Vaginal agenesis (Mayer–Rokitansky–Kuster–Hauser Syndrome) associated with the N314D mutation of galactose-1-phosphate uridyl transferase (GALT)

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Introduction

Mayer–Rokitansky–Kuster–Hauser Syndrome (M-R-K-H) or vaginal agenesis occurs in about one in 4000 female births and is the second most common cause of primary amenorrhea in women. While it is appreciated that familial occurrence of vaginal agenesis may occur, no simple inheritance pattern is discernible and multifactorial inheritance has been postulated (Carson et al., 1983). In 1987, we described two of four women with vaginal agenesis who carried variants of the enzyme galactose-1-phosphate uridyl transferase (GALT), including the Duarte and the Los Angeles variants (Cramer et al., 1987). One of the mothers also carried the Duarte variant and another mother was a carrier for classic galactosaemia. We speculated that maternal or fetal errors of galactose metabolism might lead to high fetal exposure to galactose and mimic an experimental model in which daughter mice whose mothers were Duarte carriers or Duarte and Los Angeles variant carriers demonstrated in rodents to cause decreased oocyte survival and delayed vaginal opening in offspring.

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

Materials and methods

Women with vaginal agenesis were identified from current patients of several physicians treating this condition (see Acknowledgements). None of the women who participated in our previous series were included in this study. The diagnosis of M-R-K-H was based upon absence of the vagina in women with normal breasts and hair pattern and variable remnants of uterine tissue. In a protocol approved by the Human Subjects Committee of the Brigham and Women’s Hospital, mothers and daughters were asked to complete questionnaires related to their medical and family history and to contribute blood for quantitative and qualitative assays of galactose metabolism.

GALT activity was measured in red cells by a carbon 14 labelling method and its electrophoretic pattern characterized (Lee and Ng, 1982). GALT genotype has previously been inferred on the basis of the electrophoretic pattern and GALT activity level: normal individuals have a single band and activity above 17 micromoles of hexose conversion per h per gram of haemoglobin; galactosaemic individuals have a single band and activity <6, and galactosaemia carriers have a single band and activity of 6–17; and carriers or homozygotes for the Duarte or Los Angeles variants are recognized by a three band electrophoretic pattern and either low or high activity levels respectively.

More precise genotypic characterization was performed using extracted DNA from whole blood to detect common GALT mutations including the Q188R mutation associated with classic galactosaemia and the N314D mutation which appears to be associated with both the Duarte and Los Angeles variant of GALT. Briefly, this methodology involved phenol–chloroform extraction of DNA, amplification of ~0.1 µg of the DNA template with primers specific for exons 6 and 10 of GALT (Reichardt et al., 1991; Reichardt and Woo, 1991; Lin et al., 1994), and restriction enzyme digestion with HpaII for Q188R or AvaI for N314D. The restricted products were electrophoresed on 4% NuSieve 3:1 agarose gel for separation of Q188R or N314D genotypes.
Referenced against the ~14% frequency of controls who carried at least one N314D allele, the frequency of the N314D mutation of GALT was 23.1 (± 0.32) for the daughters of individuals with vaginal agenesis and their mothers. Six out of 13 (46.2%) of the vaginal agenesis group were N314D homozygotes for the N314D mutation of GALT.

The average (± SE) GALT activity for the 113 control subjects was 20.5 (± 1.05) which were both significantly reduced (P < 0.05 and 0.01 respectively). Of the 113 control subjects, 15 (13.3%) were heterozygous and one (0.95%) was homozygous for this mutation. In this study, we found that six of 13 daughters with vaginal agenesis (46%) who did not have the N314D mutation, two (29%) had mothers who were carriers for the N314D mutation which, although double the 14% control frequency, was not significant in this small series. None of the mothers or daughters in this series possessed a Q188R mutation compared to one out of the 113 controls found to be heterozygous for this mutation.

Comparison values for the frequency of the Q188R and N314D genotypic variants of GALT or medical traits were available from a population-based study of 113 women of reproductive age selected from the general population of Boston who had also served as controls in a study of women with a family history of ovarian cancer (Cramer et al., 1994). Exclusion criteria required that they had no family history of ovarian cancer, had not undergone a hysterectomy or bilateral oophorectomy, and were not currently using oral contraceptives. None of these women reported any Müllerian anomalies. In both the general population study and the vaginal agenesis study, women were asked about general consumption during their childbearing years of four common types of dairy products (whole and skimmed milk, yogurt, ice cream, and cottage cheese). From this, the daily intake of lactose could be crudely estimated. A glass of milk (240 ml) contains ~12 g lactose (or 6 g galactose).

**Statistical analysis**

This was based upon comparison of frequencies using χ² or Fisher’s exact test or comparison of quantitative variables using a non-paired t-test.

