Advance Access publication on March 10, 2008

Interleukin-1 receptor antagonist gene (IL-1RN) polymorphism is a predictive factor of clinical pregnancy after IVF

S. Gremlich[1,3], S. Fratta[2], E. Rebellato[2], R. Uras[2], D. Reymondin[1], F. Damnon[1], M. Germond[2] and S. Gerber[1,3]

[1]*Department of Gynecology and Obstetrics, CHUV Hospital/Maternity, Avenue Pierre Decker 2, 1011 Lausanne, Switzerland;*
[2]*Reproductive Medicine Unit, CHUV Hospital, 1011 Lausanne, Switzerland*

[3]Correspondence address. E-mail: sandrine.gremlich-irrausch@chuv.ch; stefan.gerber@chuv.ch

BACKGROUND: Only 25% of IVF transfer cycles lead to a clinical pregnancy, calling for continued technical progress but also more in depth analysis of patients' individual characteristics. The interleukin-1 (IL-1) system and matrix metalloproteinases (MMPs) are strongly implicated in embryo implantation. The genes coding for IL-1Ra (gene symbol IL-1RN), IL-1β, MMP2 and MMP9 bear functional polymorphisms. We analysed the maternal genetic profile at these polymorphic sites in IVF patients, to determine possible correlations with IVF outcome. METHODS: One hundred and sixty women undergoing an IVF cycle were enrolled and a buccal smear was obtained. The presence of IL-1RN variable number of tandem repeats and IL-1B + 3953, MMP2-1306 and MMP9-1562 single nucleotide substitutions were determined. Patients were divided into pregnancy failures (119), biochemical pregnancies (8) and clinical pregnancies (33). RESULTS: There was a 40% decrease in IL-1RN*2 allele frequency ($P = 0.024$) and a 45% decrease in IL-1RN*2 carrier status in the clinical pregnancy group as compared to the pregnancy failure group ($P = 0.017$). This decrease was still statistically significant after a multivariate logistic regression analysis. The likelihood of a clinical pregnancy was decreased accordingly in IL-1RN*2 carriers: odds ratio = 0.349, 95% confidence interval = 0.2–0.8, $P = 0.017$. The IL-1B, MMP2 and MMP9 polymorphisms showed no correlation with IVF outcome. CONCLUSIONS: IL-1RN*2 allele carriage is associated with a poor prognosis of achieving a pregnancy after IVF.

Keywords: interleukin-1; receptor antagonist; polymorphism; IVF; clinical pregnancy

Introduction

Epidemiological studies have shown that despite continued improvements in assisted reproductive technologies, the clinical pregnancy rate after IVF has remained at about 25% since 1993 (Van den Bergh *et al*., 2005; Wunder *et al*., 2005). Clinical and fundamental research efforts are, therefore, still necessary to understand reasons for this limitation and to improve outcomes.

Achievement of pregnancy relies both on embryo quality and the implantation capability of the maternal endometrium (Chung *et al*., 2006). Therefore, in addition to technical advances leading to the improved embryo quality, preliminary biochemical studies have opened the way to an evaluation of patients' chances to become pregnant after IVF (for review, Broekmans *et al*., 2006). Likewise, genotypic studies have been conducted on several genes such as p53 (Kay *et al*., 2006), HLA-G (for review Hviid, 2006) or the FSH receptor (Jun *et al*., 2006), and suggest that certain genetic profiles may be more advantageous than others in achieving a clinical pregnancy after IVF.

Embryo implantation is composed of two processes, an inflammation phase and an invasion phase. The interleukin-1 (IL-1) system is an important part of the innate immune system. It consists of two agonists (IL-1α and IL-1β), one antagonist (IL-1Ra) and two receptors (IL-1RI and IL-1RII), of which only IL-1RI is biologically active (for review: Sunder and Lenton, 2000). The IL-1β/IL-1Ra ratio defines the overall IL-1 pro-inflammatory response. Animal studies have demonstrated that mice injected with IL-1Ra exhibited a decreased blastocyst implantation frequency, whereas transfer of the same blastocysts to pseudo-pregnant animals reversed this tendency. The hypothesis was that IL-1 produced by the embryo, by interacting with receptors expressed in the maternal endometrium, mediated implantation by inducing production of vascular endothelial growth factor and integrins along with other mediators (for review: Sunder and Lenton, 2000).

Matrix metalloproteinase (MMP)-2 and -9 are associated with syncytiotrophoblast cell penetration into the maternal endometrium and establishment of the placental barrier via spiral arteries invasion (for review: Curry and Osteen, 2003). Knockout mice studies showed that the absence of MMP2 resulted in offspring that were smaller at birth and had a decreased growth rate (Itoh *et al*., 1997), whereas the absence of MMP9 resulted in a decreased breeding efficiency (Vu *et al*., 1998; Dubois *et al*., 1999, 2000).

