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Original Research

Melatonin Receptor 1B and Corticosteroid Receptor Polymorphisms in Infertile Women with Implantation Failure and Miscarriages

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Abstract

Background: The development of assisted reproductive techniques has significantly improved fertility chances in many women, but recurrent implantation failure (RIF) and miscarriages (RM) might preclude successful pregnancy. Alterations in the intrinsic secretory patterns of melatonin and cortisol influence reproduction in humans, and imperfection of receptor — dependent signaling may additionally compromise the hormonal effects. Therefore, the present study aims to investigate the influence of certain melatonin and cortisol receptor polymorphisms in infertile women. **Methods**: A total of 111 female infertile patients suffering from implantation failure and/or miscarriages were genotyped for *MTNR1B* rs1562444, *MTNR1B* rs10830962, *GCCR* rs41423247, and *GCCR* ER22/23EK variants. Additionally, 106 female volunteers were genotyped for the same polymorphisms. **Results**: The allele and genotype distribution of the investigated polymorphisms did not differ between infertile women and the control group. Significantly more women with history of RIF have *MTNR1B* rs1562444 G-allele-containing genotypes in comparison to AA carriers (19.3% vs. 3.6%, $p = 0.004$). The minor allele of the ER22/23EK variant was more frequent in infertile patients with three or more unsuccessful implantation attempts than in other women (12.5% vs. 2.4%, $p = 0.025$). **Conclusions**: Melatonin receptor 1B polymorphisms might affect embryo implantation and early pregnancy loss, while their influence on late pregnancy complications needs further evaluation. The possible association between the cortisol receptor ER22/23EK variant and recurrent implantation failure might help to differentiate women who could benefit from corticosteroid treatment.

Keywords: MTNR1B polymorphism; GCCR polymorphism; infertility; implantation failure

1. Introduction

The prevalence of pregnancy loss varies between 10 and 25% in the common female population, and often it may occur even before its recognition [1,2]. Additionally, some women experience two or more pregnancy losses leading to a significant psychological burden on the affected patients and their families [3,4]. The development of assisted reproductive techniques has significantly improved the fertility chance of many couples, but recurrent implantation failure (RIF) emerges as a new obstacle precluding successful reproduction [5].

Miscarriage is defined as spontaneous pregnancy termination before 24 weeks of gestation [3]. The presence of two or more pregnancy losses is considered recurrent miscarriages, while recurrent implantation failure (RIF) corresponds to three or more unsuccessful *in vitro* fertilization-embryo transfer cycles (IVF-ETs) [3,6]. Recurrent miscarriages and RIF are distinct processes caused by different gynecological, endocrine, and immune factors [5, 7]. Moreover, genetic disturbances, including aneuploidy, copy number variations, skewed X inactivation, or single-gene mutations in the embryo, might lead to its demise [8]. Different maternal genetic polymorphisms modulating coagulation, metabolism, immune response, placental function, and estrogen receptor sensitivity have been thoroughly investigated regarding reproduction failure [9–11]. However, the role of certain hormonal receptor polymorphisms involved in physiological adaptation to environmental challenges has not been studied in detail.

Recently, a large population-based study in Denmark revealed that two or more work night shifts weekly increased the risk of miscarriage by more than 30% [12]. In addition, circadian rhythm alterations in night-shift workers are associated with hormonal disturbances such as decreased melatonin excretion and increased cortisol secretion, which might contribute to reproductive failure [13,14]. However, it is still unclear if receptor genetic variants of these hormones might influence the RIF prevalence in infertile couples.

Our previous pilot study showed a possible protective effect of the melatonin receptor 1B gene (*MTNR1B,* OMIM* 600804) rs1562444 genotype AA on recurrent miscarriages [15], but other reports on the topic are lacking. The role of glucocorticoid receptor (*GCCR*, OMIM* 138040) polymorphisms is also unclarified since only one study has investigated glucocorticoid receptor variants in women with recurrent miscarriages, while data about different ethnic groups are lacking [16]. Therefore, the present study aimed to investigate the relevance of *MTNR1B* rs1562444, *MTNR1B* rs10830962, *GCCR* rs41423247, and *GCCR* ER22/23EK variants to the reproductive failure in infertile women with a history of RIF.

