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The Genetics and Genomics of Endometriosis

Endometriosis is chronic, inflammatory, and estrogen-dependent gynecological disease that affects ten to fifteen percent of women of childbearing age and is a major promoter of pain and infertility, diagnosed in thirty to forty percent of women with pelvic pain and infertility. It's characterized by the implantation and growth of endometrial tissue, or womb lining tissue, at ectopic locations, normally within the peritoneum. The ectopic endometrial glands may be found at several sites, including the uterus, ovaries, posterior cul-de-sac, vagina, gastrointestinal tract, urinary bladder, liver, kidneys, ureters peritoneum, and uterosacral ligaments. There are four stages of endometriosis. Stage I or minimal, stage II, mild, stage III, moderate, and stage IV, severe. The diagnosis for stages is based on the amount and location of ectopic endometrial tissue present and the amount of scarring in the pelvis. Women with endometriosis may be asymptomatic, but patients often experience severe menstrual problems including dysmenorrhea (painful menstruation), chronic pelvic pain, dysuria (painful urination), dyspareunia (painful sexual intercourse), urinary tract problems, and gastrointestinal symptoms, including dyschezia (painful defecation). (Dun, E.C., et al.) The diagnosis of endometriosis is based on clinical manifestations, imaging, and surgical approaches. Laparoscopic exploration of visible lesions on tissues and organs allows for confirmation of endometriosis and treatment. Infertility is a prevalent struggle in those with the disease, as much as in up to forty percent of patients. Endocrine and ovulatory disorders are also observed in a small fraction of patients. This disease

is complex and has an important genetic component and heritability estimated to be around fifty percent. (Borghese, B., et al.)

The causation of endometriosis is unknown, but there is a considerable amount of evidence showing that genetic susceptibility is a major risk factor in developing the disease. Endometriosis is thought to be a complex where multiple gene loci interact with each other and the environment to produce the phenotype of the disease. Evidence of this is that there is familial clustering in humans and rhesus monkeys, concordance reported in monozygotic twins, the age of onset symptoms is similar in non-twin concordant sisters, prevalence of the disease is six to nine times greater in first-degree relatives than in the general population, the prevalence determined by an MRI can be as high as fifteen percent in sisters of women with severe forms of endometriosis, some genetic polymorphisms are more prevalent affected women than controls, and exposure to dioxin, a pollutant in the environment, may be a risk factor in disease development. The impression that endometriosis is genetically influenced was first gained from uncontrolled studies on apparent familial clustering. The prevalence of endometriosis is found to be higher in first-degree relatives of women affected than in those that are controls, which is evidence of a genetic basis for the disease in humans. (Kennedy, S.) For complex diseases such as endometriosis, it's hard to characterize the allelic architectures because they cannot show classical Mendelian inheritance, as it is a multifactorial and polygenic disorder that is concluded by multiple genes with allelic variations. Endometriosis research began in the 1940s, and it included several reports of relatives that were affected with the disease, which suggested familial occurrence. From a questionnaire in a 1971 study of three hundred and fifty women with endometriosis, twenty-two-point-four percent of respondents said they had a first-or-second-degree relative with the disease. The clinical characteristics of patients with confirmed

endometriosis who had affected relatives in one study were compared to patients without affected relatives. The results showed a primary difference in the finding that women with relatives with endometriosis had more severe cases, defined as stage III or IV. This result supported the role of genetics in the pathophysiology of endometriosis and established a seven-percent chance of getting the disease for first-degree relatives, and later a one-point nine percent risk for second-degree relatives. (Dun, E.C., et al.)

Endometriosis has been linked to familial clustering in studies, although it does not appear to be inherited in a Mendelian fashion. Endometriosis has been studied in the rhesus monkey, which has provided unique insight into the disease's inheritance. The rhesus monkey is a good model for endometriosis since it develops the disease on its own and the ectopically implanted tissue looks like endometrial implants. Because they have a large population of rhesus monkeys and well-documented pedigrees for these monkeys, the Wisconsin Regional Primate Research Center is a good venue for genetic studies. They found 142 animals with endometriosis and were able to create a multigenerational pedigree for each of them. With a considerably greater kinship coefficient for diseased animals and a higher occurrence risk in complete siblings, this study proved the familial character of endometriosis. B. Ranney was one of the first researchers to use a survey to demonstrate the family basis of endometriosis in humans. In this study, 350 women with surgically diagnosed endometriosis were issued a questionnaire asking about close relatives who had endometriosis. The findings suggested that endometriosis is inherited. Several studies have found that endometriosis runs in families, with first-degree relatives of diseased women being 5 to 7 times more likely to have surgically confirmed disease. In 1980, J. L. Simpson et al examined 123 women with surgically proved endometriosis and found that 5.9% of their mothers and 8.1 percent of their sisters had endometriosis, compared to

