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Association between single nucleotide polymorphisms of MUTYH, hOGG1 and NEIL1 genes, and depression.

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ABSTRACT

Background: An elevated levels oxidative modified DNA bases and a decreased efficiency of oxidative DNA damage repair were found in patients with depression disorders, including recurrent type (rDD). The glycosylases are involved in base excision repair (BER), which eliminates oxidative DNA damage. Therefore, we genotyped the single nucleotide polymorphisms (SNPs) of genes encoding three glycosylases: hOGG1, MUTYH and NEIL1.

Methods: We selected three polymorphisms: c.977C > G – *hOGG1* (rs1052133), c.972G > C – *MUTYH* (rs3219489) and c.*589G > C – *NEIL1* (rs4462560). A total of 555 DNA samples (257 cases and 298 controls) were genotyped using TaqMan probes.

Results: The C/C genotype and allele C of the c.*589G > C decreased the risk of rDD occurrence, while the G/G genotype and allele G of the same SNP increased the risk. This polymorphism had a stronger association with early-onset depression (patients with first episode < 35 years of age) than with late-onset depression (first episode ≥ 35 years of age). We did not find any significant differences in distribution of alleles and genotypes of other SNPs; however, the G/G genotype of the c.972G > C increased the risk of late-onset rDD. We also found that combined genotype C/C–C/C of c.977C > G and c.*589G > C significantly reduced the risk of rDD.

Limitations: Limited sample size and ethnic homogeneity of the studied population.

Conclusion: This is the first study to show that SNPs of genes involved in DNA repair, particularly in BER pathway, may modulate the risk of rDD. These results further support the hypothesis on the involvement of DNA repair mechanisms in pathogenesis of depression.

1. Background

A growing body of evidence indicates that inflammation may play an important role in pathogenesis of depression disorder (including recurrent depressive disorder [rDD]) (Gardner and Boles, 2011). Increased levels of pro-inflammatory cytokines were found in the depressed patients (Maes et al., 1993; Rawdin et al., 2013). Activation of two of those cytokines, interleukin-1b (IL-1β) and interleukin-8, is done by the inflammasome – a complex of proteins containing Nod-like receptor (NLR) – and elevated expression of one of the NLRs – NLRP3 – was detected in peripheral blood mononuclear cells (PBMCs) of depressed patients (Leemans et al., 2011; Alcocer-Gómez et al., 2014).

Abbreviations: RDD, recurrent depression disorder; IL-1β, interleukin-1b; IL-8, interleukin-8; NLR, Nod-like receptor; PBMCs, peripheral blood mononuclear cells; mtROS, mitochondrial reactive oxygen species; 8-oxoG, 8-oxoguanine; SNPs, single nucleotide polymorphisms; hOGG1, human 8-oxoguanien glycosylase 1; MUTYH, MutY E. coli homolog; NEIL1, nei endonuclease VIII-like 1; HDRS, Hamilton Depression Rating Scale; CIDI, Composite International Diagnostic Interview; NCBI dbSNP, National Center for Biotechnology Information the Single Nucleotide Polymorphisms database; RT-PCR, real-time polymerase chain reaction; HWE, Hardy–Weinberg equilibrium; OR, odds ratio; CI, confidence interval; AP, apurinic/apyrimidinic site; FapyG, 6-diamino-4-hydroxy-5-formamidopyrimidine; FapyA, 4,6-diamino-5-formamidopyrimidine; NTH1, neutral trehalase 1

