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AMNIOTIC FLUID INSULIN-LIKE GROWTH FACTOR BINDING-PROTEIN 3 CONCENTRATION AS EARLY INDICATOR OF FETAL GROWTH RESTRICTION

THESE

préparée sous la direction du Docteur Stefan Gerber,
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par

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Amniotic fluid insulin-like growth factor binding protein 3 concentration as early indicator of fetal growth restriction

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<div align="center">Rapport de synthèse</div>

La concentration d'IGFBP-3 dans le liquide amniotique, un indicateur précoce de la croissance intra-utérine

Introduction :

La croissance fœtale intra-utérine dépend d'un grand nombre de facteurs maternels, placentaires et fœtaux. Une inadéquation d'un ou plusieurs de ces facteurs peut induire un retard de croissance intra-utérin (RCIU) ou au contraire une macrosomie. Les principales causes de RCIU comprennent les infections maternelles, l'éclampsie, les cardio-vasculopathies maternelles, la toxicomanie, les malformations fœtales et les insuffisances placentaires. Les facteurs endocriniens constituent un petit pourcentage des causes de RCIU, mais méritent que l'on s'y intéresse de plus près. Les facteurs hormonaux les plus importants pour la croissance fœtale sont l'insuline et les insuline-like growth factors (IGFs) et non l'hormone de croissance (GH) qui joue un rôle majeur dans la croissance postnatale.

Notre attention s'est portée sur IGF-1 qui joue un rôle important dans la croissance intra-utérine. Sa biodisponibilité dépend de plusieurs protéines plasmatiques, les IGF-binding proteins (IGFBP 1 à 9). IGFBP-3 est la principale de ces IGFBPs, autant d'un point de vue quantitatif que fonctionnel.

Nous avons cherché à déterminer si les concentrations d'IGF-1 et d'IGFBP-3 dans le liquide amniotique au début du deuxième trimestre étaient prédictives de la croissance intra-utérine.

Les gènes codant pour IGF-1 et IGFBP-3 contenant certaines séquences polymorphiques, nous avons également étudié leur influence sur la croissance foetale.

L'analyse du liquide amniotique présente l'avantage de pouvoir être effectuée dès la 14ème semaine d'aménorrhée alors que la biométrie foetale échographique ne permet pas à ce stade de déceler des anomalies de la croissance intra-utérine.

Méthode :

Nous avons analysé des échantillons de liquide amniotique prélevés entre la 14ème et la 18ème semaine de grossesse chez 196 patientes. Les concentrations d'IGF-1 et d'IGFBP-3 ont été dosées par ELISA, les polymorphismes analysés par PCR. Ces résultats ont été ensuite analysés en fonction du poids de naissance des nouveaux-nés, répartis en trois groupes : normal pour l'âge gestationnel (AGA), petit pour l'âge gestationnel (SGA) et grand pour l'âge gestationnel (LGA).
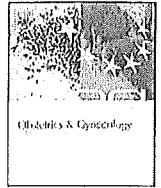
Résultats :

Les concentrations d'IGFBP3 dans le liquide amniotique sont significativement plus élevées (p = 0.030) dans le groupe SGA par rapport au groupe AGA, d'autant plus quand les taux sont ajustés en fonction de paramètres tels que l'âge gestationnel lors de l'amniocentèse (ANCOVA analysis : p = 0.009). La distribution du polymorphisme VNTR (variable number of tandem repeat) dans la région promotrice d'*IGF-1* au sein du groupe SGA est significativement différente de celle du groupe AGA (p = 0.029). En effet, la fréquence de l'association allélique 19CA/20CA est diminuée dans le groupe SGA.

Nous n'avons pas identifié de différence de distribution des séquences polymorphiques d'*IGFBP-3* entre les différents groupes.

Conclusion :

Une concentration élevée d'IGFBP-3 dans le liquide amniotique au début du deuxième trimestre est associée à un risque plus élevé de retard de croissance alors que l'association allélique 19CA/20CA dans la région polymorphique *IGF-1* VNTR est un facteur protecteur.

