H. Beydoun
A.F. Saftlas

Review Article

Association of human leucocyte antigen sharing with recurrent spontaneous abortions

Key words:
human leucocyte antigen; immunogenetics; major histocompatibility complex; maternal–foetal sharing; recurrent spontaneous abortion

Acknowledgment:
This study was supported by grant RO1-HD-325790 from the National Institute of Child Health and Human Development.

Abstract: An estimated 15% of clinically recognized pregnancies abort spontaneously. Recurrent spontaneous abortion (RSA) is defined as three or more consecutive miscarriages conceived with the same partner in the absence of uterine, genetic or autoimmune abnormalities. Evidence points to human leucocyte antigens (HLA) as playing a role in the successful development of the foetus. In particular, HLA compatibility is more prevalent in couples experiencing reproductive failure, especially RSA couples, compared to fertile couples. According to the immunological hypothesis, an adequate immune response is necessary for proper implantation of the embryo; conversely, a depressed response of maternal lymphocytes to the stimulation by paternal antigens because of HLA sharing can result in disorders, such as RSA. The genetic hypothesis implicates homozygosity for recessive lethal alleles in linkage disequilibrium with specific HLA haplotypes. The specificity of HLA alleles or haplotypes responsible for or linked to other RSA susceptibility genes remains unclear. In this study, we identified 40 observational studies (32 case-control, five cohort, one cross-sectional, one case series and one basic science) that examined the associations between HLA and RSA, focusing on HLA allele couple and maternal–foetal sharing, and the special role of HLA-G. We sought to identify consistent findings among studies examining similar questions. Evidence remains divided concerning the role of HLA allele couple sharing. Of major concern is the focus of many studies on couple sharing as a proxy measure of maternal–foetal sharing. Therefore, adequately powered studies are needed, which employ standard case definitions and reproducible methodologies to directly assess the role of maternal–foetal HLA sharing on the risk of RSA.

Authors' affiliation:

H. Beydoun
A.F. Saftlas

Department of Epidemiology, University of Iowa, Iowa City, IA, USA

Correspondence to:
Dr Audrey F. Saftlas
University of Iowa College of Public Health
Department of Epidemiology
GH C21-F
200 Hawkins Drive
Iowa City
IA 52242
USA
Tel.: +1 319 384 5013
Fax: +1 319 384 5004
e-mail:
audrey-saftlas@uiowa.edu

Received and accepted for publication 7 December 2004

doi: 10.1111/j.1399-0039.2005.00367.x

Tissue Antigens 2005: **65:** 123–135

Successful human reproduction requires the coexistence of mother and foetus, two antigenically dissimilar beings (1, 2). Thus, under ideal conditions, mothers do not reject their semiallogeneic foetuses, despite differences in genetic makeup (3). In the 1950s, Medawar was the first to address the unique immunology of the maternal–foetal interface and its potential relevance to the field of transplantation (4). Many studies, thereafter, have highlighted the important contribution of the placenta to immune tolerance (2, 5–8). The placenta is recognized as the structure that provides oxygen and nutrition to the developing foetus (5). In addition, placental trophoblasts act as an

immunological barrier, capable of protecting the foetus from potential rejection by the maternal immune system, mainly through their lack of expression of highly polymorphic HLA antigens (5, 7–11).

Recent studies have demonstrated that maternal recognition of paternally derived foetal antigens occurs at the time of mating and conception, as well as implantation and throughout pregnancy, which may be beneficial for the establishment and maintenance of gestation (7, 9, 12). Disruption of the maternal–foetal immune relationship is often implicated in the pathophysiology of pregnancy complications, such as preeclampsia, eclampsia, intrauterine growth retardation and recurrent foetal miscarriage (9, 13–15).

An estimated 15% of clinically recognized pregnancies abort spontaneously (16). Recurrent spontaneous abortions (RSA), wherein a definite cause – genetic or environmental – cannot be established, affects one in 400 couples (16). RSA is defined as the occurrence of three or more consecutive aborted pregnancies conceived with the same partner in the absence of uterine, genetic or autoimmune abnormalities (16–20). A number of studies have distinguished three RSA subgroups: (i) primary (three or more consecutive abortions and no history of live births), (ii) secondary (three or more consecutive abortions after one live birth) and (iii) potential (two consecutive abortions) aborters (16–18). In 30% of RSA patients, no demonstrable reason has been identified (17, 20). In addition, the chances of a subsequent loss of pregnancy in a woman who has suffered three or more such losses previously have been reported to be more than 30% (16).

Current evidence suggests that human leucocyte antigen (HLA) expression in tissues at the maternal–foetal interface almost certainly plays a role in the successful development of the foetus (21). According to Thomas and co-workers, considerable evidence points to HLA compatibility or sharing being more prevalent in couples experiencing reproductive failure, especially RSA couples, compared to couples having normal childbearing capabilities (17). It is presumed that when the foetus expresses paternally derived HLA that is shared with maternal HLA, the initial maternal immune response to foetal antigens will be deficient (22). Therefore, HLA incompatibility between mother and foetus, rather than histocompatibility as hypothesized in earlier studies, appears to confer a selective advantage in terms of fertility and reproductive success (22–28).

Two distinct hypotheses have been advanced. According to the immunological hypothesis, an adequate maternal immune response is necessary for proper implantation; by contrast, a depressed response of maternal lymphocytes to the stimulation by paternal antigens in couples can result in disorders, such as RSA (17). The genetic hypothesis attributes reproductive failure to homozygosity for recessive lethal alleles that are in linkage disequilibrium with specific HLA haplotypes (17). Thus, sharing of HLA antigens may be just the detectable marker for the segment of chromosome that carries these genes (17, 29) (Fig. 1).

Materials and methods

Using serological methods and genotyping, numerous observational studies have examined the associations between classical or nonclassical HLA loci and the recurrence risk of spontaneous abortion, focusing on HLA sharing (30). The current study is an update of a qualitative review previously performed by Ober and van Der Ven (30), which has summarized the results from 32 observational studies on HLA couple sharing and unexplained primary or secondary RSA. In this study, we re-examine the role of HLA couple sharing as well as maternal–foetal sharing and the special role of HLA-G.

