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Original Article

Genetic Mutation that May Contribute to Failure of Prolapse Surgery in White Women: A Case-Control Study

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ABSTRACT **Objective:** To identify a potential genetic basis for early failure after prolapse surgery.
Design: Case-control study (Canadian Task Force classification II).
Setting: This study was carried out in 1 academic community medical center referral practice, and all patients had surgery at 1 of 2 hospitals.
Patients: Ten women with early, multicompartment prolapse recurrence after robotic sacrocolpopexy compared with 40 control subjects with known success after the same procedure.
Interventions: Patients were treated with robotic sacrocolpopexy.
Measurements and Main Results: DNA was isolated and initially genotyped on a single nucleotide polymorphism (SNP) array to direct more detailed exome analyses. Exome sequences were mapped to the Human Genome Reference Sequence (GRCh37), and variants were compared between groups and to participants in the 1000 Genomes Project. Statistical analyses were performed using a software package commonly used in genetics research. TaqMan assay was used for verification, and p values were adjusted using the false discovery rate. Demographics of groups were compared using χ^2, Mann-Whitney U, and *t* tests. A SNP [rs171821] located near the *ZFYVE16* gene was associated with patients but not control subjects, and the false discovery rate–adjusted p value was .046 (odds ratio, 45.2; 95% confidence interval, 5.06–403). Exome analyses of this gene yielded another SNP [rs249038 (G/A)] in 6 of 10 patients and none of the control subjects ($p = .02$). This SNP causes a heterozygous missense mutation of glycine to serine predicted to be deleterious by the Protein Variation Effect Analyzer and was also very rare among participants in the 1000 Genomes Project ($p < .001$).
Conclusions: Two SNPs located near the *ZFYVE16* gene on chromosome 5 may have played a role in the early, multicompartment sacrocolpopexy failure experienced by our patients. (www.clinicaltrials.gov Identifier: NCT01614587). Journal of Minimally Invasive Gynecology (2016) 23, 726–730 © 2016 AAGL. All rights reserved.

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In the United States alone millions of women suffer from pelvic organ prolapse, the prevalence of which is expected to increase nearly 50% by 2050 [1]. The term "pelvic organ prolapse" refers to any descent of the anterior vaginal wall, the vaginal apex, the posterior vaginal wall, or any combination of these defects. The etiology is multifactorial, and risk factors include vaginal childbirth, frequent increases in intra-abdominal pressure, aging, and genetic predisposition to connective tissue abnormalities [2]. In terms of the genetic predisposition, several studies have reported variations in the expression of certain genes that could lead to the development of pelvic organ prolapse [3–6].

Sacrocolpopexy is considered by many to be the "gold standard" procedure for correction of prolapse involving

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the apex, with reported success rates between 80% and 100% depending on techniques and definitions of success used [7]. Originally, an open abdominal procedure designed primarily for the correction of posthysterectomy vaginal vault prolapse, the sacrocolpopexy is now often performed via the laparoscopic approach—with or without robotic assistance for virtually any variety of prolapse—whether or not the patient still has a uterus. Using our standardized robotic techniques, we have reported cure rates of 97% at 1 year, with our few failures typically occurring in the distal most anterior or posterior segments [8,9].

However, over the years a very small group of our patients experienced objective overt failures in the early postoperative period. These failures occurred within 6 months from the time of surgery and could not be explained by differing surgical techniques, poor compliance with postoperative restrictions, or complicated perioperative courses. The aim of this study was to determine whether a genetic basis may exist for the early overt surgical failures seen within this "extreme phenotype" group.

Methods

This was a case-control study approved on October 26, 2011 by the Institutional Review Board at the Atlantic Health System (R11-10-004), and all subjects gave written consent to participate after a detailed informed consent process. The study was posted on the website www.clinicaltrials.gov before enrollment of our first subject (Identifier: NCT01614587).

For the purposes of this study we defined "early overt failure" after robotic-assisted laparoscopic sacrocolpopexy to be development of stage III or IV prolapse based on the pelvic organ prolapse quantification system [2] occurring in more than 1 compartment within 6 months of surgery. This study was carried out within an academic community medical center referral practice, and all patients had surgery at 1 of 2 hospitals. Any combined surgical procedures included hysterectomy, salpingectomy, oophorectomy, or suburethral sling. Our clinical records were reviewed to find any patients who were found to have stage II or greater prolapse after undergoing our standardized robotic sacrocolpopexy with 1 of 2 attending surgeons between 2005 and 2013. The medical records of this group were reviewed to identify those patients who required downstream surgical or nonsurgical pelvic organ prolapse treatments. Resultant potential cases were then reviewed by the urogynecology attendings to select only those patients deemed true clinical outliers. By these methods we identified 10 patients (our cases) who experienced early overt surgical failure and thus made up our extreme phenotype group.

