Significant Linkage Evidence for a Predisposition Gene for Pelvic Floor Disorders on Chromosome 9q21

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Predisposition factors for pelvic floor disorders (PFDs), including pelvic organ prolapse (POP), stress urinary incontinence (SUI), urge urinary incontinence (UUI), and hernias, are not well understood. We assessed linkage evidence for PFDs in mostly sister pairs who received treatment for moderate-to-severe POP. We genotyped 70 affected women of European descent from 32 eligible families with at least two affected cases by using the Illumina 1 million single-nucleotide polymorphism (SNP) marker set. Parametric linkage analysis with general dominant and recessive models was performed by the Markov chain Monte Carlo linkage analysis method, MCLINK, and a set of SNPs was formed, from which those in high linkage disequilibrium were eliminated. Significant genome-wide evidence for linkage was identified on chromosome 9q21 with a HILOD score of 3.41 under a recessive model. Seventeen pedigrees (53%) had at least nominal evidence for linkage on a by-pedigree basis at this region. These results provide evidence for a predisposition gene for PFDs on chromosome 9q.

Pelvic floor disorders (PFDs), including pelvic organ prolapse (POP) (MIM 176780), stress urinary incontinence (SUI), urge urinary incontinence (UUI), and hernias, are seen in women of all ages and are a major public health concern. One in nine women will undergo surgery for these disorders in her lifetime,1 and of these, one third will undergo repeated surgeries. Many more women are affected by POP and incontinence but manage their symptoms without surgery.2

The etiology of PFDs is most likely multifactorial. Risk factors typically focus on defects in the pelvic floor musculature or connective tissue weakness. Childbirth is the most studied risk factor;3 vaginal delivery, especially with forceps, increases the risk of urinary incontinence, but cesarean delivery is not entirely protective.4 Other risk factors include increased age, smoking, and chronic increased intra-abdominal pressures such as occupational lifting, obesity, and chronic constipation.3 However, these environmental risk factors fail to fully explain the pathophysiology of PFDs. Severe POP and SUI have been observed in nulliparous women,5 whereas other highly parous women do not develop POP or SUI.

Several studies have investigated a possible genetic predisposition for PFDs. Epidemiology surveys have found that women with urinary incontinence were more likely to have family members with incontinence,6–9 and genetic effects may account for as much as 40% of the total variance of SUI and POP.10 Recent candidate gene studies have focused on genes related to skeletal muscle myosin,11 p27/kip1 (cyclin-dependent kinase inhibitor 1B [MIM 600778]),12 and the basement membrane protein laminin (LAMC1 [MIM 150290]).13

To better understand the genetics of POP, this study was undertaken with the purpose of determining whether there is linkage evidence for a predisposition gene(s) for POP in a large set of affected sister pairs with PFDs. To our knowledge, this study is the first linkage study of high-risk POP pedigrees.

Women who underwent surgical repair of a PFD in the urogynecology service at the University of Utah from 1996 to 2006 were invited to participate in this study (n = 533 women). Of these, 297 had surgery for POP only and 256 had surgery for both POP and SUI. These probands completed a demographic questionnaire about age at onset of symptoms, childbirth history, family history, and potential clinical markers such as striae and joint mobility. The probands contacted their sisters for permission to receive a mail questionnaire for evidence of a PFD. Sister recruitment began in 2002. Sisters who agreed to participate completed the same demographic questionnaire as the probands and completed a 43-question standardized survey for PFDs, the pelvic floor distress inventory (PFDI).14 The PFDI has been validated as a PFD symptom screen in at-risk populations.15 The University of Utah Institutional Review Board approved this study prior to enrollment, and informed consent was obtained for every participating subject.

Sisters were identified as “probably affected” or “unlikely affected” on the basis of questionnaire results or surgical histories. In a few families, other close female relatives were screened. Women who were termed “probably affected” were examined for POP via a standardized clinical assessment for POP, the POP-Q,16 as well as for SUI and UUI via the full bladder standing cough stress test (CST) or diary as appropriate. If a relative was unable to travel to complete the POP-Q or CST, documentation of treatment for a PFD, which was usually surgery for the condition, was required. Finally, all affected individuals underwent blood sampling for DNA analysis.

Of the 553 total female probands, 195 had no living sisters; 140 had died, could not be contacted, or did not

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respond to the study invitation; and 94 declined, withdrew, or did not have a sister who agreed to participate. Our final sample included 124 probands who provided information on 1–8 sisters per family and additional female relatives. A total of 209 women were sampled and phenotyped.