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Amniotic fluid insulin-like growth factor binding protein 3 concentration as early indicator of fetal growth restriction

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ABSTRACT

Objective: Insulin-like growth factor-I (IGF-I) is an important regulator of fetal growth and its bioavailability depends on insulin-like growth factor binding proteins (IGFBPs). Genes coding for IGF-I and IGFBP3 are polymorphic. We hypothesized that either amniotic fluid protein concentration at the beginning of the second trimester or genotype of one of these two genes could be predictive of abnormal fetal growth.
Study design: Amniotic fluid samples (14–18 weeks of pregnancy) from 123 patients with appropriate for gestational age (AGA) fetuses, 39 patients with small for gestational age (SGA) fetuses and 34 patients with large for gestational age (LGA) were analyzed. Protein concentrations were evaluated by ELISA and gene polymorphisms by PCR.
Results: Amniotic fluid IGFBP3 concentrations were significantly higher in SGA compared to AGA group ($P = 0.030$), and this was even more significant when adjusted to gestational age at the time of amniocentesis and other covariates (ANCOVA analysis: $P = 0.009$). Genotypic distribution of IGF-I variable number of tandem repeats (VNTR) polymorphism was significantly different in SGA compared to AGA group ($P = 0.029$). 19CA/20CA genotype frequency was threefold decreased in SGA compared to AGA group and the risk of SGA occurrence of this genotype was decreased accordingly: OR = 0.289, 95%CI = 0.1–0.9, $P = 0.032$. Genotype distribution of IGFBP3(A-202C) polymorphism was similar in all three groups.
Conclusions: High IGFBP3 concentrations in amniotic fluid at the beginning of the second trimester are associated with increased risks of SGA while 19CA/20CA genotype at IGF-I VNTR polymorphism is associated with reduced risks of SGA. Neither IGFBP3 concentrations, nor IGF-I/IGFBP3 polymorphisms are associated with modified risks of LGA.

1. Introduction

Intrauterine growth is a complex process depending on maternal, fetal and placental factors, but also on environmental conditions. It is principally controlled by growth hormone-independent insulin-like growth factor (IGF) production until just before birth [1–4].

There are two forms of IGF, IGF-I and -II, in which none is able to cross the placental barrier. Animal experiments have shown that IGF-I was principally involved in fetal and postnatal growth/development and IGF-II in placental development [5–8]. 75–80% of IGF is present as a complex with ALS (acid-labile subunit) and insulin-like growth factor binding protein 3 (IGFBP3), 20–25% is associated with other IGFBPs, and less than 1% is free [9]. IGFBPs

increase IGF half-life, control IGF transport, regulate IGF–IGF receptor interaction and also prevent cell growth overstimulation [5]. But IGFBPs also act through their own receptors, inducing pathways sometimes opposed to IGF actions such as cellular growth inhibition or apoptosis stimulation [2,5].

Previous studies in mice have shown that IGF-I KO resulted in intrauterine growth retardation and post-weaning growth defect [10,11], and ALS KO in a modest reduction in postnatal growth [12].

No clear association between birth weight and IGF family members levels in amniotic fluid (AF) at the beginning of the second trimester could be demonstrated [4,13,14], although Tisi et al. reported a significant association between AF IGFBP3 level and newborn birth weight in macrosomes [15].

Genes coding for IGF-I and IGFBP3 are polymorphic. IGF-I gene was shown to contain a Variable Number of Tandem Repeats (VNTR) in the promoter region and IGFBP3 gene a single nucleotide polymorphism (SNP) (A → C) at position −202 in the promoter region.

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If some controversy exists on the association between IGF-I VNTR and IGF-I serum values, most studies showed a significant reduction of IGF-I concentrations in non-carriers of the most common allele, 19CA [16–20]. IGFBP3 SNP was shown to correlate with adult IGFBP3 serum concentrations, the mutated C allele being associated with decreased circulating concentrations [21,22].