2. Materials and Methods

2.1 Subjects and Study Protocol

A total of 111 female infertile patients (27–49 years) with reproductive failure - implantation unsuccess and/or miscarriages, were recruited at Assisted Reproduction Clinic. A total of 35 patients from the group had at least one miscarriage, while 81 patients had at least one failed IVF-ET with five of women suffering from both unsuccessful IVF-ET and at least one miscarriage (Fig. 1). Recurrent pregnancy loss (two or more pregnancy losses) was established in 19 patients (17.1% of all infertile patients). A total of 34 women were with three or more unsuccessful IVF-ETs (defined as recurrent implantation failure, RIF) (Fig. 1).

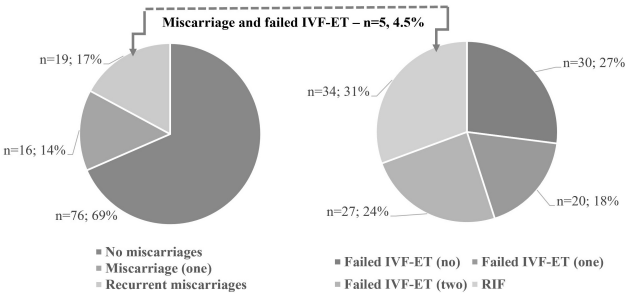


Fig. 1. Prevalence of failed *in vitro* fertilization-embryo transfer cycles (IVF-ETs), recurrent implantation failure (RIF) and miscarriages in the investigated group of infertile women.

A population-based control group of 106 female volunteers of a similar age (22–55 years) and from a similar Caucasian ethnic origin (East Europeans) was investigated. A total of 36 infertile women and 31 individuals from the control group were older than 40 years, while other participants were of younger reproductive age.

The experimental protocol was explained to all participants, and written informed consent for participation in the study and publication of the results was provided by each of them. The investigation was approved by the Ethics Committee of Sofia University, Bulgaria, and was performed in compliance with the WMA Declaration of Helsinki. Patients and controls provided blood samples, and genomic DNA was extracted using GeneMATRIX Quick Blood DNA Purification Kit (EURx, Gdańsk, Poland). A total of 206 participants were genotyped for *MTNR1B* rs1562444 A/G, 199 — for *MTNR1B* rs10830962 G/C, 203 — for *GCCR Bcl*I rs41423247 C/G, and 198 women — for *GCCR* ER22/23EK G/A. A PCR-RFLP analysis for polymorphism determination was performed according to previously described methods [15,17,18]. Standard PCR technique for amplification was used with the following sets of primers: *MTNR1B* rs1562444 A/G – F: 5′-GAAAACACTCTTGGTGGTGTCTT-3′, R: 5′-GATGTGGTGGCTATGTGTGTGTGTA-3′; *MTNR1B* rs10830962 G/C – F: 5′-TACTAGATATTAGCTGTGTGCTAGTGACT-3′, R: 5′-TCTGGGCAACTCAGTGAAACC-3′; *GCCR Bcl*I rs41423247 C/G – F: 5′-GAGAAATTCACCCCTACCAAC-3′, R: 5′-AGAGCCCTATTCTTCAAACTG-3′; *GCCR* ER22/23EK G/A-F: 5′-TTGATTCGGAGTTAACTAAAAG-3′, R: 5′-ATCCCAGGTCATTTCCCATC-3′. The amplified fragments were subsequently digested with the restriction endonucleases HinfI, NlaIII, BclI and MnlI respectively and 2.5% agarose gel electrophoresis with Ethidium bromide was used for the separation of the fragments received. The espected profiles of the different genotypes is presented on Table 1.

