[Frontiers in Bioscience 6, e23-29, August 1, 2001]

ROLE OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS III GENES IN RECURRENT SPONTANEOUS ABORTIONS

Marja-Liisa Lokki[1] and Tarja Laitinen[2]

[1] The Finnish Red Cross Blood Transfusion Service, Tissue Typing Laboratory, Finland, [2] Department of Medical Genetics, University of Helsinki, Finland

TABLE OF CONTENTS

1. ABSTRACT

Increased parental Human Leukocyte Antigen (HLA) sharing has been repeatedly reported in recurrent spontaneous abortions (RSA). Parental HLA sharing increases the chance of feto-maternal histocompatibility and potentially affects maternal allo-recognition of the fetus. However, strong linkage disequilibrium across the whole Major Histocompatibility Complex (MHC) region makes it difficult to interpret parental HLA sharing conclusively. It is not known whether the shared HLA gene as such or an unknown gene(s) in linkage disequilibrium or a combination of several loci are causing the disease. Interestingly, in mouse and rat MHC-linked, recessive genes are known to control the reproduction, development and growth of the fetus. Human analogs have not been identified. Compared to HLA genes, MHC Class III has been studied much less in RSA. However, there are some observations of an increased number of unexpressed complement C4 alleles in RSA spouses. Complement C4 genes are located in a chromosomal region characterized by extremely high gene density and frequent gene rearrangements. C4 "null" alleles can act as markers of gene rearrangements in Class III unfavorable for pregnancy outcome. Many of the novel genes located in this region by sequencing serve as new candidates for RSA, since they have housekeeping functions and some of them are highly expressed in human reproductive organs.

2. INTRODUCTION

Recurrent spontaneous abortions (RSA) are defined as a clinical condition in which consecutive (>3) miscarriages occur in early pregnancy without evidence of anatomical abnormalities of female genital tract, maternal autoimmune or endocrinological disease, or parental chromosomal abnormalities. According to frequency estimations, RSA affects approximately one percent of couples (10). Primary RSA occurs in couples with no children, and secondary RSA in couples with child(ren) before the episode of abortions.

It is likely that some of the pregnancy related disorders are caused by a failure in maternal immune tolerance towards semi-allogenic fetus. Traditionally, the pregnancy has been compared to allogenic organ transplantation. Lately it has become obvious that the outer cell layers of placenta do not express class I HLA-A and -B or class II HLA-DR, HLA-DQ antigens (1, 2). However, cytotrophoblasts may express HLA-C (3) and they do express class I related HLA-G antigen (4, reviewed in 5). Differences in the expression patterns of placental HLA antigens may be the main reason why a semi-allogenic fetus can escape maternal rejection. HLA-G shows only few allelic variants (and thus, is less antigenic for the mother) in contrast to HLA-A and –B antigens (6) and the amino acid changes are not essential for antigen binding and T-cell recognition (7). However, it is believed that HLA-G can elicit maternal allogenic response. In a successful pregnancy the balance between placental HLA-G and soluble HLA-G can be critical. Soluble HLA-G antigens can protect fetal tissues against maternal natural killer (NK) cell and allo-reactive T cell attacks. Since placenta is missing classical antigen presenting molecules, HLA-G may have an important role in the protection against infections *in utero* (8). Recent findings show that HLA-G can also regulate HLA-E expression and angiogenesis in embryogenesis or placental development. Both innate and specific immunity are present at the maternal-fetal interface (9). Because of the relatively suppressed adaptive immunity, innate immunity is highly activated with an increased number of mono- and granulocytes and increased production of cytokines and complement factors. Innate immunity is important in maternal defense against infections, but has to become compromised not to damage the fetus. Obviously, maternal

recognition of the fetus occurs and is needed for successful pregnancy, but the harmony between advantageous and disadvantageous allo-recognition is delicate and can be disturbed at many levels.