The genes coding for IL-1Ra (gene symbol IL-1RN), IL-1B, MMP2 and MMP9 all possess genetic polymorphisms in their sequences: the IL-1B gene has three single nucleotide polymorphisms (SNPs) at positions −511 and −31 in the promoter region and +3953 in the coding region; the IL-1RN gene has a variable number (1–6) of tandem repeats (VNTR) of an 86 bp motif in an intronic region; the MMP2 gene has a SNP at position −1306 in the promoter region, and the MMP9 gene has a SNP at position −1562 and a (CA)*n* microsatellite at position −90 in the promoter region.

Fetal carriage of IL-1RN allele 1 (IL-1RN*1) was reported to be linked with an increased rate of spontaneous abortion in women (Perni *et al*., 2004), and maternal carriage of IL-1RN*2 was 3-fold more frequent in women with a recurrent pregnancy loss (Unfried *et al*., 2001). Maternal genetic variants at IL-1B-511C and IL-1B-31T were shown to be associated with an increased rate of recurrent pregnancy loss (Wang *et al*., 2002); the IL-1B+3953 polymorphism was not examined. Finally, MMP2 and MMP9 polymorphisms have never been evaluated in a pregnancy outcome context. We recently showed that the MMP2 polymorphism was associated with a higher risk of intrauterine growth retardation in Caucasian fetuses (Gremlich *et al*., 2007). It has to be emphasized that none of the above studies were done on an IVF population.

We, therefore, decided to investigate a possible link between the maternal genetic profile and pregnancy outcome in an IVF population, to determine if maternal carriage of any particular allele could influence IVF success. Such a finding would be of value as a prognostic indication of a woman's likelihood of a successful IVF outcome.

Material and Methods

Patients and treatment

The patient cohort in this prospective study was composed of women consulting at the Reproductive Medicine Unit of the CHUV hospital between September 2003 and September 2005. Pregnancy follow-up was done by the Reproductive Medicine Unit.

Inclusion criteria were the following of a standard IVF protocol without any modification and providing written informed consent to participate in the study. Exclusion criteria were maternal use of an immunosuppressive drug (e.g. cortisone), maternal pathology or genetic anomaly, maternal inflammatory disease, uterine malformation, diabetes or lupus erythematosus.

Patients either followed a fresh embryo transfer protocol (stimulated-cycle IVF), or a frozen–thawed embryo transfer protocol (natural-cycle IVF) (embryo frozen at the two pronuclei zygote stage). In fresh cycles, for controlled ovarian stimulation (Germond *et al*., 2002), patients were desensitized with GnRH agonist (Decapeptyl; Ferring, Zürich, Switzerland) and follicular growth stimulated with recombinant FSH (Gonal-F; Serono, Switzerland; Puregon, Organon, Switzerland). When at least two follicles reached ≥17 mm in diameter, HCG (Profasi; Serono, Switzerland) was administered. In frozen–thawed cycles, patients monitored their own urine using a commercially available qualitative LH test (OVU-LH; Intex, Muttenz, Switzerland). Transfers were planned 3 days after the LH peak. Thawing of the zygotes was planned 24–26 h earlier, in order to allow for at least one mitotic division to occur. In cases of anovulation, embryos were replaced in artificial cycles using pituitary desensitization in association with estradiol (Progynova; Schering, Baar) and progesterone (Utrogestan; Vifor, Fribourg, Switzerland) administration (de Ziegler and Frydman, 1990). Fourteen days after the transfer, a blood sample was retrieved for a pregnancy test. Pregnancy failure was defined by a βHCG level <2 UI/L. Biochemical pregnancy was defined by a βHCG level between 11 and 1000 UI/L, followed by a decrease of the value and associated with the absence of gestational sac at embryo transfer day 28 (ET28) assessed by ultrasound. Clinical pregnancy was defined by a βHCG level >1000 UI/L, doubling every 48 h, and associated with the presence of a gestational sac at ET28 and presence of cardiac activity at ET35 assessed by ultrasound.

The study protocol was approved by the clinical research ethical committee of the Biology and Medicine Faculty of the University of Lausanne.

Genetic analysis

Buccal smears were frozen at −80°C until use. Upon thawing, they were resuspended in 240 μl NID buffer (50 mM KCl, 10 mM Tris–HCl pH 8.3, 2.5 mM $MgCl_2$, 1% Brij 35) and, after the addition of 10 μl proteinase K (5 mg/ml), incubated 1 h at 56°C to allow protein digestion. Proteinase K was then inactivated by a 10 min heating at 95°C, and DNA samples used for PCR analysis as described below. Primers are listed as forward, then reverse.

