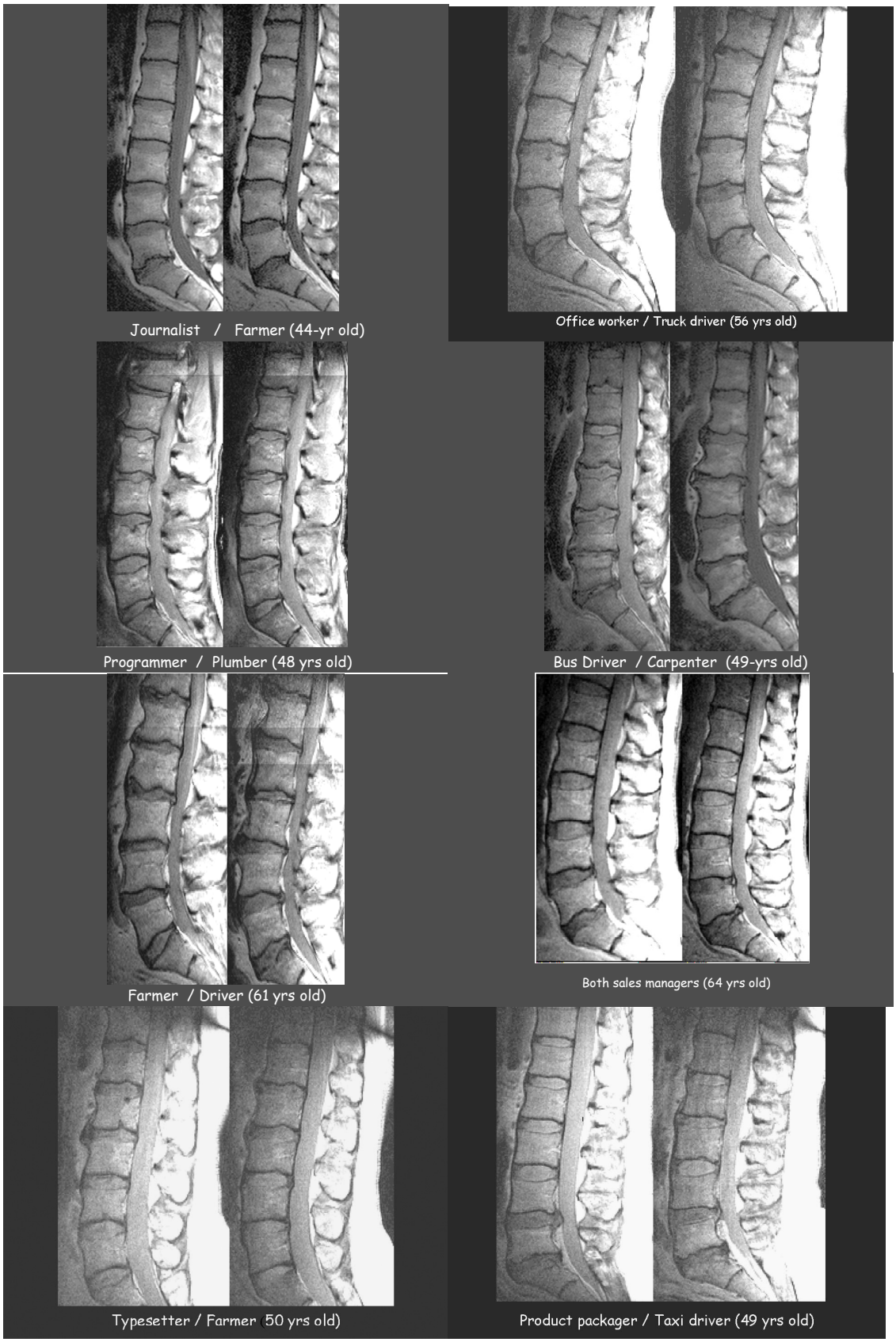

Figure 6. High degrees of similarities in disc degeneration were noted between twin siblings, often despite high discordance in lifetime physical loading exposures. (In part from *Spine*, Battié et al 2004[55])

levels, fifty-seven percent remained unexplained in the lower lumbar region. These study findings led us to the conclusion that lumbar disc degeneration may be explained primarily by genetic influences, early environmental exposures and yet unidentified factors, which may include complex interactions, such as between environmental factors and individual spinal anthropometrics.[1]