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It has been suggested that NLRP3 may be involved in DNA damage response (DDR). Its knock-out increased expression of BER and double-strand repair genes, and decreased apoptosis in murine dendritic cells exposed to genotoxic and oxidative stress, thus NLRP3 can suppress DNA damage repair and induce apoptosis mediated by p53 (Licandro et al., 2013). In agreement with this, an elevated level of 8-oxoguanine (8-oxoG), which is a marker of oxidative DNA damage, was found in serum, urine and lymphocytes of the patients with clinical depression as well as depression coexisting with other non-mental diseases (Irie et al., 2001; Forlenza and Miller, 2006; Irie et al., 2003; Maes et al., 2009; Wei et al., 2009; Kupper et al., 2009). On the other hand, urinary levels of 8-oxoG of Japanese office workers were not associated with mild depression symptoms (Yi et al., 2012). Our results obtained by using comet assay showed that PBMCs isolated from patients with rDD had more DNA damage, including oxidative modification of purine and pyrimidines, when compared to PBMCs of the control group (Czarny et al., 2015). Moreover, we also revealed that the patients' cells repair of oxidative DNA damage induced by hydrogen peroxide in less efficient way than the controls' cells.

The impairment of DNA base excision repair (BER) pathway, which is responsible for repair of oxidative DNA damage, might be associated with pathological neurophysiology of depression. Therefore, in this paper we examine the relationship between single nucleotide polymorphisms (SNPs) of glycosylases involved in BER: c.977C > G (rs1052133) of *hOGG1* (human 8-oxoguanien glycosylase 1), c.972G > C (rs3219489) of *MUTYH* (MutY *E. coli* homolog, encoding MUTYH protein), c.*589G > C (rs4462560) of *NEIL1* (nei endonuclease VIII-like 1) and incidence of depression, as well as age at which the first episode occurred.

2. Methods

2.1. Study subjects and data collection

The study was carried out in a group of 555 subjects: patients with rDD ($n=257$, age 51.9 ± 12.9) and a matched group of healthy controls ($n=298$, age 49.1 ± 10.4).

All patients were hospitalized at the Department of Adult Psychiatry of the Medical University of Lodz, Poland. The selection of individuals for the study group was performed randomly without replacement sampling.

The patients were selected based on the inclusion criteria for ED and rDD outlined in ICD-10 (F32.0-7.32.2, F33.0-F33.8) (World Health Organization, 1992). The presence of axes I and II disorders, other than depressive episodes, and the diagnosis of somatic diseases and injuries of the central nervous system were regarded as exclusion criteria. Other exclusion criteria included: inflammatory or autoimmune disorders and unwillingness to give informed consent. For all the subjects, a case history was obtained prior to participation using the standardized Composite International Diagnostic Interview (CIDI) (Patten, 1997).

All the subjects were free from medical illnesses, including infectious and inflammatory or allergic reactions. None of the control subjects or depressed patients was treated with drugs known to influence lipid metabolism, immune response or endocrine function. None of the participants were drinkers or heavy smokers, and none had ever taken psychotropic drugs.

An informed, written consent for participation in the study was obtained from each subject, according to the protocol approved by the Bioethics Committee of the Medical University of Lodz (No. RNN/70/14/KE).

2.2. Selection of single-nucleotide polymorphisms

To choose the SNPs we used the public domain of National Center for Biotechnology Information the Single Nucleotide Polymorphisms database (NCBI dbSNP) at http://www.ncbi.nlm.nih.gov/snp (Bethesda, MD, USA). We selected polymorphisms that have known distribution in European population, their minor allele frequency is larger than 0.05 (submitter population ID: HapMap-CEU) and are localized either in the coding or regulatory region of the genes: c.977C > G is localized in coding region of *hOGG1* gene and causing serine to cysteine substitution at codon 326 of the protein, c.972G > C is localized in exon of *MUTYH* and causing glutamine to histidine substitution in codon 324, and c.*589G > C is located near 3′ end of *NEIL1*. Another choosing reason

Table 1
Distribution of genotypes and alleles of c.977 C > G, c.972 G > C and c.*589 G > C and the risk of rDD.