IL-1RN VNTR (86-bp repeat in intron 2)

IL-1RN gene polymorphism was analysed as already described (Genc *et al*., 2002). The polymorphic region was amplified by PCR: 35 cycles (30 s at 94°C, 45 s at 60°C, 45 s at 72°C) with primers 5′-CTCAGC AACACTCCTAT-3′ and 5′-TCCTGGTCTGCAGGTAA-3′. The PCR products were analysed by agarose gel electrophoresis. IL-1RN*1 allele corresponded to a 410 bp fragment (four copies of the 86-bp repeat), IL-1RN*2 to a 240-bp fragment (two copies), IL-1RN*3 to a 325-bp fragment (three copies), IL-1R*4 to a 500-bp fragment (five copies), IL-1RN*5 to a 585-bp fragment (six copies) and IL-1RN*6 to a 155-bp fragment (one copy).

IL-1B(C+3953T) polymorphism

IL-1B gene polymorphism was analysed as already described (Genc *et al*., 2002). Polymorphism region was amplified by PCR: three cycles (2 min at 94°C, 2 min at 55°C, 1 min at 74°C) followed by 32 cycles (30 s 94°C, 45 s at 55°C, 45 s at 72°C) with primers 5′-GTTGTCATCAGACTTTGACC-3′ and 5′-TTCAGTTCATATG GACCAGA-3'. PCR products were digested 2 h at 65°C with TaqI restriction enzyme and analysed by agarose gel electrophoresis. Undigested PCR fragment was 249-bp in size (allele T, allele E2), whereas digested fragment yielded two sub-fragments of 114 and 135 bp (allele C, allele E1).

MMP2(C-1306T) polymorphism

MMP2 gene polymorphism was analysed as already described (Gremlich *et al*., 2007). Polymorphism region was amplified by

PCR: 40 cycles (30 s at 94°C, 30 s at 64°C, 30 s at 72°C) with primers 5′-ATATTCCCCACCCAGCAGTC-3′ and 5′-TTGGGAACGCCTG ACTTCAG-3′. Original sequence of the forward primer was modified to create an artificial AccI recognition sequence at the site of the mutation (underlined nucleotide). PCR products were digested one hour at 37°C with AccI and analysed by agarose gel electrophoresis. If there was a C to T transition, the 122 bp PCR fragment generated two sub-fragments of 19 and 103 bp.

MMP9(C-1562T) polymorphism

MMP9 gene polymorphism was analysed as already described (Gremlich *et al*., 2007). Mutation region was amplified by PCR: 30 cycles of 1 min at 94°C, 1 min at 66°C, 1 min at 72°C with primers 5′-GCCTGGCACATAGTAGGCCC-3′ and 5′-CTTCCTAGCCAGC CGGCATC-3′. Polymorphism analysis was done by restriction enzyme digestion (1 h at 37°C with SphI). If there was a C to T transition, the 436 bp PCR fragment generated two sub-fragments of 244 and 192 bp.

Statistical analyses

Differences in means were analysed using Fisher exact test, χ^2-test, Mann–Whitney *U*-test or independent samples *t*-test, depending on the type of variable (continue, discrete or categorical) and the homogeneity of variances between the two data sets. Differences in genotype/allele distribution were evaluated using Fisher exact tests. Associations between IVF outcome and potential confounding factors were evaluated by logistic regression analysis. Values of $P \leq 0.05$ were considered as statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 14.0 software (SPSS Inc., Chicago, IL, USA).

Results

Patients

One hundred and sixty-nine women initially signed the consent form, but nine subsequently withdrew. The 160 women remaining were ultimately classified as pregnancy failures (119), biochemical pregnancies (8) and clinical pregnancies (33). The biochemical pregnancy rate was 5% and the clinical pregnancy rate was 21%.

Patients' characteristics are presented in Table I. Age and body mass index (BMI) were comparable in all three groups, except for a non-significant tendency among women experiencing a biochemical pregnancy to have a mean age and BMI values higher than those in the other groups. Ethnic origin and mean gravidity/parity were also comparable in all three groups. The causes of infertility were in similar proportions for all three groups, as was the mean number of transfers at the time of the study and mean number of oocytes collected, mature oocytes and the ratio between both. The only significant difference was related to the type of transfer (fresh or frozen). The proportion of fresh transfers was greater in women experiencing a biochemical pregnancy (87.5%) than in the other groups (43.7 and 60.6%, respectively, for pregnancy failure and clinical pregnancy groups; $P = 0.024$ for biochemical pregnancy versus pregnancy failure group). However, the biochemical pregnancy group encompassed only eight cases so the significance of this finding remains questionable. The clinical pregnancy rate was 16% for frozen embryo transfers, compared

Table I. Patients characteristics.

