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MELATONIN RECEPTOR 1B POLYMORPHISMS AND REPRODUCTIVE FAILURE – A PILOT STUDY

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Abstract

The present study aimed to investigate the possible associations between the melatonin receptor 1B (*MTNR1b*) genetic polymorphisms and the presence of reproductive disturbances due to spontaneous abortion and implantation failure leading to pregnancy loss. The patients group included 35 women presenting with one or more miscarriages and/or implantation failure. Another 36 healthy women without history of implantation failure or miscarriages and with at least one birth served as a control group. Genomic DNA was extracted and genotyping for the *MTNR1b* polymorphisms rs1562444, rs10830962 and rs10830963 was performed by PCR-RFLP analysis. The genotype distribution of the investigated *MTNR1b* polymorphisms in patients with reproductive failure did not differ significantly to the group of women with at least one birth ($p > 0.05$ for all). Nevertheless, the presence of a haplotype rs1562444 AA rs10830962 GG was significantly less common in women with miscarriages or implantation failure than in women with at least one birth (2.9% vs. 22.2%, $p = 0.028$). We suggest that the haplotype might be protective against implantation failure and spontaneous miscarriage. Additionally, rs1562444 AA genotype was not found in women with recurrent pregnancy loss. Studies in

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larger populations are necessary to reveal the proper associations between different *MTNR1b* haplotypes, ovarian function and reproductive failure.

Key words: *MTNR1b*, SNP, rs1562444, rs10830962 and rs10830963, implantation failure, recurrent pregnancy loss

Introduction. A miscarriage is defined as the spontaneous pregnancy termination before the conceptus reaches viability, i.e. before the 24 weeks of gestation [1,2]. Recurrent pregnancy loss (RPL) might be considered in case of two or more unsuccessful clinical pregnancies, or in case of three or more pregnancy losses according to different scientific groups [1–3]. The main causes for pregnancy loss include genetic, anatomical, haemostatic, endocrine and immune factors, though the cause for more than half of recurrent miscarriages could not be explained [4–6]. Therefore, the present study aimed to investigate the possible associations between three genetic variants in the melatonin receptor 1B gene (*MTNR1b*, OMIM*600804) and the presence of spontaneous abortion and implantation failure.

Materials and methods. ***Subjects and study protocol.*** Thirty-five women with reproductive failure due to one or more miscarriages and/or repeated implantation failure (RIF) were recruited at Assisted Reproduction Clinic. Recurrent pregnancy loss (RPL) has been defined as the presence of two or more unsuccessful pregnancies [1,3]. After informed consent the patients provided blood samples for DNA analysis in K2EDTA tube. The genomic DNA was extracted through a standard salt extraction procedure. Genotyping for the *MTNR1b* polymorphisms rs1562444, rs10830962 and rs10830963 was performed by PCR-RFLP analyses employing methods described in detail elsewhere [7].

Thirty-six healthy women without history of implantation failure or miscarriages and with at least one birth were selected and served as a control group. Additionally the frequency distribution of the *MTNR1b* polymorphisms rs1562444, rs10830962 and rs10830963 in women with reproductive failure was compared to the published data of a population group of 101 clinically healthy Bulgarian women irrespective of their reproductive history [7]. The distribution of all investigated genotypes in healthy females was in agreement with the Hardy–Weinberg equilibrium. The experimental protocol was explained to all participants and written informed consent was obtained.

Statistical analysis. The results were presented as a frequency (%) for categorical variables. The data were analyzed through χ^2 test or Fisher's exact test. All results were considered significant at the 0.05 level. Calculations were made through the software package MedCalc Statistical Software ver. 19.2.6 (MedCalc Software bv, Ostend, Belgium; `https://www.medcalc.org`; 2020).