Genotype /Allele	Control ($n=298$)		Depression ($n=257$)		Crude OR (95% CI)	p	Adjusted OR*(95% CI)	p
	Number	Frequency	Number	Frequency				
***hOGG1*c.977C > G (rs1052133)**								
C/C	190	0.638	146	0.568	0.748 (0.531–1.052)	0.095	0.744 (0.529–1.048)	0.091
C/G	95	0.319	98	0.381	1.317 (0.928–1.870)	0.123	1.060 (0.758–1.481)	0.120
G/G	13	0.044	13	0.051	1.168 (0.531–2.567)	0.699	1.175 (0.534–2.584)	0.689
$\chi^2=2.795$; $p=0.247$								
C	475	0.797	390	0.759	0.799 (0.600–1.064)	0.124	0.796 (0.597–1.060)	0.119
G	121	0.203	124	0.241	1.252 (0.940–1.667)	0.124	1.257 (0.943–1.675)	0.119
***MUTYH*c.972G > C (rs3219489)**								
C/C	198	0.664	176	0.685	1.097 (0.768–1.567)	0.609	1.098 (0.769–1.568)	0.608
C/G	93	0.312	71	0.276	0.841 (0.583–1.215)	0.357	0.841 (0.583–1.215)	0.356
G/G	7	0.023	10	0.039	1.683 (0.631–4.487)	0.298	1.680 (0.630–4.481)	0.300
$\chi^2=1.986$; $p=0.371$								
C	489	0.820	423	0.823	1.017 (0.746–1.386)	0.914	1.018 (0.747–1.387)	0.911
G	107	0.180	91	0.177	0.983 (0.721–1.340)	0.914	0.983 (0.721–1.339)	0.911
***NEIL1*c.*589G > C (rs4462560)**								
C/C	195	0.654	144	0.560	**0.673 (0.478–0.949)**	**0.024**	**0.669 (0.474–0.944)**	**0.022**
C/G	95	0.319	94	0.344	1.232 (0.867–1.752)	0.245	1.238 (0.870–1,763)	0.235
G/G	8	0.027	19	0.074	**2.894 (1.245–6.728)**	**0.014**	**2.896 (1.246–6.733)**	**0.014**
$\chi^2=9.181$; $\mathbf{p=0.010}$								
C	485	0.814	382	0.743	**0.661 (0.496–0.883)**	**0.005**	**0.658 (0.493–0.879)**	**0.005**
G	111	0.186	132	0.257	**1.512 (1.133–2.017)**	**0.005**	**1.519 (1.137–2.028)**	**0.005**

$p < 0.05$ along with corresponding ORs are in bold.

* OR adjusted for sex.

was fact that c.977C > G and c.972G > C are the most frequently studied polymorphism of *hOGG1* and *MUTYH*, respectively.

2.3. DNA extraction

Genomic DNA was extracted from venous blood using Blood Mini Kit (A&A Biotechnology, Gdynia, Poland) and stored at −20 °C in Tris buffer (10 mM Tris–HCl pH 8.5). The purity of the DNA samples was measured spectrophotometrically by calculating ration between absorbance at 260 nm and 280 nm.

2.4. Genotyping

Genotyping of the chosen SNPs was done using TaqMan® SNP Genotyping Assay and TaqMan Fast Universal PCR Master mix (Life Technologies, Carlsbad, CA, USA) according to manufacturer's instruction. The reaction was carried out in Bio-Rad CFX96 Real-Time PCR Detection System and analyzed in the CFX Manager Software (Bio-Rad Laboratories Inc., Hercules, California, USA).

2.5. Statistical analysis

Statistical calculation was done by the usage of two statistical software packages: SigmaPlot 11.0 and Statisitca 12 (Systat Software Inc., San Jose, CA, USA and Statsoft, Tulsa, OK, USA, repsectively). Chi-square test was used to check if the studied SNPs had genotype frequencies in agreement with Hardy–Weinberg equilibrium (HWE). Unconditional multiple logistic regression model was used to evaluate association between case/control and each polymorphism by measuring the odds ratio (OR) and its corresponding 95% confidence interval (CI). Additionally the OR was adjusted for sex, since women have doubled risk of depression in comparison to men (Kessler, 2003).