Most of the studies looking at the fetal somatotropic axis used cord blood at delivery as starting material. In this study, we analyzed AF samples at the beginning of the second trimester and focused on IGFBP3 protein quantification and *IGF-I/IGFBP3* polymorphisms analysis in three different groups: SGA, AGA, and LGA. We hypothesized that one of these biochemical/genetic markers could be predictive of abnormal fetal growth although an increase in IGFBP3 levels could lead both to an increase in IGF-I stability (stimulatory effect) and an increase in sequestration of the growth factor (inhibitory effect), depending on the context.

2. Materials and methods

2.1. Subjects

AF samples were obtained from women with singleton pregnancies admitted for amniocentesis at the Lausanne University Hospital in 2003–2004. Amniocentesis indications were maternal anxiety, maternal age >35 years, pathological T21 screening tests (predictive value >1:380) or family history of chromosomal abnormality.

Inclusion criteria were amniocentesis at 14–18 weeks of gestation and delivery after 37 weeks. Exclusion criteria were maternal HIV[+], HBV[+], systemic disease or primo-infection (cytomegalovirus, toxoplasmosis, rubella, syphilis and varicella), and fetal malformation or chromosomal abnormality.

SGA was defined by ultrasonographic follow-up during the second/third trimester showing a biometry < 10th percentile and a newborn birth weight < 10th percentile [23], AGA by a newborn birth weight between 10th and 90th percentile and LGA by a newborn birth weight >90th percentile. Gestational age was based

exclusively on first trimester ultrasound (8–10 weeks of gestation). 378 samples were obtained. Out of them, the first 123 AGA only were analyzed, plus all SGA (39 samples) and all LGA (34 samples), to obtain the 180 samples necessary for our analysis following our sample size calculation. Three SGA cases with uterine pathological Doppler were excluded, in order to have a homogeneous SGA population.

This protocol was approved by IRB of Biology/Medicine Faculty of Lausanne University. All patients were informed and asked for their consent before the study. All maternal/fetal data were prospectively recorded and assigned a number to ensure patient anonymity.

2.2. Samples

One milliliter AF was centrifuged 5 min at 2500 rpm. Supernatant was stored at −80 °C and cell pellet used for DNA extraction by adding 240 μl NID (50 mM KCL, 10 mM Tris–HCl pH 8.3, 2.5 mM MgCl$_2$, 1% Brij 35) and 10 μl proteinase K (5 mg/ml). Sample was incubated 1 h at 56 °C, 10 min at 95 °C, and stored at −80 °C. Supernatant was used for protein quantification and pellet for polymorphism studies.

2.3. IGFBP3 quantification

IGFBP3 AF concentrations were measured by ELISA (Biosource Europe SA, Belgium). Detection limit was 10.5 ng/ml, and there was no cross-reactivity for IGF-I, IGFBP1 and IGFBP2.

2.4. IGF-I VNTR polymorphism

Polymorphic region (−841 in the promoter) was amplified by PCR with primers: 5′-GCTTCTATTGCTAGCCAGCTGGTGT-FAM-3′ and 5′-GGGCAAACAATTTTCCTGT-3′. Reaction was carried out in 25 μl: 10 μl DNA, 2.5 μl 10× PCR buffer, 0.5 μl 10 mM dNTPs, 0.15 μl Taq DNA polymerase (Roche), 0.5 μl each primer (50 μM) and 1.5 μl DMSO. Amplification was: 2 min at 94 °C, 35 cycles of 1 min at 94 °C, 30 s at 62 °C and 45 s at 72 °C, and 7 min at 72 °C.

Table 1
Maternal, newborn and amniotic fluid characteristics in SGA, AGA and LGA patient groups.