2.2 Statistical Analysis

The distribution of all investigated genotypes in the control group was in agreement with the Hardy-Weinberg equilibrium. The results were presented as mean ± SD [median] for continuous variables or frequency (%) for dichotomous variables. Categorical data were analyzed through the χ^2 test or Fisher's exact test. Logistic regression was used to establish the influence of different polymorphisms on the RIF after adjustment for age. All results were considered significant at the 0.05 level. Additionally, the Bonferroni adjustment for multiple testing was used, and the significance of the *p*-value was set at 0.0125 (0.05/4 considering the four investigated polymorphisms). Analyses were performed using software package MedCalc® Statistical Software version 20.009 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021).

3. Results

The infertile patients and control group of women were of a similar age (37.85 ± 5.08 [38] years vs. 36.71 ± 9.11 [36] years, $p = 0.260$). The distribution of *MTNR1B* rs1562444, *MTNR1B* rs10830962, *GCCR* rs41423247, and *GCCR* ER22/23EK polymorphisms did not differ between the two groups (Table 2).

The *MTNR1B* rs1562444 AA genotype tended to be less frequent in patients with two or more failed IVF-ET than in other women (17.9% vs. 31.3% $p = 0.057$). Sig-

Table 1. Restriction enzymes used for PCR-RFLP analysis for polymorphism determination and espected genotype profiles on 2.5% agarose gel electrophoresis.

Gene variant	Restriction endonuclease	Genotype profiles on 2.5% agarose gel electrophoresis
MTNR1B rs1562444 A/G	HinfI	G/G: 319 bp + 81 bp G/A: 319 bp + 163 bp + 156 bp + 81 bp A/A: 163 bp + 156 bp + 81 bp
MTNR1B rs10830962 G/C	NlaIII	GG: 210 bp GC:210 bp + 184 bp + 26 bp CC: 184 bp + 26 bp
*GCCR Bcl*I rs41423247 C/G	BclI	GG Wt: 263 bp + 151 bp GC Het: 418 bp + 262 bp + 151 bp CC Minor: 418 bp
GCCR ER22/23EK G/A	MnlI	GG: 149 bp + 163 bp (+ 50 bp + 49 bp + 35 bp) GA: 184 bp + 149 bp + 163 bp (+ 50 bp + 49 bp + 35 bp) AA: 184 bp + 163 bp (+ 50 bp + 49 bp)

Table 2. Genotype distribution of the investigated polymorphisms.

Polymorphism	Genotypes	Frequency (controls)		Frequency (patients)		*p*
		n	%	n	%	
MTNR1B (Melatonin receptor 1B)						
rs1562444	AA	29	28.4%	27	26.0%	0.787
	GA	54	52.9%	60	57.7%	
	GG	19	18.6%	17	16.3%	
rs10830962	GG	30	30.6%	24	23.8%	0.260
	GC	42	42.9%	55	54.5%	
	CC	26	26.5%	22	21.8%	
GCCR (Glucocorticoid receptor)						
ER22/23EK	GG	91	96.8%	99	95.2%	0.724
	GA	3	3.2%	5	4.8%	
	AA	0	0%	0	0%	
rs41423247 (*BclI*)	GG	41	44.1%	57	51.8%	0.243
	GC	39	41.9%	45	40.9%	
	CC	13	14.0%	8	7.3%	

p — differences between genotype frequencies.

nificantly more women with *MTNR1B* rs1562444 G-allele-containing genotypes suffered from RIF than AA carriers (Fig. 2). The presence of *MTNR1B* rs1562444 AG or GG genotype was associated with a significantly increased risk of RIF after adjustment for age in the group of infertile women (OR 9.199 [1.955–43.278], $p = 0.005$). However, the same variant was not related to the number of miscarriages in the investigated groups.

