Endometriosis associated with the N314D mutation of galactose-1-phosphate uridyl transferase (GALT)

Daniel W. Cramer1,2, M.D. Hornstein1, W.G. Ng2 and R.L. Barbieri1

1Department of Obstetrics and Gynecology and the OB-GYN Epidemiology Center, Brigham and Women’s Hospital, 221 Longwood Avenue, Boston, MA 02115, and 2Division of Medical Genetics, Children’s Hospital of Los Angeles, 4650 Sunset Boulevard, Los Angeles, CA 90027, USA

To explore a possible connection between endometriosis, Müllerian anomalies, and possession of the N314D allele of the gene for galactose-1-phosphate uridyl transferase (GALT), we studied 33 women with endometriosis attending a fertility clinic. Patients completed questionnaires and had DNA tested for the N314D mutation of GALT. A previously completed general population survey of 111 women which obtained the same information was available for comparison. Women with endometriosis were more likely to carry at least one N314D allele (30% compared with 14%) and more likely to report a medical history of scoliosis (21% compared with 2%) compared to general population controls: two features we have described in women with vaginal agenesis. Compared with endometriosis cases without the N314D allele, those cases with the allele tended to have more advanced disease and a family history of endometriosis. We speculate that endometriosis may arise due to defects of canalization of the cervix leading to cervical stenosis and retrograde menstruation. The relevance of the N314D mutation, via this model, may derive from an association between abnormalities of galactose metabolism and vaginal agenesis which represents a canalization defect of the vaginal plate of the Müllerian tubercle, the same structure which gives rise to the cervix.

Key words: endometriosis/galactose-1-phosphate uridyl transferase/Müllerian anomalies/scoliosis

Materials and methods

Women with surgically diagnosed and staged endometriosis were selected from consecutive attenders at the Fertility and Endocrinology Unit of the Brigham and Women’s Hospital. In a protocol approved by the Human Subjects Committee, subjects were asked to complete questionnaires related to their medical and family history and to provide a blood specimen for quantitative and qualitative assay of their GALT enzyme. Activity was measured in red cells by a carbon 14 labelling method and its electrophoretic pattern characterized (Lee and Ng, 1982). GALT genotype was investigated using extracted DNA from whole blood for determination of the presence of the N314D mutation. Briefly, this methodology involved phenol-chloroform extraction of DNA, amplification of ~0.1 μg of the DNA template with primers specific for exon 10 of GALT (Reichardt and Woo, 1982), and restriction enzyme digestion with AvaiI. The restricted products were electrophoresed on 4% NuSieve 3:1 agarose gel for separation of N314D genotypes. Comparison values for the frequency of genotypic variants of GALT and various medical traits were available from a population-based study of these traits in 113 pre-menopausal women selected from the general population who had also served as controls in a study of women with a family history of ovarian cancer (Cramer et al., 1994). This control group excluded women with a family history of ovarian cancer, current oral contraceptive users, or women with hysterectomy or bilateral oophorectomy. Two of the 113 women who reported a history of endometriosis were also excluded.

Statistical analysis

Analysis was based upon comparison of frequencies using χ2, Fisher’s exact test, or Student’s t-test for unpaired samples.

Results

Of the endometriosis patients, 32 were Caucasian and one was Asian compared to the ethnic distribution in the controls of 107 Caucasians, two blacks, and two Asians. Table 1 shows the characteristics of the 33 patients with endometriosis. The average (± SE) for GALT activity was 22.9 (± 0.88) which...
procedures such as conization or cryotherapy. In utero exposure to diethylstilboesterol (DES) exposure in 33 women with endometriosis (27%) said they had a family history of endometriosis in the N314D negative group (P = 0.08). There was also a tendency for cases carrying an N314D allele who were stage II or greater compared with seven out of 10 (70%) who had the N314D allele who were stage II or greater (P = 0.24). Finally, nine out of 33 women with endometriosis (27%) said they had either diethylstilboesterol (DES) exposure in utero or cervical procedures such as conization or cryotherapy.

Discussion

The N314D mutation of the GALT gene is a common polymorphic variant with a carrier frequency of 5–15%. We have described a greater occurrence of this mutation among women with endometriosis. A total of 30% of women with endometriosis carried this mutation compared to a 14% frequency in our general population sample. This is the same mutation that appeared with high frequency in daughters with vaginal agenesis and their mothers including three mothers who had endometriosis (Cramer et al., 1996). An explanation for a connection between errors of galactose metabolism and endometriosis follows from two premises: that metabolic errors of GALT are associated with certain Mullerian anomalies and that endometriosis is associated with Mullerian anomalies.

A key premise is that an association between errors of galactose metabolism and Mullerian anomalies may exist. We have previously reported that about half of women with class I Mullerian anomalies involving vaginal agenesis and/or their mothers have an N314D mutation of GALT (Cramer et al., 1996). Biological plausibility for a link between vaginal agenesis and metabolic errors of GALT is based upon experiments involving high galactose feeding of pregnant rodents which caused delayed vaginal opening in...
female offspring (Chen et al., 1981). GALT mutations in the mother or fetus which lead to decreased metabolism of galactose could produce high intrauterine levels of this carbohydrate and mimic the rodent model.

