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MICA-129 A/G dimorphism, its relation to soluble mica plasma level and spontaneous preterm birth: A case-control study

Renate Von Linsingen[a,*], Geórgia Fernanda Gelmini[b], Maria da Graça Bicalho[b], Newton Sérgio De Carvalho[a]

[a] Department of Gynecology and Obstetrics, Infectious diseases in Gynecology and Obstetrics Sector, Clinics Hospital of University of Paraná (UFPR), Post Graduate Program of Gynecology and Obstetrics, Rua General Carneiro, 181, Alto da Glória, CEP80060-900 Curitiba, Paraná, Brazil
[b] Genetics Department of Federal University of Paraná. LIGH- Immunogenetics and Histocompatibility Laboratory, Avenida Coronel Francisco H. dos Santos, 100. Centro Politécnico - Jardim das Américas, CEP: 80050-540 Curitiba, Paraná, Brazil

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ABSTRACT

The aim of this case- control study was to investigate the association between preterm birth (PTB), *MICA*-129 A/G dimorphism and sMICA levels. Fifty pregnant women with singleton pregnancy and previous PTB, or clinic diagnostic of threatened preterm labor in the actual pregnancy, or cervical length less than 25 mm and 50 healthy pregnant women were enrolled. DNA was extracted for genotyping for *MICA*-129 A/G by real-time PCR and sMICA plasma level was quantified by sandwich ELISA assay. Clinical and socioeconomic characteristics, results of TaqMan® genotyping and ELISA quantification were compared between the groups using qui-square, Fisher´s exact or Mann-Whitney test. A binary logistic regression model was used to predict PTB. The correlation between *MICA*-129 A/G genotypes and sMICA levels was investigated. There were not statistically significant differences between *MICA*-129 A/G polymorphism and sMICA plasma level.There was found a correlation between *MICA*-129 val/val genotype and higher levels of sMICA (ρ: -0.342; p:0.001). The presence of *MICA*-129 val/val genotype may be influencing sMICA expression.

1. Introduction

Spontaneous preterm birth (PTB) < 37 weeks of gestation, affects 5–18% of pregnancies; and it is the leading cause of neonatal death and the second cause of childhood death below the age of 5 years (Blencowe et al., 2013; Liu et al., 2012). Brazilian population has a high rate of PTB (11.5%), and this constitutes a significant public health challenge. PTB is a complex disease and several factors have been associated with its etiology and pathophysiology (Romero et al., 2014). Bacteria and virus were identified in amniotic fluid (Behnia et al., 2015; McCormick, 1985; Menon, 2008; Nguyen et al., 2004; von Linsingen et al., 2017; Zhao et al., 2011) and maternal–fetal membranes (Nien et al., 2006; Wenstrom et al., 1998) but despite this fact, most of these women delivered healthy babies at term. Mechanisms beyond infection have recently been reviewed by us (von Linsingen et al., 2017). Intra-amniotic inflammation associated with PTB, for example, occurs in the absence of demonstrable microorganisms, indicating a role for sterile intra-amniotic inflammation (Yoon et al., 2001). At the maternal fetal interface there are some modifications in the maternal immune system to allow the development of a fetus that contain 50% of paternal alleles. In the decidua, uterine Natural Killer cells (uNK) are the dominant lymphocyte population and these cells exhibit a unique functional and phenotypic characteristic the CD56bright CD16− phenotype, which has low cytotoxicity. They produce a variety of cytokines and the uNK have a positive role in placental formation by limiting excessive trophoblast invasion. NKG2D is a C-type lectin-like molecule that is an activating receptor expressed by NK cells and T cell subsets (Raulet, 2003). NKG2D ligands (NKG2DL) MHC class-I related (MIC) and UL-16 binding proteins (ULBP) (Eagle and Trowsdale, 2007) are constitutively expressed by cells, such as the gut epithelium (Groh et al., 1996) or its expression can be induced in other tissues by stresses, such as infection or transformation (Groh et al., 2001; Gasser et al., 2005).