Many studies have reported that RSA is associated with some HLA antigens and/or with increased HLA sharing between the spouses (reviewed in 11 and 12). The earlier studies have been done using HLA serological typing methods and more recently, DNA based typing methods. It has also been reported that children born later to RSA couples have smaller birth weight than children in the families with no history of RSA (13). In RSA, both direct immunological and genetic factors can be of great importance, and therefore these HLA sharing studies can be interpreted in two different ways. Firstly, parental HLA sharing increases the chance of feto-maternal histocompatibility that can influence maternal allo-recognition of the fetus, and thus induce the growth of placenta and fetus via an immune-endocrinological pathway. In case of an HLA association, some of fetuses may be able to raise only weak/too strong maternal allo-recognition, or some pregnant women may act as low responders/high responders in allo-recognition during the pregnancy, or maybe the combination of these two that is important (14). However, HLA sharing has not been found in all of the studies and in most of the studies, sharing is rather weak. Furthermore, HLA genes associated with RSA differ from study to study, and so far, there is no evidence of HLA-G or -E (the only HLA antigens in immediate contact with maternal tissues) allele associations in RSA (15, 16). Secondly, the results can be interpreted based on special features of MHC genetics combined with the data of reproduction deficiencies in rodent models. Since significant linkage disequilibrium occurs across the whole MHC region (=specific allele combinations are favored throughout the region), HLA associations may merely reflect the presence of other than HLA genes involved in reproduction. The most frequently studied antigens are HLA-A, -B, -DR, and –DQ, giving a clue that genes responsible for reproductive defects may be mapped to the HLA-B to HLA-DR region rather than anywhere else in MHC (17). A genetic model is further supported by the finding that RSA has an increased familial risk (18). In rat (grc=growth and reproduction complex) and in mouse (t-complex and Ped locus) conserved regions containing recessive genes involved in fertility, growth, developmental defects and susceptibility to cancer have been identified (19). Based on localization and functional similarities, HLA-G was thought to represent the human analog to the mouse Qa-2 antigen encoded by the Ped locus. However, according to more recent reports the human Ped gene is presumably HLA-G-linked, but distinct from it. Noteworthy structural homologies could not be found between the rat grc region and human MHC by hybridization suggesting that either the human grc does not exist MHC-linked or its structural homology with rat is rather low (20, 21). Interestingly, recent transfection studies of individual grc genes in rat suggest that the changes in conformation of DNA (22), and thus the presence of large deletions, may be more critical than the functions of a single gene for defects in reproduction and embryonal development. In human, similar extended effects of the changed DNA 3D structure have been previously described in the regulation of various globin genes.

3. ORGANIZATION OF MHC CLASS III GENES

At least 70 out of about 200 MHC genes or gene fragments are localized in MHC class III (1.1 Mb) in between classes II and I (Figure 1A and 1B). By sequencing the number of novel genes increased rapidly, many of them remain, however, yet functionally uncharacterized (23). It is well known that class II genes are associated with many immune-related diseases. However, it does not exclude the possibility that susceptibility alleles may also be found simultaneously in class III and class I regions. Despite numerous studies on HLA genes in RSA, much less is known about class III genes in RSA. In class III, genes with putative immune functions have been mapped throughout the region. Many of them are expressed by a number of immune cells and belong to clusters of related genes (24). MHC class I chain-related MICA and MICB are closely related genes. MICA is expressed on the surface of epithelial and endothelial cells as a ligand for gamma/delta T cells and may contribute to the development of coeliac disease (25, 26). MICA is also a stress protein and has functions that are related to those of heat shock genes HSP70-1, -2, and –HOM. Some genes, such as IκB-Like and HLA-B associated transcript 1 (BAT1), regulate transcription of cytokine genes. Cytokines Tumor Necrosis Factor (TNF), Lymphotoxin-A (LTA) and Lymphotoxin-B (LTB) are involved in pro-inflammatory response (reviewed in 24) (Figure 1B). It has been reported that patients with repeated miscarriages have shown higher serum TNF-α levels compared to those with a successful pregnancy (27).

In the centromeric end of class III are located genes for complement proteins C4, factor B and C2. The region shows considerable amount of variation in gene numbers, sizes, and allele polymorphism. The size of the gene cluster can vary from 142 to 214 kb depending on the number of deletions or duplications frequently found in this region (reviewed in 28). Duplicated C4 genes, C4A and C4B, and the partially duplicated serine/threonine kinase genes, RP1 and RP2, steroid 21-hydroxylase genes, CYP21A and CYP21B, and tenascin-X genes, TNXA and TNXB, are arranged in the order of 5'-RP1-C4A-CYP21A-TNXA-RP2-C4B-CYP21B-TNXB-3'. Extensive molecular biological studies have established that these four genes arranged in tandem, RP-C4-CYP21-TNX, form a genetic unit (RCCX module) (Figure 1C). The number of modules can vary, bimodular haplotype, however, is the most frequent. Deletions and duplications of the C4 genes are accompanied by deletions and duplications in the neighboring RCCX genes (29). The intergenic region between C4A and C4B contains the pseudogene CYP21A and the truncated sequences of TNXA and RP2 form a hybrid structure, resulting from the fusion of the 3' regions of the RP1 and TNXB genes (30). The functional consequences of gene arrangements in this region are not