only 0.9 percent of controls. Following research has backed up J.L. Simpson et al's conclusions. S. Kennedy et al found a 15-fold greater relative risk for an afflicted sister in a proband with severe endometriosis in research where endometriosis was identified using magnetic resonance imaging. Other findings support the polygenic and multifactorial findings of endometriosis. When opposed to sporadic occurrences, the first endometriosis that arises in a family is more severe. This shows that those with severe disease have a higher genetic proclivity or tendency, and hence are more likely to have affected siblings or kids. The similar and early age of beginning of symptoms in affected families is another indication that suggests a genetic susceptibility to endometriosis. Endometriosis concordance was shown to be higher in monozygotic twins than in dizygotic twins in twin studies, implying stronger heredity. In an Australian twin registry, Treloar et al mailed questionnaires to 3298 monozygotic and dizygotic twin pairs. 3096 (94%) of the twins completed the questionnaires, and 215 (7%), including 2% of monozygotic and 0.6 percent of dizygotic twins concordant for endometriosis, reported having the condition. These researchers discovered that hereditary factors account for 51% of the disease's latent propensity. Endometriosis heritability has also been studied utilizing big population-based genealogical databases. Stefansson et al conducted the first investigation in Iceland, identifying 750 women with surgically diagnosed illness who had a statistically significant greater kinship coefficient than unaffected women. Sisters (5.20) and cousins (1.56) were also shown to have a significantly higher relative probability of being affected. Farrington et al in Utah confirmed the Icelandic research in that area. In these studies, endometriosis was found to be clustered in families. Familial clustering does not always imply a genetic cause, according to a recent study. In fact, familial clustering could signify other risk factors for the

disease in issue, such as lifestyle and diet, or an inherited intermediary factor related with endometriosis development, such as menarche age.

Gene mapping is used to investigate gene mutations or polymorphisms associated with endometriosis. These methods include candidate gene studies, genome-wide association studies, and linkage mapping. Multiple candidate genes have been studied in association with endometriosis and include ones involved in steroid synthesis, inflammation, detoxification, estrogen metabolism, hormone receptors, growth factors, adhesion molecules, apoptosis, cell-cycle regulation, oncogenes, other enzymes, and metabolic systems. Most have failed to confirm an association with few exceptions. Endometriosis, being an estrogen-dependent disease, has sparked interest in studying several genes involving steroid hormone synthesis and their signaling pathways. A recent meta-analysis showed an association between endometriosis and progesterone receptor (PGR)-PROGINS polymorphism with a pooled OR: (95% CI, 1.31-2.88) and estrogen receptor 1-PvuII polymorphism with a pooled OR: (95% CI, 1.20-3.68). However, these studies are suspected to be spurious for they are all experiments that leave concern for bias including selection and publication bias and lack of replication. Investigators in the UK and Australia have used linkage mapping on recruited individuals with endometriosis and their families for sibling-pair analysis to analyze the genome for excess sharing of informative polymorphic microsatellite markers in affected siblings. These investigators, as part of the International Endogene Consortium, investigated DNA extracted DNA from one thousand one hundred and seventy-six affected sister pairs for linkage evidence, in analysis describing a peak of linkage on chromosome 10 and suggestive peak on chromosome 20, further mapping on ten confirmed linkages. Another analysis on a subset of families with at least three or more affected individuals, showed linkage analysis demonstrating another peak of linkage on chromosome 7p.

This may represent a susceptibility allele with high penetrance for endometriosis. Genome Wide Association Scans are used in investigating the inheritance of endometriosis that uses single nucleotide polymorphisms (SNPs) to evaluate the genome for risk-associated variations.

(Hansen, K.A. et al.) The mapping of genetic risk factors in endometriosis is an ongoing study from earlier studies on women's health, which was initiated by Nick Martin and Susan Treloar. Their recruitment of endometriosis cases and their family members got a major boost and then became a flagship project for the Cooperative Research Centre for the Discovery of Common Human Disease. Martin and Treloar surveyed the health of female twins which resulted in the genesis of the endometriosis mapping project. A result from analyses of the data that was found surprising was the high heritability of hysterectomies, which is, in turn, for the relatively high heritability for endometriosis and uterine fibroids, which are risk factors for hysterectomy.