	Pregnancy failure $N = 119$	Biochemical pregnancy $N = 8$	Clinical pregnancy $N = 33$	Total population $N = 160$
Mother				
Maternal age (years)	34 ± 4 (25–44)	37 ± 4 (31–42)	33 ± 4 (26–42)	34 ± 4 (25–44)
Maternal body mass index (kg/m^2)	22.9 ± 3.9	26.4 ± 9.2	23.8 ± 4.1	23.3 ± 4.4
Ethnic origin: caucasian (%)	95	100	94	95
Gravidity (%):				
0	57	63	40	53
1	23	12	20	23
≥2	20	25	40	24
Parity (%):				
0	70	75	52	67
1	20	25	33	23
≥2	20	0	15	10
Infertility history				
Infertility origin (%):				
Paternal	51	37.5	55	51
Maternal	24	37.5	30	26
Both conjugated	18	25	12	18
Unknown	7	0	3	5
Infertility (%):				
Primary	83	75	70	80
Secondary	17	25	30	20
Previous transfers (fresh and frozen)	3.4	6.3	3.6	3.6 ± 3.1
Current IVF				
Transfer type (%)				
Fresh	43.7	87.5*	60.6	49.4
Frozen	56.3	12.5	39.4	50.6
Oocytes (%)				
Collected	12.6 ± 6.5	11.0 ± 6.0	12.9 ± 7.1	12.6 ± 6.6
Ripe	10.4 ± 5.7	8.9 ± 5.6	10.7 ± 5.7	10.4 ± 5.7
Ripe/collected	0.83	0.80	0.85	0.83

No significant difference was observed between biochemical or clinical pregnancy groups, and pregnancy failure group by independent sample *t*-test, χ^2-test, Mann–Whitney *U*-test or Fisher exact test, except $*P = 0.024$ by Fisher exact test.

to 25% for fresh embryo transfers and 24 and 22%, respectively, for maternal and paternal infertility origin, compared to 15% for combination of both. However, none of these differences was statistically significant.

Maternal genetic profile

All four gene polymorphisms studied were compatible with Hardy–Weinberg equilibrium.

IL-1RN VNTR (86-bp repeat in intron 2)

In the pregnancy failure group, allelic frequencies were as follows: 63% for the IL-1RN*1 allele, 34% for the IL-1RN*2 allele and 3% for the combined IL-1RN*3, *4 and *6 alleles (Table II, Fig. 1). In the biochemical pregnancy group, no significant differences were observed compared to the pregnancy failure group, except that the minor alleles other than IL-1RN*1 or *2 were absent. In the clinical pregnancy group, however, the IL-1RN*1 allele frequency was increased to 80% and the IL-1RN*2 allele frequency decreased to 20% ($P = 0.026$ compared with the pregnancy failure group). Thus, IL-1RN*2 carriage decreased the chances of clinical pregnancy compared with the IL-1RN*1 allele as follows: odds ratio (OR) = 0.472, 95% confidence interval (CI) = 0.2–0.9, $P = 0.024$. Similarly, IL-1RN*1 homozygotes were almost 2-fold increased and IL-1RN*1/IL-1RN*2 heterozygotes 2-fold decreased, compared with the pregnancy failure group ($P = 0.016$). This modified the chances of IVF success of IL-1RN*1/IL-1RN*2 carriers compared to the IL-1RN*1 homozygote genotype as follows: OR = 0.275, 95% CI = 0.1–0.7, $P = 0.006$. The global pregnancy rate was 29% for IL-1RN*1 homozygotes, versus 13% for IL-1RN*2 carriers (OR = 0.354, 95% CI = 0.2–0.8, $P = 0.018$).

The pregnancy rate linked with IL-1RN*2 carriage was different according to the number of previous IVF attempts. When the number of previous attempts was ≤ 3, the rate of IL-1RN*2 carriers was 54% (39 out of 72) in the pregnancy failure group versus 23% (5 out of 22) in the clinical pregnancy group, but when the number of previous attempts was >3, it was 58% (27 out of 47) in pregnancy failure group versus 46% (5 out of 11) in clinical pregnancy group, modifying clinical pregnancy likelihood in the following way: OR = 0.249, 95% CI = 0.1–0.7, $P = 0.014$ and OR = 0.617, 95% CI = 0.2–2.3, $P = 0.517$, respectively. It is interesting to note that in our study, the pregnancy rate of IL-1RN*2 carriers was decreased in the same way whether following fresh or frozen embryo transfers. Indeed, in fresh embryo transfers, the pregnancy rate was 15% (5 out of 34) in IL-1RN*2 carriers compared with 33% (15 out of 45) in non-carriers, whereas in frozen embryo transfers it was 11% (5 out of 46) compared with 23% (8 out of 35) in non-carriers, a difference which was not statistically significant. The choice of one or the other transfer type, therefore, did not seem to matter for IL-1RN*2 carriers.

IL-1B(C+3953T) polymorphism

For the pregnancy failure group, allele frequencies were 79% for allele 1 and 21% for allele 2, not much different from the 81 and 19% measured in the biochemical pregnancy group and 80 and 20% measured in the clinical pregnancy group

Table II. Genotype and allele distribution of interleukin-1 (IL-1) receptor antagonist (IL-1RN), IL-1B, matrix metalloproteinase (MMP)2 and MMP9 polymorphisms in pregnancy failure, biochemical pregnancy and clinical pregnancy groups.