known. The 30 kb genomic region between the RCCX

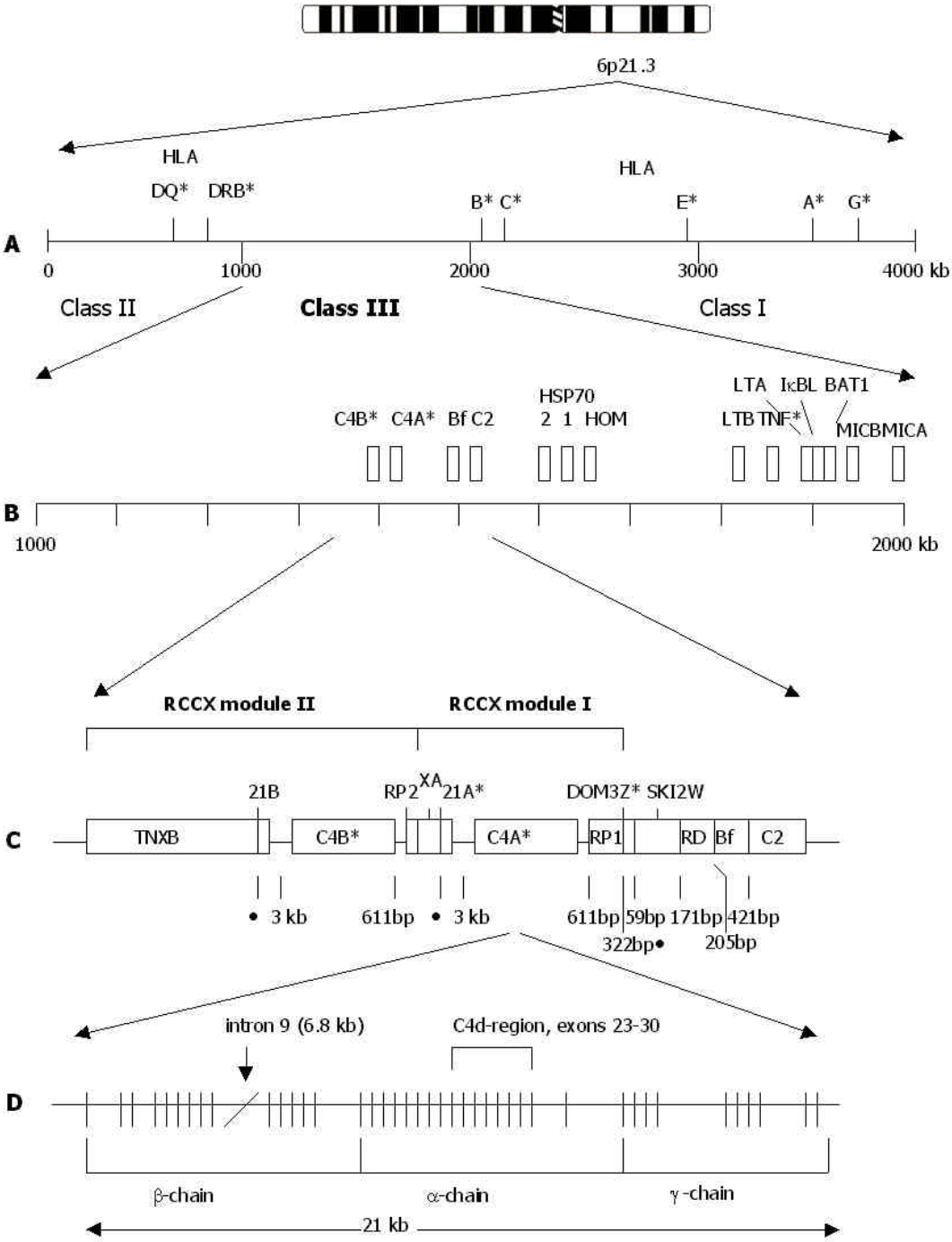

Figure 1. The map of HLA genes in MHC in the chromosome 6p21 (A), some of the Class III genes (B), genes in complement C4 cluster (C), and exon-intron structure of complement C4A gene (D). A black dot in RCCX modules indicates an overlap of the neighboring genes (29). The genes putatively involved in RSA are shown with an asterisk.

module and factor B carries three novel genes: DOM3Z, Superkiller viralicidic activity 2 (SKI2W), and RNA binding protein (RD) (Figure 1C). RD is located only 205 bp downstream of the factor B gene and is arranged with SKI2W in a head-to-head orientation with a very short intergenic distance of 171 bp. Also the next two genes downstream, DOM3Z and RP1, form a similar head-to-head gene complex (31).

