Published in final edited form as:
Fertil Steril. 2011 August ; 96(2): 512–515. doi:10.1016/j.fertnstert.2011.06.001.

SNPs in the Lysyl Oxidase-like Protein 4 (LOXL4) and Complement Component 3 (C3) genes are Associated with Increased Risk for Endometriosis and Endometriosis-Associated Infertility

Lynnette A. Ruiz, PhD[1], **Julie Dutil, PhD**[2], **Abigail Ruiz, BS**[1], **Jessica Fourquet, MPH**[1], **Sonia Abac, RN**[1], **Joaquín Laboy, MD**[3], and **Idhaliz Flores, PhD**[1]

[1] Department of Microbiology, Ponce School of Medicine and Health Sciences, Ponce, PR

[2] Department of Biochemistry, Ponce School of Medicine and Health Sciences, Ponce, PR

[3] Department of Ob-Gyn, Ponce School of Medicine and Health Sciences, Ponce, PR

Abstract

This study was conducted to assess genetic associations to endometriosis in a Puerto Rican population. Significant differences in allelic frequencies and genotype distribution of genetic variants in *LOXL4* and *C3* were documented in patients with endometriosis-associated infertility vs controls, and in patients with endometriosis vs controls, respectively, and in women having the risk genotype at both SNPs, the estimated risk for endometriosis nearly doubled.

Keywords

endometriosis/genetics; single nucleotide polymorphisms (SNPs); lysyl oxidase-like 4; complement factor 3; *LOXL4*; *C3*; infertility/genetics; genetic variants

Endometriosis is characterized by chronic painful symptoms and infertility (1-4). There is strong evidence suggesting that endometriosis is a multifactorial disease resulting from interactions of multiple susceptibility genes and environmental factors (5-12). Following recent reports of genetic associations to endometriosis, there has been a surge in the interest to uncover the role of genetic variants in this disease (13, 14). Significant associations to endometriosis have been documented in dozens of genes thus far (15, 16) but for most of these genes association was highly dependent on ethnicity (17). None of these studies have been conducted in a Hispanic population.

We have previously identified genes that were upregulated in human and experimental endometriosis, including lysyl oxidases (LOXs) and complement factor 3 *(C3)* (18, 19). LOXs are a family of copper-dependent amine oxidases that are involved in collagen and elastin crosslinking at the extracellular matrix (20), and play roles in inflammation, cell migration and invasion (21). *LOX* and *LOXL1* expression is upregulated in endometriotic lesions (18, 19) and *LOXL1* is downregulated during secretory phase in endometrium from

healthy women (22). Interestingly, *LOXL4* gene is located near regions shown to be linked to endometriosis (13, 23, 24). The important roles of LOXs in extracellular matrix stability make these proteins potential candidates to be studied in the context of infertility (25, 26)

C3 is also dysregulated in women and animal models of endometriosis (19, 27, 28). Infertile patients with endometriosis expressed high C3 levels in the peritoneal fluid (29). In normal endometrium, high expression of *C3* was observed during the secretory phases of the menstrual cycle (30). Moreover, *C3* expression is upregulated in endometrium of baboons treated with hCG compared to controls, and C3 levels in eutopic endometrium predict pregnancy success, which indicates that regulation in *C3* expression is critical for implantation (31-33). Since *C3* is marker of chronic inflammation, a hallmark of endometriosis, we hypothesized that SNPs in *C3* could play a role in the pathogenesis of endometriosis and infertility (34).

Study subjects were recruited by the Endometriosis Research Program at PSMHS. Patients were premenopausal women with surgically-confirmed endometriosis. Controls were women without endometriosis or known infertility who had surgery for benign gynecologic conditions. Documentation of the revised American Fertility Society criteria severity (rAFS) was obtained during surgery, and demographic, gynecologic, obstetric and clinical data were collected (35). Subjects (n=384) were categorized as follows: 1) Control group: subjects without endometriosis or infertility (n=147); 2) Endometriosis group: subjects with endometriosis, with or without infertility (n=214; 3) Endo-only: subjects with endometriosis without known infertility problems (n=72); 4) Endo-associated infertility: subjects with endometriosis-associated infertility (n=73). Women were considered “infertile” if they answered positively to “Have you experienced problems getting pregnant?”. Most infertile women were nulliparous (73.4%), although some have had children (n=26). These were surveyed for the specific cause of their perceived infertility. The majority of them (19/26) reported suffering from either miscarriages and/or a history of infertility (average: 4.9 yrs; range: 1-10yrs). One reported being infertile due to endometriosis and six did not report a specific reason or problem. All participants read and signed an informed consent form prior to enrollment. Protocols were approved by the PSMHS IRB Committee.