	SGA (n = 39)	AGA (n = 123)	LGA (n = 34)
Mother			
Age (years)	35.0 ± 4.8 (21–43)	35.7 ± 4.3 (23–44)	35.7 ± 5.0 (24–45)
Gravidity	2.0 ± 0.9 (1–4)[‖]	2.6 ± 1.5 (1–8)	2.8 ± 1.9 (1–10)
Parity	0.6 ± 0.8 (0–3)[*]	1.0 ± 0.9 (0–4)	1.4 ± 1.1 (0–5)[*]
BMI (kg/m^2)	23.2 ± 3.5	23.5 ± 3.8	24.9 ± 3.9
Ethnic origin: caucasian (%)	87.2	86.8	88.2
Smoking (%)	5.3	5.0	2.9
PIH/GD (%)	10.5	8.4	2.9
Newborn			
Birth weight (BW) (g)	2526 ± 257[*]	3349 ± 313	4075 ± 249[*]
Placenta weight (PW) (g)	438 ± 91[*]	607 ± 116	697 ± 115[*]
PW/BW ratio	0.17	0.18	0.17
GA at time of delivery (weeks)	38.9 ± 1.2	39.3 ± 1.2	39.0 ± 1.2
Method of delivery ratio (% CS)	43.6[‡]	21.1	32.4
Gender (% male)	28.2[‡]	52.0	79.4[‡]
Amniotic fluid			
GA at time of amniocentesis (weeks)	16.5 ± 0.8	16.5 ± 0.8	16.5 ± 0.7
IGFBP3 (ng/ml)	6359 ± 384	5384 ± 219	5015 ± 288
Independent Sample *t*-test	P = 0.030		NS
ANCOVA analysis[a]	P = 0.009		NS

PIH = pregnancy-induced hypertension; GD = gestational diabetes; GA = gestational age; CS = caesarean section; NS = not significant. Data are presented as mean ± s.d. except for IGFBP3 values (mean ± s.e.m.). Minima–maxima are indicated in brackets.
[*] P < 0.05 versus AGA by independent Sample t-test.
[‖] P < 0.05 versus AGA by Mann–Whitney U-test.
[‡] P < 0.05 versus AGA by Fisher's exact test.
[a] Covariates include gestational age at the time of amniocentesis, maternal BMI, gravidity, parity, ethnic origin, age, smoking and PIH/GD status, and fetal gender.

PCR product size was determined by Genescan analysis (Microsynth, Balgach, Switzerland). Product size correlated with CA repeat number. Ten samples were sequenced to check Genescan analysis accuracy.

2.5. IGFBP3(A-202C) polymorphism

Polymorphic region (−202 in the promoter) was amplified by PCR with primers: 5′-CCGAGAGCGGAAGGGGTAAG-3′ and 5′-CCCGTGCTTCGCCCTGAGCA-3′. Reaction was carried out as above but amplification was: 2 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 65 °C and 15 s at 72 °C, and 7 min at 72 °C. Avi II restriction enzyme (Roche) was used for PCR digestion (1 h at 37 °C) and DNA fragments were analyzed on 2% agarose gels. Non-mutated allele yielded a 244 bp fragment, and mutated allele two fragments of 164 and 80 bp.

2.6. Statistical analyses

Results are expressed as mean ± standard deviation (s.d.) for patient characteristics and mean ± standard error of means (s.e.m.) for IGFBP3 concentrations. Student's t, Mann–Whitney U, Kruskall–Wallis H, Fisher's exact or Chi^2 tests were used for maternal/newborn characteristics (depending on the type of variable and of variance between groups). ANCOVA analysis was used for IGFBP3 concentrations, using gestational age at the time of amniocentesis, maternal BMI, gravidity, parity, ethnic origin, age, smoking and PIH/GD status and fetal gender as covariates. Binary logistic regression analysis was used to evaluate genotype results versus other parameters potentially influencing fetal growth. Receiver operating characteristic curve analysis was used to evaluate correlation between IGFBP3 concentration and fetal growth phenotype. All statistical analyses were performed using SPSS 14.0 software (SPSS Inc., Chicago, IL) and differences were significant when $P < 0.05$.