**Results**

All the daughters with vaginal agenesis and their mothers were Caucasian. The control group included 109 whites, two blacks, and two Asians. Table I describes daughters with vaginal agenesis and their mothers with respect to key study variables. The average (± SE) GALT activity for the 113 control subjects was 23.1 (± 0.32); this compares with an average (± SE) of 21.1 (± 1.11) and for their mothers of 20.5 (± 1.05) which were both significantly reduced (P = 0.05 and 0.01 respectively). Of the 113 control subjects, there were 15 (13.3%) who were heterozygous and one (0.95%) who was homozygous for the N314D mutation. Although no N314D homozygotes for the N314D mutation were found among the vaginal agenesis group, six out of 13 (46.2%) daughters with vaginal agenesis and six out of 13 of their mothers were heterozygous for the N314D mutation of GALT. Referenced against the ~14% frequency of controls who carried at least one N314D allele, the frequency of the N314D mutation among daughters with vaginal agenesis (or their mothers) was significantly elevated (P = 0.004). In four out of the six instances in which daughters had the N314D mutation, the trait was inherited from the mother. Of seven daughters who did not have the N314D mutation, two (29%) had mothers who were carriers for the N314D mutation which, although double the 14% control frequency, was not significant in this small series. None of the mothers or daughters in this series possessed a Q188R mutation compared to one out of the 113 controls found to be heterozygous for this mutation.

There were several phenotypic or historical features which appeared to distinguish the daughters with vaginal agenesis or their mothers. Three out of the 13 (23%) daughters with vaginal agenesis had scoliosis compared to a control frequency of two out of 113 (2%) (< 0.01, Fisher’s exact). Eight out of 13 (61.5%) daughters had either café-au-lait birthmarks or freckling in non-sun exposed areas compared with 38 out of 113 (33.6%) controls (P = 0.04). Although these same phenotypic features did not significantly distinguish mothers in this series, we did observe that three of the 13 (23%) mothers had a history of endometriosis compared with two out of the 113 controls (P < 0.01, by Fisher’s exact). A family history of melanoma was reported by three out of 13 (23%) mothers which was about double that reported by controls (10%) but not significant in this small series.

Considerable variation was observed in the estimated consumption of lactose by the mothers during their childbearing years. Overall consumption averaged 15.2 (± 3.7) g which, although greater, did not differ significantly from the 12.1 (± 1.1) g reported by controls.

**Discussion**

In this study, we found that six of 13 daughters with vaginal agenesis (46%) carried an N314D mutation of their GALT gene and two mothers of the remaining seven daughters (29%) also carried this mutation compared to a background frequency in our control population of about 14%. The frequency of the N314D mutation does appear to vary by ethnicity with the lowest frequency reported in blacks (Elsas et al., 1994).
Virtually all of the subjects in this study were Caucasian, indicating that ethnic differences in the study populations did not account for the differences observed. These findings confirm our earlier report (Cramer et al., 1987), based upon electrophoretic pattern of GALT, that two out of four daughters with vaginal agenesis were carriers for the Duarte or Los Angeles variants of GALT. Subsequently these variants have been shown to have the same molecular genetic basis, namely a substitution of aspartic acid at the 314 amino acid position for asparagine leading to the altered electrophoretic pattern and generally lower GALT activity (Lin et al., 1994).

In the earlier series, one of the mothers was a presumed carrier for classic galactosaemia based upon low GALT activity and single band electrophoretic pattern. Aughton (1993) recently described a grandmother, mother, and daughter who were carriers for classic galactosaemia (based upon electrophoretic pattern and GALT activity) and were unique because the daughter had streak ovaries and vaginal agenesis and the mother had a Müllerian fusion anomaly. In the current series no mothers or daughters who carried classic galactosaemia were identified using more precise molecular genetic techniques to detect the Q188R mutation associated with classic galactosaemia. Since the background frequency of this mutation in our population is only 1%, the power to rule out an association between this genotype and vaginal agenesis is low in this series of patients.

Biological plausibility for an association between errors of galactose metabolism and vaginal agenesis is based upon rodent experiments in which high galactose feeding of pregnant animals caused delayed vaginal opening in female offspring (Chen et al., 1981). As opposed to humans in whom the vagina is canalized during fetal life, the vagina does not open until after birth in rodents. Thus, delayed vaginal opening in rodents and vaginal agenesis in humans could be equivalent defects related to intrauterine exposure to galactose. In the rodent experiment, the offspring also had oocyte depletion indicating that galactose had a toxic effect on oocytes. Our previous report of premature menopause and that of Aughton (1993) of streak ovaries in women with vaginal agenesis suggest that germ cell depletion may be a pathogenetically related event to intrauterine exposure to galactose. Since the events of interest occurred many years in the past. Accuracy of such information is seriously affected by the fact that the events of interest occurred many years in the past. Further investigation of the role of maternal galactose consumption is important because it might offer a strategy for preventing this disorder in pregnancies of women known to have GALT mutations.

In conclusion, this report confirms and extends our original observations on a potential link between vaginal agenesis and errors of galactose metabolism. Approximately half of daughters with vaginal agenesis display the N314D mutation of GALT associated with the Duarte variant of galactosaemia and about one third of daughters with vaginal agenesis not having this mutation have mothers who carry this mutation; frequencies which are higher than the 14% rate of this mutation in our general population. Future investigations should attempt to clarify whether families with vaginal agenesis not found to have N314D or Q188R mutations may have other GALT mutations or defects of additional enzymes involved in galactose metabolism including galactokinase or epimerase.

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References