MTNR1B rs10830962 polymorphism did not influence the prevalence of miscarriages or RIF among all investigated women ($p > 0.05$ for all). In younger women (age $\leq$40 years), the prevalence of *MTNR1B* rs10830962 GC heterozygous state was increased in infertile patients compared to the other women (60.0% vs. 40.3%, $p = 0.026$), but no other associations were established.

The minor allele of the ER22/23EK was found very rarely (only in 4% of all investigated women), and no homozygous CC samples were found. Nevertheless, the prevalence of the minor allele was significantly increased in patients with three or more unsuccessful implantation attempts than in other women (12.5% vs. 2.4%, $p = 0.025$).

Corticosteroid receptor rs41423247 polymorphism was not significantly related to the increased prevalence of miscarriages or implantation failure in the whole group and considering younger women only ($p > 0.05$ for all) (Table 2). Interestingly, the same polymorphism was significantly associated with the *MTNR1B* rs1562444 polymorphism ($p = 0.024$), and the heterozygous *MTNR1B* rs1562444 AG carriers have variant rs41423247 alleles significantly more often than AA and GG carriers ($p = 0.001$).

Considering the Bonferroni correction for multiple comparisons, only the association between the *MTNR1B* rs1562444 G allele and RIF was statistically significant.

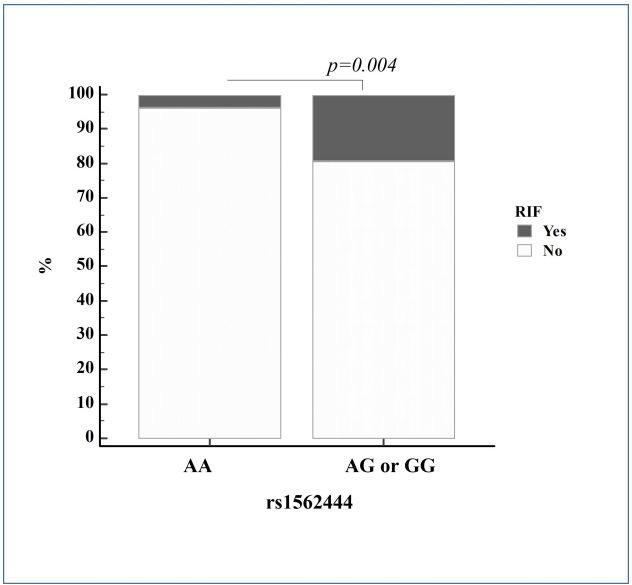


Fig. 2. Prevalence of recurrent implantation failure (RIF) according to rs1562444 genotype.

4. Discussion

The present study showed that melatonin receptor 1B gene polymorphisms might influence the implantation success in infertile patients. The rs1562444 G allele was associated with an increased risk of RIF, but it did not influence the number of miscarriages. In women under the age of 40 years, the prevalence of *MTNR1B* rs10830962 GC heterozygous state was more common in infertile patients with failed IVF-ETs or miscarriages than in women from the common population. Thus, the melatonin receptor polymorphisms could have impacted the embryo–endometrium relationships, at least in some population groups. RIF and recurrent miscarriages are distinct categories sharing common ground and overlapping etiology [19]. In the case of RIF, there was a failure in the embryo transplantation, while in RM, the implantation could not be sustained [20]. However, it is challenging to distinguish implantation failure from early occult pregnancy loss, since similar embryonic and maternal factors might cause unsuccessful IVF-ET and early post-implantation loss of the conceptus [19]. Therefore, we can speculate that melatonin receptor 1B gene polymorphisms might influence embryo implantation and early pregnancy loss, while their influence on late pregnancy complications needs further evaluation.

Melatonin plays a vital role in normal pregnancy through different mechanisms. As a potent antioxidant, the hormone protects the cytotrophoblasts from oxidative stress and apoptosis [21]. In addition, it increases progesterone secretion and exerts immunomodulatory effects, which could promote trophoblast survival [22,23]. Furthermore, the maternal pineal indolamine provides photoperiodic signals that are crucial for adjusting the embryonic circadian rhythms [24]. Melatonin treatment of IVF patients has been associated with an increased biochemical and clinical pregnancies rate, though it does not influence the miscarriage or live birth rate [25].