Isolation of genomic DNA from lymphocytes was conducted using standard procedures (QIAamp DNA Blood kit, Qiagen, Valencia, CA), genotyping was conducted on 12 SNPs in *C3* and *LOXL4*, on a custom-made Golden Gate chip from Illumina, Inc. (San Diego, CA) as previously described (36). Genotyping was conducted at the Southern California Genotyping Consortium, UCLA, CA.

All statistical analyses were performed using SYSTAT 13.0 (Cranes Software Int. Ltd.). Demographic, gynecologic and clinical parameters are expressed as mean ± standard errors for quantitative variables or as percentages for frequency data. Differences in clinical characteristics between groups were compared using chi-square (χ^2) for frequencies or Student's t-test for quantitative variables. SNP genotypes of controls were assessed for departures from Hardy-Weinberg equilibrium (HWE) using χ^2 test. Associations between SNP and risk of endometriosis and/or endometriosis-associated infertility were performed by χ^2 test. Odds ratios (OR) and 95% confidence interval (CI) were calculated using a logistic regression model adjusted for confounding factors.

Gene-gene interaction was modeled using the multifactor dimensionality reduction (MDR) approach. MDR is a non-parametric genetic model-free approach for detecting epistatic interactions between SNPs that combines a cross-validation and permutation procedure to assess the significance of the detected interactions. Data were first divided into a training set (9/10 of the data) and a testing set (1/10) (10-fold cross-validation). Performance was

estimated by cross-validation consistency and model's prediction accuracy. Subsequently, permutation analysis was performed in which case-control status was randomized 1000 times to assess significance of the modeled interaction.

Group characteristics, allelic frequencies and genotype distribution of significant SNPs associated to endometriosis or endometriosis-associated infertility are shown in Table 1. The prevalence of symptoms such as pain and infertility was significantly higher in patients than controls (37). Thirty percent of patients with endometriosis reported having fertility problems, further demonstrating that infertility commonly coexists with endometriosis (2, 38). Several studies suggest a possible link between endometriosis and miscarriages, but there is insufficient evidence for this association (39, 40). In our population, 40% of patients with endometriosis reported having at least one miscarriage, which was significantly different from controls. These data add to the current state of knowledge in this controversial issue by providing support for a possible link between pregnancy loss and endometriosis in a Hispanic population.

Genotypes of 12 SNPs were tested for associations using allelic, recessive, dominant and additive models. Genotype distributions were in HWE in the control group for all SNPs tested. The GG genotype at rs737657 in *LOXL4* was more frequent in patients compared to controls, indicating an increased risk for endometriosis in individuals homozygous for the G allele (OR=1.68; 95%CI: 1.049-2.700; p=0.031). This risk increased when patients with endometriosis-associated infertility were compared to controls (OR=2.34; 95%CI: 1.273-4.286; p=0.006). Also, significant differences were observed in the frequency of the AA genotype at rs17524355 in *LOXL4* when patients with endometriosis-associated infertility were compared to controls (OR=5.23; 95%CI:1.434-19.072; p=0.012). In conclusion, these two polymorphisms in the *LOXL4* gene (GG genotype at rs737657 and AA genotype at rs17524355) are associated with higher risk for infertility in the context of endometriosis. SNP rs737657 was also shown in this study to be associated with a diagnosis of endometriosis, suggesting that some of the risk factors or molecular mechanisms underlying endometriosis and infertility may be linked. Because our population was recruited for an endometriosis case-control study, and we did not focus on recruiting infertility cases, our study design would not allow distinguishing between infertility alone and infertility in the context of endometriosis. Further studies would be needed to assess whether the observed associations remain significant in patients suffering from infertility only.