3. Results

3.1. Single-nucleotide polymorphisms of genes encoding DNA glycosylases and the risk of recurrent depression disorder

Table 1 presents the distribution of genotypes and alleles of c.977C > G – *hOGG1*, c.972G > C – *MUTYH* and c.*589G > C – *NEIL1* in the depressed patients and the controls. We did not find any statistically significant difference in distribution of genotypes and alleles of *hOGG1* and *MUTYH* SNPs between those two groups. However, the C/C genotype and C allele of c.*589G > C – NEIL1 were negatively correlated with depression while genotype G/G and allele G of the same SNP were positively correlated with the disease. The distribution of genotypes was in agreement with HWE.

3.2. Single-nucleotide polymorphisms of genes encoding DNA glycosylases and the age of the first episode of recurrent depression disorder

To assess whether the studied polymorphisms are associated with age at which the first episode of depression occurs, we divided the patients into two groups – those which had their first episode before 35 years of age (marked as early onset depression) and those which had their first episode at or after 35 years of age (marked as late onset depression). The results are shown in Table 2. We did not find any significant differences in distribution of genotypes and alleles of c.977C > G – *hOGG1*. The G/G genotype of c.972G > C – *MUTYH* was associated only with late onset depression. In case of the c.*589G > C – *NEIL1* we found that both early onset and late onset were positively correlated with allele G and negatively correlated with allele C. However only early onset depression was associated with G/G genotype and negatively correlated with C/C genotype.

3.3. Gene-gene interactions and the risk of recurrent depression disorder

We also assessed whether the combined genotypes of studied polymorphisms are associated with occurrence of rDD and the results are present in Table 3. We found that the C/C–C/C genotype of the c.977C > G – hOGG1 and c.*589G > C – *NEIL1* decreased the risk of depression, while genotype C/C–G/G of the same polymorphisms combination increased the risk of the disease. In case of c.972G > C – *MUTYH* and c.*589G > C – *NEIL1* combined genotypes C/C–C/G and C/G–G/G were associated with occurrence of rDD. There were no statistical differences in distribution of combined genotype of c.977C > G – *hOGG1* and c.972G > C – *MUTYH*. We also did not find any statistical correlation between combined genotypes of all three studied SNPs and occurrence of depression (data not shown).

4. Discussion

This is the first study to show that SNPs of genes involved in DNA repair, particularly in BER pathway, may modulate the risk of rDD. Our team and others found oxidative modification of DNA bases and other types of DNA damage in patients with clinical depression and/or with depression coexisting with other non-mental diseases (Forlenza and Miller, 2006; Irie et al., 2003; Maes et al., 2009; Wei et al., 2009; Kupper et al., 2009; Irie et al., 2001; Czarny et al., 2015). We also revealed that the patients repaired oxidative DNA damage in less efficient way than the controls, which may indicate impairments of BER pathway (Czarny et al., 2015). In this study we genotyped SNPs of three genes encoding glycosylases, which are essential enzymes for adequate operation of BER. They recognize the damaged or chemically modified DNA base and excise it leaving the apurinic/apyrimidinic (AP) site. This AP site is then modified by AP-endonuclease or by glycosylase with such activity to create a free 3′–OH end suitable for DNA polymerase. After the repair synthesis, DNA ligase seals the nick on the strand, thus finishing the repair process. What is important is the fact that some variants of SNPs of genes encoding proteins involved in BER may have a negative impact on the oxidative DNA damage repair (Erčulj et al., 2010; Zielinska et al., 2011).