Our control subjects were randomly selected (by means of a random number generator) from our research database that included greater than 500 patients who underwent robotic-assisted laparoscopic sacrocolpopexy with polypropylene mesh during the same time period and had documented objective and subjective surgical success at $\geq$12 months. The definition of success included an absence of prolapse symptoms, pelvic organ prolapse quantification system stage 1 or better on examination, and no reoperation or treatment for pelvic organ prolapse at the 12-month mark. In addition to having documented surgical success, eligible control subjects had to have experienced uneventful perioperative courses. Because all of our patients were white, we decided to include only whites as control subjects as well. These control subjects were contacted via telephone 1 at a time regarding study participation. Those who verbally agreed were mailed buccal swab kits and consent forms. Each time a potential control patient declined enrollment, another was chosen at random from the database. This process was repeated until 40 control subjects were enrolled.

Demographics and perioperative details were retrospectively reviewed, collected, and compared between patients and control subjects. All patients and control subjects underwent our standardized technique for robotic-assisted laparoscopic sacrocolpopexy by 1 of 2 surgeons at a single center. The details of our surgical technique have been previously published [8,9]. Briefly, we perform extensive dissection in the vesicovaginal and rectovaginal spaces to the level of the trigone and perineum, respectively. We attach a preformed polypropylene "Y-mesh" to the full length and width of the anterior vaginal wall (down to the level of the trigone) and to the full length and width of the posterior vaginal wall (down to the level of the perineal body) using interrupted polytetrafluoroethylene sutures (Gore-Tex; WL Gore, Flagstaff, AZ). The mesh is then attached to the anterior longitudinal ligament of the sacrum using a permanent suture of the surgeon's choice.

DNA from the 10 patients and 40 control subjects was isolated from buccal swabs and genotyped on a single nucleotide polymorphism (SNP) array that contains probes for approximately 262,000 SNPs (NspI 250K SNP array; Affymetrix Inc., Santa Clara, CA). Statistical analyses were performed using a statistical software package commonly used in genetics research (SVS; GoldenHelix, Bozeman, MT). Genotype models tested (D minor allele, d major allele) were basic allele (D vs d), genotypic (DD vs dd vs Dd), additive (dd $\rightarrow$Dd $\rightarrow$ DD), dominant ((DD and Dd) vs dd), and recessive (DD vs (Dd and dd)). Association analysis and quality control filtering was performed using GoldenHelix SVS, and p values were adjusted for multiple testing using the false discovery rate to control for the expected proportion of incorrectly rejected null hypotheses ("false discoveries"). Baseline demographic and clinical descriptors for the patients and control subjects were compared using χ^2, Mann-Whitney U, and t tests, and principal component analysis testing for genotype stratification was performed to identify any patients as outliers.

Candidate genetic loci identified by the SNP array based genome-wide association analyses were further investigated by specifically evaluating the sequence around the SNP using whole exome sequence data from the same

sample set. Whole exome sequencing was performed using the Ion AmpliSeq Exome Solution (Thermo Fischer Scientific Inc., Waltham, MA) as recommended by the supplier with 2 samples per P1 chip. Exome sequences were mapped to the Human Genome Reference Sequence (GRCh37) using Bowtie2 version 2.1.0 [10] and processed with samtools version 0.1.19-44428cd [11]. Variants were compared between patients and control subjects and called with VarScan 2.3.5 [12]. Effects of variants on gene structure were estimated with snpEFF 3.5 [13], and the protein function effect analysis was estimated with the bioinformatic tool Protein Variation Effect Analyzer [14]. Variants with putative functional significance identified by next-generation sequencing were independently verified using TaqMan quantitative real-time polymerase chain reaction–based allelic discrimination, as recommended by the supplier (Thermo Fischer Scientific Inc.). To evaluate the robustness of our findings and to verify the rare nature of the SNPs in question, we further compared DNA from our cases to DNA from participants in the 1000 Genomes Project [15]. There are 379 whites within that database, so only that group was used as comparison DNA with our cases. All data used in our study were deposited in NCBI's Gene Expression Omnibus [16] and are accessible through GEO Series accession number GSE63236 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE63236).