The beneficial effects of melatonin on progesterone secretion, implantation, and early pregnancy development might be at least partly receptor-mediated since melatonin receptors *MTNR1A*, and *MTNR1B* are widely expressed in the ovaries, uterus, placenta, and fetal tissues [26,27]. Thus, the melatonin receptor gene polymorphisms are possible modulators of reproduction, though they have been studied mostly in regard to metabolic and autoimmune disturbances [28–31].

The *MTNR1B* rs10830962 risk allele has been associated with a higher fasting glucose and decreased insulin secretion after glucose load in non-diabetic individuals [28]. Additionally, it might increase the risk of gestational diabetes mellitus in pregnant women [29]. At the same time, it determines an optimal response to increased physical activity in the same population [29]. The *MTNR1B* rs1562444 polymorphism has been associated with increased serum rheumatoid factor positivity in patients with rheumatoid arthritis, and it could modulate the daily melatonin levels in patients with systemic lupus erythematosus [30,31]. Thus, the influence of *MTNR1B* polymorphisms on reproductive failure might be associated with metabolic alterations, increased antibody production, and changes in melatonin concentrations. Interestingly, in our group, the *MTNR1B* rs10830962 heterozygous state was related to an increased infertility risk only in younger women but not in the whole group. The implantation success and rate of miscarriages depend strongly on maternal age, and in late reproductive years, ovarian aging and increased frequency of chromosomal anomalies are among the leading causes of pregnancy loss [32–34]. Thus, the weak influence of the melatonin receptor polymorphism on embryo-endometrial interrelations might be clinically significant only for younger women without other severe causes for implantation failure. Further studies are needed to explore these hypotheses.

Glucocorticoids represent essential hormones exerting pleiotropic physiological effects through the glucocorticoid receptor [35]. They are involved in the regulation of cell homeostasis, carbohydrate, fat, protein metabolism, and immunity; however, their influence on reproduction is still contradictory [35,36]. According to different authors, decreased and increased cortisol concentrations in the follicular fluid have been associated with better fertilization rates [36–38]. The glucocorticoid rise could directly suppress the gonadotropin-releasing hormone transcription through glucocorticoid receptor signaling leading to decreased gonadotropin secretion and ovulatory dysfunction [39]. On the other hand, optimal cortisol levels might exert immunosuppressive effects, which could facilitate embryo implantation and prevent immunological rejection [39,40]. The action of glucocorticoids might depend on glucocorticoid receptor gene polymorphisms, which could modu-

late the cortisol biological effects in the tissues [41,42]. The ER22/23EK polymorphism of the glucocorticoid receptor gene consists of two linked single-nucleotide genetic variants in codons 22 and 23 of the exon 2 [42,43]. The ER22/23EK minor allele was related to decreased corticosteroid sensitivity estimated through the low dexamethasone suppression test [43,44]. In opposite, the rs41423247 *Bcl*I variant located in intron 2 of the glucocorticoid receptor gene was associated with an increased corticosteroid sensitivity [44]. According to our results, *Bcl*I polymorphism was not associated with reproductive failure risk, while the ER22/23EK minor allele was related to the increased prevalence of RIF. Only one study has investigated both polymorphisms with respect to recurrent miscarriages and did not find statistically significant associations [16]. Nevertheless, the minor rs41423247 C allele tended to be less frequent in women with recurrent miscarriages than in the control group [16].

It has been suggested that the ER22/23EK minor variant carriers differ from non-carriers in terms of longevity due to improved metabolic and inflammatory profiles [43, 44]. At the same time, the ER22/23EK minor allele determines increased predisposition to some autoimmune diseases, and it might modulate the luteinizing hormone levels in women with the polycystic ovarian syndrome [45,46]. The possible link between the same variant and RIF might help to differentiate women who might benefit from corticosteroid treatment. Thus, further research on other ethnic groups is essential.