			Pregnancy failure *N* (%)	Biochemical pregnancy *N* (%)	Odds ratio	95% CI	*P*	Clinical pregnancy *N* (%)	Odds ratio	95% CI	*P*
IL-1RN	Genotype	*1/*1	47 (39)	4 (50)	1.000			23 (70)	1.000		
		*1/*2	52 (44)	3 (38)	0.678	0.1–3.2	NS	7 (21)	0.275	0.1–0.7	0.006
		*2/*2	14 (12)	1 (12)	0.839	0.1–8.1	NS	3 (9)	0.438	0.1–1.7	NS
		Other	6 (5)	0 (0)	NA	NA	NA	0 (0)	NA	NA	
		*2−	53 (44)	4 (50)	1.000			23 (70)	1.000		
		*2+	66 (56)	4 (50)	0.803	0.2–3.4	NS	10 (30)	0.349	0.2–0.8	
	Allele	*1	151 (63)	11 (69)	1.000			53 (80)	1.000		
		*2	81 (34)	5 (31)	0.864	0.3–2.6	NS	13 (20)	0.472	0.2–0.9	0.024
		Other	6 (3)	0 (0)	NA	NA	NA	0 (0)	NA	NA	NA
IL-1B	Genotype	11	78 (65)	5 (63)	1.000			20 (61)	1.000		
		12	33 (28)	3 (37)	1.418	0.3–6.3	NS	13 (39)	1.536	0.7–3.4	NS
		22	8 (7)	0 (0)	NA	NA	NA	0 (0)	NA	NA	NA
	Allele	1	189 (79)	13 (81)	1.000			53 (80)	1.000		
		2	49 (21)	3 (19)	0.890	0.2–3.2	NS	13 (20)	0.946	0.5–1.9	NS
MMP2	Genotype	CC	90 (76)	5 (63)	1.000			25 (76)	1.000		
		CT	26 (22)	3 (37)	2.077	0.5–9.3	NS	7 (21)	0.969	0.4–2.5	NS
		TT	3 (2)	0 (0)	NA	NA	NA	1 (3)	1.200	0.1–12.0	NS
	Allele	C	206 (87)	13 (81)	1.000			57 (86)	1.000		
		T	32 (13)	3 (19)	1.486	0.4–5.5	NS	9 (14)	1.016	0.5–2.3	NS
MMP9	Genotype	CC	83 (70)	5 (63)	1.000			23 (70)	1.000		
		CT	30 (26)	3 (37)	1.660	0.4–7.4	NS	8 (24)	0.962	0.4–2.4	NS
		TT	5 (4)	0 (0)	NA	NA	NA	2 (6)	1.443	0.3–7.9	NS
	Allele	C	196 (83)	13 (81)	1.000			54 (82)	1.000		
		T	40 (17)	3 (19)	1.131	0.3–4.2	NS	12 (18)	1.089	0.5–2.2	NS

NA, not applicable; NS, not significant; CI, confidence interval.

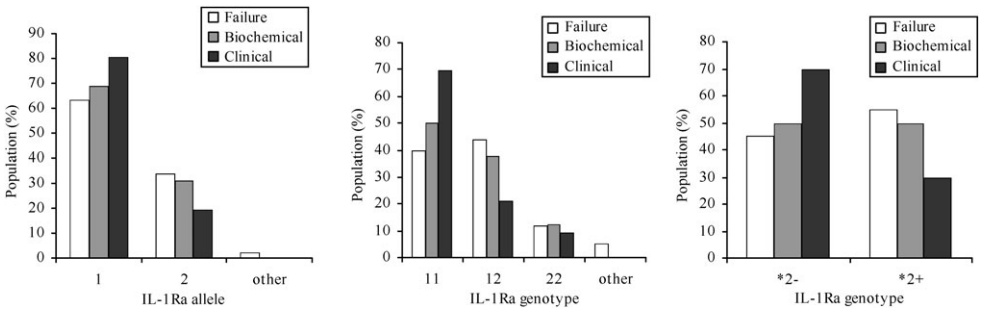

Figure 1: Interleukin-1 (IL-1) receptor antagonist IL-1RN*2 allele and genotype frequencies are decreased in the clinical pregnancy group, compared to the pregnancy failure group. The IL-1RN variable number of tandem repeats genotype was assessed in a cohort of 160 IVF patients classified as: pregnancy failures (119), biochemical pregnancies (8) and clinical pregnancies (33). Results are presented as mean ± SEM

(Table II). Genotype frequencies were also unchanged: 65:28:7% for genotype 1,1, 1,2 and 2,2, respectively, in the pregnancy failure group, compared with 63:37:0% in the biochemical group, and 61:39:0% in the clinical pregnancy group. The combination of both IL-1RN VNTR and IL-1B+3953 SNP did not show a further at risk group.