The polymorphism of – c.977C > G – is localized in exon of *hOGG1*. This gene encodes glycosylase that recognizes and removes oxidatively modified DNA bases, mainly 8-oxoG paired with cytosine, but also 8-oxoadenine paired with cytosine, as well as foramidopyrimidine (fapy)-guanine and methy-fapy-guanine (Bjorâs et al., 1997; Boiteux and Radicella, 1999). It has been reported that HeLa cells expressing the Cys326 variant under the condition characteristic for inflammation had significantly lower repair rates of oxidative DNA damage than the cells expressing the Ser326 variant (Moritz et al., 2014). Moreover, other study showed that Cys326 variant repaired the damage 3 to 4 times slower than the second hOGG1 variant (Zielinska et al., 2011). This SNP was also associated with occurrence of various types of cancers, including lung and stomach cancers (Hunget al., 2005; Takezaki et al., 2002). According to our knowledge there are now reports concerning association between the c.977C > G and mental diseases.

The second positive signal in this paper is c.972G > C causing the glutamine to histidine substitution in Codon 324 of MUTYH. This glycosylase removes 8-oxoG, when paired with adenine after the replication of DNA. It was found that the H324 variant is 36%

Table 2
Distribution of genotypes and alleles of c.977C > G, c.972G > C and c.*589G > C and the risk of early onset rDD or late onset rDD.

Genotype /Allele	Control ($n=298$) N (Freq.)	Early onset depression ($n=123$) N (Freq.)	Crude OR (95% CI)	p	Adjusted OR* (95% CI)	p	Late onset depression ($n=134$) N (Freq.)	Crude OR (95% CI)	p	Adjusted OR* (95% CI)	p
***hOGG1*c.977C > G (rs1052133)**											
C/C	190 (0.64)	69 (0.56)	0.73 (0.47–1.11)	0.142	0.73 (0.48–1.12)	0.152	77 (0.58)	0.78 (0.51–1.17)	0.229	0.76 (0.50–1.16)	0.203
C/G	95 (0.32)	51 (0.42)	1.51 (0.98–2.34)	0.061	1.51 (0.98–2.33)	0.065	47 (0.35)	1.14 (0.74–1.76)	0.544	1.16 (0.75–1.78)	0.504
G/G	13 (0.04)	3 (0.02)	0.53 (0.15–1.96)	0.355	0.54 (0.15–1.93)	0.344	10 (0.07)	1.77 (0.76–4.16)	0.187	1.80 (0.77–4.13)	0.178
$\chi^2=3.984$; $p=0.097$											
C	475 (0.80)	189 (0.77)	0.84 (0.58–1.21)	0.345	0.84 (0.59–1.22)	0.364	201 (0.75)	0.78 (0.55–1.09)	0.137	0.77 (0.55–1.07)	0.120
G	121 (0.20)	57 (0.23)	1.19 (0.83–1.72)	0.345	1.19 (0.82–1.71)	0.364	67 (0.25)	1.29 (0.92–1.81)	0.137	1.31 (0.93–1.83)	0.120
***MUTYH*c.972 G > C (rs3219489)**											
C/C	198 (0.66)	87 (0.71)	1.22 (0.77–1.93)	0.393	1.21 (0.77–1.91)	0.413	89 (0.66)	1.00 (0.65–1.54)	0.996	0.98 (0.64–1.52)	0.944
C/G	93 (0.31)	35 (0.28)	0.88 (0.55–1.39)	0.577	0.88 (0.56–1.41)	0.602	36 (0.27)	0.81 (0.51–1.28)	0.362	0.82 (0.52–1.29)	0.396
G/G	7 (0.02)	1 (0.01)	0.34 (0.04–2.80)	0.316	0.34 (0.04–2.80)	0.316	9 (0.07)	**2.93 (1.09–8.22)**	**0.033**	**3.00 (1.09–8.25)**	**0.033**
$\chi^2=3.984$; $p=0.097$											
C	489 (0.82)	209 (0.85)	1.26 (0.82–1.92)	0.290	1.25 (0.82–1.90)	0.305	214 (0.80)	0.87 (0.61–1.25)	0.447	0.86 (0.60–1.24)	0.413
G	107 (0.18)	37 (0.15)	0.80 (0.52–1.22)	0.290	0.80 (0.53–1.22)	0.305	54 (0.20)	1.15 (0.80–1.65)	0.447	1.16 (0.81–1.67)	0.413
***NEIL1*c.*589 G > C (rs4462560)**											
C/C	195 (0.65)	69 (0.56)	0.68 (0.44–1.04)	0.072	**0.64 (0.41–0.98)**	**0.039**	75 (0.56)	0.67 (0.44–1.02)	0.061	0.66 (0.43–1.00)	0.051
C/G	95 (0.32)	42 (0.34)	1.12 (0.71–1.73)	0.652	1.18 (0.76–1.84)	0.468	52 (0.39)	1.36 (0.89–2.07)	0.161	1.38 (0.90–2,11)	0.140
G/G	8 (0.03)	12 (0.10)	**3.92 (1.56–9.84)**	**0.004**	**3.94 (1.57–9.90)**	**0.004**	7 (0.05)	2.00 (0.71–5.63)	0.190	2.02 (0.72–5.69	0.185
$\chi^2=10.513$; $p=0.005$											
C	485 (0.81)	180 (0.73)	**0.63 (0.44–0.89)**	**0.009**	**0.60 (0.42–0.86)**	**0.005**	202 (0.75)	**0.69 (0.48–0.98)**	**0.040**	**0.68 (0.47–0.97)**	**0.033**
G	111 (0.19)	66 (0.27)	**1.59 (1.12–2.26)**	**0.009**	**1.66 (1.17–2.36)**	**0.005**	66 (0.25)	**1.46 (1.02–2.08)**	**0.040**	**1.48 (1.03–2.12)**	**0.033**