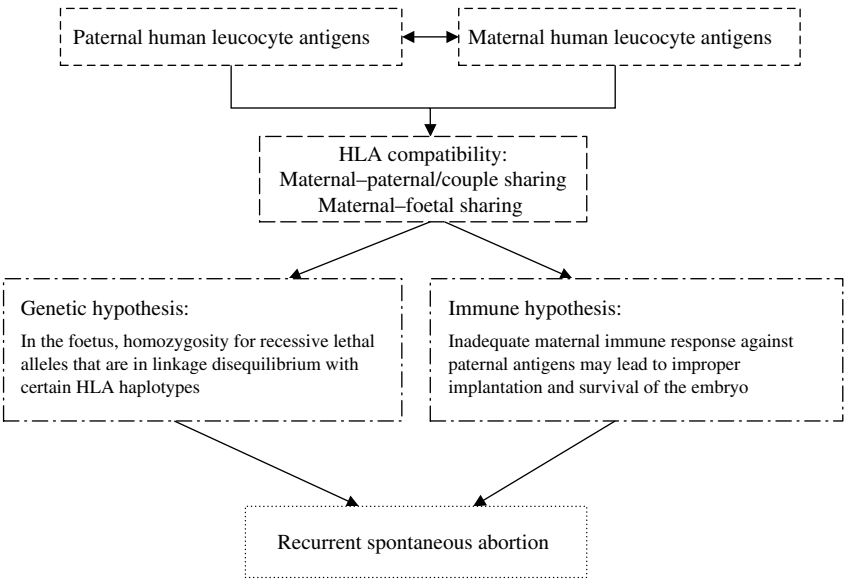

Fig. 1. **Conceptual framework for the immunological and genetic hypotheses regarding the effect of human leucocyte antigen (HLA) sharing on recurrent spontaneous abortion (RSA).**

A series of PUBMED searches were performed in order to identify all relevant studies within the period (1975–2004) that contain MESH terms, such as 'major histocompatibility complex', 'human leucocyte antigen' and 'recurrent spontaneous abortion' and cross-referenced studies were obtained. A total of 42 studies were identified (17–20, 31–68). Of those, two were later excluded (38, 48), because their main objective was different from testing an effect of HLA on RSA. Nineteen of the selected studies were not reviewed previously by Ober and van Der Ven (19, 20, 27, 30, 32, 36, 37, 39, 40, 49, 51, 57, 60–63, 65–68). Of the 40 selected studies, the vast majority had adopted a case-control design, five a cohort approach (27, 32, 61, 64, 66), one was cross-sectional (19), another was a case series (34) and one had a basic sciences (65) design. Most studies reviewed in this study examined couple sharing (16–19, 27, 31–37, 39–42, 44–47, 49–57, 59–61), three were concerned with maternal–foetal sharing of HLA genotype (19, 58, 59) and nine relatively recent studies examined HLA-G characteristics (20, 60, 62–68) as potential risk factors for RSA.

A meta-analysis was performed among a subset of case-control studies that tested the relationship between primary RSA and classical (class IA and II) HLA couple sharing. Inclusion criteria for meta-analysis were specified in order to select studies that were most consistent in terms of outcome and exposure definitions. These criteria were (i) case definition: three or more consecutive abortions of unknown aetiology with the same partner and no previous live births; (ii) control group selection: fertile couples; (iii) HLA sharing assessment: compatibility between partners at HLA-A, HLA-B, HLA-C, HLA-DR or HLA-DQ; (iv) effect size calculation: data are available in a 2×2 table format for the computation of odds ratios (OR) and 95% confidence intervals (95% CI) for each HLA locus separately.

The effect of HLA-DQ allele sharing on RSA was reported in only two of the selected studies (53, 59), prohibiting us from performing a meta-analysis at this locus. In addition, some studies did not report locus-specific allele sharing (16, 33, 36, 41, 43, 59), and were thereby excluded from the meta-analysis. Of the 32 studies that examined HLA couple sharing in the context of RSA, 13 met all inclusion criteria (31, 35, 37, 39, 40, 42, 44, 46, 47, 51–53, 55). The Q-statistic was used in order to assess heterogeneity among the studies. Both fixed and random effects Mantel-Haenszel OR and their 95% CI are reported. Random effects estimates were used, if the Q-statistic was significant (HLA-A and HLA-B) at an alpha level of 5%. Otherwise, fixed effects estimates were deemed appropriate (HLA-C and HLA-DR) (69–71).

Results

HLA couple sharing

The interest in HLA couple sharing was fuelled by the belief that the identification of the specific HLA locus associated with an increased RSA susceptibility may be helpful in targeting those couples that would benefit from immunotherapeutic blood transfusions (72, 73). Although the effectiveness of immunotherapy in treating RSA remains controversial, the use of various forms of immunotherapy has become widespread (73). A systematic review of 19 randomized trials of various forms of immunotherapy (paternal cell immunization, third-party donor leucocytes, trophoblast membranes and intravenous immune globulin) in women with three or more prior miscarriages and no more than one live birth showed no significant beneficial effect of such treatments over placebo in preventing further miscarriages. Further research is needed in order to clarify the effect or the lack of effect of specific immunotherapeutic treatments in the RSA population.

Empirical evidence from 32 studies (16 positive and 16 negative) does not implicate couple sharing at any one particular HLA locus or allele as a significant risk marker for RSA. In fact, couple sharing at the HLA-A (16, 31, 32, 34, 37, 39, 40, 53), HLA-B (16, 17, 31, 32, 34, 39, 40, 53, 61), HLA-C (61), HLA-DR (16–18, 34, 36, 41, 45, 53) and HLA-DQ (18, 47, 53, 59) loci has been reported to be positively associated with the risk of RSA. At least three studies identified couples at risk of RSA, if they shared alleles at any of a number of HLA loci (16, 39, 53), and one reported an increased risk among couples who shared the entire 16-loci HLA haplotype (61). By contrast, other studies have failed to identify a relation between RSA and couple sharing at HLA-A (17–19, 27, 33, 35, 36, 46, 49–52, 54, 55), HLA-B (18, 19, 27, 33, 35–37, 46, 49–52, 54, 55), HLA-C (18, 49, 54), HLA-DR (19, 27, 33, 35, 37, 46, 49–52, 54–56) or HLA-DQ (57) (Table 1).

Table 2 and Figs 2–5 present the results from a meta-analysis of 13 studies that met our inclusion criteria and assessed the impact of couple sharing at HLA-A (31, 35, 37, 39, 40, 42, 44, 46, 47, 51–53), HLA-B (31, 35, 37, 39, 40, 42, 44, 46, 47, 51–53), HLA-C (39, 42, 44, 47, 53) and HLA-DR (35, 37, 42, 44, 46, 47, 51, 53, 55) loci on primary RSA. Q-values showed significant heterogeneity among the studies of HLA-A and HLA-B, but not HLA-C and HLA-DR. Therefore, the random effects summary OR is appropriate in the studies of couple sharing at HLA-A and HLA-B, whereas the fixed effects summary OR is used in meta-analyses of sharing at HLA-C and HLA-DR. The pooled OR for the association of HLA-A with RSA risk was suggestive of an increased risk, although it was not statistically significant (OR = 1.392, 95% CI = 0.945–2.049) (Fig. 2). Meta-analyses of sharing at HLA-B and HLA-C on the risk of RSA showed essentially no evidence of an effect (Figs 3 and 4). Pooled analyses suggested a significantly increased risk of RSA (OR = 1.330, 95% CI = 1.013–1.748) among couples who shared at least one allele at the HLA-DR locus (Fig. 5).