3. Results

3.1. Subjects

A total of 196 patients were included in this study, which were 39 SGA, 123 AGA and 34 LGA. Maternal characteristics were comparable in all three groups, except gravidity and parity, which were lower in SGA and higher in LGA compared to AGA group ($P < 0.05$ versus AGA group, except for gravidity in LGA group) (Table 1). For newborn characteristics, rate of caesarean section was significantly higher in SGA than in AGA group ($P = 0.006$), probably due to patient management in case of fetal growth restriction (Table 1). Birth weights were of course significantly different in the three groups, with mean values of 2526 ± 257 g in SGA, 3349 ± 313 g in AGA and 4075 ± 249 g in LGA group ($P < 0.001$ versus AGA group in both cases). Placenta weights were also significantly higher in LGA (697 ± 115 g) and lower in SGA (438 ± 91 g) compared to AGA group (607 ± 116 g) ($P = 0.002$ and $P < 0.001$, respectively), while placenta to birth weight ratio was not significantly modified (0.17 in SGA and LGA, versus 0.18 in AGA group, NS in both cases). Finally, there was a 1.5-fold higher percentage of males in LGA and a 1.8-fold lower percentage of males in SGA, compared to AGA group ($P = 0.004$ and 0.010, respectively).

3.2. IGFBP3 concentration

IGFBP3 AF concentrations were significantly higher in SGA than in AGA group (6359 ± 384 versus 5384 ± 219 ng/ml, $P = 0.030$), while there was no statistical difference between AGA and LGA groups (5384 ± 219 ng/ml versus 5015 ± 288 ng/ml, respectively) (Fig. 1A, Table 1). However, IGFBP3 AF concentration also increased
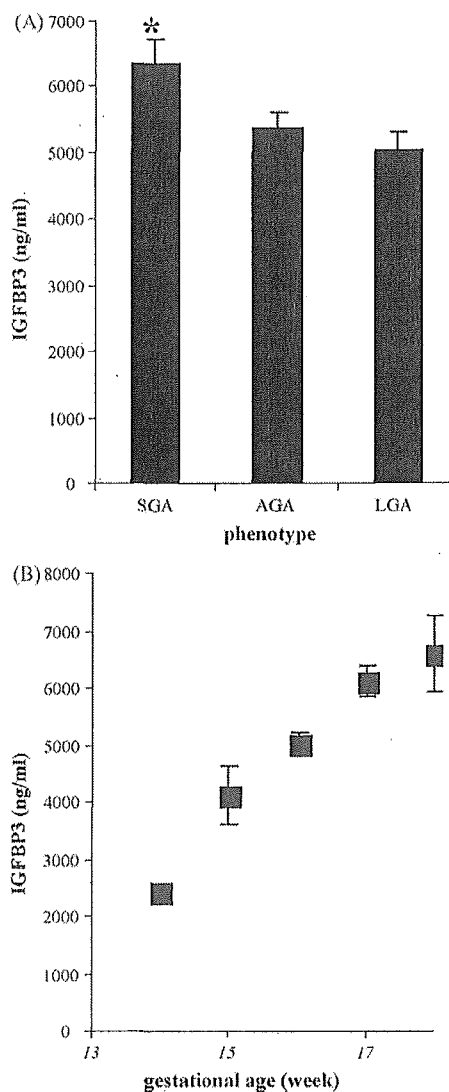

Fig. 1. (A) Amniotic fluid IGFBP3 concentrations are higher in SGA than in AGA group. (B) Amniotic fluid IGFBP3 concentrations proportionally increase with gestational age. Results are expressed as mean ± s.e.m. AF IGFBP3 concentrations were measured by ELISA. Statistical analysis were done by independent Sample t-test and ANCOVA analysis and results were considered significant when $P < 0.05$. *$P < 0.05$ vs. AGA group.