4. CLASS III COMPLEMENT GENE CLUSTER

C2 (OMIM#217000, http://www.ncbi.nlm.nih.gov/Omim/), factor B (OMIM#138470), C4A (OMIM#120810), and C4B (OMIM#120820) are important humoral components of innate immune system, forming the complement C3 and C5 convertases (Figure 1). C2 and factor B are structural homologs of genes encoding serine proteases. They share a domain related to the von Willebrand factor type A that may function as an adhesion target to collagen during inflammation. C2 contains a composite retroposon SVA with a SINE element, multiple intragenic tandem repeats and an *Alu* element (32-34). C4 proteins function in defense against infectious agents and they are involved in the elimination of immune complexes. C4 is complex both at the protein and at the gene level (35, 36). C4A and C4B express together more than 40 allotypes with different functional activities. It is well documented that the C4 deficiencies are associated with many autoimmune diseases. This may relate to immune complex clearance and to self-tolerance maintained by negative selection of self-reactive B cells (37). The activation peptide of C4 may inhibit inflammation by preventing the infiltration of monocytes to the site of lesion (38), suggesting that C4 has distinct roles in immunological defence. In the RCCX module, usually the C4 gene in locus I encodes for C4A and in locus II for C4B. However, many chromosomes contain only C4B genes (16-19%) or C4A genes (10-15%) and some have three (1-2%) or even four C4 genes (two for C4A and two for C4B). C4 genes show dichotomous size variation mediated by an endogenous retrovirus, HERV-K(C4) in intron 9 of the C4A and C4B genes (39) (Figure 1D). HERV-K(C4) is organized in the opposite transcriptional orientation in relation to C4 and may produce the antisense proviral RNA during transcription of the C4 gene and therefore produce a neutralizing agent to inhibit retroviral proliferation. CYP21A and CYP21B (OMIM#201910) have been mapped downstream of the complement C4 genes (40, 41). CYP21B is a functional gene and is transcribed in the adrenal cortex, whereas CYP21A is a pseudogene as a consequence of an eight base pair deletion. The steroid 21-hydroxylase enzyme regulates mineralocorticoid and glucocorticoid biosynthesis. A deletion or mutation of the CYP21B gene causes congenital adrenal hyperplasia (42). Tenascin genes encode extracellular matrix proteins. TNXB (OMIM#600985) has both its 5' and 3' ends buried in the flanking genes. TNXA (OMIM#600261) contains a gene segment from intron 32 to exon 45 of TNXB. It has a 120 bp deletion at exon 36 and intron 36 resulting in the premature termination of translation by a frame shift mutation. The large hexameric TNXB proteins contain eighteen epidermal growth factor repeats, thirty-two fibronectin type III repeats and a fibrinogen domain (43). As an embryonic protein TNXB has strongest expression in fetal adrenal cortex, testis and muscle as well as in developing connective tissue participating in tissue cell migration. Tenascin-like proteins are assumed to play a part in tumor metastasis. TNXB deficiency is associated with the Ehlers-Danlos syndrome, an inherited connective tissue disorder producing hyperextensible and friable tissues (44). The genomic segment with C4, CYP21 and TNX genes have an extraordinary and intriguing feature: each of these genes show adrenal expression of smaller transcripts with still unknown functions (45).

The RP2 gene contains the last two and a half exons of RP1. The RP1 gene has nine exons and in intron 4 both *Alu* elements and a composite retroposon similar to that in C2 located 20 kb upstream of RP1. The deduced amino acid sequence suggests that RP1 codes for a nuclear protein functionally related to a serine/threonine kinase (31, 46). RP1 (GenBank accession number L26260) and DOM3Z (GenBank accession number AF059252) genes have 5' regulatory regions probably overlapping to some extent. They both show multiple transcripts in many tissues, with higest expression levels in reproductive tissues and pancreas. The function of the DOM3Z gene is unknown but homologous genes have been detected both in the genomes of the yeast and the nematode *Caenorhabditis elegans* (47). In *C. elegans*, the function of the DOM3 gene may be related to those of the upstream the MES-3 gene encoding for a maternal effect component required for the normal postembryonic development of the germ line. Both the genomic and mRNA structures of the SKI2W (OMIM#600478) and RD (OMIM#154040) genes have been resolved and reviewed by Yu *et al*. (31). SKI2W encoded proteins are localized in the nucleoli and cytoplasmic polysomes indicating fundamental involvement in RNA turnover (48). Also the putative RD gene product has a potential to bind RNA molecules. These four human genes RP1, DOM3Z, SKI2W and RD form an intriguing complex of related genes showing similar genomic motifs and their importance in reproductive diseases and in autoimmunity will be of interest.