According to Ober and van Der Ven's qualitative review (30), studies relating HLA couple sharing to RSA often varied on several

Classical human leucocyte antigen (HLA) couple sharing and the risk of recurrent spontaneous abortion (RSA): empiric evidence

Author	Study design	Patients	Controls	HLA biomarker	Study findings		
					+	ND	–
Gerencer et al. (31)	Case-control	303 RSA women + 45 husbands (45 couples)	79 normal unrelated couples	A, B	A, B	–	–
Schacter et al. (32)	Cohort	77 couples with adverse obstetric history (SA or NTD)	17 fertile couples	A, B	A, B (*RSA, NTD)*	–	–
Caudle et al. (33)	Case-control	12 primary and secondary RSA couples	77 fertile couples	A, B, C, DR (# of antigens shared)	–	A, B, C, DR (# of antigens shared)	–
Unander and Olding (34)	Case series	8 RSA couples	European general population	A, B, DR	A, B, DR	–	–
Oksenberg et al. (35)	Matched case-control (age, ethnicity)	60 (class I) RSA 30 (class II) RSA	30 fertile couples (class I) 30 fertile couples (class II) 60 random panel couples	A, B, DR	–	A, B, DR	–
Reznikoff-Etievant et al. (36)	Case-control	20 RSA couples	32 fertile couples 100 normal controls	A, B, C, DR	DR	A, B	–
Schacter et al. (37)	Case-control	(RSA (primary + secondary); NTD) couples	Fertile couples	A, B, DR	A (primary and secondary RSA)	B, DR (primary and secondary RSA)	–
Bolis et al. (39)	Case-control	28 RSA couples	28 fertile couples	Overall (class I & II)	Overall (Class I & II)	–	–
Casciani et al. (40)	Case-control	18 RSA couples	23 fertile couples	A, B	A, B (low frequency antigens)	–	–
Taylor et al. (41)	Case-control	139 RSA couples	103 fertile couples	HLA	HLA (at least 2 loci)		
Thomas et al. (17)	Case-control	21 Caucasian RSA couples	388 unrelated Caucasians (HLA-A, -B) 180 unrelated Caucasians (HLA-DR)	A, B, DR	B, DR (A, B), (A, B, DR)	A	–
Vanoli et al. (42)	Case-control	47 primary URSA couples	65 fertile couples Control panel: 98 males, 92 females	Overall HLA	–	Overall HLA	–
McIntyre et al. (43)	Case-control	161 primary and secondary URSA couples	103 fertile couples	Overall HLA	Overall HLA (Primary URSA)	Overall HLA (Secondary URSA)	–
Takakuwa et al. (44)	Case-control	20 URSA couples	10 fertile couples	HLA	D/DR	–	–

Coulam et al. (45)	Case-control	59 primary and secondary RSA couples 79 unexplained infertility couples	51 fertile couples	DR, DQ	DR: primary *vs* control DQ: secondary *vs* control	–	–
Cauchi et al. (46)	Case-control	85 RSA (43 primary; 42 secondary) couples	Fertile control couples	A, B, DR	–	(A, B, DR)	–
Johnson et al. (47)	Case-control	80 primary URSA couples 33 secondary URSA couples	Fertile couples	HLA	–	HLA	–
Ober et al. (27)	Cohort	111 Hutterite couples	–	A, B, DR	–	A, B, DR	–
Sciorelli et al. (49)	Case-control	96 RSA couples	124 fertile couples 204 randomly paired males and females	A, B, C, DR	–	A, B, C, DR	–
Smith and Cowchock (50)	Case-control	115 URSA couples	41 ERSA couples	A, B, DR (# of antigens shared)	–	A, B, DR (# of antigens shared)	–
Balasch et al. (51)	Case-control	57 RSA couples	57 fertile couples	A, B, DR	–	A, B, DR	–
Christiansen et al. (52)	Case-control	56 RSA couples	33 fertile couples	A, B, DR	–	A, B, DR (single locus OR combination of loci)	–
Ho et al. (53)	Case-control	91 primary RSA couples 32 secondary RSA couples	51 normal fertile couples	A, B, DR, DQ	Primary: A, DQ Primary & secondary: Three or more of (A, B, DR, DQ)	–	–
Chang et al. (54)	Case-control	25 primary RSA couples 11 secondary RSA couples	35 multiparous couples	A, B, C, DR (A, B), (A, B, DR)	–	A, B, C, DR (A, B), (A, B, DR)	–
Koyama et al. (18)	Case-control	99 RSA couples (primary, secondary, potential)	Fertile couples: 1578 (class I MHC) 1492 (class II MHC)	A, B, C, DR, Drw52/53, DQ	DR, DQ (primary RSA)	A, B, C	
Eroglu et al. (55)	Case-control	60 RSA couples	60 normal fertile couples	A, B, DR	–	A, B, DR	–
Ito et al. (56)	Case-control	URSA: 82 primary aborters 21 secondary aborters	Normal fertile couples	DR	–	DR	–

Continued

Author	Study design	Patients	Controls	HLA biomarker	Study findings		
					+	ND	–
Takakuwa et al. (57)	Case-control	22 RSA couples	20 fertile couples	DQB	–	DQB	–
Purandare et al. (16)	Case-control	103 RSA couples	1342 (Class I) 430 (Class II) fertile couples	A, B, DR	(A, B, DR) (overall)	–	–
Kilpatrick and Liston (19)	Cross-sectional	36 RSA couples	–	A, B, DR	–	A, B, DR (birthweight)	–
Ober et al. (59)	Case-control	40 abortuses (RSA couples who had undergone leucocyte immunization)	31 liveborn children (RSA couples who had undergone leucocyte immunization)	DQA1, DQB1	–	DQA1, DQB1	–
Ober et al. (61)	Cohort (10-year)	111 Hutterite couples; 251 pregnancies; outcome: abortion	–	A, B, C, DR, DQ	(entire 16-loci haplotype) B, C, C4	A, DR, DQ	–

HLA, human leucocyte antigen; ERSA, explained recurrent abortion; NTD, neural-tube Defects RSA, recurrent spontaneous abortion; SA, spontaneous abortion; URSA, unexplained recurrent abortion; ND, no difference between patients and controls. +: higher among patients; –: higher among controls.