with gestational age (Fig. 1B) and when gestational age and other factors such as maternal BMI, gravidity, parity, ethnic origin, age, smoking and PIH/GD status, and fetal gender were taken into account, the difference between SGA and AGA values was even more significant (ANCOVA analysis: $P = 0.009$) (Table 1). A receiver operating characteristic curve analysis was performed on a sub-group of patients (16–17 weeks of gestation) to minimize the effect of gestational age but to not significantly affect the size of the sample. Area under the curve (AUC) indicated a fair predictive value of IGFBP3 AF concentrations for SGA (AUC = 0.7, 95%CI = 0.6–0.8, $P = 0.005$), and the cut-off value giving the best sensitivity (70%) and specificity (61%) was 5445 ng/ml (data not shown).

3.3. IGF-I VNTR polymorphism

IGF-I VNTR allele distribution was wide, varying from 16 to 22 CA repeats, 19 CA being the most common allele (Table 2). Allele distribution was not statistically modified in SGA, compared to

Table 2
Amniotic fluid IGFBP3 concentration by genotype, and IGF-I/IGFBP3 polymorphisms results in SGA, AGA and LGA patient groups.

| | AF concentration | | Phenotype groups | | | | | | | |
| | IGFBP3 | | SGA | | | | AGA | LGA | | |
	ng/ml	P*	n (%)	Odds ratio (95%CI)	P		n (%)	n (%)	Odds ratio (95%CI)	P
IGF-I VNTR										
Genotype										
19CA/19CA	5344 ± 212		19 (48.7)	1.000			55 (44.7)	16 (47.1)	1.000	
19CA/20CA	5641 ± 312	NS	4 (10.3)	0.289 (0.1–0.9)	0.032		40 (32.5)	7 (20.6)	0.602 (0.2–1.6)	NS
19CA/21CA	7080 ± 939	0.050	4 (10.3)	1.053 (0.3–3.7)	NS		11 (8.9)	3 (8.8)	0.938 (0.2–3.8)	NS
18CA/19CA	4815 ± 561	NS	4 (10.3)	2.895 (0.7–12.7)	NS		4 (3.3)	1 (2.9)	0.859 (0.1–8.2)	NS
Other	5047 ± 388	NS	8 (20.5)	1.781 (0.6–5.0)	NS		13 (10.6)	7 (20.6)	1.841 (0.6–5.4)	NS
Allele										
16CA			1 (1.3)	NA			0 (0.0)	0 (0.0)	NA	
17CA			0 (0.0)	NA			4 (1.6)	0 (0.0)	NA	
18CA			6 (7.7)	3.288 (1.0–10.6)	NS		6 (2.4)	2 (2.9)	1.213 (0.2–6.2)	NS
19CA			52 (66.7)	1.000			171 (69.5)	47 (69.1)	1.000	
20CA			11 (14.1)	0.754 (0.4–1.6)	NS		48 (19.5)	13 (19.1)	0.985 (0.5–2.0)	NS
21CA			7 (9.0)	1.771 (0.7–4.7)	NS		13 (5.3)	3 (4.4)	0.840 (0.2–3.1)	NS
22CA			1 (1.3)	0.822 (0.1–7.5)	NS		4 (1.6)	3 (4.4)	2.729 (0.6–12.6)	NS
IGFBP3 SNP										
Genotype										
AA	5237 ± 265		12 (31.6)	1.000			32 (26.0)	7 (21.2)	1.000	
AC	5667 ± 230	NS	18 (47.4)	0.706 (0.3–1.6)	NS		68 (55.3)	21 (63.6)	1.412 (0.5–3.7)	NS
CC	5523 ± 168	NS	8 (21.1)	0.928 (0.3–2.6)	NS		23 (18.7)	5 (15.2)	0.994 (0.3–3.5)	NS
Allele										
A			42 (55.3)	1.000			132 (53.7)	35 (53.0)	1.000	
C			34 (44.7)	0.937 (0.6–1.6)	NS		114 (46.3)	31 (47.0)	1.026 (0.6–1.8)	NS

NA = not applicable; NS = not significant; *ANCOVA analysis with covariates including gestational age at the time of amniocentesis, maternal BMI, gravidity, parity, ethnic origin, age, smoking and PIH/GD status, and fetal gender; PIH = pregnancy-induced hypertension; GD = gestational diabetes; GA = gestational age; NS = not significant.