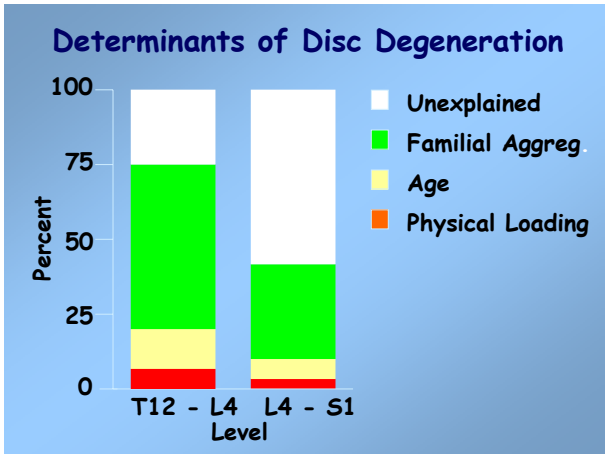

Figure 7. The variability explained (adj. R^2) in qualitative summary scores explained by physical loading, age, familial aggregation (proxy of heredity) demonstrating that significantly more of the variability remained unexplained in the L4-S1 disc levels.[1] (Modified from *Spine*, Battié et al 1995[1])

Later, in a sample composed primarily of women from the U.K. and Australia, Sambrook et al (1999) reported on heritability estimates for lumbar disc degeneration of 73%, supporting a substantial genetic influence.[44] Heritability estimates refer to the proportion of population variance in a trait attributable to genetic variation. Interestingly, while heritability estimates were high for disc bulging and narrowing (65% and 79%, respectively) a genetic influence on disc signal intensity was not apparent. Our preliminary analyses from a classic twin study of 300 pairs of MZ and DZ male twins indicate substantial but somewhat lower heritability estimates closer to 50%, more in line with expectations from our earlier study of MZ twins. Contrary to Sambrook et al's finding of no genetic influence on disc signal intensity using a qualitative 4-point rating system, we found similar heritability estimates for disc height narrowing and signal intensity, when using the more reliable, precise measure of CSF-adjusted disc signal intensity. This provides an example of the importance of phenotype measurements.

Our research results and those of Sambrook et al of high heritability estimates for different degenerative findings in spine MRI provide motivation for identifying associated genes. Yet, disc degen-

eration and associated pathology likely represent complex conditions with multifactorial inheritance, presenting challenges to mapping out the genetic architecture of disc degeneration.

The Search for Susceptibility Genes

Common diseases generally have a genetic contribution from multiple gene loci. For each gene locus we are interested in how many alleles exist and their frequencies. Allele frequencies and average effects associated with the alleles determine the contribution of allelic variation to the trait of interest, which can then be partitioned into additive genetic variance (gene "dosage") and variance due to gene dominance.[70] Candidate genes may be used as targets, with potential genetic variation leading to differences in the proteins encoded by these genes. These proteins are part of the physiological system that, when disturbed, gives rise to the condition. Thus, the identification of associated genes, given their basic role in determining cell structure and function and hence tissue structure and function, can provide insights into mechanisms underlying disease. The candidate gene approach is promising for the analysis of common diseases, which are complex in their etiology and development, and has been utilized in most 'gene hunting' studies of disc degeneration and associated pathology to date. However, undoubtedly gene-gene and gene-environment interactions are present in common polygenic conditions, such that simple linear models are unlikely to grasp the complexity. Thus, unraveling the contribution of genes and environment to etiology will be a difficult task.

Following the discovery of a substantial genetic influence on disc degeneration there has been considerable effort focused on identifying associated genes. The first gene polymorphisms associated with disc degeneration were identified through the Twin Spine Study in 1998.[45] They were two polymorphisms of the Vitamin D Receptor Gene, TaqI and FokI identified in 170 MZ male twins. The associations were revealed with the phenotype of CSF-adjusted disc signal intensity. Signal intensities were 12.9% lower (more desiccation) in men with the TaqI tt genotype and 4.5% lower with the Tt genotype as compared with signal intensities in men with the TT genotype. A similar pattern was seen between

disc signal and FokI genotypes. Associations with degenerative scores using qualitative, gross ordinal scales did not reach statistical significance, emphasizing the value of more precise phenotype definition and measurement, particularly with small samples. As was written in the BackLetter (Vol. 13(7): 73-80, 1998) following presentation of the findings at the annual International Society of the Study of the Lumbar Spine meeting in 1998, the study *"confirmed for the first time the existence of genetic susceptibility to this progressive, age related degenerative process…This is the first step in a long process. However, this research opens the door to more accurate assessment of susceptibility to degenerative problems, and perhaps even prevention of these problems."*

Since that time there have been over 30 studies of genes associated with disc degeneration and associated pathology (Table 1). Among 23 studied genes, including aggrecan (AGC), collagen (COL), vitamin D receptor (VDR), inflammatory (IL) degradative (MMP) and some other genes, 17 have been associated with disc degeneration or associated pathology in at least one study. However, many observed associations were based on small sample sizes and have not been replicated in other studies. Phenotypes also vary; in one quarter of studies the phenotype was based on X-ray images, which can provide only indirect evidence of disc degeneration through disc space measurements. In the studies based on spine MRI the specific findings of disc degeneration have been assessed visually (in most studies using a 4-point qualitative scale). Despite the challenges with sample sizes and phenotype definitions and associated misclassification, there is reasonable evidence suggesting associations of disc degeneration with the VDR gene (7/8 studies), with COL9A2 (8/10) and with COL9A3 (4/8). Yet, the available findings indicate that each gene has only modest effects.