Table 1

Effect of classical human leucocyte antigen (HLA-A, HLA-B, HLA-C or HLA-DR) couple sharing on the risk of recurrent spontaneous abortion (RSA): a meta-analysis of 12 case-control studies

	HLA-A[a]			HLA-B[b]			HLA-C[c]			HLA-DR[d]		
Author	Sample size	OR	95% CI	Sample size	OR	95% CI	Sample size	OR	95% CI	Sample size	OR	95% CI
Gerencer et al. (31)	168	2.408	1.248–4.645	168	1.824	0.939–3.540	–	–	–	–	–	–
Oksenberg et al. (35)	90	0.704	0.287–1.725	90	0.476	0.189–1.195	–	–	–	60	1.144	0.414–3.166
Schacter et al. (37)	412	1.910	1.211–3.014	412	1.644	0.911–2.965	–	–	–	300	1.611	0.914–2.840
Bolis et al (39)	56	9.750	1.927–49.333	56	2.179	0.624–7.611	56	7.364	0.824–65.833	–	–	–
Casciani et al. (40)	41	2.946	0.821–10.580	41	0.260	0.0590–1.152	–	–	–	–	–	–
Vanoli et al. (42)	113	1.354	0.635–2.884	107	1.303	0.530–3.203	51	1.778	0.320–9.885	50	5.120	0.584–44.9101
Takakuwa et al. (44)	31	0.167	0.0173–1.602	31	1.571	0.242–10.217	31	0.600	0.119–3.032	31	8.667	1.662–45.208
Cauchi et al. (46)	146	0.541	0.265–1.107	146	0.303	0.128–0.716	–	–	–	146	1.632	0.806–3.302
Johnson et al. (47)	131	0.982	0.486–1.985	131	2.160	1.000–4.667	98	0.846	0.383–1.871	98	1.090	0.453–2.623
Christiansen et al. (52)	89	0.750	0.313–1.797	89	0.947	0.379–2.367	–	–	–	–	–	–
Balasch et al. (51)	114	1.677	0.786–3.581	114	0.621	0.283–1.363	–	–	–	114	1.074	0.512–2.255
Ho et al. (53)	142	2.429	1.174–5.022	142	0.947	0.438–2.048	142	1.146	0.562–2.337	142	1.642	0.810–3.329
Eroglu et al. (55)	–	–	–	–	–	–	–	–	–	120	0.500	0.229–1.091
Fixed effects	1533	1.441	1.161–1.788	1527	1.046	0.825–1.327	378	1.150	0.727–1.819	1061	1.330	1.013–1.748
Random effects	1533	1.392	0.945–2.049	1527	0.992	0.668–1.475	378	1.105	0.674–1.813	1061	1.366	0.921–2.026

[a] Q-value = 28.945; d.f. = 11; P = 0.00231.
[b] Q-value = 26.037; d.f. = 11; P = 0.00641.
[c] Q-value = 4.201; d.f. = 4; P = 0.37947.
[d] Q-value = 14.186; d.f. = 8; P = 0.07704.

Table 2

methodological issues, such as case definition, the locus or the combination of loci examined and tissue typing methods, all of which might account for the inconsistent findings among these studies. In addition, many of these studies were plagued by small sample sizes. Of major concern, however, is the focus of most studies on couple sharing, which does not directly address foetal histocompatibility, because couple sharing of one antigen at a locus will produce both maternal compatible and incompatible foetuses at relatively equal frequencies (30).

HLA maternal–foetal sharing

Despite the fact that sharing of HLA alleles between mother and foetus is the primary exposure of interest for which couple sharing is merely a proxy measure, very few studies have directly assessed maternal–foetal HLA sharing as a predictor of RSA (19, 58, 59). A case series by Kilpatrick and Liston reported an increased incidence of RSA in women who shared HLA-A, HLA-B or HLA-DR alleles with their offspring (19). It is worth noting that the latter study found no significant association between RSA and couple sharing of HLA

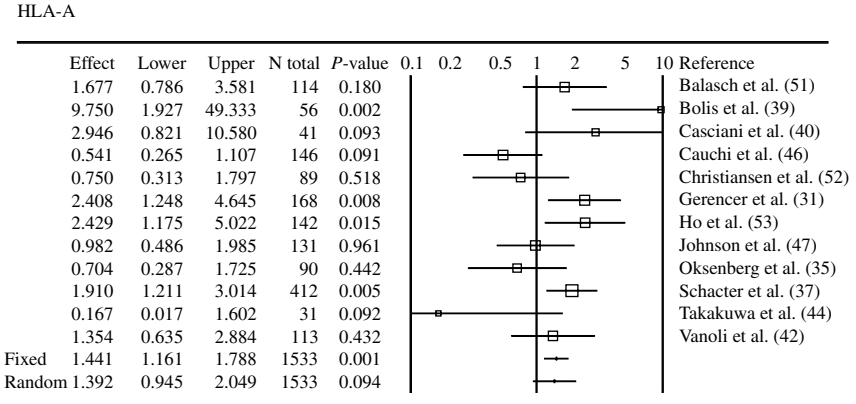

Fig. 2. **Meta-analysis of recurrent spontaneous abortion (RSA) and human leucocyte antigen-A (HLA-A) sharing.** Effect = odds ratio (OR) estimate; lower = lower limit of 95% confidence interval (95% CI) for OR; upper = upper limit of 95% CI for OR; N total = total sample size; P-value = significance of OR estimate.

HLA-B

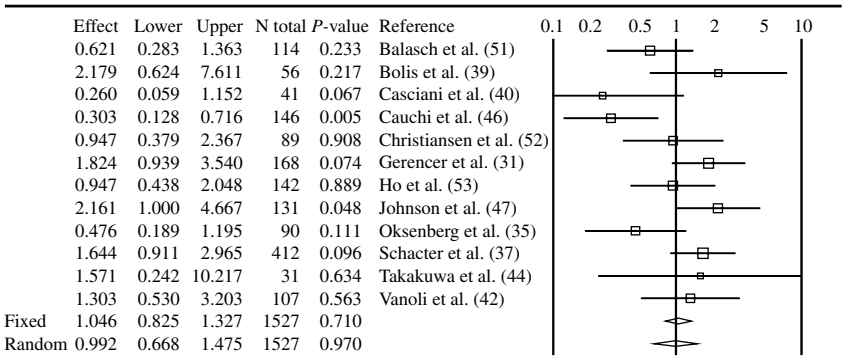

Fig. 3. **Meta-analysis of recurrent spontaneous abortion (RSA) and human leucocyte antigen-B (HLA-B) sharing.** Effect = odds ratio (OR) estimate; lower = lower limit of 95% confidence interval (95% CI) for OR; upper = upper limit of 95% CI for OR; N total = total sample size; *P*-value = significance of OR estimate.

HLA-C

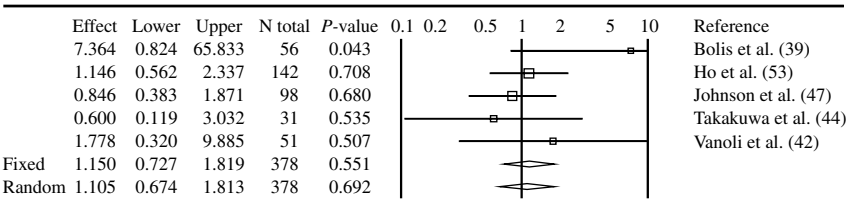

Fig. 4. **Meta-analysis of recurrent spontaneous abortion (RSA) and human leucocyte antigen-C (HLA-C) sharing.** Effect = odds ratio (OR) estimate; lower = lower limit of 95% confidence interval (95% CI) for OR; upper = upper limit of 95% CI for OR; N total = total sample size; *P*-value = significance of OR estimate.

alleles (19). On the other hand, Laitinen and co-workers (58) reported no association between RSA and foetal–maternal sharing at the HLA-DQ locus. Finally, Ober and co-workers reported a non-significant deficit of aborted foetuses and a significant deficit of liveborn children who were compatible at the HLA-DQA1 locus (59). The authors suggest that this deficit of HLA-DQA1 compatible foetuses after nearly 8-week gestation may show that HLA-DQA1 compatible foetuses are aborted early in pregnancy, before the time when foetal tissue can be recovered for genetic studies (59) (Table 3).