Results

All surgical procedures for patients and control subjects were uncomplicated, and perioperative courses were unremarkable. No patient or control subject required conversion from laparoscopy to laparotomy, and there were no perioperative complications in either group. The mean estimated blood loss for patients and control subjects was 52.5 ± 53.3 mL and 55.5 ± 50.1 mL, respectively (p = .87). Average operative time for patients and control subjects were 167 ± 27.9 and 151 ± 30.7 minutes, respectively (p = .15). All 50 patients were discharged home the day after surgery with no readmissions. There were no baseline demographic or clinical differences between the patients and control subjects (Table 1), and principal component analysis testing did not identify any patients as outliers. Time to failure for the group of patients was between 3 and 6 months, with an average of 4.6 months. Initial analysis via SNP array yielded a SNP (dbSNP ID rs171821) located near the *ZFYVE16* gene (also known as *Endofin*) on chromosome 5 that was associated with the group of patients but not the control subjects, and correlation/trend testing on the basic allele model yielded a false discovery rate–adjusted p value of .046 (odds ratio, 45.2; 95% confidence interval, 5.06–403). In addition, we found 3 other SNPs within the *ZFYVE16* gene near dbSNP ID rs171821 with raw p < .001, but after false discovery rate adjustment these 3 SNPs were not significantly associated. These 3 SNPs were dbSNP IDs rs259043, rs1423113, and rs16877757, and adjusted p values were .078, .701, and .351, respectively. These findings prompted us to perform an exome analysis in the region of the *ZFYVE16* gene. A Manhattan plot illustrates the genome wide association significance (Fig).

This exome analysis yielded the SNP dbSNP ID rs249038 (G/A), which was present in 6 of 10 patients and none of our control subjects (Fisher exact 2-tailed p = .02). This SNP is rare in European populations and is a heterozygous (G/A) missense mutation that results in formation of serine rather than glycine by the *Endofin* gene. The remaining patients and all control subjects expressed the expected homozygous (G/G) pattern. Genotypes for this locus were confirmed with 100% concordance using TaqMan allelic discrimination. A significant difference was recognized when data from the 1000 Genomes Project was used to compare our patients with 379 whites (p < .001) [15]. This change from glycine to serine was predicted to be deleterious by the bioinformatic tool Protein Variation Effect Analyzer [14].

Table 1

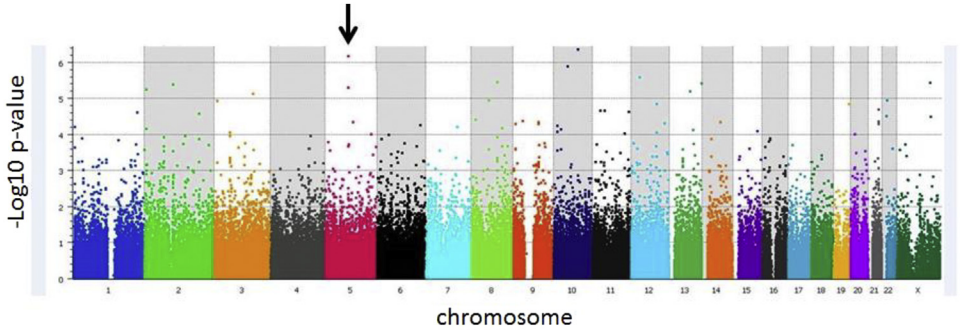
Characteristics and demographics of the study population

Characteristic	Patients (n = 10)	Control subjects (n = 40)	p value
Mean age, yr (± standard deviation)	58.5 ± 5.5	59.9 ± 8.2	.61
Mean body mass index, (± standard deviation)	26.6 ± 3.1	24.9 ± 4.4	.26
Median parity (range)	2.5 (1–4)	2.0 (1–4)	.76
Tobacco use	1 (10)	2 (5)	.50
Menopausal	8 (80)	30 (75)	.39
Hormone replacement therapy	1 (10)	4 (10)	.47
Prior hysterectomy	2 (20)	8 (20)	.48
Prior pelvic organ prolapse surgery	2 (20)	4 (10)	.22
Pelvic organ prolapse quantification system leading edge preoperatively, cm, median (range)	3.5 (0–10)	1.75 (–1 to 10)	.18
Total	10	40	

Values are number of incidences with percents in parentheses, unless otherwise indicated.

