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

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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

Introduction
Cramer et al. (1996) have reported that a higher than expected proportion of daughters with the class I Müllerian anomaly, vaginal agenesis, carry the N314D variant of the gene for galactose-1-phosphate uridyl transferase (GALT) and that their mothers, particularly those who themselves had the N314D variant, were more likely to report endometriosis. The N314D mutation of GALT generally causes decreased GALT activity and might be relevant to vaginal agenesis because errors of galactose metabolism in a mother or fetus could lead to intrauterine exposure to galactose and mimic a rodent model in which high galactose feeding during pregnancy produced offspring with delayed vaginal opening (Chen et al., 1981). However, no basis for a possible connection between endometriosis, Müllerian anomalies, and N314D mutations was immediately apparent, prompting further exploration in an independent series of women with endometriosis.

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 I shows the characteristics of the 33 patients with endometriosis. The average (± SE) for GALT activity was 22.9 (± 0.88) which
did not differ significantly from the 23.2 (± 0.32) observed for controls. However, 10 out of 33 (30%) of the endometriosis patients carried at least one N314D allele of GALT compared with 15 out of 111 controls (14%) who carried at least one N314D allele (P = 0.02) and the GALT activity for these 10 patients was 19.04 (± 2.18).

There were several traits which distinguished women with endometriosis, in particular those with an N314D allele. Seven out of 33 women (21%) with endometriosis said they had scoliosis compared to two out of the 111 controls who reported such a history (P < 0.001). Seven of the 33 women with endometriosis (27%) said they had a family history of the disease, although medical records of the relatives were unavailable to confirm this. Four of these women (40%) were among the 10 who were N314D positive which was three times greater than the three out of 23 (13%) who 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 to have higher staged disease. Eleven of the 23 cases without an N314D allele (48%) 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 Müllerian anomalies and that endometriosis is associated with Müllerian anomalies.

A key premise is that an association between errors of galactose metabolism and Müllerian anomalies may exist. We have previously reported that about half of women with class I Müllerian 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
proposed that the development of endometriosis in patients
with Müllerian anomalies principally depends upon the co-
ocurrence 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 particular-
ly 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 tube (Jiraseks, 1976).
Since the cervix also arises from the Müllerian tunicle,
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 congen-
tital 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,
pigmentary 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 but 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, retro-
grade menstruation due to cervical stenosis is, probably, the
theory most compatible with experimental and epidemi-
ological 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 hypo-
thesis 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.

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Acknowledgements
The authors acknowledge the support of Phaedra Thomas, R.N. in assisting with recruitment of patients with endometriosis. This research was supported in part by grant no. 90 BW25, from the American Institute for Cancer Research.

References

Received on October 16, 1995; accepted on January 12, 1996