HLA-G characteristics

The HLA-G gene, primarily expressed in placental cells that invade the maternal decidua during pregnancy, encodes multiple isoforms that fulfil a variety of functions at the maternal–foetal interface throughout pregnancy (66). The effect of HLA-G expression, polymorphism or sharing on the risk of RSA and related pathologies of pregnancy was reported by eight studies. Of those, one study found an inverse association between RSA risk and HLA-G expression (65), whereas five studies implicated HLA-G polymorphism as a risk factor for RSA (20, 64, 66–68). Two studies hypothesized that HLA-G couple sharing was a determinant of RSA, but found no significant association (60, 67).

A Finnish study was performed among 38 RSA couples and 26 random fertile couples in order to investigate an association between HLA-G locus and habitual abortion (60). Results showed that parental sharing of HLA-G, extended HLA-G/A haplotypes and frequencies of HLA-G alleles were similar within the two groups (60). In a Hungarian study of HLA-G polymorphism and the risk of RSA, 21 RSA couples were compared to 72 randomly selected healthy people (62). Three HLA-G alleles were identified (HLA-G*01011, HLA-G*01003 and HLA-G*01013) (62). However, the results showed no significant difference in the allele frequencies between patient and control groups (62). Yamashita and co-workers performed a case-control study in order to clarify whether there is a difference in the

HLA-DR

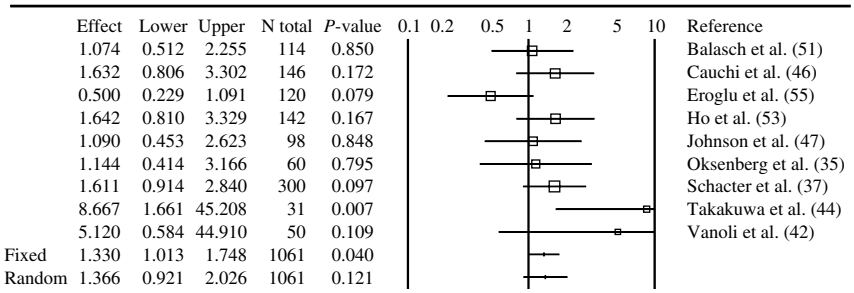

Fig. 5. **Meta-analysis of recurrent spontaneous abortion (RSA) and human leucocyte antigen-DR (HLA-DR) sharing.** Effect = odds ratio (OR) estimate; lower = lower limit of 95% confidence interval (95% CI) for OR; upper = upper limit of 95% CI for OR; N total = total sample size; *P*-value = significance of OR estimate.

Classical human leucocyte antigen (HLA) maternal–foetal sharing and the risk of recurrent spontaneous abortion (RSA): empirical evidence[a]

Maternal–foetal sharing	Study design	Patients	Controls	HLA biomarker	Study findings		
					+	ND	–
Kilpatrick and Liston (19)	Cross-sectional	36 RSA mother, father, baby triads	–	A, B, DR	A, B, DR (birthweight)	–	–
Laitinen et al. (58)	Case-control	35 primary RSA triads 15 secondary RSA triads	40 randomly selected Finnish families	DRB, DQA, DQB, DPA	–	DQ	–
Ober et al. (59)	Case-control	40 abortuses (RSA couples who had undergone leucocyte immunization)	31 liveborn children (RSA couples who had undergone leucocyte immunization)	DQA1, DQB1	–	DQA1, DQB1	–

RSA = Recurrent spontaneous abortion; ND, no difference between patients and controls; +: higher among patients; –: higher among controls.

Table 3

allele frequency of HLA-G among 20 RSA couples *vs* 54 healthy couples (63). The results suggested that the frequency of each allele in the affected women and their husbands did not significantly differ from that of the healthy people (63). Pfeiffer and co-workers studied 78 RSA women and 52 normal fertile controls. The results showed that HLA-G allele frequencies in women who had suffered five or more RSA differed significantly from those in fertile controls and in women who had undergone three or four RSA (20). In addition, there was a significantly higher frequency of HLA-G alleles – HLA-G*01013 and HLA-G*0105N – among all RSA women *vs* the fertile controls (20). The effects of seven HLA-G polymorphisms on the risk of subsequent miscarriage were analysed by Aldrich and co-workers (64) among 113 couples with unexplained RSA. The presence of an HLA-G*0104 or HLA-G*0105N allele in either partner was significantly associated with an increased risk for miscarriage, after adjustment for maternal age, the number of previous miscarriages, history of a previous birth and treatment with paternal mononuclear cells (64). In a recent study by Hviid and co-workers, 61 RSA couples and 47 fertile controls were genotyped for specific HLA-G polymorphisms. Although no statistically significant differences were observed in the distribution of HLA-G alleles between the study groups, 15% of RSA women carried HLA-G*0106 allele, compared to only 2% of control women (67). These data did not support HLA-G histocompatibility as a determinant of RSA (67). Emmer and co-workers showed that, in contrast to normal pregnancy tissue, recurrent miscarriage tissue had sustained natural killer cell marker expression paralleled by the decreased expression of HLA-G (65). Recently, Ober and co-workers found a wide variation in HLA-G promoter region, which is likely to influence miscarriage rates (66). Finally, in a case-control study by Hviid and co-workers, a 14-bp deletion/insertion HLA-G polymorphism was significantly associated with infertility measured as unsuccessful *in vitro* fertilization (IVF) as well as RSA (68) (Table 4).

Despite the diverse properties and functions attributed to HLA-G, which may prevent maternal rejection of the foetal allograft, the number of published studies is at the moment very small, and thus no definitive conclusions can be drawn.

Discussion

In this study, we have highlighted some of the major issues and findings of studies that attempt to relate HLA characteristics to adverse pregnancy outcomes, specifically RSA. In the process, we sought to identify some consistencies among similar studies. Difficulties arise when trying to evaluate and compare studies that (i) test a wide range of hypotheses, (ii) adopt various classifications for the same disease (primary *vs* secondary; unexplained *vs* explained RSA), (iii) use various control groups, including normal fertile women and couples, unrelated patients or people from the general population, (iv) examine various HLA loci as biomarkers of interest, (v) perform various laboratory techniques for HLA typing, (vi) use various methods of statistical analysis and (vii) fail to adjust for potentially confounding factors (74).

Empirical evidence appears to be ambiguous as to whether one or several specific HLA alleles may be involved in the pathogenesis of RSA. Moreover, it is not clear whether these HLA antigens are themselves the susceptibility factors or are linked to other genes that are the main causative agents for the onset of RSA.