We have recently analyzed DNA data for SNPs and haplotypes in 26 candidate genes, including 14 structural (AGC, 12 COL, VDR), 8 interleukin and 4 matrix metalloproteinase genes, selected for lumbar degenerative phenotypes.[71] For genotype-phenotype associations we used the FBAT (Family-Based Association Tests in genetic analyses) program package.[72] These tests are based on the classic transmission/ disequilibrium test (TDT),[73] and permit testing of the hypotheses of no linkage and

no association and linkage but no association. We used a strict statistical method (including 1000 permutations) to accept an 'overall gene association'. The main phenotype was quantitative CSF-adjusted disc signal, in addition to the typical qualitative ordinal scores of disc height reduction and bulging in 579 MZ and DZ twin subjects.

Candidate Gene Studies to date seeking associations with disc degeneration, sciatica, 'lumbar disc disease' or spinal stenosis in general population sample and patients

Authors	Genes	Sample size	Phenotype	Ethnicity
Videman et al 1998	**VDR**	170 population	MRI	Finnish
Jones et al 1998	**VDR**	**282** elderly subject	X-ray (K/L) *)	Australian
Jordan et al 2005	**VDR**	**291** subjects	X-ray (K/L)	UK
Videman et al 2001	**VDR**	142 population	MRI	Finnish
Kawaguchi et al 2002	**VDR**	**205** subjects	MRI	Japanese
Cheung et al 2006	**VDR**	**804** population	MRI	Chinese
Kawaguchi 1999	**AGC**	64 mix	MRI	Japanese
Roughley 2006	**AGC**	44 patients	MRI/X-ray	Canadian
Annunen et al 1999	**COL9A2**	157 patients +101 controls	MRI	Finnish
Paassilta et al 2001	**COL9A1-3**	171 patients	MRI /CT	Finnish
Solovieva et al 2002	**COL9A3**	135 subjects	MRI	Finnish
Karppinen et al 2002	**COL9A2**	159 patients +22 families	MRI	Finnish
Matsui et al 2004	**COL9A 2-3**	107 spondylolisthesis patients	X-ray/MRI ?	USA
Kales et al 2004	**COL9A2-3**	105 patients 102 controls	X-ray (K/L)	European
Jim et al 2005	**COL9A2**	**804** population	MRI	Chinese
Seki et al 2006	**COL9A2**	**470** LDD patients, 658 controls	MRI	Japanese
Higasheno 2006	**COL9A2, COL9A3**	84 herniation patients	MRI	Japanese
Solovieva et a 2006	**COL9A2-3; COL2A1**; **COL11A2 IL-1β**	135 subjects	MRI	Finnish
Noponen-H. et al 2003	**COL9A1-2-3; COL11A1; AGC1; VDR; MMP-3**	29 stenosis,56 controls	MRI/CT	Finnish
Pluijm et al 2004	**COL1A1**	**966** subjects	X-ray (K/L)	Dutch
Tilkeridis et al 2005	**COL1A1**	36 subjects	X-ray (K/L)	European
Takahashi et al 2001	**MMP-3**	103 subjects	MRI	Japanese
Valdes et al 2005	**MMP-3; TIMP1; COX2**; **VDR** ;**THSD2**	**720** subjects	X-ray (K/L)	UK
Solovieva et al 2006	**IL-1β, COL9A2, COL9A3,COL11A2, COL2A1**	135 subjects	MRI	Finnish
Solovieva et al 2004	**IL-1α, IL-1β**	133 subjects	MRI	Finnish
Le Maitre et al 2005	**IL-1α, IL-1β; IL1Ra–RI**	30 tissue samples or	MRI	UK
Noponen-H. et al 2005	**IL6, IL1A,IL1B, TNFA**	155 patients,179 controls	MRI	Finnish
Min et al 2006	**MATN3**	**809** subjects + **382** OA patients	X-ray (K/L)	Dutch, Icelandic
Seki at al 2005	**CILP**	**467** patients 664 controls	MRI Surg. patients	Japanese
Virtanen et al 2007	**CIL**	**602** LDD patients/ 602 controls	MRI	Finnish, Chinese
Koshizuka et al 2007	**ER, PTH,IL-1b,VDR**	**381** spondylosis population	X-ray (K/L)	Japanese