For linkage analysis, we defined POP cases by a rigorous phenotype definition. “Affected” individuals were those who received treatment for moderate-to-severe POP, usually stage III or IV, and in most cases the treatment was surgery. In the POP-Q staging system, stage III represents prolapse of the pelvic organs more than 1 cm beyond the hymeneal ring or vaginal opening; stage IV represents complete prolapse of the vagina and pelvic organs.

Eligible families had to have at least two affected relatives meeting our strict phenotype definition. Although probands were identified by having a PFD surgery at the University of Utah, relatives were scattered across the United States.

We identified 32 eligible families informative for linkage analysis in which the largest number of affected, genotyped individuals per family was three. These families included a total of 70 affected individuals meeting our strict phenotype criteria, all of whom were of European descent. The majority of families (n = 25, 78%) contained two affected sisters, five families had three affected sisters, and two families contained more distant relatives. One of the extended pedigrees consisted of an aunt-niece pair. The niece has a sister who is also affected with POP, but the sister never received treatment for POP. The other extended pedigree consisted of two affected sisters and their affected aunt. All defined POP cases used for this study received a physical exam by a physician at the University of Utah Hospital and Clinics for confirmation of diagnosis.

Table 1 summarizes the clinical characteristics of the sampled women. Most women had given birth to at least one child; only one woman in this sample was nulliparous. Approximately half (n = 33 of 64 with body mass index [BMI] information) of the women were overweight or obese (BMI > 25 kg/m²). A substantial percentage of the women meeting our strict phenotype criteria for POP had also received treatment for SUI (64.3%), UUI (31.4%), and/or hernias (5.7%).

Samples for participating affected women in eligible families were genotyped with the Illumina 1 million single-nucleotide polymorphism (SNP) set. If markers are in strong linkage disequilibrium, linkage analysis of densely typed markers can lead to an inflation of linkage statistics and an excess of false positive results. Therefore, we analyzed a pruned set of SNP markers (n = 27,157) in which markers in high linkage disequilibrium were eliminated. These 27,157 markers had a minimum spacing of 0.1 cM, a minimum heterozygosity of 0.3, and a maximum r² of 0.16 over a sliding 500,000 bp window in the publicly available HapMap CEPH/Utah (CEU) data and exceeded an individual call rate of 98% for genotyped subjects.

We used the integrated genetic map of Duffy as our backbone map and linearly interpolated additional SNPs with Build 35.1 of the human genome sequence to derive our genetic map. Allele frequencies were estimated directly from observation of all genotyped individuals at each locus.

We performed a linkage analysis on the linkage subset of markers with the multipoint Markov chain Monte Carlo (MCMC) linkage method MCLINK. MCLINK allows for fully informative multilocus linkage analysis on large extended pedigrees and has been used previously for identification of candidate genomic regions for a number of complex diseases. Because the mode of inheritance of POP is unknown, we performed a parametric analysis with both general dominant and recessive models. Phenotypes of all individuals not termed “affected” were considered “unknown,” and these individuals were not genotyped in the present study. Although individuals termed unknown did not meet strict study criteria for POP, many of the women were affected with other types of PFDs. Males were also considered unknown because, though they cannot express the phenotype, they may be genetic carriers of a predisposition gene(s).

Because PFDs are most likely complex diseases and probably arise from multiple heterogeneous loci, we report heterogeneity LOD (HLOD) scores rather than LOD scores. HLOD scores more accurately reflect the true position of a linkage peak and have been shown to be more powerful than homogeneity LOD scores and model-free methods under conditions of heterogeneity.

Significance was determined by the Lander and Kruglyak genome-wide criteria. Suggestive linkage evidence was defined by a LOD score ≥ 1.86, and significant evidence was defined by a LOD score ≥ 3.30. Specific pedigrees were also identified for chromosomal regions that exceeded HLOD ≥ 3.30 and had individual LOD scores > 0.5, which represents a nominal, uncorrected p < 0.05 for an individual pedigree.

Genome-wide linkage results for all pedigrees are displayed in Figure 1. The maximum HLOD statistic observed was 3.41 (rs11139451) at chromosome 9q21,
assuming a recessive model. This meets the Lander and Kruglyak genome-wide significance criterion accounting for a dense marker map. All markers between rs4077632 (80,353,293 bp) and rs10868525 (88,807,848 bp) in the chromosome 9q21 region exceeded HLOD > 1.86. Seventeen of the 32 pedigrees attained nominal linkage evidence (i.e., HLOD > 0.5) on a by-pedigree basis for the region. The highest HLOD score per pedigree was 1.18, which was observed for two pedigrees, each with three affected sisters. Suggestive linkage evidence was also noted on chromosome 9q31 (maximum HLOD = 1.90), which is approximately 17 Mb away from 9q21, and chromosome 1q42 (maximum HLOD = 1.89), assuming a recessive model.