On the other hand, the vast majority of studies that focused on HLA sharing were mainly concerned with couple sharing as a proxy of foetal–maternal sharing. Studies examining the association of HLA couple sharing with the risk of RSA have yielded inconsistent results, in terms of whether or not couple sharing is significantly related to the outcome of interest and which particular HLA genes may be responsible. Our meta-analysis of selected case-control studies suggested a slightly increased and significant risk of RSA

Human leucocyte antigen-G (HLA-G) characteristics and the risk of recurrent spontaneous abortion (RSA): empirical evidence[a]

HLA characteristic	Study design	Patients	Controls	biomarker	Study findings		
					+	ND	–
HLA-G expression/polymorphism							
Karhukorpi et al. (60)	Case-control	38 RSA couples	26 random fertile couples	G	–	G (alleles, extended G/A haplotypes)	–
Penzes et al. (62)	Case-control	21 RSA couples	72 normal couples	G	–	*01011, *01013	–
Yamashita et al. (63)	Case-control	20 RSA couples	54 normal couples	G	–	G*01011 G*01012 G*01013 G*0104	–
Aldrich et al. (64)	Cohort	113 RSA couples	–	G	G*0104 G*0105 N	–	–
Pfeiffer et al. (20)	Case-control	78 RSA women (≥5 abortions; ≥3 abortions)	52 normotensive women	G	G*01013, G*0105 N	–	–
Emmer et al. (65)	Basic Science	9 RSA tissue sections	11 non-RSA tissue sections	G	Low HLA-G expression	–	–
Hviid et al. (67)	Case-control	61 RSA couples	47 non-RSA couples	G	G*0106	–	–
Ober et al. (66)	Cohort (15-year)	42 Hutterite women	–	G	Promoter region SNP	–	–
Hviid et al. (68)	Case-control	29 IVF, 61 RSA women	93 fertile women	G	14-bp deletion/insertion in exon 8 of HLA-G	–	–
HLA-G sharing							
Karhukorpi et al. (60)	Case-control	38 RSA couples	26 normal couples	G	–	G (alleles, extended G/A haplotypes)	–
Hviid et al. (67)	Case-control	61 RSA couples	47 non-RSA couples	G	–	G	–

RSA, recurrent spontaneous abortion; ND, no difference between patients and controls; +: higher among patients; –: higher among controls.

Table 4

among couples who shared at least one allele at the HLA-DR locus, but not at other HLA loci.

The genes controlling virtually all immune responses in humans are situated in the HLA complex, a tightly linked region on chromosome 6, which also contains many non-HLA 'passenger' genes, including tumour necrosis factor-α (TNF-α) and other Th1 and Th2 cytokines, which have been associated with pregnancy complications, such as preeclampsia (75, 76). It was postulated that in normal pregnancy, the ratio of Th1 (pro-inflammatory T-helper) to Th2 (suppressor T-helper) cells is shifted towards the suppressor phenotypes (77), which is believed to facilitate immune tolerance. Whereas Th1 cells function in allograft rejection and secrete pro-inflammatory cytokines – such as TNF-α, interleukin-2 (IL-2) and INF-γ – Th2 cells induce antibody production and synthesize suppressor cytokines – such as IL-4, IL-5, IL-6 and IL-10 (78). Recent studies have showed that Th1 cytokines predominate in pregnancies complicated by preeclampsia and RSA, possibly accounting for the poor placentation common to both conditions (77, 79). This makes us question whether or not any particular HLA allele is, in fact, playing a direct role in the pathogenesis of pregnancy disorders, or whether it is merely an indicator of other linked genes (e.g., TNF-α) embedded in the HLA haplotype.

Further epidemiological research is needed in order to clarify the role of HLA antigens that may be either responsible for or linked to other genes that increase the risk of RSA and other adverse outcomes

of pregnancy. Adequately powered studies that employ larger sample sizes, standard case definitions and reproducible methodologies should be adopted for comparative purposes. Some ethical, cultural and logistical barriers need to be overcome in order to facilitate the implementation of maternal–paternal–foetal triad studies in the context of RSA. As previously recommended by Ober and van Der Ven (30), overcoming these barriers would allow a more direct assessment of the effect of maternal–foetal HLA sharing on the risk of RSA to advance the understanding of this troubling reproductive condition.