Interestingly, the *LOXL4* gene is mapped to 10q24.2, close to two regions of interest in endometriosis: 10q23.3 which had significant linkage to endometriosis in families from Puerto Rico, and 10q26 identified by the Endogene study of 1,176 families as associated with endometriosis (13, 23). Noteworthy, in a recent report, linkage analysis on families grouped by fertility status not only validated the previously observed linkage peak at 10q26 but also detected association of endometriosis to a SNP in that genetic region (24). Our study, thus, confirms that there is a region of interest in chromosome 10 that is associated with endometriosis-associated infertility and, moreover, independently identified a genetic polymorphism in this region associated to increased risk for this disease.

These studies also showed that the frequency of the GG genotype at rs2241392 in *C3* was significantly different between patients with endometriosis and controls (OR=0.369; 95%CI: 0.144-0.942) indicating a decreased risk for endometriosis associated with this genotype in this population. Significance for this particular SNP was lost when all endometriosis patients (including those with infertility) or when patients with endometriosis-associated infertility were compared to controls; therefore, we speculate that association is driven by the endometriosis phenotype and not by infertility. It is known that C3 levels are dysregulated in

patients with the condition and aberrant expression of this inflammatory molecule could explain some of the symptoms of endometriosis.

In order to determine if more than one SNP can interact to increase risk, we compared prevalence of endometriosis in the study population grouped by the number of risk genotypes at *LOXL4* and/or *C3* loci. The prevalence of endometriosis was of 44.7% in women carrying neither of the risk genotype at the *C3* and *LOXL4* loci. In a group of individuals bearing a risk genotype at either locus, *C3* or *LOXL4*, the prevalence of endometriosis increased to 52.7%, (OR=1.361; 95%CI:0.674-2.748; p=0.39) in comparison to women not carrying any risk genotype. Interestingly, in the group of women having the risk genotype at both SNPs, the prevalence of endometriosis reached 66.1% and the risk for endometriosis nearly doubled (OR=2.247; 95%CI:1.106-4.785; p=0.0362). These results suggest that, in combination, SNPs in different candidate genes may improve the probability of correctly predicting genetic susceptibility to endometriosis. More studies are necessary to identify a possible functional effect of these SNPs in gene expression or protein levels/ activity in particular during the window of implantation; alternatively, these SNPs may be in linkage disequilibrium with other SNP(s) that would prove to be causative of this disease.

Some of the p values we report here did not reach statistical significance after applying Bonferroni correction. Also, the SNPs analyzed did not cover all of the potential LD blocks in the genes. We also performed a multifactor dimensionality reduction (MDR) analysis to search for possible SNP-SNP interactions between rs737657 and rs2241392. The testing accuracy was 0.5134 with a cross-validation consistency of 10/10. However, when submitted to a permutation analysis, this interaction did not reach statistical significance (p=0.5). For those reasons, it would be important to validate these findings in a larger sample size and in other populations. In addition, future studies should incorporate a tag-SNP strategy to provide a better coverage of the whole genes.

Our results suggest that polymorphisms in *LOXL4* and *C3* can be associated with risk for endometriosis and for infertility in the context of endometriosis. To our knowledge, this is the first report of a genetic association between SNPs in these genes and risk of endometriosis or endometriosis-associated infertility, and between any SNP and these two diagnoses in a Hispanic population. Since the prevalence of SNPs may vary among ethnic groups, it is important to ascertain effects due to ethnic differences and validate these findings in other populations (41, 42).

Acknowledgments

NIH-NICHD R01 HD050559; NIH-MBRS S06 GM08239; NIH-NICHD R01 HD050559 S1A1

References

1. Bulun SE. Endometriosis. N Engl J Med. 2009; 360:268–79. [PubMed: 19144942]
2. Paris K, Aris A. Endometriosis-associated infertility: a decade's trend study of women from the Estrie Region of Quebec, Canada. Gynecol Endocrinol. 2010
3. Vercellini P, Fedele L, Aimi G, Pietropaolo G, Consonni D, Crosignani PG. Association between endometriosis stage, lesion type, patient characteristics and severity of pelvic pain symptoms: a multivariate analysis of over 1000 patients. Hum Reprod. 2007; 22:266–71. [PubMed: 16936305]
4. Giudice LC, Kao LC. Endometriosis. Lancet. 2004; 364:1789–99. [PubMed: 15541453]
5. Montgomery GW, Nyholt DR, Zhao ZZ, Treloar SA, Painter JN, Missmer SA, et al. The search for genes contributing to endometriosis risk. Hum Reprod Update. 2008; 14:447–57. [PubMed: 18535005]
6. Child TJ, Tan SL. Endometriosis: aetiology, pathogenesis and treatment. Drugs. 2001; 61:1735–50. [PubMed: 11693463]

