ORIGINAL ARTICLE: GENETICS

Follow-up to genome-wide linkage and admixture mapping studies implicates components of the extracellular matrix in susceptibility to and size of uterine fibroids

Brahim Aissani, Ph.D.,[a] Kui Zhang, Ph.D.,[b] and Howard Wiener, Ph.D.[a]

[a] Department of Epidemiology and [b] Department of Biostatistics, University of Alabama at Birmingham, Birmingham, Alabama

Objective: To conduct a follow-up association mapping to independent genome-wide linkage and admixture mapping studies of uterine leiomyoma.
Design: Case-control, cross-sectional study.
Setting: Not applicable.
Patient(s): A total of 1,045 premenopausal North American participants in the National Institute of Environmental Health Sciences Uterine Fibroid Study.
Intervention(s): None.
Main Outcome Measure(s): We genotyped 2,772 single-nucleotide polymorphisms from candidate genes located in peaks of linkage (2q37, 3p21, 5p13, 10p11, 11p15, 12q14, and 17q25) or admixture linkage disequilibrium (2q37, 4p16.1, and 10q26) mapping and reported to have regulated expression in uterine fibroids.
Result(s): We report significant associations of variant members of the collagen gene family with risk and tumor size, including missense variants in *COL6A3* and *COL13A*, with replications in African American and European American study groups. Furthermore, the cell-matrix Rho GTPase-encoding *ARHGAP26* gene, and *MAN1C1*, a gene encoding a Golgi mannosidase involved in the maturation of procollagens, emerged as new candidate uterine leiomyoma genes affecting both risk and tumor size.
Conclusion(s): Our data converge onto a possible model of uterine leiomyoma pathogenesis resulting from altered regulation, maintenance, and/or renewal of the extracellular matrix. (Fertil Steril® 2014;■:■–■. ©2014 by American Society for Reproductive Medicine.)
Key Words: Polymorphism, fibroids, collagen, susceptibility, extracellular matrix, NIEHS cohort

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Uterine leiomyomas (ULs) are benign neoplasms arising from the smooth muscle cells of the uterus. These tumors are believed to develop in the majority of American women by the time they reach menopause, and become symptomatic in about 25% (1). Despite their benign nature, ULs are responsible for significant gynecologic morbidities, including excessive bleeding, pelvic pain, urinary incontinence, infertility, and pregnancy complications (2, 3). As a consequence of this morbidity, uterine fibroids are the primary indication for hysterectomy, accounting for over 600,000 hysterectomies annually in the United States (4). Cumulative exposure to estrogen is believed to be a major etiologic factor (5), and factors that may influence the hormonal milieu,

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Reprint requests: Brahim Aissani, Ph.D., University of Alabama at Birmingham, School of Public Health, 1665 University Blvd., Birmingham, Alabama 35294-0022 (E-mail: baissani@uab.edu).

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such as obesity, are also believed to be associated with the risk (6). However, the clearly established risk factors are age (increasing risk with increasing premenopausal age), menopause (risk decreases with menopause), and African American ethnicity (higher risk compared with that of non-Hispanic whites) (7).

No candidate susceptibility genes have consistently emerged from the few linkage scans and genome-wide association studies reported so far (8–11). Exome sequencing in 18 UL tumors and matched normal myometria has implicated the mediator complex subunit 12 (*MED12*) gene (12). Further examinations of exomes and gene-expression profiling in a set of 38 ULs and matched myometria led to the hypothesis that a chromothripsis-like event drives the pathogenesis of ULs, leading to translocations of the *HMGA2* and *RAD51B* loci and to other chromosomal aberrations, including the *COL4A5-COL4A6* locus (13).

In contrast to these somatic mutations, germline mutations affecting highly conserved amino acids have been reported for the fumarate hydratase–encoding gene (*FH*) in band q43 on chromosome 1, but only in rare cases of nonsyndromic ULs (14, 15). Furthermore, a genome-wide linkage scan in the "affected sister study" (8), and fine association mapping across the *FH*-linked region in the National Institute of Environmental Health Sciences (NIEHS) cohort (16), suggested the presence of a candidate 1q43 gene affecting the risk and size of ULs. More recently, epidemiologic studies of ULs in American women enrolled in the Right from the Start cohort, and the BioVU DNA repository, replicated the results of a genome-wide association study in a Japanese population for 2 of the 3 most significantly associated genes encoding BET1L (blocked early in transport 1 homolog) and TNRC6B (trinucleotide repeat containing 6B) (17, 18).