References

1. Redman CWG, Bodmer JG, Bodmer WF, Beilin LJ, Bonnar J. HLA antigens in severe pre-eclampsia. *Lancet* 1978: **2**: 397–9.
2. De Luca Brunori I, Battini L, Simonelli M et al. HLA-DR in couples associated with preeclampsia: background and updating by DNA sequencing. *J Reprod Immunol* 2003: **59**: 235–43.
3. Hunt JS, Orr HT. HLA and maternal-fetal recognition. *FASEB J* 1992: **6**: 2344–8.
4. Medawar PB. Some immunological and endocrinological problems raised by the evolution of vivparity in vertebrates. *Symp Soc Exp Biol* 1953: **7**: 320–38.
5. Colbern GT, Chiang MH, Main EK. Expression of the nonclassic histocompatibility antigen HLA-G by preeclamptic placenta. *Am J Obstet Gynecol* 1994: **170**: 1244–50.
6. McMaster MT, Librach CL, Zhou Y et al. Human placental HLA-G expression is restricted to differentiated cytotrophoblasts. *J Immunol* 1995: **154**: 3771–8.
7. De Luca Brunori I, Battini L, Simonelli N et al. Increased HLA-DR homozygosity associated with pre-eclampsia. *Hum Reprod* 2000: **15**: 1807–12.
8. Hunt JS, Jadhav L, Chu W, Geraghty DE, Ober C. Soluble HLA-G circulates in maternal blood during pregnancy. *Am J Obstet Gynecol* 2000: **183**: 682–8.
9. Sibai BM. Immunologic aspects of preeclampsia. *Clin Obstet Gynecol* 1991: **34**: 27–34.
10. Colbern GT, Main EK. Immunology of the maternal-placental interface in normal pregnancy. *Semin Perinatol* 1991: **15**: 196–205.
11. Le Bouteiller P, Mallet V. HLA-G and pregnancy. *Rev Reprod* 1997: **2**: 7–13.
12. Cowan LD, Hudson L, Bobele G, Chancellor I, Baker J. Maternal-fetal HLA sharing and risk of newborn encephalophathy and seizure: a pilot study. *J Child Neurol* 1994: **9**: 173–7.
13. Verp MS, Sibul M, Billstarnd C, Bellen G,Hsu M, Ober L. Maternal-fetal histocompatibility in intrauterine growth retarded and normal weight babies. *Am J Reprod Immunol* 1993: **29**: 195–8.
14. Hara N, Fujii T, Yamashita T, Kozuma S, OkaiT, Taketani Y. Altered expression of human leukocyte antigen G (HLA-G) on extravillous trophoblasts in preeclampsia: immunological demonstration with anti-HLA-G antibody '87G' and anti-cytokeratin antibody 'CAM5.2'. *Am J Reprod Immunol* 1996: **36**: 349–58.
15. Takakuwa K, Honda K, Ishii K, Hataya I, Yasuda M, Tanaka K. Studies on the HLA-DRB1 genotypes in Japanese women with severe preeclampsia positive and negative for anticardiolipin antibody using a PCR-RFLP method. *Hum Reprod* 1999: **14**: 2980–6.
16. Purandare AS, Smith DS, Wilson PJ. HLA frequency, HLA sharing and immunotherapy in the management of recurrent miscarriage. *Int J Fertil Menopausal Stud* 1993: **38**: 219–24.
17. Thomas ML, Harger JH, WagenerDK, Rabin BS, Gill TJ III. HLA sharing and spontaneous abortion in humans. *Am J Obstet Gynecol* 1985: **151**: 1053–8.
18. Koyama M, Saji F, Takahashi S et al. Probabilistic assessment of the HLA sharing of recurrent spontaneous abortion couples in the Japanese population. *Tissue Antigens* 1991: **37**: 211–7.
19. Kilpatrick DC, Liston WA. Parental HLA sharing, feto-maternal compatibility and neonatal birthweight in families with a history of recurrent spontaneous abortion. *Dis Markers* 1993: **11**: 125–30.
20. Pfeiffer KA, Fimmers R, Engels G et al. The HLA-G genotype is potentially associated with idiopathic recurrent spontaneous abortion. *Mol Hum Reprod* 2001: **7**: 373–8.
21. Aldrich C, Verp MS, Walker MA, Ober C. A null mutation in HLA-G is not associated with preeclampsia or intrauterine growth retardation. *J Reprod Immunol* 2000: **47**: 41–8.
22. Beer AE, Quebbeman JF, AyersJWT, Haines RF. Major histocompatibility antigens, maternal and paternal immune response, and chronic habitual abortions in humans. *Am J Obstet Gynecol* 1981: **141**: 987–99.
23. Lauritsen JG, Jorgensen J, Kissmeyer-Nielson F. Significance of HLA and blood-group incompatibility in spontaneous abortion. *Clin Genet* 1976: **7**: 575–82.
24. Komlos L, Zamir R, Joshua H, Halbrecht I. Common HLA antigens in couples with repeated abortions. *Clin Immunol Immunopathol* 1977: **7**: 330–5.
25. Gill TJ III. Immunogenetics of spontaneous abortions in humans. *Transplantation* 1983: **35**: 1–6.
26. Beer AE, Need JA. Immunological aspects of preeclampsia/eclampsia. *Birth Defects Orig Artic Ser* 1985: **21**: 131–54.
27. Ober C, Elias S, O'Brien E et al. HLA sharing and fertility in Hutterite couples: evidence for prenatal selection against compatible fetuses. *Am J Reprod Immunol Microbiol* 1988: **18**: 111–5.
28. Hauck WW, Ober C. Statistical analysis of outcomes from repeated pregnancies: effects of HLA sharing on fetal loss rates. *Genet Epidemiol* 1991: **8**: 187–97.
29. Hedrick PW. HLA-sharing, recurrent spontaneous abortion and the genetic hypothesis. *Genetics* 1988: **119**: 199–204.
30. Ober C, van Der Ven K. Immunogenetics of reproduction: an overview. In: Hunt JB, ed. HLA and the Maternal-Fetal Relationship. Austin: RG Landes 1995: 133–56.
31. Gerencer M, Drazancic A, Kuvacic I, Tomaskovic Z, Kastelan A. HLA antigen studies in women with recurrent gestational disorders. *Fertil Steril* 1979: **31**: 401–4.
32. Schacter B, MuirA, Gyves M, Tasin M. HLA-A, B compatibility in parents of offspring with neural-tube defects or couples experiencing involuntary fetal wastage. *Lancet* 1979: **1**: 796–9.
33. Caudle MR, Rote NS, Scott JR, DeWitt C, Barney MD. Histocompatibility in couples with idiopathic recurrent spontaneous abortion and normal fertility. *Fertil Steril* 1983: **39**: 793–7.
34. Unander AM, Olding LB. Habitual abortion: parental sharing of HLA antigens, absence of maternal blocking antibody, and suppression of maternal lymphocytes. *Am J Reprod Immunol* 1983: **4**: 171–8.