MMP2(C-1306T) polymorphism

For the pregnancy failure group, the allele frequency was 87% for allele C and 13% for allele T. This was comparable to the 81 and 19% frequencies in the biochemical pregnancy group, and the 86 and 14% frequencies in the clinical pregnancy group (Table II). Genotype distribution was 76% CC, 22% CT and 2% TT for pregnancy failure group, 63% CC, 37% CT and 0% TT for biochemical pregnancy group and 76% CC, 21% CT and 3%TT for clinical pregnancy group, not significantly different in one group compared to the others.

MMP9(C-1562T) polymorphism

In the pregnancy failure group, the allele frequency was 83% for allele C and 17% for allele T, similar to that observed in the clinical pregnancy (82 and 18%) and biochemical pregnancy (81 and 19%) groups (Table II). Genotype frequencies were also similar in all three groups: 70, 26 and 4%, respectively, for CC, CT and TT genotypes in pregnancy failure group, 63, 37 and 0% in biochemical pregnancy group and 70, 24 and 6% in clinical pregnancy group.

Logistic regression analysis

A general model comprising all the factors potentially influencing a pregnancy (personal and IVF-related maternal characteristics) plus our present results was created using the logistic regression model analysis, to evaluate each parameter's influence on the final IVF outcome (Table III). Only maternal age, transfer type (fresh or frozen) and IL-1RN polymorphism (especially the presence of one or two IL-1RN*2 allele), were associated with IVF outcome.

Discussion

Despite ongoing efforts to increase IVF efficiency, the clinical pregnancy rate still remains at ~25% after each transfer cycle (Van den Bergh *et al*., 2005). Therefore, a more precise identification of patients' individual biochemical/genetic profiles may aid in the evaluation of each woman's intrinsic capacity to become pregnant, even prior to any IVF attempt.

In this study, the maternal genetic profile at four gene polymorphisms associated with inflammation and/or invasion were evaluated in a cohort of IVF patients and IL-1RN VNTR was shown to be associated with IVF outcome.

The genotype distributions of all four polymorphisms were similar to previous reports. Indeed, for the MMP2 and MMP9 polymorphisms, allele T frequencies were 13 and 17%, respectively, in accordance with the 12 and 16% frequencies recorded in our previous study on intrauterine growth restriction (IUGR) (Gremlich *et al*., 2007). For the IL-1B and IL-1RN polymorphisms, allele 2 frequencies were 20 and 31%, respectively, comparable to the 21–25 and the 22–29% frequencies recorded in a previous meta-analysis report (Camargo *et al*., 2006).

In this study, we found that the IL-1RN VNTR, but not IL-1B+3953, polymorphism, was associated with IVF outcome. Indeed, the clinical pregnancy rate was more than doubled in the absence of IL-1RN*2 allele, compared with the rate in the presence of one or two IL-1RN*2 alleles. The IL-1RN*2 allele frequency was essentially unchanged when the number of previous IVF attempts was >3, which probably means that beyond three attempts, other factors probably contribute to outcome. This result is consistent with a previous study where Unfried *et al*. (2001) had shown that there was a 3-fold increase in IL-1RN*2 allele frequency in women with recurrent pregnancy loss (three or more consecutive pregnancy losses). We also found that the presence of the IL-1RN*2 allele was associated with an absence of the IL-1B+3953 allele 2 ($P = 0.008$). Santtila *et al*. had previously observed similar findings utilizing DNA from peripheral blood mononuclear cells in a non-pregnant population. Moreover, they had shown that it was the presence or absence of the IL-1RN*2 allele, more than the presence or absence of the IL-1B + 3953 allele 2, that was responsible for the rate of *in vitro* IL-1β production, and that the effect of the IL-1RN*2 allele was to increase IL-1β production (Santtila *et al*., 1998). The IL-1RN*2 allele has also been associated with moderately higher circulating levels of IL-1Ra protein (Hurme and Santtila, 1998), and higher IL-1β production (Santtila *et al*.,

Table III. Logistic regression model of the contribution of the different parameters to the IVF outcome.

		IVF outcome: clinical pregnancy			
		B	Exp(*B*) = odds ratio	95% CI	*P*
Maternal age		−0.148	0.863	0.8–1.0	0.029
Body mass index		0.093	1.097	1.0–1.3	NS
Ethnic origin	Caucasian	1.051	2.860	0.4–19.9	NS
Past history of surgery		0.242	1.274	0.2–6.6	NS
Gravidity	1	0.164	1.178	0.2–5.6	NS
	≥2	1.414	4.113	0.6–26.1	NS
Parity	1	0.860	2.362	0.5–11.9	NS
	≥2	0.557	1.745	0.2–14.4	NS
Infertility origin		−0.411	0.663	0.4–1.3	NS
Previous transfers		0.093	1.098	0.9–1.3	NS
Transfer type		−1.155	0.315	0.1–1.0	0.045
Oocytes collected		−0.005	0.995	0.8–1.3	NS
Oocytes ripe		0.069	1.071	0.8–1.4	NS
IL1RN VNTR polymorphism	*1/*2	−1.948	0.143	0.0–0.5	0.002
	*2/*2	−1.734	0.177	0.0–0.9	0.042
	other	−21.746	0.000	0.0	NS
IL1B+3953	12	0.088	1.092	0.4–3.1	NS
Polymorphism	22	−19.670	0.000	0.0	NS
MMP2-1306	CT	0.181	1.198	0.3–4.5	NS
Polymorphism	TT	1.664	5.280	0.1–202.0	NS
MMP9-1562	CT	−0.404	0.668	0.2–2.3	NS
Polymorphism	TT	0.161	1.175	0.1–12.0	NS
Constant		0.912	2.489		NS