The second premise is that endometriosis is associated with Müllerian anomalies. Olive and Henderson (1987) proposed that the development of endometriosis in patients with Müllerian anomalies principally depends upon the co-occurrence of outflow obstruction, functioning endometrium, and patent tubes. Ten out of 13 women (77%) with Müllerian anomalies having such characteristics had endometriosis. However, more than a third of the women with 'non-obstructive' anomalies (16 out of 43) also had endometriosis. Olive and Henderson concluded that a 'relative outflow obstruction' may exist among women with non-obstructive Müllerian anomalies and predispose to endometriosis particularly when the woman's immune system is inadequate to handle the menstrual debris.

Our hypothesis is that some cases of endometriosis may arise as a consequence of errors of galactose metabolism that lead to subtle Müllerian defects predisposing to relative outflow obstruction and retrograde menstruation. Vaginal agenesis, which we have linked to N314D mutations, arises from a defect of canalization and elongation of the vaginal plate component of the Müllerian tubercle (Jiraseks, 1976). Since the cervix also arises from the Müllerian tubercle, we propose that embryological events leading, in some instances, to a failure of canalization of the vagina might, in other circumstances, lead to defective canalization of the cervix; i.e. cervical stenosis. Barbieri et al. (1992) have postulated that, as the diameter of the cervical os decreases, retrograde flow through the tubal ostia becomes more likely. This hypothesis is supported by an experimental model in baboons in which partial cervical occlusion by supracervical ligation produced endometriosis in animals within 3 months of the procedure (D’Hooghe et al., 1994).

Since our hypothesis regarding endometriosis and congenital cervical stenosis was developed after collection of the data, no systematic assessment of the cervix was conducted as part of this study. It is unlikely that a simple description of the diameter of the external os would suffice to confirm our hypothesis. Rather, novel techniques may need to be developed to assess the anatomical and physiological features of the endocervical canal and internal os.

The proposition that endometriosis and class I Müllerian anomalies are related is further supported by demonstrating common phenotypic features. One such feature is scoliosis which occurred in about 20% of women with either endometriosis or vaginal agenesis (Cramer et al., 1996) compared with our control rate of ~2%. Although our studies did not include clinical confirmation of the scoliosis, we believe the validity of using patient reports of scoliosis is demonstrated by the fact that our ‘control’ frequency of 2% is close to the 4% frequency found in general population screening in girls (Willner and Uden, 1982). Another phenotypic feature found in vaginal agenesis patients included freckling and café-au-lait birthmarks (Cramer et al., 1996). Although we did not observe a clear excess of such birthmarks among women with endometriosis, dysplastic naevi have been described in women with endometriosis (Frisch and Wyshak, 1992). The connection between scoliosis, pigmented skin changes and GALT defects may reflect the ability of galactose to affect development of the neural crest.

There are also case reports that premature ovarian failure may be associated with vaginal agenesis (Aughton, 1993; Cramer et al., 1987) and is a prominent component of the animal model (Chen et al., 1981). This raises the possibility that decreased oocyte reserve could be a co-morbid event associated with endometriosis and might be relevant to the debate of why infertility is associated with early staged endometriosis not producing tubal obstruction or extensive adhesions.

The major limitation of our study is, of course, its small size leading to possible selection factors accounting for the genotypic or phenotypic differences observed. The prevalence of the N314D mutation varies by ethnicity and there were no ethnic differences between the endometriosis patients and controls which could account for the observed variation in N314D frequencies. The fact that the majority of women with endometriosis in this study did not have the N314D mutation does not contradict our theory of a link between GALT mutations, Müllerian defects, and endometriosis. The full spectrum of defects of galactose metabolism that may be associated with Müllerian defects has not yet been defined; nor did we investigate whether maternal GALT defects might have played a role as they apparently do in vaginal agenesis (Cramer et al., 1996). Finally, there may be other congenital or acquired causes of cervical stenosis as observations concerning DES exposure and cervical procedures suggest.

Our emphasis on cervical stenosis as the pathway for a connection between GALT mutations and endometriosis is not meant to exclude the possibility of a role for coelomic metaplasia. If indeed metabolic errors of GALT can cause canalization defects of the Müllerian tract, they might also predispose to embryological rests on the pelvic peritoneum capable of undergoing Müllerian differentiation. Still, retrograde menstruation due to cervical stenosis is, probably, the theory most compatible with experimental and epidemiological data. Indeed, Meig’s observations (1953) on early pregnancy in the prevention of endometriosis could well be explained by the simple fact that a vaginal delivery produces cervical dilation.

In conclusion we have described a high occurrence of the N314D mutation of GALT among women with endometriosis. We have hypothesized that errors of galactose metabolism may be associated with canalization defects of the cervix producing relative outflow obstruction, retrograde menstruation, and endometriosis. Confirmation of this hypothesis will require assessment of anatomical and physiological characteristics of the cervical os among women with endometriosis or GALT mutations. Endometriosis is a major cause of morbidity in women accounting for about 190,000 hospitalizations annually in the US. Ideas about the pathogenesis of this disorder that could lead to a strategy for its prevention should be vigorously pursued.
D.W. Cramer et al.

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