Early-onset depression – the first occurred episode before 35 years of age
Late-onset depression – the first episode occurred at or after 35 years of age
$p < 0.05$ along with corresponding ORs are in bold

* OR adjusted for sex.

Table 3
Gene-gene interactions of studied polymorphisms and the risk of rDD.

Combined genotype	Control ($n=298$)		Depression ($n=257$)		Crude OR (95% CI)	p	Adjusted OR*(95% CI)	p
	Number	Frequency	Number	Frequency				
***hOGG1*c.977 C > G (rs1052133) and*NEIL1*c.*589G > C (rs4462560)**								
C/C–C/C	128	0.430	81	0.315	**0.611 (0.431–0,867)**	**0.006**	**0.609 (0.429-0.864)**	**0.005**
C/C–C/G	58	0.195	52	0.202	1.050 (0.691–1.595)	0.820	1.048 (0.690–1.592)	0.826
C/C–G/G	4	0.013	13	0.051	**3.916 (1.261–12.164)**	**0.018**	**3.936 (1.267–12.233)**	**0.018**
C/G–C/C	58	0.195	60	0.233	1.260 (0.839–1.894)	0.265	1.259 (0.838–1.892)	0.267
C/G–C/G	33	0.111	33	0.128	1.183 (0.707–1.979)	0.522	1.194 (0.712–2.003)	0.501
C/G–G/G	4	0.013	5	0.019	1.458 (0.387–5.489)	0.577	1.448 (0.384–5.456)	0.585
G/G–C/C	9	0.030	3	0.012	0.379 (0.102–1.416)	0.149	0.377 (0.101–1.410)	0.147
G/G–C/G	4	0.013	9	0.035	2.667 (0.812–8.766)	0.106	1.072 (0.767–1.500)	0.101
G/G–G/G	0	–	1	0.004	–	–	–	–
***MUTYH*c.972 G > C (rs3219489) and*NEIL1*c.*589G > C (rs4462560)**								
C/C–C/C	130	0.436	96	0.374	0.771 (0.548–1.084)	0.134	0.763 (0.541–1.076)	0.123
C/C–C/G	61	0.205	71	0.276	**1.483 (1.002–2.196)**	**0.049**	**1.490 (1.006–2.208)**	**0.047**
C/C–G/G	7	0.023	9	0.035	1.509 (0.554–4.110)	0.421	1.518 (0.557–4.139)	0.415
C/G–C/C	61	0.205	42	0.163	0.759 (0.492–1.172)	0.213	0.761 (0.493–1.176)	0.218
C/G–C/G	31	0.104	21	0.082	0.766 (0.429–1.370)	0.369	0.768 (0.430–1.374)	0.374
C/G–G/G	1	0.003	8	0.031	**9.542 (1.185–76.814)**	**0.034**	**9.498 (1.179–76.521)**	**0.034**
G/G–C/C	4	0.013	6	0.023	1.757 (0.490–6.296)	0.387	1.756 (0.490–6.295)	0.387
G/G–C/G	3	0.010	2	0.008	0.771 (0.128–4.652)	0.777	0.774 (0.128–4.673)	0.780
G/G–G/G	0	–	2	0.008	–	–	–	–