NS, not significant; VNTR, variable number of tandem repeat.

1998; Witkin *et al*., 2003). The net effect of IL-1RN*2 allele carriage is a higher IL-1β/IL-1Ra ratio and an elevated pro-inflammatory response. Our genetic study also corroborates a previous biochemical study where Spandorfer *et al*. found that a relative antagonism of the IL-1 system, i.e. a low level of IL-1β associated with a high level of IL-1Ra in maternal serum, was associated with a higher clinical pregnancy rate in an IVF population (Spandorfer *et al*., 2003).

The IL-1RN VNTR has also been studied in the fetal genome. Perni *et al*. (2004) were able to show that fetal carriage of IL-1RN*2 was associated with higher amniotic fluid concentrations of IL-1β and to a lesser extent IL-1Ra, and with a decreased history of spontaneous abortions. Altogether, IL-1RN*2 maternal carriage seems to have a negative influence on pregnancy while fetal carriage of the same allele may have a positive one. The inflammatory episode associated with embryo implantation is, however, subjected to a very tight equilibrium: a too low inflammation process could result in implantation failure, whereas too high inflammation could signify rejection of the embryo. It is possible that the combined fetal and maternal genetic profile at IL-1RN VNTR is important. The potentially antagonistic contribution of both maternal and the fetal IL-1RN genotypes can be paralleled to the situation of graft acceptance. Indeed, IL-1 gene cluster polymorphisms, including IL-1RN VNTR and IL-1B+3953 SNP, are associated with different graft-versus-host diseases, when present in the donor or the host (for review, Cullup and Starck, 2005).

In the present study, we found no association between MMP2 or MMP9 SNPs and IVF outcome. Previous indirect evidence (animal studies and *in vitro* studies) had highlighted roles for MMP2 and MMP9 in embryo implantation (for review: Curry and Osteen, 2003) and more recently, our group had shown that fetal carriage of MMP2-1306 polymorphism allele T was linked to an increased risk of IUGR (Gremlich *et al*., 2007). Our present negative result does not mean, however, that maternal MMPs production is not important in embryo implantation in IVF. Fetal carriage of MMP polymorphisms was not evaluated and could be more important than maternal profile in this context. We also did not evaluate for tissue inhibitor of metalloproteinase which would influence the extent of MMP activity. Lastly, the actual MMP protein concentration at the site of implantation and at the time of implantation was not measured.

In conclusion, this study confirms a predominant role of inflammation in embryo implantation, and also adds a genetic marker to a list of favorable/unfavorable indications for IVF success. In the future, IL-1RN genetic profiling may be useful to screen prospective patients prior to their initial IVF cycle. In addition, knowledge of IL-1RN*2 carriage may eventually result in individualized medicinal intervention prior to any IVF attempt to modulate the maternal inflammatory response, or a supplementation of the embryo culture medium in order to minimize its influence.

References

Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. *Hum Reprod Update* 2006;**12**:685–718 (Review).

Camargo MC, Mera R, Correa P, Peek RM, Jr, Fontham ET, Goodman KJ, Piazuelo MB, Sicinschi L, Zabaleta J, Schneider BG. Interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms and gastric cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2006;**15**:1674–1687.

Chung K, Coutifaris C, Chalian R, Lin K, Ratcliffe SJ, Castelbaum AJ, Freedman MF, Barnhart KT. Factors influencing adverse perinatal outcomes in pregnancies achieved through use of in vitro fertilization. *Fertil Steril* 2006;**86**:1634–1641.

Cullup H, Stark G. Interleukin-1 polymorphisms and graft-vs-host disease. *Leuk Lymphoma* 2005;**46**:517–523 (Review).

Curry TE, Jr, Osteen KG. The matrix metalloproteinase system: changes, regulation, and impact throughout the ovarian and uterine reproductive cycle. *Endocr Rev* 2003;**24**:428–465.

de Ziegler D, Frydman R. Different implantation rates after transfers of cryopreserved embryos originating from donated oocytes or from regular in vitro fertilization. *Fertil Steril* 1990;**54**:682–688.

Dubois B, Arnold B, Opdenakker G. Gelatinase B deficiency impairs reproduction. *J Clin Invest* 2000;**106**:627–628.