Table 1. We found 31 studies on the associations of genes and spine degeneration; no association was found with genes in read. There were 12 studies with sample sizes of more than 200 subjects or cases. Half of the studies were based on population and half of patients with spinal disorders. The phenotypes were based visual gradings of MRI in 14 and of X-ray in 8-10 studies and on back pain histories in 8 studies. Quantitative MRI measures were used in two studies. *) K/L = Kellgren/Lawrence osteophyte – disc height classification.[74]

Disc signal, bulging and disc height reduction at both upper and lower lumbar discs were associated with AGC1 gene in preliminary analyses. Disc signal of upper lumbar discs was associated with the COL9A1 gene, and in the lower lumbar discs with COL9A2 gene. Associations were found using multi-allele testing of COL5A1, COL9A2, IL1RL2 and IL18R1 genes with disc signal (p = 0.037 -

0.0006). Bulging in lower lumbar discs was associated with the COL1A2 gene. In addition, various polymorphisms of the collagen and interleukin genes were significantly associated with the degenerative phenotypes, but the overall gene associations were not statistically significant after permutation tests. None of studied SNPs of COL9A3, COL10A1, IL1R1, IL1R2, MMP3, MMP8, MMP9 and MMP13 genes was associated with disc signal, bulging or disc height reduction.

The specific interests in this study were variations in 'durability' of structural proteins (disc matrix synthesis and degradation) and in inflammatory and degradative reactions. However, other mechanisms in disc degeneration may exist, such as those related to anthropometrics, muscularity and lifting strength, which all have genetic correlations and are also included in the genetic component of disc degeneration.[49] Some of these findings support those of earlier analyses, while others will await replication.

Is the Disc a Pathway Through Which Genes Influence Back Pain Problems?

Disc degeneration and back pain are clearly not synonymous and the association between the two is routinely debated. Yet, if disc degeneration does influence back pain problems, and both have a substantial genetic component, disc degeneration may be one pathway through which genes influence back pain. We were interested in examining the hypothesis that genetic influences on back pain are mediated through genetic influences on disc degeneration. Thus, we conducted a classic twin study of 300 MZ and DZ twin pairs of the Twin Spine Study using multivariate quantitative genetic models to estimate the degree to which genetic effects on back pain are correlated with genetic effects on disc degeneration.[75] Disc height narrowing was used to index disc degeneration as it was the finding most associated with back pain in earlier analyses of MZ twins.[58] In support of our hypothesis, statistically significant genetic correlations were found for various definitions of back pain and disc height narrowing. A substantial minority (up to ¼) of the genetic influences on pain was due to the same genetic influences affecting disc height narrowing. Yet, the substantial portion of genetic influences on pain left

unexplained suggests an important role for other genetic influences that may affect pain processing, reporting or other underlying pathological conditions.

In contrast, less than 5% of the variance in back pain outcomes explained by environmental factors was due to the same environmental factors influencing disc height narrowing. This is concordant with our earlier exposure-discordant twin studies revealing negligible or modest effects on disc degeneration of occupational activities associated with back pain complaints. This raises the question, do some of the particular environmental physical loading exposures serve primarily to exacerbate symptoms rather than cause the underlying pathology? It is also important to note that while little overlap was found between environmental factors influencing pain reporting and disc narrowing, environmental factors do appear to have a substantial role in disc height narrowing as do genetics. The challenge is to refine or reconceptualize influential environmental exposures, such as biomechanical forces, which may include hypotheses of interactions with other systems and the pathways through which they may affect lumbar disc degeneration and associated pathology.

In Summary

Knowledge gained through the Twin Spine Study and others' efforts over the past decade have substantially enhanced our understanding of disc degeneration and have provided a new paradigm. Disc degeneration is now considered a condition that is genetically determined in large part, with environmental factors, although elusive, also playing an important role. This advance in the understanding of disc degeneration provides a foundation from which to develop new hypotheses and more fruitful research that may help to elucidate the etiology of disc degeneration and associations with pain.

Our earlier work on disc degeneration in MZ twins[1,46,75] established a substantial role for heredity in disc degeneration through the identification of high degrees of familial aggregation, suggesting a substantial genetic influence. This has been further substantiated by Sambrook et al's and our own classic twin studies of MZ and DZ twins. Our discovery of two gene forms associated with disc degeneration[45] ushered in the current wave of studies to identify genes associated with disc degenera-

tion, with the hope of better understanding important pathways leading to pathology. Yet, the investigation of genetic influences on disc degeneration is still in its infancy. Future research will aim to clarify the genetics of disc degeneration, identify influential environmental factors, and explore the interplay between the two.

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