Results. The genotype distribution of the *MTNR1b* polymorphisms rs1562444, rs10830962 and rs10830963 did not differ between patients with reproductive failure (miscarriages or implantation failure) and women with at least

Table 1

Genotype frequency of the *MTNR1b* polymorphisms rs1562444, rs10830962 and rs10830963 in patients with reproductive failure (miscarriages or implantation failure) and women with at least one birth (p – differences between women with reproductive failure and women with at least one birth)

MTNR1b polymorphism	Genotype	Women with reproductive failure ($N = 35$)	Women with at least one birth ($N = 36$)
rs1562444	AA	30.3%	33.3%
$p = 0.961$	AG	51.5%	50.0%
	GG	18.2%	16.7%
rs10830962	CC	20.6%	29.4%
$p = 0.470$	CG	55.9%	41.2%
	GG	23.5%	29.4%
rs10830963	CC	55.9%	42.9%
$p = 0.517$	CG	35.3%	48.6%
	GG	8.8%	8.6%

one birth (Table 1). The genotype distribution of the *MTNR1b* polymorphisms rs1562444, rs10830962 and rs10830963 in patients with reproductive failure (miscarriages or implantation failure) was similar to that described in healthy Bulgarian women [7] ($p > 0.05$ for all).

Women with one or more miscarriages ($n = 15$) did not differ from women with implantation failure ($n = 20$) in regard to the investigated *MTNR1b* polymorphisms ($p > 0.05$ for all). All the patients with RPL ($n = 7$) were with rs1562444 (AG) or (GG) but no (AA) genotype, while in other female patients the prevalence of rs1562444 AA genotype was 38.5% ($p = 0.073$). No differences in the genotype distribution of the *MTNR1b* polymorphisms rs10830962 or rs10830963 were established between women with RPL and other patients ($p > 0.05$ for all).

The distribution of the three polymorphisms showed linkage disequilibrium in healthy women. Women with rs1562444 AA genotype had concomitant rs10830962 GG genotype significantly more often than other genotypes, while women with rs1562444 GG genotype were usually with rs10830962 CC genotype ($p < 0.001$) (Fig. 1). The presence of a haplotype rs1562444 AA/rs10830962 GG was significantly less common in women with miscarriages or implantation failure than in women with at least one birth (2.9% vs. 22.2%, $p = 0.028$). The presence of haplotype rs1562444 GG /rs10830962 CC was not significantly related to the reproductive failure ($p > 0.05$).

The presence of rs1562444 GG genotype was usually associated with the presence of rs10830963 CC genotype ($p = 0.003$). The combined haplotype rs1562444 GG/rs10830963 CC was not related to the reproductive failure ($p > 0.05$). The genotype distribution of rs10830962 and the genotype distribution of rs10830963 were also related ($p = 0.001$), but no associations of the combined genotypes