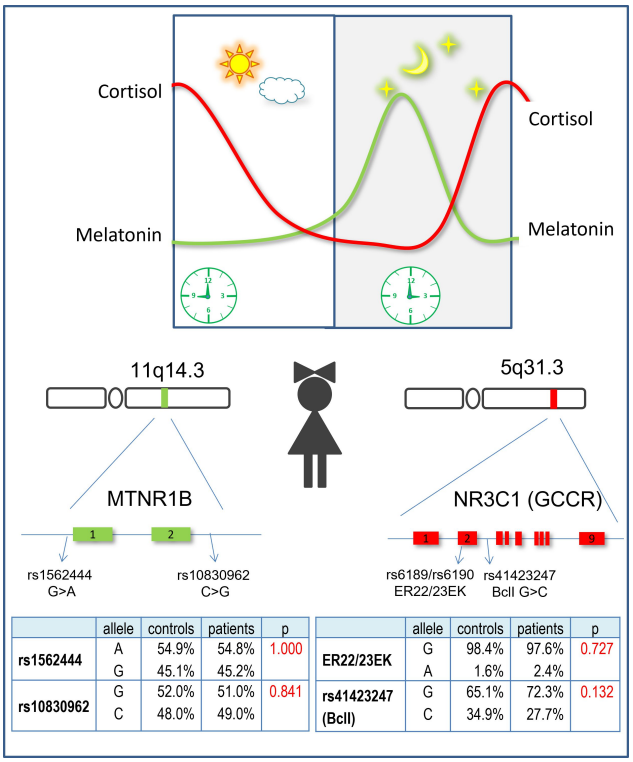
Melatonin and cortisol are hormones with opposite circadian rhythms (Fig. 3), e.g., melatonin secretion peaks during the night, when the cortisol levels are low, and decreases after that, when cortisol levels increase reaching their maximal values early in the morning [47]. Melatonin might increase cortisol secretion in postmenopausal women, and the interrelations between the two hormones depend on peripheral estrogen concentrations [48,49].

5. Conclusions

We have found an association between *MTNR1B* and *GCCR* polymorphisms, but the physiological meaning or the observed receptor interrelations remain obscure. The study has several limitations, such as a relatively low number of patients, heterogeneity of the investigated infertile group, and the lack of hormonal measurements of melatonin and cortisol secretion. Nevertheless, the results show that *MTNR1B* and *GCCR* polymorphisms might be associated with recurrent implantation failure. Further studies on the topic might help understand underlying pathophysiological mechanisms.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.



	allele	controls	patients	p
rs1562444	A	54.9%	54.8%	1.000
	G	45.1%	45.2%	
rs10830962	G	52.0%	51.0%	0.841
	C	48.0%	49.0%	

	allele	controls	patients	p
ER22/23EK	G	98.4%	97.6%	0.727
	A	1.6%	2.4%	
rs41423247 (BclI)	G	65.1%	72.3%	0.132
	C	34.9%	27.7%	

Fig. 3. Melatonin and cortisol circadian rhythms of secretion and genetic polymorphisms in their receptors. Melatonin secretion peaks during the night, and decreases during the day, while cortisol levels are strongly increased in the morning and decrease in the evening. Variant location and allele frequencies of the investigated melatonin receptor 1B (*MTNR1B*) and glucocorticoid receptor (GCCR) polymorphisms are shown.

Author Contributions

RR, RK, G.Nikolaev and G.Nikolov designed the research study. EM, SA, AS and DT performed the research. RR and analyzed the data. RR, G.Nikolaev, G.Nikolov and RK wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The experimental protocol was explained to all participants, and written informed consent for participation in the study and publication of the results was provided by each of them. The investigation was approved by the Ethics Committee of Sofia University "St. Kliment Ohridski", Bulgaria (Ref. No. 93-G-8), and was performed in compliance with the WMA Declaration of Helsinki.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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