$p < 0.05$ along with corresponding ORs are in bold.

* OR adjusted for sex.

less active than the wild type one (Ali et al., 2008). Moreover, the c.972G > C increased the risk of lung and colorectal cancer, as well as end stage renal disease (Miyaishi et al., 2009; Tao et al., 2008; Cai et al., 2012). As far as we are concern, this SNP was not previously correlated with any of mental disorders. Our results showed no significant associated with rDD (Table 1). However we found some association between late onset depression and the G/G genotype, while such association did not exist in early onset depression (Table 2, $p=0.033$ and $p=0.316$, respectively). Thus one can speculate that its involvement in the pathogenesis is rather minor and only together with other factors it may contribute to development of depression later in life.

Finally, the significant polymorphism of – c.*589G > C – is localized near 3′ end of the gene encoding NEIL1. This mammalian ortholog of Nei enzyme was recently found to be an important glycosylase participating in repair of oxidative DNA damage (Hazra et al., 2002). NEIL1 detects and removes 8-oxoG, 5-5-hydroxyuracil, hydroxycytosine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), 4,6-diamino-5-formamidopyrimidine (FapyA) and (5S-6R) thymine glycol, the last two, which cannot be excised by OGG1 or neutral trehalase 1 (NTH1) (Grin et al., 2010; Rosenquist et al., 2003). Moreover, embryonic stem cells with knock-out *NEIL1* were sensitive to even low levels of gamma-irradiation (Rosenquist et al., 2003). This glycosylase was also found to be important in preserving cognitive functions, and protecting from brain dysfunction or death after ischemic stroke in a mice model comparing wild type and NEIL1 deficient mice (Canugovi et al., 2012). The reason for this may be increased levels of 5-hydroxyuracil in a brain mitochondrial lysate and Fapy lesions in brain tissue of *NEIL1*$^{-/-}$ mice (Canugovi et al., 2012; Chan et al., 2009). Furthermore, in the same model increased levels of mitochondrial DNA damage was detected in liver (Vartanian et al., 2006). Since brain cells demand vast amount of energy for proper functioning, any damage to mitochondria or their DNA may lead to abnormalities. The results obtained on NEIL1 deficient mice suggest that this protein is important component of mitochondrial DDR, and its impairment may result with increased vulnerability to oxidative stress and brain damage. Although the exact influence of the studied SNP on expression or folding of the protein is unknown, it was revealed that it may serve as a risk predictor of the radiation-induced toxicities during definitive radiotherapy of esophageal cancer patients (Chen et al., 2013). According to our knowledge none of the *NEIL* polymorphisms were studied in context of mental illnesses. We found that the C/C genotype and the allele C of the c.*589G > C decreased risk of depression occurrence (Table 1; $p=0.022$ and $p=0.005$, respectively), while the G/G genotype and the allele G increased this risk (Table 1; $p=0.014$ and $p=0.005$, respectively). This results are consistent with previously cited work of Chen et.al., as the G/G genotype had significantly higher risk of acute radiation-induced esophageal toxicity and acute radiation pneumonitis, when compared to the C/C and C/G genotypes (Chen et al., 2013). This may indicate that the G variant somehow affects the functionality of NEIL1. We also studied the possible correlation between the c.*589 G > C – NEIL1 and onset of depression, and discovered higher correlation of the alleles with early onset than with the late onset one (Table 2; $p=0.005$ and $p=0.033$, respectively). What is more, the C/C genotype slightly decreased the risk of early onset rDD, while the G/G genotype strongly increased this risk (Table 2; $p=0.039$ and $p=0.004$, respectively). These two genotypes were not associated with late onset depression (Table 2; $p=0.051$ and $p=0.185$, respectively). This results suggest, that this polymorphisms are more involved in pathogenesis of rDD, than the studied SNP of *MUTYH*. Because the c.*589G > C – *NEIL1* is more associated with early onset depression than with late onset one, it can be speculated that it is responsible for earlier occurrence of depression. Thus it is possible, that this SNP does not need other, non-genetic factors, which may induce depression in later life.

