syndrome, the concentrations of SSA/SSB antibodies do not correlate with exacerbations *(20)*. Perhaps IgG anti-tTG has clinical value in monitoring these individuals.

Our results are only the first step in exploring the clinical value of IgG anti-tTG assays in patients with autoimmune diseases. In addition to the more fundamental aspects concerning the link between apoptosis and autoimmunity, its role in diagnosis, including sensitivity and specificity, and in the monitoring of patients still has to be elucidated and is the object of further investigations.

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S100B Protein Concentrations in Amniotic Fluid Correlate with Gestational Age and with Cerebral Ultrasound Scanning Results in Healthy Fetuses, *Diego Gazzolo,[1] Matteo Bruschettini,[1] Valentina Corvino,[2] Renzo Oliva,[5] Rossana Sarli,[3] Mario Lituania,[4] Pierluigi Bruschettini,[1] and Fabrizio Michetti[2*]* (Departments of [1] Pediatrics and [4] Obstetrics and Gynecology, Giannina Gaslini Children's University Hospital, I-16147 Genoa, Italy; [2] Institute of Anatomy, Catholic University, I-00168 Rome, Italy; [3] Department of Obstetrics and Gynecology, Genoa University Hospital, I-16121 Genoa, Italy; [5] Laboratory of Immunohematology, Liguria, I-16142 Genoa, Italy; * address correspondence to this author at: Institute of Anatomy, Catholic University, Largo Francesco Vito 1, I-00168 Rome, Italy; fax 39-0630154813, e-mail fabrizio.michetti@rm.unicatt.it)

S100B is an acidic calcium-binding protein of the EF-hand family present in the central nervous system, where it is concentrated mainly in glial cells *(1)*. It has been suggested that this protein is involved in various cellular functions (e.g., cell-cell communication, cell growth, cell structure, energy metabolism, and intracellular signal transduction) and that it may also act as a cytokine with neurotrophic effects at physiological concentrations. In this regard, studies in experimental models on laboratory animals and cell cultures have shown that decreased S100B expression in the nervous tissue correlates with neurobehavioral abnormalities and with microcephaly as a result of in utero cocaine exposure *(2, 3)*. In humans, umbilical blood cord concentrations of S100B have been shown to be inversely correlated with gestational age, suggesting a neurotrophic role for this protein in the third trimester of pregnancy *(4)*. On the other hand, its appearance at high concentrations in biological fluids has been shown to be a reliable marker of brain lesion in adults and pediatric patients and, recently, in the perinatal period *(5–8)*. In particular, the appearance of S100B protein in the amniotic fluid of anencephalic fetuses is considered an indicator of damage in the central nervous system associated with neural tube defects *(9)*.

This study provides reference values of S100B amniotic fluid concentrations during the second trimester of pregnancy.

We investigated, between the 15th and 18th weeks of gestation (mean, 16.5 weeks), 322 women (mean age, 35.5 ± 2.7 years; <35 years, n = 121; >35 years, n = 199) with consecutive physiological singleton pregnancies, who underwent amniocentesis for chromosomal abnormality exclusion (from June 1995 to November 1997). Appropriate fetal growth was defined by the presence of ultrasonographic signs (when the biparietal diameter and abdominal circumference were between the 10th and 90th centiles) according to the nomograms of Campbell and Thoms *(10)* and by postnatal confirmation of a birth weight between the 10th and 90th centiles according to our population standards after correction for the mother's height, weight, and parity and the sex of the newborn. Exclusion criteria were multiple pregnancies; intrauterine

growth retardation; gestational hypertension, diabetes, and infections; fetal malformations; chromosomal abnormalities; maternal exposure to alcohol, cocaine, and tobacco smoke; perinatal asphyxia; and dystocia. The study protocol was approved by the local ethics committee, and the parents of the subjects examined gave informed consent.

At the indicated times, ranging between the 15th and 18th weeks of gestation, 500-μL heparin-treated amniotic fluid samples taken from the amniotic cavity were immediately centrifuged at 900*g* for 10 min, and the supernatants were stored at −70 °C before measurement. The S100B protein concentration was measured in all samples by a commercially available two-site IRMA (Sangtec 100; AB Sangtec Medical, Bromma, Sweden). This method is specific for the β subunit of the protein, which is known to be the predominant form (80–96%) in the human brain *(11, 12)*. Each measurement was performed in duplicate according to the manufacturer's recommendations, and the averages were reported. The detection limit of the assay was 0.2 μg/L. The within-run assay imprecision (CV) was <5%, and the between-run imprecision was <10%.