We conducted an independent follow-up fine mapping of extended candidate regions that were identified in a genome-wide linkage scan in the affected sister study (11) and through mapping by admixture linkage disequilibrium (MALD) in the Black Women's Health Study (10). We report data from both the African American (AA) and European American (EA) study groups showing association of UL outcomes with variant members of the collagen gene family encoding components of the extracellular matrix suspected in fibrosis (19).

MATERIALS AND METHODS

Study Population

Detailed characteristics of the study population have been reported elsewhere (20); only those relevant to the present study are described here. A random sample of women, aged 35 to 51 years, was selected from a computerized list of members of a prepaid urban health plan for enrollment in the NIEHS Uterine Fibroid Study (NIEHS-UFS) (7). Of the enrolled women who were premenopausal and had a diagnosis of UL (n = 1,119), 1,045 (93%) had available DNA specimens and were self-identified as AA (n = 574), non-Hispanic EA (n = 394), and Other (0; n = 77). The NIEHS-UFS and the present substudy were approved by the human subjects review boards at the NIEHS, George Washington University, and the University of Alabama at Birmingham, respectively. Participants gave written informed consent in accordance with the requirements of these review boards.

Covariates and Ascertainment

The covariates included age, age at menarche, parity, number of pregnancies after age 25 years (giving birth at younger ages was not significantly related to fibroid development in the NIEHS-UFS [21, 22]), body mass index, and physical activity. Fibroid status was assessed by ultrasound screening at baseline or by medical record review in 80% and 90% of the AA and EA participants, respectively. For women who had a pelvic ultrasound examination recently through the health plan, radiology records from that examination were used to assess fibroid status. The remaining premenopausal participants were asked to have a pelvic ultrasound examination at the primary care site. Women for whom neither ultrasound nor medical record review could be conducted were excluded from the analyses.

Both a transabdominal and a transvaginal ultrasound examination were performed. The abdominal portion evaluated fibroid change arising from the upper uterus that would not be seen readily with the transvaginal approach alone. Tumor size was classified into 3 categories, according to tumor diameter (small: ≤ 2 cm; medium: >2–<4 cm; large: ≥ 4 cm). For participants diagnosed with multiple tumors, the largest tumor determined the size category.

Target Positional Candidate Regions and Genes

Genes identified as being in extended peaks of linkage (2q37, 3p21, 5p13, 10p11, 11p15, 12q14, and 17q25) with ULs, in the affected sister study (11), and of admixture linkage disequilibrium (2q37, 4p16.1, 10q26), in the Black Women's Health Study (10), were selected on the basis of regulated expression (down- or up-regulated) in ULs. The criterion for inclusion was a differential expression ($P<.01$) in 5 pairs of fibroids and matched myometria in at least 2 independent studies reported in the GeoProfile database (National Center for Biotechnology Information). Overall, this strategy identified 93 positional, and 4 nonpositional, candidate genes. In addition, we included 3 positional genes (*CYP27B1, PRLHR*, and *HMGA2*) that were not reported in the Geoprofile database as being strongly regulated in ULs but are considered strong candidates.

Single-Nucleotide Polymorphism Selection and Typing

Within the selected candidate genes, tagging single-nucleotide polymorphisms (tag SNPs) were selected from the International HapMap Project (HapMap) reference populations (www.hapmap.org), as described elsewhere (23). Because the participants in the NIEHS-UFS are predominantly of AA or EA descent, we downloaded SNP genotype data from the reference HapMap II and III populations relevant to these ethnic backgrounds and used the NIEHS TAGster (24) to select tag SNPs, as described elsewhere (16). We selected 537 tag SNPs for the top 15 candidate positional genes, and 652 SNPs for the remaining 85 positional candidates. The top 15