We also checked whether the combined genotypes of studied polymorphisms are associated with the depression. Even though we did not find statistically significant correlation between the c.972G > C – *hOGG1* and depression, the combined genotype C/C–C/C of this SNP and the c.*589G > C – *NEIL1* strongly decreased the risk of depression (Table 3; $p=0.005$), while the genotype C/C–G/G of the same SNP combination moderately increased this risk (Table 3; $p=0.018$). This indicates, that the c.972G > C – *hOGG1* may have limited impact on the risk of rDD occurrence, but genotype C/C together with others genetics factors may significantly decrease this risk. If we assume – especially in light of reports indicating mitochondria dysfunction in depression – that mtDNA damage may play more important role in the pathogenesis of the disease than the damage of its nuclear counterpart, our results

may support this thesis due to fact that the c.972 G > C causes amino acid substitution only in isoform of hOGG1involved in nuclear BER (Blasiak et al., 2012; Hashiguchi et al., 2004, Alcocer-Gómez et al., 2014). Since brain cells are especially prone to ROS due to their high metabolic rate, their DDR must work at peak efficiency. Thus variants of gene encoding proteins with altered efficacy involved in maintaining mitochondrial genome, including NEIL, may lead to accumulation of mtDNA damage, mutation of genes encoding proteins of respiratory chain, increased mtROS production and mitochondrial oxidative vicious cycle (Hiona and Leeuwenburgh, 2008). In case of combined genotypes of c.972G > C – *MUTYH* and c.*589G > C – *NEIL1* the C/C–C/G and C/G–G/G variants slightly increased the risk of rDD (Table 3, $p=0{,}047$ and $p=0{,}034$), however the former one represented borderline association. We did not find any correlation between combined genotypes of c.972G > C – *hOGG1* / c.972G > C – *MUTYH* and depression (data not shown).

We are fully aware of the limitations of our work. First and foremost, the sample size was relatively small, though it must be noted that similar studies regarding depression had comparable study groups (van West et al., 2006; Szczepankiewicz et al., 2014). The second limitation is ethnic homogeneity of the studied population, thus our results cannot be extrapolated to general world population.

5. Conclusion

We showed for the first time that SNPs of genes involved in DNA repair, particularly BER pathway, may modulate the risk of depression occurrence. We found that C/C genotype and C allele of the c.*589G > C – *NEIL1* decreased the risk of rDD, while G/G genotype and allele G of the same SNP increased this risk. We also found stronger association of this polymorphism with early onset depression than with the late onset one. Therefore, this polymorphism in the *NEIL1* gene may be considered as an independent marker of depression occurrence. The two other SNPs – c.972G > C – *hOGG1* and c.972G > C – *MUTYH* – may modulate the risk of rDD only in minor degree, if any. Our results also support the hypothesis that DNA damage and repair may be involved in pathogenesis of depression.

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