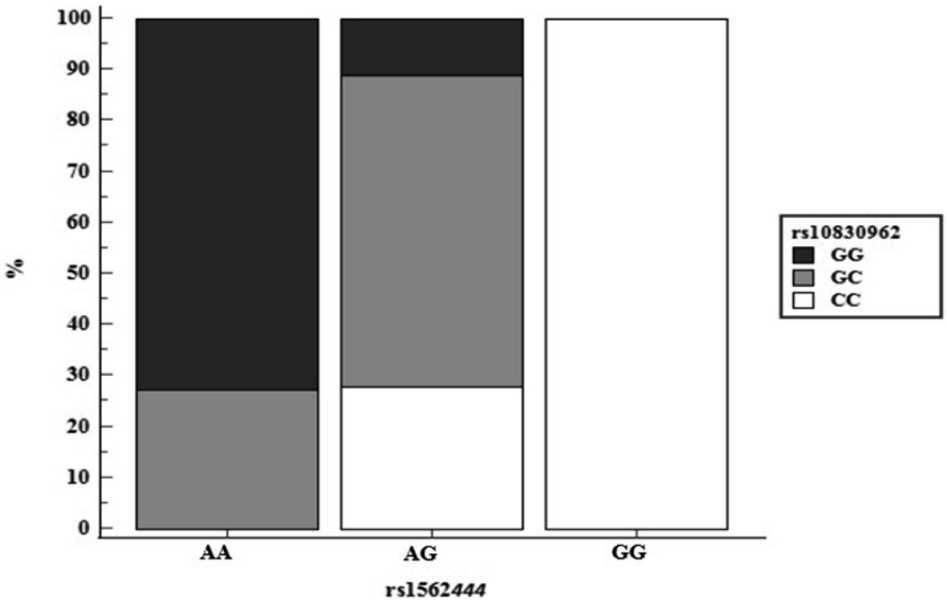

Fig. 1. Associations between the *MTNR1b* polymorphisms rs1562444 and rs10830962 in healthy women ($p > 0.001$)

with the prevalence of miscarriages and/or implantation failure were established ($p > 0.05$ for all).

Discussion. The present study shows that the prevalence of *MTNR1b* genetic polymorphisms rs1562444, rs10830962 and rs10830963 is similar in patients with reproductive failure (miscarriages or implantation failure) compared to women with at least one birth. It is also similar to those found in the common female population in a previous study on the same ethnic group [7]. Considering the small number of investigated women we could not generalize this conclusion. However, we can speculate that the haplotype rs1562444 AA/rs10830962 GG might be protective against implantation failure and spontaneous miscarriage. Moreover, rs1562444 AA genotype was not determined among women with recurrent pregnancy loss suggesting in addition a protective influence of the same genotype.

The selected *MTNR1b* genetic variants in our study have been investigated in association with different metabolic and autoimmune diseases. The genotype rs1562444 AA was significantly more common than rs1562444 AG and rs1562444 GG genotypes in patients with rheumatoid arthritis and positive rheumatoid factor compared to controls but not in those with negative rheumatoid factor [8]. The rs1562444 genotype distribution did not differ between patients with systemic lupus erythematosus (SLE) and diabetes mellitus type 2 in comparison to healthy controls from the same population [7,9,10], but it could influence some of the clinical manifestations of the SLE [7,9]. Interestingly, Wang et al. [9] found

significantly different plasma melatonin levels in SLE patients according to the rs1562444 genotypes, but the result could not be linked to a healthy population.

Many reports consider the rs10830962 and rs10830963 polymorphisms in association with metabolic diseases. Homozygous GG carriers of both polymorphisms have shown increased fasting glucose level and decreased insulin secretion in comparison to the carriers of other genotypes [11]. The rs10830962 and rs10830963 polymorphisms have been related to increased risk of type 2 diabetes and gestational diabetes in most studies considering the minor G allele as diabetogenic [12–14]. *MTNR1b* polymorphisms have not been investigated yet in regard to pregnancy loss despite the possible pathophysiological associations. Melatonin in its virtue of a circadian regulator might exert an important role for the uterine homeostasis, implantation process and placentation (reviewed in [15]). Moreover, melatonin treatment increases significantly the clinical pregnancy rate in assisted reproductive technology cycles [16]. Twenty years ago, Sandyk et al. [17] suggested that melatonin deficiency might be related to miscarriages considering the observed increase of melatonin levels during gestation and its systemic endocrine and non-endocrine effects. Melatonin might decrease uterine contractions and prostaglandin synthesis, and stimulate progesterone secretion [17]. Additionally, the antioxidant, cell protective and immune modulating features of melatonin probably favour the successful pregnancy and hamper the possible complication (reviewed in [18, 19]). Melatonin could up-regulate several genes involved in the progesterone synthesis of the corpus luteum through the melatonin receptor type 1B [20]. Thus, we could speculate that the *MTNR1b* polymorphisms might modulate the corpus luteum function and the risk of miscarriages.

In conclusion, our pilot study suggests that rs1562444 AA/rs10830962 GG haplotype might be protective against implantation failure and miscarriages. There is a need for further studies in larger groups to reveal the proper associations between different *MTNR1b* haplotypes, corpus luteum function and reproductive failure.

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