The Duarte (N314D) variant in the *GALT* gene has no effect on in vitro fertilization outcome

We assessed the effect of the Duarte (N314D) variant in the *GALT* gene on in vitro fertilization outcome measures. Our data do not definitely exclude variants in the *GALT* gene as factors influencing outcome, but the lack of suggestive evidence makes it difficult to justify a larger, more definitive study. (Fertil Steril® 2006; 85:502–4. ©2006 by American Society for Reproductive Medicine.)

Predicting poor response to gonadotrophin stimulation in in vitro fertilization (IVF) is an important aim. Protocols could be more individualized and the mechanisms responsible for poor response might be better understood were it possible to identify poor responders before treatment. Numerous parameters have been studied, although in a meta-analysis only the basal FSH level emerged as a predictor of poor outcome (1). More recently, interest has focused on a pharmacogenetic approach as variants in genes involved in ovarian steroidogenesis may influence the response to gonadotrophin stimulation. Thus, there is some evidence to suggest that the *PvuII* polymorphism in the estrogen receptor α (*ER*α) gene may be a marker for a reduced mean ratio of follicles to oocytes harvested (2) as well as the number of follicles, oocytes and embryos obtained, and pregnancy rates (3). There are also reports that functional variants in the FSH receptor gene may influence the gonadotrophin doses required in ovarian stimulation (4, 5).

We speculated that functional mutations in the galactose-1-phosphate uridyltransferase (*GALT*) gene might affect ovarian responsiveness. Galactose-1-phosphate uridyltransferase is an enzyme that catalyzes the conversion of galactose-1-phosphate to glucose-1-phosphate via transfer of uridine monophosphate. Some mutations in the *GALT* gene switch off enzyme activity causing classical galactosemia which is associated with premature ovarian failure (POF) (6), probably because the biological activity of FSH is dependent on its glycosylation pattern (7). Other mutations reduce *GALT* activity, without causing galactosemia. One example, an A-to-G substitution in exon 10 at amino acid 314 (N314D), reduces enzyme activity to 40%–50% in some patients (Duarte variant); it is associated with raised FSH levels in pre-menopausal women (8). In others, carrying an N314D mutation with a C-to-T substitution in exon 7 has no effect on, or may increase, enzyme activity by 110%–130% (Los Angeles variant).

Received March 9, 2005; revised and accepted July 18, 2005.
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To test the effect of these *GALT* variants on IVF outcome, we studied a group of 152 patients undergoing IVF treatment who were genotyped for the Duarte and Los Angeles variants, as well as the much rarer Q188R mutation, which is the most common cause of galactosemia. Over a 3-month period, blood samples from 152 consecutive Caucasian women undergoing IVF were obtained with informed consent for DNA studies. IRB approval, from our local ethics committee, allowed the use of these anonymised, linked DNA samples to investigate genetic factors involved in infertility. The standard IVF treatment protocol used in Oxford has been described previously by Lockwood et al (9).

In brief, all patients had a long protocol cycle with luteal phase start using the GnRH agonist, nafarelin (Synarel; Searle, High Wycombe, UK; 400 μg intranasal twice a day) followed by stimulation with recombinant FSH. The dose in the initial treatment cycle was determined on an individual basis according to age, body mass index, and follicular phase FSH level. Upon ultrasonographic (Toshiba, Crawley, UK) detection of at least three follicles, $\geq$18 mm in diameter, 10,000 IU of human chorionic gonadotrophin (hCG) (Profasi; Serono, Feltham, UK) was administered. Oocyte retrieval (OR) was performed transvaginally using intravenous propofol, midazolam, and fentanyl for sedation and analgesia. The practice in the unit is to aspirate all follicles $\geq$12 mm diameter. Embryo transfer (ET) was performed approximately 48 hours after the oocyte retrieval.

Genomic DNA was extracted from 9 mL of EDTA anticoagulated whole blood using the QIAmp DNA extraction kit (Qiagen).A 311-bp fragment of exon 10 of the *GALT* gene was amplified by polymerase chain reaction (PCR). It was then subjected to restriction enzyme analysis with Ava II (Boehringer Mannheim, Bracknell, UK) with visualization on a NuSieve® 3:1 agarose gel (FMC, Rockland, ME). The region of exon 10 amplified contains one Ava II restriction site normally and two restriction sites if the polymorphism is present, thereby providing an internal control of enzyme digestion. The outcome measures in the cases and controls were compared using *t*-tests and the chi-square test as appropriate.

0015-0282/06/$32.00

doi:10.1016/j.fertnstert.2005.07.1307

Among the 152 women recruited, 27 (17.8%) carried at least one allele with the N314D mutation and one woman was heterozygous for the Q188R mutation. These data are consistent with previously reported findings in U.K. population controls (10). Of the 27 women with the N314D mutation, 20 had the Duarte variant and 7 the Los Angeles variant; the only woman who was homozygous for the N314D mutation had the Duarte variant. For the purposes of comparison, the cases consisted of the 21 women with genotypes associated with reduced *GALT* activity (Duarte variant = 20; Q188R = 1); the 124 women with the wild type genotype acted as controls. The results for the women with the Los Angeles variant, which is associated with increased *GALT* activity, are reported but without statistical analysis because of the small numbers.