As secondary confirmation of the significant chromosome 9q21 result, we reanalyzed the data by using the linkage package Merlin, which computes exact HLOD scores via sparse binary trees. We obtained a maximum HLOD score of 3.42, which is nearly identical to our reported MCLINK results. We also empirically validated our results by using the recently proposed latent p value method as implemented by Alun Thomas with 5000 independent simulations. The median distribution of the p value via the latent p method was 0.051 under the general recessive model on a genome-wide basis, which is in close agreement with the Lander and Kruglyak genome-wide significance criterion.

There are only a limited number of previous studies that have examined the role of genetics for PFDs. We are aware of only one other linkage analysis that has been reported to date. Nikolova et al. studied a single Filipino family with six affected individuals of mixed PFD phenotypes (i.e., POP and/or hernia) aged < 55 years. They identified ten linkage regions with LOD scores near 1.5, the maximum expected for this family size and marker density. However, none of the linkage signals were on chromosome 9q.

Several other studies have compared differential gene expression between women with and without a PFD via biopsy specimens from one of the pelvic floor muscles. Caution must be exercised when interpreting these studies, because many are underpowered. Visco and Yuan compared gene expression in pubococcygeus muscle biopsy specimens from five patients with stage III or stage IV POP and five control subjects without prolapse. They identified 111 genes that were underexpressed and 120 genes that were overexpressed after correcting for background intensity levels; however, none were in the chromosome 9q21 region. Hundley and colleagues
observed significant differences in gene expression in the pubococcygeus muscle for two specific genes (MYH3 [myosin, heavy chain 3, skeletal muscle, embryonic] [MIM 160720] on chromosome 17p13 and MyBP-h [myosin-binding protein h] [MIM 160795] on chromosome 1q32) in 17 women with stage III or stage IV POP and 23 controls with minimal to no prolapse. Bukovsky and coworkers\textsuperscript{12} observed differences in p27kip1 (chromosome 14q) expression levels from the levator ani muscle in 22 symptomatic patients with SUI and/or POP compared to nine asymptomatic women. Results from these gene expression studies are all limited to the specific tissue studied and cannot be generalized to all pelvic floor muscles. Our present study focuses on germline mutations from DNA obtained from blood; thus, our results are not in discrepancy with these previous reports.

There are a number of genes in the chromosome 9q21 region that have a known protein function in muscle or have been observed to be highly expressed in muscle. They include: TLE4 (Transducin-like Enhancer of Split 4 [MIM 605132]), TLE1 (Transducin-like Enhancer of Split 1 [MIM 600189]), UBQLN1 (Ubiquilin 1 [MIM 605046]), MAK10, and GOLM1. None of these genes has been previously studied as a candidate gene for PFDs.

In conclusion, we have found significant linkage evidence for a predisposition gene for PFDs on chromosome 9 by using the largest collection of families with POP reported to date. These linkage results are remarkable for the strength of signal obtained on chromosome 9q21 and a large percentage of our eligible families (n = 17, 53%) showing evidence of linkage to the chromosome 9q21 region. Although this study focused on defining cases by POP severity status, many of the women were also affected with varying degrees of SUI, UUI, and/or hernias. Hence, these results cannot definitively identify a chromosomal region that predisposes one to POP. We also note that our results are premature to suggest that all PFDs share a common genetic predisposition, although epidemiological studies suggest overlap of the PFD subcomponents.\textsuperscript{29}

We are in the process of collecting and genotyping additional high-risk PFD pedigrees and plan in the future to examine each PFD subcomponent separately, and we will include in our analysis older, unaffected females in these high-risk pedigrees to strengthen our conclusion that a presumed disease locus causes disease in familial cases and not in unaffected cases. Our results do not dispute that PFDs are probably multifactorial diseases with contributions from both genetic and environmental factors; we note that not all of our pedigrees linked to the chromosome 9q21 region. Environmental factors and more common risk alleles identified through association analysis or other techniques may also play a role in PFD predisposition. If our results are confirmed, they can direct future gene localization efforts. Knowledge of genetic susceptibility may provide insight into the pathogenesis, prevention, and intervention of this condition through a genetic screen.

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Web Resources

The URLs for data presented herein are as follows:


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