5. CLASS III COMPLEMENT GENES IN RECURRENT SPONTANEOUS ABORTIONS

During the last years a number of novel class III genes have been identified. Based on their sequence homologies, expression patterns and functions known so far, they can be considered potential candidates in pregnancy related diseases. A exceptionally high density of genes, a great number of highly polymorphic genes, overlapping gene structures, the shared regulatory regions of genes, and variation in the number of units of the genes give us a possibility to study how gene rearrangements can be involved in morbidity. We studied 14 polymorphic markers across the MHC region among Finnish primary (N=41 pairs) and secondary (N=18 pairs) RSA couples. They were carefully clinically studied to exclude other causes for RSA. During the follow-up period, 29 RSA women gave birth to a child. That made the haplotype analysis possible in 37 families with children born either before or after a period of abortions. The most significant finding was the increased frequency of complement C4A "null" alleles among primary RSA wives and husbands (32%) compared to that among the Finnish controls at large (18%) (49). In couples with the secondary RSA, C4B "null" alleles were increased (56% in wives and 50% in husbands) compared to 29% in controls. This increased the risk of the fetus to inherit several paternal and maternal C4 "null" alleles. The same observation has been made also in

another Caucasian population (50). C4 rearrangements in the Finnish families were genetically heterogeneous (51). Some of the C4 "null" alleles associated with extended haplotypes such as A1, B8, C4AQ0, C4B1, DR3 (a large deletion in the C4A and CYP21A genes) or A2,Cw3,B40,BFS,C4AQ0,C4B2,DR13 (a 2-bp insertion at codon 1213 leading to thr premature termination of translation) (52, 53). In most of the cases the molecular genetic background of the mutations remained unknown. One of the born children had a *de novo* C4 mutation generating a hybrid C4A/C4B gene as specified by RFLP, cloning and sequencing of isotype specific fragments, monoclonal antibodies, hemolytic activity, and electrophoretic migration (54). Based on the results in the Finnish RSA families, we cannot conclude whether the C4 concentration or variation in expression of functionally different C4 allotypes as such is important. Complement is an important part of the maternal immune defense from which placenta is protected by the high expression of complement regulatory proteins throughout gestation. (55). As likely, however, is the option that the C4 "null" alleles are just markers for deletions/rearrangements of neighboring "reproduction/fertility/fetal growth/tumorigenesis" genes.

6. CONCLUSION

During evolution, the polymorphism of the MHC region has been under strong selective pressures. It is possible that not only factors after birth, such as resistance for infectious diseases in childhood, but also prenatal factors favor the polymorphism of the region. Lethal or semilethal genes acting in a recessive fashion would prevent the birth of far-reaching MHC homozygous individuals. Better diagnostic methods of maternal gynecological and systemic diseases as well as the better understanding of the functional genomics of MHC have provided new challenges for MHC genetics in RSA. One interesting aspect for the future studies is gene rearrangements in Class III. Obviously, RSA is a multifactorial disorder in which combinations of genes, MHC-linked or unlinked, affect the outcome of a pregnancy in a concerted fashion.

7. REFERENCES

1. Faulk W. P, B. L. Hsi, J. A. McIntyre, C. J. G. Yeh & A. Mucchielli: Antigens of the human extra-embryonic membranes. *J Reprod Fertil* Suppl 31,181-189 (1982)

2. Johnson P. M. & P. I. Stern: Antigen expression at the human materno-fetal interfaces. *Prog Immunol* 6,1056-1069 (1986)

3. King A, C. Boocock, A. M. Sharkey, L. Gardner, A. Beretta, A. G. Siccardi & Y. W. Loke: Evidence for the expression of HLA-C class I mRNA and protein by human first trimester trophoblast. *J Immunol* 156, 2068-2076 (1996)

4. McMaster M, C. L. Librach, Y. Zhou, K. H. Lim, M. J. Janatpour, R. DeMars, S. Kovats, C. Damsky & S. J. Fisher: Human placental HLA-G expression is restricted to differentiated cytotrophoblasts. *J Immunol* 154, 3771-3778 (1995)