7. Seli E, Arici A. Endometriosis: interaction of immune and endocrine systems. Semin Reprod Med. 2003; 21:135–44. [PubMed: 12917783]
8. Tempfer CB, Simoni M, Destenaves B, Fauser BC. Functional genetic polymorphisms and female reproductive disorders: part II--endometriosis. Hum Reprod Update. 2009; 15:97–118. [PubMed: 18805939]
9. Guo SW. Epigenetics of endometriosis. Mol Hum Reprod. 2009; 15:587–607. [PubMed: 19651637]
10. Vigano P, Somigliana E, Vignali M, Busacca M, Blasio AM. Genetics of endometriosis: current status and prospects. Front Biosci. 2007; 12:3247–55. [PubMed: 17485295]
11. Kennedy S. Genetics of endometriosis: a review of the positional cloning approaches. Semin Reprod Med. 2003; 21:111–8. [PubMed: 12917780]
12. Simpson JL. Where are the genes that cause endometriosis? J Soc Gynecol Investig. 2005; 12:143–4.
13. Treloar SA, Wicks J, Nyholt DR, Montgomery GW, Bahlo M, Smith V, et al. Genomewide linkage study in 1,176 affected sister pair families identifies a significant susceptibility locus for endometriosis on chromosome 10q26. Am J Hum Genet. 2005; 77:365–76. [PubMed: 16080113]
14. Painter JN, Anderson CA, Nyholt DR, Macgregor S, Lin J, Lee SH, et al. Genome-wide association study identifies a locus at 7p15.2 associated with endometriosis. Nat Genet. 2011; 43:51–4. [PubMed: 21151130]
15. Falconer H, D'Hooghe T, Fried G. Endometriosis and genetic polymorphisms. Obstet Gynecol Surv. 2007; 62:616–28. [PubMed: 17705887]
16. Guo SW. Glutathione S-transferases M1/T1 gene polymorphisms and endometriosis: a meta-analysis of genetic association studies. Mol Hum Reprod. 2005; 11:729–43. [PubMed: 16291859]
17. Garcia-Martin E. Interethnic and intraethnic variability of NAT2 single nucleotide polymorphisms. Curr Drug Metab. 2008; 9:487–97. [PubMed: 18680468]
18. Flores I, Rivera E, Mousses S, Chen Y, Rozenblum E. Identification of molecular markers for endometriosis in blood lymphocytes by using deoxyribonucleic acid microarrays. Fertil Steril. 2006; 85:1676–83. [PubMed: 16759924]
19. Flores I, Rivera E, Ruiz LA, Santiago OI, Vernon MW, Appleyard CB. Molecular profiling of experimental endometriosis identified gene expression patterns in common with human disease. Fertil Steril. 2007; 87:1180–99. [PubMed: 17478174]
20. Molnar J, Fong KS, He QP, Hayashi K, Kim Y, Fong SF, et al. Structural and functional diversity of lysyl oxidase and the LOX-like proteins. Biochim Biophys Acta. 2003; 1647:220–4. [PubMed: 12686136]
21. Csiszar K. Lysyl oxidases: a novel multifunctional amine oxidase family. Prog Nucleic Acid Res Mol Biol. 2001; 70:1–32. [PubMed: 11642359]
22. Talbi S, Hamilton AE, Vo KC, Tulac S, Overgaard MT, Dosiou C, et al. Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. Endocrinology. 2006; 147:1097–121. [PubMed: 16306079]
23. Ledet EM, Flores I, Bailey-Wilson JE, Mandal DM. Genetic Analysis of Hereditary Endometriosis Families in Puerto Rico. Genetic Epidemiology. 2008; 32:702.
24. Painter JN, Nyholt DR, Morris A, Zhao ZZ, Henders AK, Lambert A, et al. High-density fine-mapping of a chromosome 10q26 linkage peak suggests association between endometriosis and variants close to CYP2C19. Fertil Steril. 2011
25. Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, et al. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell. 2009; 139:891–906. [PubMed: 19931152]
26. Ng MR, Brugge JS. A stiff blow from the stroma: collagen crosslinking drives tumor progression. Cancer Cell. 2009; 16:455–7. [PubMed: 19962663]
27. Tao XJ, Sayegh RA, Isaacson KB. Increased expression of complement component 3 in human ectopic endometrium compared with the matched eutopic endometrium. Fertil Steril. 1997; 68:460–7. [PubMed: 9314915]
28. Sherwin JR, Hastings JM, Jackson KS, Mavrogianis PA, Sharkey AM, Fazleabas AT. The endometrial response to chorionic gonadotropin is blunted in a baboon model of endometriosis. Endocrinology. 2010; 151:4982–93. [PubMed: 20668030]

