These data suggest that the most cost-effective algorithm in the diagnosis of MG is testing for ACHR binding and blocking antibodies with reflex testing for modulating antibodies only in the presence of one or both of these other ACHR antibodies.

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References


Identification of the Hormone Kisspeptin in Amniotic Fluid

To the Editor:

Kisspeptin is the product of the KISS1 gene (KISS-1 metastasis-suppressor) gene and is the ligand for the G-protein-coupled receptor, now known as the KISS1 receptor (KISS1R) (1). Both kisspeptin and KISS1R play a crucial role in the regulation of reproduction and puberty (1). The KISS1 gene encodes a precursor peptide of 145 amino acid residues, which undergoes proteolytic processing to generate kisspeptins 10, 13, 14, and 54 (1). These peptides all share the common C-terminal decapeptide necessary for receptor activation (1). Inactivating mutations in the human KISS1 receptor (KISS1R) gene cause hypogonadotropic hypogonadism (1). During pregnancy, circulating plasma kisspeptin concentrations rise by

Thomas R. Haven2*
Mark E. Astill2
Brian M. Pasi3
James B. Carper3
Lily L. Wu2,4
Anne E. Tebo2,4
Harry R. Hill2,4

*Address correspondence to this author at: ARUP Institute for Clinical and Experimental Pathology 500 Chipeta Way Salt Lake City, UT 84108-1221 Fax 1-801-584-5048 E-mail haventr@aruplab.com

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1. Human genes: KISS1, KiSS-1 metastasis-suppressor; KISS1R, KISS1 receptor.
2. Nonstandard abbreviations: KISS1R, KISS1 receptor (previous symbol, GPR54); IR, immunoreactivity.
7000-fold in the third trimester, compared with the concentrations in nonpregnant women (2). Plasma markers that are altered during pregnancy, such as β human chorionic gonadotropin, have been used as markers in amniotic fluid and in certain cases may be used as markers to predict fetal outcome. Kisspeptin, however, has not previously been identified in amniotic fluid. The aim of this study was to determine whether kisspeptin is present in amniotic fluid.

After obtaining ethics approval (Hammersmith and Queen Charlotte’s and Chelsea Hospitals Research Ethics Committee no. 06/Q0406/12), we recruited 32 volunteers scheduled to undergo diagnostic amniocentesis at Queen Charlotte’s and Chelsea Hospital, London, UK. Indications for amniocentesis were screening for chromosomal abnormalities or therapeutic amniocentesis for polyhydramnios. All volunteers were in their second or third trimester [mean (SD) gestational age, 17.9 (0.9) weeks] and the mean gestational age at partum was 39.2 (0.28) weeks. Exclusion criteria were marked comorbidity and an age <18 years or >45 years. Medical records were reviewed, and amniotic fluid analysis results, pregnancy complications, and pregnancy and birth outcomes were recorded. In all cases, the amniocentesis was uncomplicated, and the outcome of the pregnancy was a healthy baby with a typical karyotype.

We collected 2 mL of amniotic fluid into sterile containers containing 5000 kallikrein inhibitor units of aprotinin (0.2 mL Trasylol; Bayer). Samples were stored at −20 °C until measurement of kisspeptin immunoreactivity (IR) as previously described (3). To reduce preanalytical factors shown to influence RIA measurement of kisspeptin (4), we collected and stored all samples in an identical fashion.

The peptide was extracted from amniotic fluid with Sep-Pak C18 cartridges (Waters) according to the manufacturer’s instructions, and kisspeptin IR was characterized in amniotic fluid by fast protein liquid chromatography, as previously described (3).

The mean (SE) kisspeptin IR in amniotic fluid was 95.9 (14) pmol/L. There was no correlation between gestational age and kisspeptin concentration in amniotic fluid (P = 0.56; Fig. 1). Amniotic kisspeptin concentrations for the 2 fetus sexes were similar [mean kisspeptin IR, 95.2 (27.8) pmol/L and 105.9 (23.2) pmol/L for male and female fetuses, respectively; P = 0.77]. There was no correlation between kisspeptin concentration in the amniotic fluid and either birth weight (P = 0.67) or gestational age at partum (P = 0.58). Kisspeptin IR eluted as a single peak at a position consistent with the elution profile of kisspeptin 54. The calculated mean chromatographic recovery was 46% (7%) (n = 3).

This report is the first to identify kisspeptin in amniotic fluid. Kisspeptin 10 has been shown to inhibit migration and invasion of trophoblast cells in placentation (2); thus, the concentration of kisspeptin in amniotic fluid may be associated with pregnancy outcomes. The concentrations of amniotic fluid kisspeptin (identified as IR in this study) were much lower than those reported for circulating maternal plasma (2). In contrast to maternal plasma concentrations, kisspeptin in amniotic fluid was not observed to increase with gestational age. These findings are perhaps unsurprising, given that a number of amniotic fluid biomarkers display different concentrations during gestation, compared with the concentrations in maternal serum (5). It is possible that the lack of an observed correlation between the kisspeptin concentration in amniotic fluid and gestational age reflects the small number of participants in this study. Amniocentesis is an invasive procedure with an associated risk of miscarriage and is most commonly performed during the second trimester; therefore, the majority of our samples were from women in the second trimester [mean gestational age, 17.9 (0.9) weeks]. Because of this risk, we performed no serial sampling of
amniotic fluid. The study therefore had a limited scope to investigate correlations between the concentration of kisspeptin in amniotic fluid, the plasma kisspeptin concentration, and gestational age.

In this study, the pregnancy outcome in all cases was a healthy baby with no chromosomal abnormalities identified. It would be interesting to investigate kisspeptin concentrations in amniotic fluid samples from a wider cohort to investigate their utility in predicting pregnancy outcome. Given the high placental expression of KISS1, it is likely that the kisspeptin IR detected in amniotic fluid was derived from the placenta (2). Thus, it would be interesting to examine any correlation between the kisspeptin concentration in amniotic fluid and placental weight.

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