5. Carosella E. D, N. Rouas-Freiss, P. Paul & J. Dausset: HLA-G: a tolerance molecule from the major histocompatibility complex. *Immunol Today* 20, 60-65 (1999)

6. Bainbrigde D. R. J, S. A. Ellis & I. L. Sargent: Little evidence of HLA-G mRNA polymorphism in Caucasian or Afro-Caribbean populations. *J Immunol* 163, 2023-2027 (1999)

7. Van der Ven K, S. Skrablin, G. Engels & D. Krebs: HLA-G polymorphisms and allele frequencies in Caucasian. *Human Immunol* 59, 302-312 (1998)

8. Pfeiffer K, V. Rebmann, H. van der Ven, M. Pässler, D. Krebs, & H. Grosse-Wilde: Soluble HLA antigens in early pregnancy. *Hum Immunol* Suppl 60, 9 (1999)

9. Sacks G, I. Sargent & C. Redman: An innate view of human pregnancy. *Immunol Today* 120, 114-118 (1999)

10. Stray-Pederson B. & S. Stray-Pederson: Etiologic factors and subsequent reproductive performance in 195 with a prior history of habitual abortion. *Am J Obstet Gynecol* 148, 140-146 (1984)

11. Gill T. J. III: Invited editorial: influence of MHC and MHC-linked genes on reproduction. *Am J Hum Genet* 50,1-5 (1992)

12. Kostyu D. D: HLA: fertile territory for developmental genes? *Critical Review Immunol* 14, 29-59 (1994)

13. Christiansen O. B, O. Mathiesen, K. Riisom, J. G. Lauritsen, N. Grunnet & C. Jersild: HLA or HLA-linked genes reduce birthweight in families affected by idiopathic recurrent abortion. *Tissue Antigens* 36, 156-163 (1990)

14. Ober C, T. Steck, K. van der Ven, C. Billstrand, L. Messer, J. Kwak, K. Beaman & A. Beer: MHC class II compatibility in aborted fetuses and term infants of couples with recurrent spontaneous abortion. *J Reprod Immunol* 25, 195-207 (1993)

15. Karhukorpi J, T. Laitinen & A. Tiilikainen: HLA-G polymorphism in Finnish couples with recurrent spontaneous abortions. *Br J Obst Gynecol* 104, 1212-1214 (1997)

16. Steffensen R, O. B. Christiansen, E. P. Bennett & C. Jersild: HLA-E polymorphism in patients with recurrent spontaneous abortion. *Tissue Antigens* 52, 569-572 (1998)

17. Gill III T. J: Role of the major histocompatibility complex region in reproduction, cancer, and autoimmunity. *Am J Reproduc Immunol* 35, 211-215 (1996)

18. Christiansen O. B, O. Mathiesen, J. G. Lauritsen & N. Grunnet: Idiopathic recurrent spontaneous abortion.

Evidence of a familial predisposition. *Acta Obstet Gynecol Scand* 69, 597-601 (1990)

19. Helou K, Q. Yan, X.-J. Yuan, H. W. Kunz, G. Levan & T. J. Gill III: Cytogenetic localization of the growth and reproduction complex (Grc) in the rat and in the mouse and its position in relation to RT1.EC and other loci in the rat MHC. *Hereditas* 130, 105-109 (1999)

20. Gill III T. J: Mechanisms of action of Major-Histocompatibility–Complex-linked genes affecting reproduction. *Am J Reprod Immunol* 41, 23-33 (1999)

21. Cao W, A. Brenner, M. Alikani, J. Cohen & C. M. Warner: Search for a human homologue of the mouse Ped gene. *Mol Hum Reprod* 541-547 (1999)

22. Yuan X.-J, B. Dixon McCarthy, S. K. Salgar, H. W. Kunz & T. J. Gill III: Biological effects of genes in the Grc and EC region of the rat Major Histocompatibility Complex. *Am J Reprod Immunol* 42, 64-69 (1999)

23. Aguado B, C. M. Milner & D. Campbell: Genes of the MHC class III region and the functions of the proteins they encode. In: Browning M, McMichael A, eds. HLA and MHC: Genes, Molecules, and Functions. Oxford, UK, BIOS Scientific Publishers (1996)