All the patients had at least one IVF treatment cycle. At the time of the first cycle, no significant differences were found in age (34.7 ± 3.7 vs. 34.0 ± 4.5 years; means ± SD) or day 1 FSH levels (6.7 ± 2.0 vs. 7.3 ± 2.3 IU/L) between the cases and controls (Table 1). The primary indications for IVF in the three groups were endometriosis (4.8%, 6.5%, and 14.3%); male factor infertility (42.8%, 24.2%, and 42.9%); polycystic ovarian syndrome (4.8%, 3.2%, and 14.3%); tubal disease (28.5%, 17.7%, and 14.3%); and unexplained/other causes (19.1%, 48.4%, and 14.3%). Table 1 also lists the outcome data consisting of the number of follicles identified on the day of oocyte retrieval; the number of oocytes obtained; the fertilization rates; the percentage of Grade A embryos; and the clinical pregnancy rates (defined as seeing a fetal heart beat on ultrasound) among the cases, controls, and women with the Los Angeles variant. No significant differences were found between the 21 cases and 124 controls, even though the fertilization rate was higher in the controls and the clinical pregnancy rate was lower in the cases.

Ten (48%) of the women with the Duarte variant, 4 of the women with the Los Angeles variant (57%), and 63 (51%) women with the wild type genotype had at least one other treatment cycle. For completeness, we report the outcome data in the second cycle, but without statistical analysis because the numbers of women are too small. The number of follicles and oocytes obtained in the three groups were 18.2 ± 10.0 vs. 14.5 ± 10.7 vs. 13.7 ± 8.5, and 10.0 ± 6.1 vs. 11.0 ± 6.3 vs. 9.0 ± 4.4, respectively. The fertilization rates, percentage of grade A embryos, and clinical pregnancy rates in the three groups were 48.9%, 13.0%, and 20.0%; 71%, 0%, and 0%; and 63.7%, 15.0%, and 22.2%, respectively. Thus, the outcome data in the second treatment cycle were also similar.

Clinicians and patients alike are searching for better ways of predicting IVF outcomes to improve ovarian stimulation regimens and to facilitate informed decision making about treatment choices. Current methods are unsatisfactory: The best predictor of outcome identified to date appears to be the antral follicle count (11). Some groups have investigated genetic variants in hormone receptors involved in the hypothalamic-pituitary-ovarian axis (2, 4, 5). These common variants are unlikely to have markedly adverse effects but alone, or in combination, they may influence reproductive processes albeit perhaps in a subtle way. It appeared reasonable, therefore, to contribute to the emerging area of pharmacogenetic research by investigating the effect of variants in the *GALT* gene on IVF outcomes.

The rationale was that galactosemia is associated with POF (6), and the functional variants studied (Duarte variant and Q188R mutation) cause reduced *GALT* activity that

TABLE 1

Baseline characteristics and IVF outcome data, in the first treatment cycle, for 21 women with the *GALT* N314D Duarte variant, 7 women with the Los Angeles variant, and 124 women with the wildtype genotype.

	Duarte variant (n = 21) (range)	Wild-type (n = 124) (range)	Los Angeles variant (n = 7) (range)
Age (y)	34.7 ± 3.7 (29.8–41.5)	34.0 ± 4.5 (21.6–44.2)	36.5 ± 2.4 (33.3–40.1)
Day 1 FSH	6.7 ± 2.0 (2.5–10.0)	7.3 ± 2.3 (3.3–15.8)	7.4 ± 1.9 (5.0–9.1)
No. of follicles	15.9 ± 8.1 (2–40)	14.2 ± 8.0 (1–49)	11.1 ± 4.7 (2–16)
No. of oocytes	10.4 ± 5.1 (2–21)	9.9 ± 5.6 (0–26)	8.0 ± 3.4 (2–11)
Fertilization rate (%)	51.0 (0–92)	67.3 (0–100)	59.5 (36.4–100)
Grade A embryos (%)	19.8 (0–100)	18.7 (0–100)	25 (0–100)
Clinical pregnancy rate (fetal heart on scan) (%)	33.3	24.2	28.6

Note: Values are mean ± SD.

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alters the biological activity of FSH. Thus, they have been associated with both altered ovarian function (8) and subfertility (12). Given that the prevalence of the Duarte variant in the U.K. population is approximately 17% and the Q188R mutation is rare (<1%), it would be expensive and time-consuming to genotype large numbers of women in a definitive study. We chose instead, in what was essentially a pilot study, to recruit and genotype a sample of 150 patients, which resulted in an expected number of women carrying the Q188R mutation (n = 1) and at least one N314D allele (n = 27). Of these 27 women, 20 (74.1%) had the Duarte variant. With these study numbers, we had approximately 80% power at the .05 significance level to detect a 30% difference in the number of oocytes/follicles obtained between the two groups, which we had arbitrarily defined as a clinically significant difference.

Despite the small numbers, the two groups were comparable in terms of their baseline characteristics (e.g., age and day 2 FSH levels). However, we detected no significant differences between the women carrying at least one N314D or Q188R allele and the wild type controls in terms of the IVF outcomes measured: numbers of follicles and oocytes obtained; fertilization rates and percentage of Grade A embryos; and clinical pregnancy rates. The Los Angeles variant similarly appeared to have no influence on outcome, although the numbers were small. These data do not definitely exclude variants in the *GALT* gene associated with reduced enzyme activity as factors influencing IVF outcome, but the lack of suggestive evidence makes it difficult to justify a larger-scale study. Nevertheless, the study highlights the potential significance of identifying functional polymorphisms in genes that might influence performance at IVF—an area of research that could profoundly affect how IVF is conducted in the future.

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