29. Kabut J, Kondera-Anasz Z, Sikora J, Mielczarek-Palacz A. Levels of complement components iC3b, C3c, C4, and SC5b-9 in peritoneal fluid and serum of infertile women with endometriosis. Fertil Steril. 2007; 88:1298–303. [PubMed: 17482181]
30. Sayegh RA, Tao XJ, Awwad JT, Isaacson KB. Localization of the expression of complement component 3 in the human endometrium by in situ hybridization. J Clin Endocrinol Metab. 1996; 81:1641–9. [PubMed: 8636381]
31. Sherwin JR, Sharkey AM, Cameo P, Mavrogianis PM, Catalano RD, Edassery S, et al. Identification of novel genes regulated by chorionic gonadotropin in baboon endometrium during the window of implantation. Endocrinology. 2007; 148:618–26. [PubMed: 17110430]
32. Isaacson KB, Galman M, Coutifaris C, Lyttle CR. Endometrial synthesis and secretion of complement component-3 by patients with and without endometriosis. Fertil Steril. 1990; 53:836–41. [PubMed: 2332059]
33. Bartosik D, Damjanov I, Viscarello RR, Riley JA. Immunoproteins in the endometrium: clinical correlates of the presence of complement fractions C3 and C4. Am J Obstet Gynecol. 1987; 156:11–5. [PubMed: 3541614]
34. Barrington R, Zhang M, Fischer M, Carroll MC. The role of complement in inflammation and adaptive immunity. Immunol Rev. 2001; 180:5–15. [PubMed: 11414363]
35. Revised American Fertility Society classification of endometriosis: 1985. Fertil Steril. 1985; 43:351–2. [PubMed: 3979573]
36. Sellers TA, Huang Y, Cunningham J, Goode EL, Sutphen R, Vierkant RA, et al. Association of single nucleotide polymorphisms in glycosylation genes with risk of epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2008; 17:397–404. [PubMed: 18268124]
37. Sinaii N, Plumb K, Cotton L, Lambert A, Kennedy S, Zondervan K, et al. Differences in characteristics among 1,000 women with endometriosis based on extent of disease. Fertil Steril. 2008; 89:538–45. [PubMed: 17498711]
38. Holoch KJ, Lessey BA. Endometriosis and infertility. Clin Obstet Gynecol. 2010; 53:429–38. [PubMed: 20436320]
39. Vercammen EE, D'Hooghe TM. Endometriosis and recurrent pregnancy loss. Semin Reprod Med. 2000; 18:363–8. [PubMed: 11355795]
40. Tomassetti C, Meuleman C, Pexsters A, Mihalyi A, Kyama C, Simsa P, et al. Endometriosis, recurrent miscarriage and implantation failure: is there an immunological link? Reprod Biomed Online. 2006; 13:58–64. [PubMed: 16820110]
41. Hinds DA, Stuve LL, Nilsen GB, Halperin E, Eskin E, Ballinger DG, et al. Whole-genome patterns of common DNA variation in three human populations. Science. 2005; 307:1072–9. [PubMed: 15718463]
42. Spielman RS, Bastone LA, Burdick JT, Morley M, Ewens WJ, Cheung VG. Common genetic variants account for differences in gene expression among ethnic groups. Nat Genet. 2007; 39:226–31. [PubMed: 17206142]

Table 1

A. Characteristics of patients with endometriosis (Endo) versus women without endometriosis (Controls). B. Allelic frequencies and genotype distribution of significant SNPs associated to endometriosis or endometriosis-associated infertility.