AGA group, but a statistically significant difference was observed between SGA and AGA groups at the genotype level (P = 0.029). 19CA/20CA genotype was three-fold less frequent in SGA than in AGA group, which corresponded to a decreased risk of SGA occurrence compared to the most common 19CA/19CA genotype: OR = 0.289, 95%CI = 0.1–0.9, P = 0.032. No statistical difference was observed in allele or genotype distribution between LGA and AGA groups.

Global analysis of the different factors potentially responsible for SGA phenotype indicated that IGF-I 19CA/20CA genotype was a statistically significant component of the equation, as was primiparity (P = 0.045 and 0.034, respectively) (Table 3).

Table 3
Logistic regression analysis of the contribution of suspected/known risk factors to the final SGA phenotype.

| | Phenotype (SGA) | | |
	Exp(B) = Odds ratio	95%CI	P
Mother			
Ethnic origin	0.969	0.3–3.1	NS
PIH/GD	1.293	0.3–5.6	NS
Smoking	1.255	0.2–9.6	NS
Primiparity	2.362	1.1–5.2	0.034
Age	0.954	0.9–1.0	NS
BMI (kg/m²)	0.992	0.9–1.1	NS
Newborn			
IGF-I polymorphism:			NS
19CA/20CA	0.290	0.1–1.0	0.045
19CA/21CA	0.794	0.2–3.0	NS
18CA/19CA	2.164	0.4–11.6	NS
Other	1.495	0.5–4.4	NS
Constant	1.547		NS

IGFBP3 AF concentrations were statistically higher in IGF-I VNTR genotype 19CA/21CA, compared to 19CA homozygotes (Table 2).

3.4. IGFBP3(A-202C) polymorphism

In AGA group, A and C alleles were present with almost the same frequency (53.7 and 46.3%, respectively) (Table 2). AC was the predominant genotype (55.3% of the population) and no significant differences in allele or genotype distribution were observed between SGA or LGA, and AGA groups.

No difference in IGFBP3 AF concentrations was observed between IGFBP3 SNP genotypes (Table 2).

4. Comment

Most of the studies looking at the fetal somatotropic axis have studied cord blood at delivery. Here, we focused on IGFBP3 concentrations at the beginning of the second trimester and studied fetal genetic profiles of *IGF-I* and *IGFBP3* gene polymorphisms in search of predictive markers of abnormal fetal growth.

Maternal and fetal characteristics showed several features already reported, like a gravidity/parity higher in LGA and lower in SGA and a newborn male gender prevalence higher in LGA [24,25], or even a placenta weight and placenta to newborn birth weight ratio smaller in SGA (significant for placenta weight only in our sample collective) [26].

AF IGFBP3 concentrations in SGA group were higher than in AGA or even in LGA groups, no matter what the gestational age. Spearman's correlation analysis indicated a highly significant relationship between IGFBP3 concentrations and newborn percentile: <5th (severe SGA) (6403 ± 559 ng/ml), 5th–10th (light SGA) (6313 ± 539 ng/ml), 10th–50th ("small" AGA) (5654 ± 294 ng/ml), 50th–90th ("large" AGA) (5151 ± 319 ng/ml), >90th percentile (LGA) (5015 ± 288 ng/ml) (P = 0.001) (data not shown). On the

contrary, Tisi et al. reported a positive correlation between AF IGFBP3 concentrations and newborn birth weight in macrosomes [15]. This discrepancy could be due to the degree of ethnic heterogeneity of their population (69% Caucasian versus 87% in ours). But it could also be due to the fact that the positive correlation reported was only found in LGA, whereas their mean IGFBP3 values did not differ between SGA, AGA and LGA [15].