35. Oksenberg JR, Persitz E, Amar A, Brautbar C. Maternal-paternal histocompatibility: lack of association with habitual abortions. *Fertil Steril* 1984: **42**: 389–95.
36. Reznikoff-Etievant MF, Edelman P, Muller JY, Pinon F, Sureau C. HLA-DR locus and maternal-fetal relation. *Tissue Antigens* 1984: **24**: 30–4.
37. Schacter B, Weitkamp LR, Johnson WE. Parental HLA compatibility in parents of offspring with neural-tube defects: evidence for a T/t-like locus in humans. *Am J Hum Genet* 1984: **36**: 1082–91.
38. Beer AE, Semprini AE, Zhu XY, QuebbemanJF. Pregnancy outcome in human couples with recurrent spontaneous abortions: HLA antigen profiles; HLA antigen sharing; female serum MLR blocking factors; and paternal leukocyte immunization. *Exp Clin Immunogenet* 1985: **2**: 137–53.
39. Bolis PF, Soro V, Martinetti Bianchi M, Belvedere M. HLA compatibility and human reproduction. *Clin Exp Obstet Gynecol* 1985: **12**: 9–12.
40. Casciani CU, Pasetto N, Forleo R, Adorno D, Valeri M, Piazza A. HLA sharing in couples with recurrent abortion. *Exp Clin Immunogenet* 1985: **2**: 65–9.
41. Taylor CG, Faulk WP, McIntyre JA. Prevention of recurrent spontaneous abortions by leukocyte transfusions. *J R Soc Med* 1985: **78**: 623–7.
42. Vanoli M, Fabio G, Bonara P et al. Histocompatibility in Italian couples with recurrent spontaneous abortions of unknown origin and with normal fertility. *Tissue Antigens* 1985: **26**: 227–33.
43. McIntyre JA, Faulk WP, Nicholas-Johnson VR, Taylor CG. Immunologic testing and immunotherapy in recurrent spontaneous abortion. *Obstet Gynecol* 1986: **67**: 169–74.
44. Takakuwa K, Kanazawa K, Takeuchi S. Production of blocking antibodies by vaccination with husband's lymphocytes in unexplained recurrent aborters: the role of successful pregnancy. *Am J Reprod Immunol Microbiol* 1986: **10**: 1–9.
45. Coulam CB, Moore SB, O'Fallon WM. Association between major histocompatibility antigen and reproductive performance. *Am J Reprod Immunol Microbiol* 1987: **14**: 54–8.
46. Cauchi MN, Tait B, Wilshire MI et al. Histocompatibility antigens and habitual abortion. *Am J Reprod Immunol* 1988: **18**: 28–31.
47. Johnson PM, Chia KV, Risk JM, Barnes RMR, Woodrow JC. Immunological and immunogenetic investigation of recurrent spontaneous abortion. *Dis Markers* 1988: **6**: 163–71.
48. Sargent IL, Wilkins T, Redman CW. Maternal immune responses to the fetus in early pregnancy and recurrent miscarriage. *Lancet* 1988: **2**: 1099–104.
49. Sciorelli G, Bontempelli M, Carella G et al. HLA sharing in Italian recurrent abortion couples. *Acta Eur Fertil* 1988: **19**: 257–61.
50. Smith JB, Cowchock FS. Immunological studies in recurrent spontaneous abortion: effects of immunization of women with paternal mononuclear cells on lymphocytotoxic and mixed lymphocyte reaction blocking antibodies and correlation with sharing of HLA and pregnancy outcome. *J Reprod Immunol* 1988: **14**: 99–113.
51. Balasch J, Coll O, Martorell J, Jove IC, Gaya A, Vanrell JA. Further data against HLA sharing in couples with recurrent spontaneous abortion. *Gynecol Endocrinol* 1989: **3**: 63–9.
52. Christiansen OB, Riisom K, Lauritsen JG, Grunnet N. No increased histocompatibility antigen sharing in couples with idiopathic habitual abortion. *Hum Reprod* 1989: **4**: 160–2.
53. Ho H-N, Gill TJ, Nsieh R-P, Hsieh H-J, Lee T-Y. Sharing of human leukocyte antigens in primary and secondary recurrent spontaneous abortions. *Am J Obstet Gynecol* 1990: **163**: 178–88.
54. Chang M-Y, Soong Y-K, Huang C-C. Comparison of histocompatibility between couples with idiopathic recurrent spontaneous abortion and normal multipara. *J Formos Med Assoc* 1991: **90**: 153–9.
55. Eroglu G, Betz G, Torregano C. Impact of histocompatibility antigens on pregnancy outcome. *Am J Obstet Gynecol* 1992: **166**: 1364–9.
56. Ito K, Obata F, Tanaka T, Tsustumi N, Kashiwagi N. Analysis of HLA-DR types of unexplained recurrent spontaneous aborters in the Japanese population by oligonucleotide-DNA typing. *Tissue Antigens* 1992: **40**: 204–9.
57. Takakuwa K, Higashino M, Ueda H et al. Significant compatibility does not exist at the HLA-DQB gene locus in couples with unexplained recurrent abortions. *Am J Reprod Immunol* 1992: **28**: 12–6.
58. Laitinen T, Koskimies S, Westman P. Foeto-maternal compatibility in HLA-DR, -DQ, and -DP loci in Finnish couples suffering from recurrent spontaneous abortions. *Eur J Immunogenet* 1993: **20**: 249–58.
59. Ober C, Steck T, van der Ven K et al. MHC class II compatibility in aborted fetuses and term infants of couples with recurrent spontaneous abortion. *J Reprod Immunol* 1993: **25**: 195–207.
60. Karhukorpi J, Laitinen R, Tiilikainen AS. HLA-G polymorphism in Finnish couples with recurrent spontaneous miscarriage. *BJOG* 1997: **104**: 1212–4.
61. Ober C, Hyslop T, Elias S, Weitkamp LR, Hauck WW. Human leukocyte antigen matching and fetal loss: results of a 10 year prospective study. *Hum Reprod* 1998: **13**: 33–8.
62. Penzes M, Rajczy K, Gyodi E et al. HLA-G gene polymorphism in the normal population and in recurrent spontaneous abortion in Hungary. *Transplant Proc* 1999: **31**: 1832–3.
63. Yamashita T, Fujii R, Tokunaga K et al. Analysis of human leukcocyte antigen-G polymorphism including intron-4 in Japanese couples with habitual abortion. *Am J Reprod Immunol* 1999: **41**: 159–63.
64. Aldrich CL, Stephenson MD, Karrison T et al. HLA-G genotypes and pregnancy outcome in couples with unexplained recurrent miscarriage. *Mol Hum Reprod* 2001: **7**: 1167–72.
65. Emmer PM, Steegers AP, Kerstens HMJ et al. Altered phenotype of HLA-G expressing trophoblast and decidual natural killer cells in pathological pregnancies. *Hum Reprod* 2002: **17**: 1072–80.
66. Ober C, Aldrich CL, Chervoneva I et al. Variation in the HLA-G promoter region influences miscarriage rates. *Am J Hum Genet* 2003: **72**: 1425–35.
67. Hviid TV, Hylenius S, Hoegh AM, Kruse C, Christiansen OB. HLA-G polymorphisms in couples with recurrent spontaneous abortions. *Tissue Antigens* 2002: **60**: 122–32.
68. Hviid TVF, Hylenius S, Lindhard A, Christiansen OB. Association between human leukocyte antigen-G genotype and success of *in vitro* fertilization and pregnancy outcome. *Tissue Antigens* 2004: **64**: 66–9.
69. Engels EA, Schmid CH, Terrin N, Olkin I, Lau J. Heterogeneity and statistical significance in meta-analysis: an empirical study of 125 meta-analyses. *Stat Med* 2000: **19**: 1707–28.
70. Stroup DF, Berlin JA, Morton SC et al. Meta-analysis of observational studies in epidemiology – a proposal for reporting. *JAMA* 2000: **283**: 2008–12.
71. Attia J, Thakkinstian A, D'Este C. Meta-analyses of molecular association studies: methodologic lessons for genetic epidemiology. *J Clin Epidemiol* 2003: **56**: 297–303.
72. Schneider K, Knutson F, Tamsen L, Sjoberg O. HLA antigen sharing in preeclampsia. *Gynecol Obstet Invest* 1994: **37**: 87–90.
73. Scott JR. Immunotherapy for recurrent miscarriage. *Cochrane Database Syst Rev* 2003: **1**: CD000112.
74. Hoff C, Peevy K, Giattina K, Spinnato JA, Peterson RDA. Maternal-fetal HLA-DR relationships and pregnancy-induced hypertension. *Obstet Gynecol* 1992: **80**: 1007–12.

75. Williams MA, Farrand A, Mittendorf R et al. Maternal second trimester serum tumor necrosis factor-alpha-soluble receptor p55 (sTNFp55) and subsequent risk of preeclampsia. *Am J Epidemiol* 1999: **149**: 323–9.
76. Chen G, Diallo JT, Volumenie JL et al. Immune suppression and Th1/Th2 balance in pregnancy revisited: a (very) personal tribute to Tom Wegmann. *Am J Reprod Immunol* 1997: **37**: 427–34.
77. Saito S, Sakai M, Sasaki Y, Tanabe K, Tsuda H, Michimata T. Quantitative analysis of peripheral Th0, Th1, Th2 and Th1:Th2 cell ratio during normal human pregnancy and preeclampsia. *Clin Exp Immunol* 1999a: **117**: 550–5.
78. Goldsby RA, Kindt TJ, Osborne BA. Kuby Immunology, 4th edn. New York: Freeman W H & Co 2000: 1–648.
79. Saito S, Umekage H, Sakamoto Y et al. Increased T-Helper-1-type immunity and decreased T-Helper-2-type immunity in patients with preeclampsia. *Am J Reprod Immunol* 1999b: **41**: 297–306.