Fig

Manhattan plot illustrating the genome-wide association significance with pelvic floor prolapse treatment response.



Discussion

Surgical procedures for pelvic organ prolapse are quite common. In the United States alone approximately 300,000 women undergo surgery for this condition yearly, and unfortunately up to 30% of these women will require a repeat operation [1,17]. Although many investigators have attempted to find specific genetic variations that might cause pelvic organ prolapse, very little evidence of such a relationship has been found. In fact, a recent systematic review and meta-analysis described only "moderate" epidemiologic credibility for the variation of *COL1A1* with the development of prolapse. That study also stressed the need for exploration of further variants to not only help explain the complex pathophysiology of prolapse but to also provide methods of prevention and treatment [18].

Our findings indicate that a candidate gene on chromosome 5, *ZFYVE16* (aka *Endofin*), may be linked to early recurrence of prolapse. Although our initial analyses identified 4 candidate SNPs near *Endofin*, only 1 of these was found to be statistically unique to our group of cases. Upon exome sequence analysis of this region, the variant known as dbSNP ID rs249038 (G/A) was identified and determined to cause a rare missense mutation predicted to be deleterious. Although the other 3 SNPs we found did not hold up against false discovery rate control, it is possible that they do play some role in prolapse surgical failures of other varieties, such as failure after native tissue repair.

The locations of the SNPs we found on or near the *Endofin* gene support the importance of our findings because *Endofin* facilitates transforming growth factor-β (TGF-β) as a scaffold protein. TGF-β plays an important role in growth and development, inflammation and repair, and host immunity, because it controls fibroblast proliferation and cellular differentiation and promotes collagen synthesis by increasing the extracellular matrix production. TGF-β levels are relatively high within tissue undergoing wound healing and remodeling [19]. Alterations in TGF-β are associated with connective tissue disorders such as Marfan syndrome and Loey-Ditz syndrome [20]. Furthermore, women with these connective tissue disorders have relatively high rates of urinary incontinence and pelvic organ prolapse [21]. Qi et al [22] demonstrated among a group of women with prolapse that the expression of TGF- β1 protein was significantly lower than that of a control group without prolapse.

Strengths of our study include the use of a standardized surgical technique for all patients and control subjects by 2 surgeons at a single center. Both surgeons were beyond the robotic learning curve at the study outset. Another strength was the systematic approach we used while identifying our patients. Every effort was made to collect only true clinical outliers as our patients, thus holding to the concept of extreme phenotype analyses [23]. Although our patients on average had a higher leading edge of prolapse initially, this was not found to be statistically significant and did not lead to a different prolapse stage between groups. Furthermore, our control subjects were properly selected at random in a 4:1 ratio from a group of similar patients with known surgical success during the same period of time.

The primary limitation of our study is the small sample size that resulted in odds ratios that were quite large. Because it is a case-control study, we cannot assume the existence of a cause-and-effect relationship. We also did not have a comprehensive family history for the patients that, if present, could have provided relevant clinical characteristics for this type of study. Examples of such history include hernias, pelvic organ prolapse, or other weak connective tissue disorders.

Our use of a SNP array could be seen as a limitation to map disease loci and determine disease susceptibility genes in individuals. A more detailed analysis (i.e., a complete exome analysis) would undoubtedly enhance our ability to explore the concept of a genetic cause for pelvic organ prolapse surgery failure. We are in the process of performing such a study in a prospective fashion. The fact that we only know the surgical results of our control group out to 12 months could be seen as a limitation; however, our prior research indicated that most failures after sacrocolpopexy happen within 12 months [24]. Our work may be criticized

because dbSNP ID rs171821 found on initial analysis, via SNP array, only yielded p = .046. A recent publication pointed out that to keep the false discovery rate below 5%, p ≤ .001 must be used; otherwise, there is a 29% chance (at least) of reporting a false positive [25]. Our reported p value is a false discovery rate–adjusted p value, and this prompted us to continue searching in the region of the gene identified, which was done with an exome analysis. This resulted in finding dbSNP ID rs249038 (G/A) in 6 of our 10 patients with a p value of .02. We also verified genotypes for this locus, which were confirmed with 100% concordance, using TaqMan allelic discrimination. With that being said, the rs249038 (G/A) polymorphism of the *Endofin* gene is possibly associated with early surgical failure of pelvic organ prolapse in white women. The issue deserves attention, and larger studies need to be performed to confirm these findings.

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