(Montgomery, G. et al.) DNA microarrays allow for the determination of gene expression under basal conditions and in the presence of endometriosis. The technology can measure changes in gene expression in response to disease, pharmaceutical agents, hormones, inflammation, growth factors, developmental changes, and deviations from homeostasis. Methods of obtaining RNA for analysis of gene expression in endometriosis have been described for intact eutopic and ectopic endometrial tissue. DNA microarray technology uses the fundamental nature of DNA and RNA to bind to complementary strands of nucleic acids. On a DNA microarray, single-stranded sequences of DNA that correspond to specific genes are arrayed on a matrix such as a glass microscope slide or cassette. RNA is taken from the tissue or cells of interest then incubated with the microarray, and the complementary base-pairing sequence structure of DNA and RNA allows the RNA to bind to the DNA on the microarray. The sequences on the microarray that the RNA is bound to are identified to develop gene expression profiles for the

tissue. Useful types of information that can be taken from DNA microarray data are lists of differentially expressed genes and groupings of differentially expressed genes into ontologies, especially lists of genes that are up or downregulated as a means of finding potential targets for diagnostic tests or treatments. Ariadne Pathway Studio, a specialty software program, uses lists of differentially expressed genes to find common regulatory factors to identify the ligands responsible for aberrant gene expression in a disease state such as endometriosis. The identification of families of genes that are up or downregulated can help show pathological etiologies. (Hansen, K.A. et al.) There are multiple theories regarding the events that provoke the development of endometriosis. The hypothesis of retrograde menstruation diminishing endometrial cells into the peritoneal cavity along the salpinges is the oldest and most prevalent theory. Whole-exome sequencing done in search of somatic mutations in the endometrial tissues in sixteen cases showed that most genes involved cell adhesions, junctions, and chromatin-remodeling complexes had mutated in both eutopic and ectopic tissues. Additional ideas on the disease pathophysiology pertain to an altered peritoneal biochemical and cellular environment, steroid function, oxidative stress, induced inflammation, and defective immune surveillance, and augmented angiogenesis. (Vassilopoulou, L. et al.)

The fact that only some women have endometriosis implies an increased susceptibility to the development of the disease in certain women, and individual susceptibility is not only influenced by genetic background but interactions of environmental factors with genes. Dioxin and compounds like it have been implicated factors for developing endometriosis. The main absorption route to human exposure is through diet, with an accumulation of high levels in lipids and membranes that are slowly released into the blood with an estimated seven-year half-life in humans. In addition to the toxic effects, it has adverse effects on the female reproductive system,

reducing reproductive success dramatically, as well as estrous cycling in rodents and primates. Rhesus monkeys were studied to show the relationship between dioxin and endometriosis, an autopsy on animals that were exposed to five to twenty-five parts-per-trillion of dioxin in their diet daily for five years showed extensive peritoneal endometriosis, in up to seventy-one percent and eighty-six percent of animals, thirty-three percent of controls having endometriosis. Several studies have shown strong environmental components in factoring on endometriosis, but this does not discredit hereditary factors. (Bischoff, F.Z. et al.) Studies on the impact of the environment, genetics, and aberrant regulation in the endometrium in women with endometriosis suggest the arising interplay between genetic variants and environmental factors. It's difficult to pinpoint the hereditary component because endometriosis susceptibility appears to be influenced by multiple genes. Techniques of the molecular component, including the linkage and genome-wide analysis, have shown candidate genes on known loci related to development, as well as regulation of the reproductive tract. As candidate genes and hereditary pathways are discovered, the possibility for treatment and prevention becomes more tangible for those affected, for as of now there is no known cure. (Dun, E.C. et al.)

Endometriosis is a neoplastic process that shares similarities with malignancy, or local invasion and angiogenesis. It has also been demonstrated that there's an increased frequency of endometroid carcinoma of the ovary in those who have endometriosis. These findings have ignited investigations of applying areas of cancer research, especially genetic changes, into the study of the disease. Loss of Heterozygosity (LOH) is a predisposing factor for the development of cancer, and several studies have evaluated endometriotic tissue with cytogenetic and molecular techniques for the presence of loss of heterozygosity. X. Jiang et al demonstrated a loss of heterozygosity at 9p, 11q, and 22q, further studies showed loss of heterozygosity at 5q,

6q, 9p, 11q, and 22q in a third of cases of ovarian cancer associated with endometriosis. Y. Kosugi et al found evidence of an increased frequency of monosomy 17 and the loss of the TP53 tumor suppressor gene locus in endometriotic samples as compared to controls. They found in sixteen endometriotic samples that twelve of sixteen had monosomy 17 and the other four had a loss of heterozygosity for the TP53 allele. Mutations of the PTEN tumor suppressor gene located on 10q23 have also been found in endometrioid and clear cell carcinomas of the ovary and endometriotic samples. These findings led to the proposition of a multi-hit strategy with the accumulation of mutations as a mechanism to explain the development of endometriosis, like a process that occurs in the development of colorectal carcinoma. In the model, the first hit, either inherited in familial disease or somatic mutation in sporadic disease, occurs in genes involved in cellular attachment or persistence in cells of menstrual effluvium. The mutated endometrial cells which reflux through the fallopian tubes then can attach to the peritoneum to survive, and following this attachment of cells, further mutations can happen which alter the metabolism and biology of the cell, which results in endometriosis. Inherited mutated genes make one more likely to experience a second hit that results in the development of endometriosis than those who require two sporadic mutations. In some situations, mutations can accumulate, possibly involving tumor suppressor genes or oncogenes, which could lead the endometriotic cells to become malignant cells. (Hansen, K. et al.)