In the female genital tract MICA molecules were found expressed in the human endometrium, on epithelial cells and in secretory phase of menstrual cycle (Basu et al., 2008). Significant mRNA expression of MICA molecules was detected in trophoblast and decidual cells, but no protein expression was demonstrated, on implantation site at 8 weeks gestation cells from normal pregnancy (Apps et al., 2008). The situation

* Corresponding author at: Rua General Carneiro, 181, Alto da Glória, CEP80060-900 Curitiba, Paraná, Brazil.
E-mail addresses: renate.linsingen@gmail.com, ligh@ufpr.br (R. Von Linsingen), georgiagel@msn.com, ligh@ufpr.br (G.F. Gelmini), mgbicalho@gmail.com, ligh@ufpr.br (M.d.G. Bicalho), newtonsdc@gmail.com, ligh@ufpr.br (N.S. De Carvalho).

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might change in pathological pregnancies and in the mid or late stages of pregnancy. In cases of intrauterine infection, MICA could be up-regulated and allow the mother to perceive and reject the fetus, or in response to stresses of pregnancy such as hypoxia or aneuploidy (Weier et al., 2005). Recently, Negishi et al. (2017) had shown that NK cells (CD56dim CD16 + NK cells) may play an important role in the onset of preterm labor.

MICA protein expression has been found in syncytiotrophoblast (STB), a fetal tissue fetal. It exists in two forms, as a membranal form at the apical and basal surfaces, and as microvesicles in cytoplasmic vacuoles. The plasma membranal ligand expression would make the STB a target for attack by NKG2D receptor-bearing maternal NK, but cell surface expression of NKG2D receptors is significantly down-regulated by elevated levels of soluble MICA (sMICA) molecules present in pregnancy sera (Hedlund et al., 2009; Mincheva-Nilsson, 2006). sMICA can be generated by protease-mediated cleavage of MICA on the cell membrane or release of placental exosomes (Clayton et al., 2008; Clayton e Tabi, 2005; Salih, 2008).

The complex genetic contribution to PTB has been investigated in gene polymorphisms association studies. *MICA* gene, located centromeric to *HLA-B*, is highly polymorphic and the dimorphism *MICA*-129 (A/G) (rs1051792) at 454 nucleotide, that is responsible for polymorphic change from Methionine (Met) to Valine (Val) at position 129 in the alfa-2 domain of the *MICA*-129 (A/G) was related to the bind NKG2D receptor affinity and downregulation of NKG2D leading to impaired function of NK cells (Steinle et al., 2001).

The *MICA*-129 polymorphism has been associated with a number of diseases related to Natural Killer cells (NK) activity, under conditions of oxidative stress, viral infection, diverse types of cancer, auto- immune diseases, as inflammatory bowel disease, ulcerative colitis, arthritis rheumatoid and allograft rejection or graft-versus-host disease (Douik et al., 2009; López-Hernández et al., 2010; Mariaselvam et al., 2017; Redman et al., 2012) (Table 1).

We tested the hypothesis that *MICA 129 A/G* polymorphism on maternal decidual cells, (or other cell in the uterus, such as, myelomonocytic, glandular or stromal cells), in presence of some pathological stimuli, could influence the downregulation of NKG2D receptor expressed in uNK cells or the NK cytotoxic cell function against the fetus, inducing preterm labor.

2. Material and methods

2.1. Patients

Between April 2106 and February 2017, 50 pregnant women with singleton pregnancy and previous PTB, with or without premature rupture of membranes, or clinic diagnostic of threatening preterm birth in the actual pregnancy, or sonographic cervical length less than 25 mm, between 22 and 28 weeks (Iams et al., 1996) who attended to prenatal care from Complex of Clinics hospital from Federal University of Parana were invited to participate the study. Gestational age was determined by dating from last menstrual period, and it was corroborated by ultrasound dating. Patients who deliver preterm (between $22^{0/7}$ and $36^{0/7}$) were included. The diagnostic of PTB was the presence of regular uterine contractions at a minimum frequency of two contractions every 10 min, followed by documented cervical changes. Only pregnant women until 32 weeks of pregnancy were included in the study. Pregnant women with fetal malformation, uterine malformation, cervical surgery or any autoimmune diseases were excluded. A total of 50 healthy, singleton pregnant women were enrolled as controls in this study. All women received information about the goals of the research, including the potential benefits for patients in the future if some new diagnostic test or drug could be discovered. The study was approved by the ethics committee of the Complex of Clinics hospital from Federal University of Parana. Informed consent was obtained from all patients participating in this study.