Dubois B, Masure S, Hurtenbach U, Paemen L, Heremans H, van den Oord J, Sciot R, Meinhardt T, Hammerling G, Opdenakker G *et al.* Resistance of young gelatinase B-deficient mice to experimental autoimmune encephalomyelitis and necrotizing tail lesions. *J Clin Invest* 1999;**104**: 1507–1515.

Genc MR, Gerber S, Nesin M, Witkin SS. Polymorphism in the interleukin-1 gene complex and spontaneous preterm delivery. *Am J Obstet Gynecol* 2002;**187**:157–163.

Germond M, Capelli P, Bruno G, Vesnaver S, Senn A, Rouge N, Biollaz J. Comparison of the efficacy and safety of two formulations of micronized progesterone (Ellios and Utrogestan) used as luteal phase support after in vitro fertilization. *Fertil Steril* 2002;**77**:313–317.

Gremlich S, Nguyen D, Reymondin D, Hohlfeld P, Vial Y, Witkin SS, Gerber S. Fetal MMP2/MMP9 polymorphisms and intrauterine growth restriction risk. *J Reprod Immunol* 2007;**74**:143–151.

Hurme M, Santtila S. IL-1 receptor antagonist (IL-1Ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1beta genes. *Eur J Immunol* 1998;**28**:2598–2602.

Hviid TV. HLA-G in human reproduction: aspects of genetics, function and pregnancy complications. *Hum Reprod Update* 2006;**12**:209–232 (Review).

Itoh T, Ikeda T, Gomi H, Nakao S, Suzuki T, Itohara S. Unaltered secretion of beta-amyloid precursor protein in gelatinase A (matrix metalloproteinase 2)-deficient mice. *J Biol Chem* 1997;**272**:22389–22392.

Jun JK, Yoon JS, Ku SY, Choi YM, Hwang KR, Park SY, Lee GH, Lee WD, Kim SH, Kim JG *et al.* Follicle-stimulating hormone receptor gene polymorphism and ovarian responses to controlled ovarian hyperstimulation for IVF-ET. *J Hum Genet* 2006;**51**:665–670.

Kay C, Jeyendran RS, Coulam CB. p53 tumour suppressor gene polymorphism is associated with recurrent implantation failure. *Reprod Biomed Online* 2006;**13**:492–496.

Perni SC, Vardhana S, Tuttle SL, Kalish RB, Chasen ST, Witkin SS. Fetal interleukin-1 receptor antagonist gene polymorphism, intra-amniotic interleukin-1beta levels, and history of spontaneous abortion. *Am J Obstet Gynecol* 2004;**191**:1318–1323.

Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. *Scand J Immunol* 1998;**47**:195–198.

Spandorfer SD, Neuer A, Liu HC, Rosenwaks Z, Witkin SS. Involvement of interleukin-1 and the interleukin-1 receptor antagonist in in vitro embryo development among women undergoing in vitro fertilization-embryo transfer. *J Assist Reprod Genet* 2003;**20**:502–505.

Sunder S, Lenton EA. Endocrinology of the peri-implantation period. *Baillieres Best Pract Res Clin Obstet Gynaecol* 2000;**14**:789–800 (Review).

Unfried G, Tempfer C, Schneeberger C, Widmar B, Nagele F, Huber JC. Interleukin 1 receptor antagonist polymorphism in women with idiopathic recurrent miscarriage. *Fertil Steril* 2001;**75**:683–687.

Van den Bergh M, Hohl MK, De Geyter Ch, Stalberg AM, Limoni C. Ten years of Swiss National IVF Register FIVNAT-CH. Are we making progress? *Reprod Biomed Online* 2005;**11**:632–640.

Vu TH, Shipley JM, Bergers G, Berger JE, Helms JA, Hanahan D, Shapiro SD, Senior RM, Werb Z. MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell* 1998; **93**:411–422.

Wang ZC, Yunis EJ, De los Santos MJ, Xiao L, Anderson DJ, Hill JA. T helper 1-type immunity to trophoblast antigens in women with a history of recurrent pregnancy loss is associated with polymorphism of the IL1B promoter region. *Genes Immun* 2002;**3**:38–42.

Witkin SS, Vardhana S, Yih M, Doh K, Bongiovanni AM, Gerber S. Polymorphism in intron 2 of the fetal interleukin-1 receptor antagonist genotype influences midtrimester amniotic fluid concentrations of interleukin-1beta and interleukin-1 receptor antagonist and pregnancy outcome. *Am J Obstet Gynecol* 2003;**189**:1413–1417.

Wunder DM, Limoni C, Birkhauser MH Swiss FIVNAT-Group. Lack of seasonal variations in fertilization, pregnancy and implantation rates in women undergoing IVF. *Hum Reprod* 2005;**20**:3122–3129.

Submitted on October 25, 2007; resubmitted on December 20, 2007; accepted on January 22, 2008