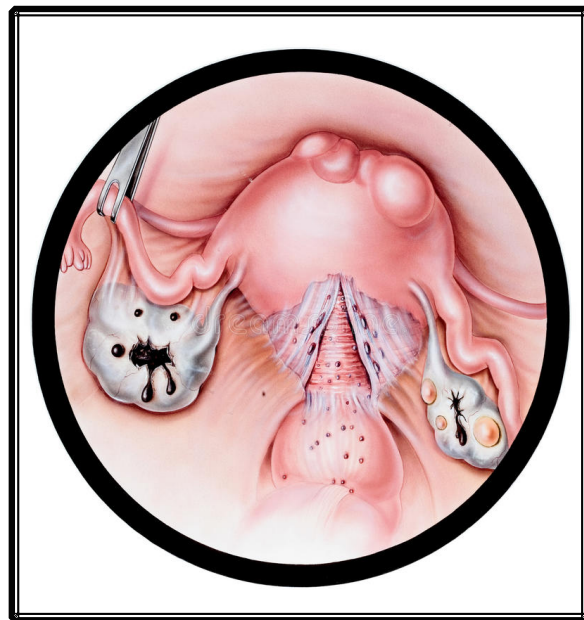
Baylor College of Medicine, the University of Oxford, the University of Wisconsin-Madison, and Bayer AG have collaborated on new research that sheds light on how to cure this severe disease. The researchers used genetic tests on humans and rhesus macaques to find a gene called NPSR1 that increases the likelihood of developing endometriosis. The findings point to a potential new nonhormonal pharmacological target that could lead to better treatment. By

analyzing DNA from families with at least three women diagnosed with endometriosis, the Oxford team, led by corresponding author Dr. Krina T. Zondervan, had previously discovered a genetic linkage to endometriosis on chromosome 7p13-15. At the Wisconsin National Primate Research Center at the University of Wisconsin-Madison, the Baylor team, led by senior author Dr. Jeffrey Rogers, confirmed this genetic linkage in the DNA of rhesus monkeys with spontaneous endometriosis. Further research was justified by in-depth sequencing analysis of endometriosis families at Oxford, which narrowed down the genetic cause to rare NPSR1 gene variants. Most of the women with these rare variants had stage III/IV disease. Similarly, Baylor researchers sequenced rhesus monkeys and found suggestive evidence in this species as well. Finally, an Oxford study of over eleven thousand women, including both endometriosis patients and healthy women, discovered a common variant in the NPSR1 gene that is also linked to stage III/IV endometriosis. This genetic study uncovered information that could lead to the discovery of a new therapeutic target. Researchers from Bayer, in collaboration with Oxford University, used an NPSR1 inhibitor to block protein signaling in cellular assays and then in mouse models of endometriosis as part of this collaboration. They discovered that this medication reduced inflammation and abdominal pain, indicating a potential focus for future endometriosis study. This is an exciting new development for treating a debilitating and underrecognized disease that affects one hundred ninety million women worldwide.

(“Researchers Identify Genetic Cause” 2021.)

It's established that the pathogenesis of endometriosis is factored in by genetic and environmental influences. The pathophysiology of endometriosis has had light shed onto it by familial studies, genetic association studies, linkage analysis, and GWAS through evidence associated with cellular procedures on disease development. However, many points and

characteristics of its etiology remain unknown. (Vassilopoulou, L. et al.) Endometriosis is a significant cause of morbidity and reduces the quality of life in many women, though the etiology of endometriosis remains a mystery, it seems to cluster in families, establishing a strong genetic factor. Studies on the disease have shown an increase in frequency in close relatives with a type of inheritance that's most likely polygenic or multifactorial. The genomic studies may lead to new diagnostic strategies and possible new therapies, and an improved understanding of the genetics and genomics of endometriosis will contribute to the understanding of the basic biology of the disease. (Hansen, K.A. et al.) This understanding is needed by a large group of women around the world who suffer regularly in multiple ways, with only a few temporary treatments that give their own negative side effects, many of which disrupt hormonal balances. With more knowledge on the disease, we can help find ways for women to improve their quality of life without completely disrupting their normal bodily functions.



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