Table 1
MICA 129 A/G alleles/haplotype and related diseases.

Disease	Allele association	Ethnic group	Risk or not	References
Ankylosing spondylitis	MICA-129 Met/Met	(Algeria)	Risk	(Amroun et al., 2005)
Psoriasis / Psoriatic Arthritis	MICA-129 Met allele	Caucasoid (North America)	Risk	(Pollock et al., 2013)
Insulin-dependent diabetes mellitus	MICA-129 Val/Val	Algerian	Risk	(Raache et al., 2012)
Systemic Lupus Erythematosus	MICA-129Met and A9	Oriental (Japanese)	Risk	(Yoshida et al., 2011)
Rheumatoid Arthritis	MICA-129Met MICA-129Val	Caucasoid (French and German) Tamal (Indians)	Protective Risk	(Kirsten et al., 2009) (Mariaselvam et al., 2017)
Ulcerative colitis	MICA-129Val	Chinese	Risk	(Zhao et al., 2011)
Inflammatory bowel disease	MICA-129Met/Val MICA-129Met/ Met	Murcians (Spanish) Murcians (Spanish)	Protective Risk	(López-Hernández et al., 2010)
Graft-versus-host disease	MICA-Val/ Val MICA129-Met	Caucasoid (French) Caucasoid (German))	Risk Protective	(Boukouaci et al., 2009) (Isernhagen et al., 2016)
Nasopharyngeal cancer	MICA-129 Val/Val	Tunisian	Risk	(Douik et al., 2009)
Severity of Chronic Chagas Heart disease	MICA-129 Met/Met MICA-129 Val/Val	Brazilian	Risk Protective	(Ayo et al., 2015)
Breast cancer	MICA-129 Val/Val			

2.2. Extraction of DNA

Five milliliters of peripheral venous blood were collected with ethylenediaminetetraacetic acid (EDTA) as anticoagulant from PTB pregnant women and controls. Genomic DNA was extracted from buffy-coat using Salting-Out method (Lahiri e Nurnberger, 1991). The plasma was separated by centrifugation within 30 min at 2400 rpm for 16 min and frozen at −80 °C until analysis.

2.3. MICA-129 polymorphism genotyping

MICA-129 genotypes were identified using a real-time polymerase chain reaction (RT-PCR) method. A customized TaqMan SNP Genotyping assay with primers, reporters dye and quencher specifically designed for *MICA* 129 A/G was purchased from Applied Biosystems. The exon 3 of the *MICA* gene was amplified using 5′-GCTCTTCCTCTC CAAAACCT-3′and 5′-CGTTCATGGCCAAGGTCTGA-3′as sense and antisense primers, respectively. The reporter 1 was AATGGACA**G**TGCCCC and reporter 2 was AATGGACA**A**TGCCCC. Then, the amplification was performed in a final volume of 10 µL containing 2 µL from 50 ng/ µL of genomic DNA, 5 µL Universal master mix, 0.5 µL from primer and reporter solution and 2.5 µL UP water. The PCR conditions (Mastercycler® RealPlex Thermal Cycler, Eppendorf Laboratories, Westbury, NY, USA) were as follows: heating at 50 °C for 2 min, heating at 95 °C for 10 min, 45 cycles of denaturing at 95 °C for 15 s, annealing and extension at 60 °C for 1 min, followed by a final endpoint step at 60 °C for 2 min. The *MICA*-129 genotyping method was established in accordance with the polymorphism (A to G) in exon 3 at position 454, which results in the amino acid change from methionine to valine in codon 129.