24. Gruen J. R. & S. M. Weissman: Evolving views of the major histocompatibility complex. *Blood* 90, 4252-4265 (1997)

25. Shiina T, G. Tamiya, A. Oka, T. Yamagata, N. Yamagata, E. Kikkawa, K. Goto, N. Mizuki, K. Watanabe, Y. Fukuzumi, S. Taguchi, C. Sugawara, A. Ono, L. Chen, M. Yamazaki, H. Tashiro, A. Ando, T. Ikemura, M. Kimura & H. Inoko: Nucleotide sequencing analysis of the 146-kilobase segment around the IkBL and MICA genes at the centomeric end of the HLA class I region. *Genomics* 47, 372 (1998)

26. Naipal A, P. Hanifi, B. Crusius, S. Pena, F. Koning, C. J. J. Mulder, F. H. J. Claas & I. I. N. Doxiadis: Positive and negative associations of MICA polymorphism with coeliac disease. *Hum Immunol* Suppl1, 3 (1999)

27. Mueller-Eckhardt G, P. Mallmann, J. Neppert, A. Lattermann, A. Melk, O. Heine, R. Pfeiffer, J. Zingsem, N. Domke & A. Mohr-Pennert: Immunogenetic and serological investigations in nonpregnant and in pregnant women with a history of RSA. *J Reprod Immunol* 27, 95-109 (1994)

28. Yu C. Y: Molecular genetics of the human MHC complement gene cluster. *Exp Clin Immunogenet* 15, 213-230 (1998)

29. Yang Z, A. R. Mendoza, T. R. Welch, W. B. Zipf & C. Y. Yu: Modular variations of the human major histocompatibility complex class III genes fir serine/threonine kinase RP, complemenr component C4, steroid 21-hydroxylase CYP 21, and tenascin TNX (the RCCX module). *J Biol Chem* 274, 12147-12156 (1999)

30. Shen L. M, L. C. Wu, S. Sanlioglu, R. Chen, A. R. Mendoza, A. Dangel, M. C. Carroll, W. Zipf & C. Y. Yu: Structure and genetics of the partially duplicated gene RP located immediately upstream of the complement C4A and C4B genes in the HLA class III region: molecular cloning, exon-intron structure, composite retroposon, and breakpoint og gene duplication. *J Biol Chem* 269, 8466-8476 (1994)

31. Yang Z, L. Shen, A. W. Dangel, L.-C. Wu & C. Y. Yu: Four ubiquitously expressed genes, RD (D6S45)-SKI2W (SKIV2L)-DOM3Z-RP1 (D6S60E), are present between complement component genes factor B and C4 in the class III region of the HLA. *Genomics* 53, 338-347 (1998)

32. Campbell R. D, D. R. Bentley & B. J. Morley: The factor B and C2 genes. *Phil Trans R Soc Lond* B306, 367-378 (1984)

33. Yosimoto I, Z. Zeng-Bian, K. Macon & J. Volanakis: Structure of the human C2 gene. *J Immunol* 151, 170-174 (1993)

34. Colombatti A. & P. Bonaldo: The superfamily of proteins with von Willebrand factor type A-like domains: one theme common to components of extracellular matrix, hemostasis, cellular adhesion, and defence mechanisms. *Blood* 77, 2305-2315 (1991)

35. Yu C. Y: The complete exon-intron structure of a human complement component C4A gene: DNA sequences, polymorphism, and linkage to the 21-hydroxylase genes. *J Immunol* 146, 1057-1066 (1991)

36. Campbell R. D, I. Dunham, E. Kendall & C. A. Sargent: Polymorphism of the human complement component C4. *Expl Clin Immunogenet* 7, 69-84 (1990)

37. Prodeus A. P, S. Goerg, L.-M. Shen, O. O. Pozdnyakova, L. Chu, E. M. Alicot, C. C. Goodnow & M. C. Carroll: A critical role for complement in maintenance of self-tolerance. *Immunity* 9, 721-731 (1998)

38. Tsuruta T, T.Yamamoto, S. Matsubara *et al*: Novel function of C4a anphylatoxin: release from monocytes of protein which inhibits monocyte chemotaxis. *Am J Pathol* 142, 1848-1857 (1993)

39. Dangel A. W, A. R. Mendoza, B. J. Baker, C. M. Daniel, M. C. Carroll, L.-C. Wu & C. Y. Yu: The dichotomous size variation of human complement C4 gene is mediated by a novel family of endogenous retroviruses which also establishes species-specific genomic patterns among Old World primates. *Immunogenetics* 40, 425-436 (1994)