A. Characteristics of patients with endometriosis and controls

Characteristics	Endo (n=214)	Controls (n=147)	*P* value
Age (years)	31.9 ± 0.5	37.8 ± 0.7	<0.001[1]
Menstrual cycle characteristics			
Age at menarche	11.9 ± 0.1	12.1 ± 0.1	0.198[1]
Regular cycles %	73.4	78.8	0.242[2]
Days cycle length	36.4 ± 3.7	30.2 ± 1.0	0.180[1]
Days menstrual flow length	6.6 ± 0.4	5.8 ± 0.2	0.164[1]
Obstetric history			
Tried to be pregnant, %	75.1	87.0	0.007[2]
Gestation, average	1.7 ± 0.1	3.0 ± 0.1	<0.001[1]
Miscarriages, average	1.2 ± 0.7	0.4 ± 0.1	0.241[1]
Miscarriages, %	40.3	24.8	0.008[2]
Age at 1st child birth	24.0 ± 1.1	21.1 ± 0.4	0.005[1]
Endometriosis-related symptoms			
Dysmenorrhea, %	84.6	64.1	<0.001[2]
Incapacitating pain, %	68.9	31.3	<0.001[2]
Dyspareunia, %	56.3	32.1	<0.001[2]
Infertility, %	34.1	-	n/a
Endometriosis Family History %	46.3	2.0	<0.001[2]

B. Allelic frequencies and genotype distribution of significant SNPs associated to endometriosis and/or endometriosis-associated infertility.

	MAF Count (%)	Genotype Count (%)			OR*	95%CI	*P* value
rs737657 (LOXL4)	A	AA	AG	GG	*Model GG vs AA+AG*		
Controls	124 (42.5)	24 (16.4)	76 (52.1)	46 (31.5)	Ref	-	-
Endometriosis	148 (34.7)	25 (11.7)	98 (46.0)	90 (42.3)	1.683	1.049-2.700	0.031
Endo-only	49 (34.0)	8 (11.1)	33 (45.8)	31 (43.1)	1.630	0.908-2.927	0.102

B. Allelic frequencies and genotype distribution of significant SNPs associated to endometriosis and/or endometriosis-associated infertility.

	MAF Count (%)		Genotype Count (%)		OR*	95%CI	*P* value
Endo-infertility	40 (27.4)	5 (6.8)	30 (41.1)	38 (52.1)	2.336	1.273-4.286	0.006
rs17524355 (LOXL4)	A	AA	AG	GG	*Model AA vs A G+GG*		
Controls	52 (17.8)	4 (2.7)	44 (30.1)	98 (67.1)	Ref	-	-
Endometriosis	84 (19.9)	11 (5.2)	62 (29.4)	138 (65.4)	2.372	0.702-8.012	0.164
Endo-only	27 (18.8)	2 (2.8)	23 (31.9)	47 (65.3)	0.944	0.190-6.033	0.939
Endo-infertility	33 (22.9)	8 (11.1)	17 (23.6)	47 (65.3)	5.230	1.434-19.072	0.012
rs2213392 (C3)	G	CC	CG	GG	*Model GG vs CC+CG*		
Controls	136 (46.3)	39 (26.5)	80 (54.4)	28 (19.0)	Ref	-	-
Endometriosis	172 (40.2)	72 (33.6)	112 (52.3)	30 (14.0)	0.631	0.344-1.159	0.138
Endo-only	54 (37.0)	25 (34.2)	42 (57.5)	6 (8.2)	0.369	0.144-0.942	0.037
Endo-infertility	66 (45.2)	23 (31.5)	34 (46.6)	16 (21.9)	1.011	0.485-2.107	0.978

*Corrected for age. MAF Minor allele frequency; OR odds ratio; CI confidence interval.