Data are expressed as the mean ± SD. The amniotic fluid concentrations of S100B and the neonatal monitoring groups were analyzed by means of Kruskal–Wallis one-way ANOVA and Mann–Whitney two-sided *U*-test when data did not follow a gaussian distribution. The relationship between the S100B amniotic fluid concentrations and weeks of gestation was analyzed by linear regression analysis. Multiple linear regression analysis was performed with the S100B concentrations as the dependent variable to analyze the influence of various clinical parameters [gestational age, sonographically estimated fetal weight, head circumference and biparietal diameter, transverse cerebellum diameter, abdominal circumference (all automatically calculated at sampling time points using an Aloka SSD-2000 duplex pulsed color Doppler ultrasonograph with built-in software), gender, and maternal age] on the value of S100B. $P < 0.05$ was considered significant.

At birth, all newborn infants showed normal clinical conditions, and no overt neurological injury was observed on discharge from the hospital. Gestational age at birth (39.4 ± 1.1 weeks), birth weight (2990 ± 109 g), and Apgar scores evaluated at the 1st and 5th min (8 ± 1 and 9 ± 1, respectively) for all infants were within the reference intervals, and the gender distribution was 164 females and 158 males. In this regard, no significant statistical gender differences were observed for mode of delivery, gestational age, birth weight, or Apgar scores ($P > 0.05$, i.e., not significant, for all).

At sampling all fetuses monitored showed appropriate growth according to ultrasound scanning parameters. No gender differences were observed for gestational age (16.5 ± 1 weeks for males vs 16.6 ± 1 weeks for females; $P > 0.05$), fetal weight (189 ± 22 g for males vs 196 ± 28 g for females; $P > 0.05$), head circumference (134 ± 9 mm for males vs 134 ± 10 mm for females; $P > 0.05$), biparietal diameter (116 ± 9 mm for males vs 115 ± 10 mm for females; $P > 0.05$), or transverse cerebellum diameter (15.9 ± 0.9 mm for males vs 15.8 ± 1 mm for females; $P > 0.05$).

S100B concentrations in the amniotic fluid increased progressively from the 15th (0.45 ± 0.11 μg/L) to the 18th (0.58 ± 0.17 μg/L; $P < 0.05$) week of gestation and were positively correlated with the gestational age ($r = 0.21$; $P < 0.001$; Fig. 1). Regarding gender, S100B amniotic fluid concentrations were similar in the two groups (0.48 ± 0.17 μg/L for males vs 0.46 ± 0.14 μg/L for females). No statistically significant differences were found in S100B concentrations when the study group was subdivided according to maternal age (≥35 years, 0.53 ± 0.30 μg/L; <35 years, 0.59 ± 0.23 μg/L; $P > 0.05$, not significant.). Multiple linear regression analysis in which S100B protein was the dependent variable and the monitoring parameters the independent variables showed a significant relationship of S100B concentrations with gestational age ($P < 0.001$), head circumference ($P < 0.001$), and biparietal diameter ($P = 0.027$), whereas no significant relationships were found with transverse cerebellum diameter ($P = 0.34$), estimated fetal weight ($P = 0.78$), gender ($P = 0.41$), and maternal age ($P = 0.23$).

The present findings constitute the first observation of detectable S100B concentrations in amniotic fluid in the second trimester of pregnancy that also show a statistically significant relationship with gestational age and ecographic parameters such as head circumference and biparietal diameter. These relationships could reflect in-