40. White P. C, D. Grossberger, B. J. Onufer, M. I. New, B. Dupont & J. Strominger: Two genes encoding steroid 21-hydroxylase are located near the genes encoding the fourth component of complement in man. *Proc Natl Acad Sci USA* 82, 1089-1093 (1985)

41. Carroll M, R. D. Campbell & R. R. Porter: Mapping of steroid 21-hydroxylase genes adjacent to complement C4 genes in HLA, the major histocompatibility complex in man. *Proc Natl Acad Sci USA* 82, 521-525 (1985)

42. Miller W. L & Y. Morel: Themolecular genetics of 21-hydroxylase deficiency. *Ann Rev Genet* 23, 371-393 (1989)

43. Matsumoto K, N. Ishihara, A. Ando, H. Inoko & T. Ikemura: Extracellular matrix protein tenscin-like gene found in human MHC class III region. *Immunogenetics* 36; 400-403 (1992)

44. Burch G. H, Y. Gong, W. Liu, R. W. Dettman, C. J. Curry, L. Smith, W. L. Miller & J. Bristow: Tenascin-X deficiency is associated with Ehlers-Danlos syndrome. *Nature Genet* 17, 104-108 (1996)

45. Tee M. K, G. O. Babalola, P. Aza-Blanc, M. Speek, S. E. Gitelman & W. L. Miller: A promoter within intron 35 of the human C4A gene initiates abundant adrenal-specific transcription of a 1 kb RNA: Location of a cryptic CYP21 promotor element? *Hum Molec Genet* 4, 2109-2116 (1995)

46. Sargent C, A, M. J. Anderson, A.-L. Hsieh , E. Kendall, N. Gomez-Escobar & R. D. Campbell: Characterization of the novel gene G11 lying adjacent to the complement C4A gene in the human major histocompatibility complex. *Hum Molec Genet* 3, 481-488 (1994)

47. Paulsen J. E, E. E. Capowski & S. Strome: Phenotypic and molecular analysis of mes-3, a maternal-effect gene required for proliferation and viability of the germ line in C. elegans. *Genetics* 141, 1383-1398 (1995)

48. Qu X, Z. Yang, S. Zhang, L. Shen, A. W. Dangel, J. H. Hughes, K. L Redman, L. C. Wu & C. Y. Yu: The human DEVH-box protein Ski2w from the HLA is localized in nucleoli and ribosomes. *Nucl Acid Res* 26, 4068-4077 (1998)

49. Laitinen T, M.-L. Lokki, M. Tulppala, O. Ylikorkala & S. Koskimies: Increased frequency of complement C4 "null" alleles in recurrent spontaneous abortion. *Hum Reprod* 6, 1384-1387 (1991)

50. Pennesi G, G. Brioli, P. Lulli, B. Mariani, M. Morellini, M. Nicotra & S. Trabace: HLA and complement factors alleles sharing in Italian couples with recurrent spontaneous abortions. *Hum Immunol* 59, 382-386 (1998)

51. Laitinen T, M.-L. Lokki, J. Partanen, M. Tulppala, O. Ylikorkala & S. Koskimies: Major histocompatibility complex located complement C4 and steroid 21-hydroxylase gene rearrangements in couples with recurrent spontaneous abortions. *Eur J Immunogenet* 19, 413-418 (1992)

52. Barba G, C. Rittner & P. M. Schneider: Genetic basis of human complement C4A deficiency: detection of a point mutation leading to nonexpression. *J Clin Invest* 91, 1681-1686 (1993)

53.Lokki M.-L, A. Circolo, P. Ahokas, K. L. Rupert, Y. C. Yu & H. R. Colten: Deficiency of complement protein C4 due to identical frameshift mutations in the C4A and C4B genes. *J Immunol* 162, 3687-3693 (1999)

54. Jaatinen T, M. Eholuoto, T. Laitinen & M.-L. Lokki: Characterization of a *de novo* mutation in C4 gene. (manuscript in preparation)

55. Tedesco F, G. Narch, O. Radillo, S. Meri, S. Ferone & C. Betterle: Susceptibility of human trophoblast to killing by human complement and the role of the complement regulatory proteins. *J Immunol* 151, 1562-1570 (1993)

Key Words: Association, Complement, Class III, Major Histocompatibility Complex, Recurrent Spontaneous Abortion, Review

Send correspondence to: Marja-Liisa Lokki, Ph.D., The Finnish Red Cross Blood Transfusion Service, Kivihaantie 7, 00310 Helsinki, Finland, Tel: 358-9-5801282, Fax: 358-9-580495, E-mail: maisa.lokki@bts.redcross.fi