The overall allele distribution of IGF-I VNTR found in our study was similar to previous reports (70% 19CA, 20% 20CA, 5% 21CA and 2% 18CA repeats) [18,19], and it was in Hardy–Weinberg equilibrium. Only one genotype, 19CA/20CA, was associated to a modified (reduced) risk of SGA occurrence. Vaessen et al. had previously described an association between the absence of 19CA in the fetal genome and a reduction in birth weight compared to 19CA homozygotes [27]. We also found a threefold increase in genotypes without 19CA in SGA compared to AGA group (15.4% versus 5.7%), but the risk of SGA occurrence linked with this absence was not statistically modified compared to the presence of one 19CA (OR = 3.731, 95%CI = 1.1–12.8, P = 0.066) or compared to 19CA homozygotes (OR = 2.481, 95%CI = 0.7–8.3, P = 0.183). This difference could be due to our sample size, as only 6–7 individuals were devoid of 19CA allele in SGA and AGA groups, but it could also be due to the fact that the reduction in birth weight reported did not take into account the birth weight percentile [27].

Genotype distribution of IGFBP3 SNP was similar to previous studies that showed a marked predominance of AC genotype (54%) [21,22], and it followed the Hardy–Weinberg law. AA genotype was shown to correlate with higher IGFBP3 circulating concentrations than AC and finally CC genotype in adults, although differences were low [21,22]. In our study, no association between IGFBP3 genotype and AF concentration was observed, even when the gestational age at the time of amniocentesis and the other maternal/fetal characteristics were controlled for (ANCOVA analysis: P = 0.337). This could be due to the fact that factors regulating IGFBP3 gene expression during intrauterine life are different from those expressed at adulthood, or to the fact that AF concentrations are not proportional to circulating concentrations.

Morimoto et al. previously reported a cross-association between the absence of 19CA allele in IGF-I VNTR and higher IGFBP3 plasma concentrations [28] that we did not find in our study, no matter what the gestational age at the time of amniocentesis or the other maternal/fetal parameters used as covariates (ANCOVA analysis: P = 0.882). However, in Morimoto et al. study, differences between 19CA homozygotes and the other genotypes were only of 5% [28], and again, AF concentrations might simply not reflect circulating concentrations.

Mouse animal studies have shown that ubiquitous transgenic expression of IGFBP3 resulted in higher IGFBP3 and IGF-I circulating concentrations and a modest reduction in size of the transgenic pups (10%) which persisted in early postnatal life [29]. On the other hand, IGFBP3 single KO showed no growth phenotype, but IGFBP3-5 triple KO exhibited the prenatal growth defect observed in IGFBP4 single KO, plus a postnatal growth defect significant from day 20 and thereafter. This was associated with a decrease in IGF-I circulating concentrations and bioactivity [30]. It is clear that the presence of multiple members in the IGFBPs family allows compensation mechanisms between members. Moreover, IGFBPs have both inhibitory and stimulatory effects on IGF-I action, depending on the situation. In our study, we found that higher IGFBP3 AF concentrations were associated with lower birth weight percentile, coherent with the transgenic animal profile and with the fact that AF concentrations are proportional to circulating concentrations.

In summary, we have shown that IGFBP3 AF concentration at 14–18 weeks of pregnancy negatively correlated with newborn percentile. We also have shown that a particular IGF-I VNTR

genotype, 19CA/20CA, was associated with a reduced risk of SGA occurrence to the same extent that primiparity was associated with an increased risk of SGA occurrence. These results taken together could help predict abnormal fetal growth in early pregnancy.

Disclosure statement

The authors have nothing to disclose.

Acknowledgment

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