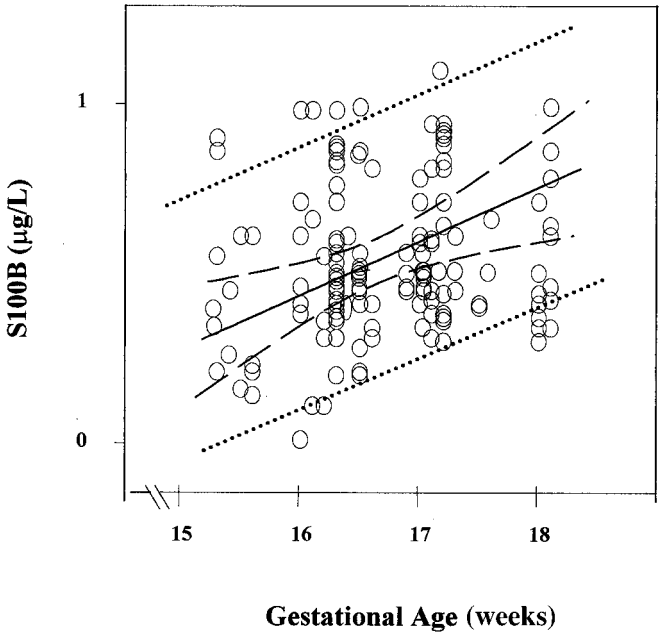

Fig. 1. Correlation of S100B protein blood cord concentrations (μg/L) with gestational age (weeks) at 15–18 weeks of gestation in healthy fetuses.

Values are expressed as median (*solid line*) and interquartile range. The *dotted lines* represent the 3rd and 97th centiles. The *dashed lines* represent the 25th and 75th centiles.

creased glial cell proliferation and S100B production. These associations also appear to be consistent with the hypothesis that, as a cytokine, S100B exerts a neurotrophic role *(1)*, although more extensive studies of the relationships of S100B with other brain constituents will be needed to support this possibility. Previous investigations have reported that amniotic fluid is devoid of detectable S100B in physiological conditions, whereas detectable concentrations can be observed in anencephalic fetuses *(9)*. The reason for the discrepancy between these findings and ours of low but measurable S100B concentrations in healthy fetuses is probably attributable to the different limits of detection of the methods used (0.2 μg/L in our study vs 1.5 μg/L for the method used in the previous study). The present data provide reference values for S100B in amniotic fluid during the second trimester of pregnancy, which could constitute a useful tool for the further study of pathological conditions of the nervous system in the early stages of pregnancy. In this respect, the source of a large part of S100B present in the amniotic fluid is probably the fetal nervous system, where the protein has been shown to be present at the ages investigated in the present study, although not at mature concentrations *(13–16)*. On the other hand, it is possible that S100B could also be released, at least in part, from other sites in which it is concentrated, such as adipose tissue, although data on the presence of the protein in adipose tissue at this age are inconclusive. Finally, the possibility that S100B is released from placental tissue as a trophic factor should be taken into account, although its presence in the placenta has not been documented. In any case, the present findings offer preliminary data supporting further investigation of S100B dynamics in vivo, with special reference to a possible role of the protein in fetal brain maturation.

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Phenotype Determination of Thiopurine Methyltransferase in Erythrocytes by HPLC, *Roselyne Boulieu,[1,2*] Martine Sauviat,[2] Thierry Dervieux,[2,3] Michelle Bertocchi,[4] and Jean-François Mornex[4]* ([1] Université Claude Bernard Lyon 1, Département de Pharmacie Clinique, de Pharmacocinétique, et d'Evaluation du Médicament, 8 avenue Rockefeller, 69373 Lyon Cedex 08, France; [2] Hôpital Neuro-Cardiologique, Service Pharmaceutique, 59 boulevard Pinel, 69394 Lyon Cedex 03, France; [3] St. Jude Children's Research Hospital, 332 N. Lauderlale St., Memphis, TN 38101; [4] Hôpital Cardiologique, Service de Bronchopneumologie, 59 boulevard Pinel, 69394 Lyon Cedex 03, France; * author for correspondence: fax 33-04-72-35-73-31, e-mail roselyne.boulieu@chu-lyon.fr)

Thiopurine methyltransferase (TPMT) is a cytosolic enzyme that catalyzes the *S*-methylation of thiopurine drugs, which are used in cancer chemotherapy and as immunosuppressive agents *(1)*. TPMT activity is controlled by a common genetic polymorphism that contributes to interindividual variability in drug response and, consequently, to implications for thiopurine therapeutic efficacy and toxicity *(2)*. Severe myelosuppression has been reported for TPMT-deficient patients treated with standard doses of thiopurines *(3–5)*, and high TPMT activity has been associated with the rejection of transplanted organs *(6)*. Because of the clinical significance of the TPMT genetic polymorphism, determination of the TPMT phenotype in red blood cells is routinely performed to optimize and individualize thiopurine treatment *(5)*. Variant alleles of the *TPMT* gene have been characterized and associated with low TPMT activity