Single-nucleotide polymorphisms of uracil-processing genes affect the occurrence and the onset of recurrent depressive disorder

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Depressive disorders (DD) are known to be associated with increased DNA damage, the impairment of DNA damage repair, and the presence of single-nucleotide polymorphisms (SNPs) in DNA damage repair genes. Some indirect evidence also suggests that uracil metabolism may be disrupted in depressed patients. Therefore, the current study genotypes three SNPs localized in genes encoding uracil-processing proteins: two glycosylases, i.e. *UNG* g.7245G>C (rs34259), *SMUG1* c.-31A>G (rs3087404), and dUTPase, i.e. *DUT* g.48638795G>T (rs4775748). The polymorphisms were analyzed in 585 DNA samples (282 cases and 303 controls) using TaqMan probes. The G/G genotype and G allele of *UNG* polymorphism decreased the risk of depression, while the G/C genotype and C allele of the same SNP increased it. It was also found that G/G carriers had their first episode significantly later than the heterozygotes. Although there was no association between the occurrence of depression and the *SMUG1* SNP, a significant difference was found between the homozygotes regarding the onset of DD. In conclusion, the SNPs localized in the uracil-processing genes may modulate the occurrence and the onset of depression, which further supports the hypothesis that impairment of DNA damage repair, especially base-excision repair, may play an important role in the pathogenesis of the disease.

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Abstract

Depressive disorders (DD) are known to be associated with increased DNA damage, the impairment of DNA damage repair, and the presence of single-nucleotide polymorphisms (SNPs) in DNA damage repair genes. Some indirect evidence also suggests that uracil metabolism may be disrupted in depressed patients. Therefore, the current study genotypes three SNPs localized in genes encoding uracil-processing proteins: two glycosylases, i.e. *UNG* g.7245G>C (rs34259), *SMUG1* c.-31A>G (rs3087404), and dUTPase, i.e. *DUT* g.48638795G>T (rs4775748). The polymorphisms were analyzed in 585 DNA samples (282 cases and 303 controls) using TaqMan probes. The G/G genotype and G allele of *UNG* polymorphism decreased the risk of depression, while the G/C genotype and C allele of the same SNP increased it. It was also found that G/G carriers had their first episode significantly later than the heterozygotes. Although there was no association between the occurrence of depression and the *SMUG1* SNP, a significant difference was found between the homozygotes regarding the onset of DD. In conclusion, the SNPs localized in the uracil-processing genes may modulate the occurrence and the onset of depression, which further supports the hypothesis that impairment of DNA damage repair, especially base-excision repair, may play an important role in the pathogenesis of the disease.

Introduction

By 2020, depression (depressive disorders – DD, including recurrent ones - rDD) is predicted to be the most profound economic and social burden next to ischemic heart disease (Murray and Lopez 1997; Greden 2001). Unfortunately, despite extensive research, its pathogenesis, etiology, diagnosis and treatment remain unclear (Gruenberg et al., 2005). No cellular or molecular biomarkers exist, and so clinical diagnosis is based only on observational manifestations. In addition, one-third of patients suffers from treatment-resistant depression (Brown et al., 1994; Parker, 2000; Gruenberg et al., 2005; Akiskal and Lewis, 2005; Starkstein et al., 2005; Iwata et al., 2013; Rodrigues et al., 2014; Chang et al., 2014).

Some factors are well known to play a crucial role in depression. Inflammation, and elevated oxidative and nitrosative stress, have recently attracted much attention (Pasco et al., 2010; Gardner and Boles, 2011; Alcocer-Gómez et al., 2014; Anderson and Maes, 2014; Lu et al., 2013). These kinds of stress may induce damage to biomolecules, including nucleic acids. Accordingly, an elevated level of 8-oxoguanine (8-oxoG), a marker of oxidative DNA damage, has been found in the serum, peripheral blood mononuclear cells (PBMCs) and urine of patients suffering from clinical depression and/or depression coexisting with other non-psychiatric diseases (Irie et al., 2001; Irie et al., 2003; Forlenza and Miller, 2006; Maes et al., 2009b; Wei et al., 2009; Kupper et al., 2009; Black et al., 2015; Lindqvist et al., 2016).

Our team revealed that the PBMCs isolated from depressed patients demonstrated slower DNA damage repair efficiency (DRE) after exposure to hydrogen peroxide than controls (Czarny et al., 2015a). Moreover, we found that the presence of single-nucleotide polymorphisms (SNPs) encoding proteins involved in base excision repair (BER), a primary pathway used to ameliorate oxidative DNA damage, also appear to affect the occurrence and onset of depression (Czarny et al., 2015b; Czarny et al., 2016). Although a genotype-phenotype analysis indicated that these SNPs did not generally influence the levels of DNA damage in the patients' cells, some may still have an impact on DRE (Czarny et al., 2017a). This leads us to believe that the increased DNA damage observed in depression may be caused not only by oxidative or/and nitrosative stress, but also by the impairment of DNA damage repair pathways, particularly BER (Czarny et al., 2017b).

67   The BER pathway is also responsible for the removal of uracil and its derivatives from

68 DNA (Barnes and Lindahl, 2004; Visnes et al., 2009). Uracil DNA glycosylases (UDGs) initiate

69 repair by recognizing and excising uracil. Several enzymes in the UDG superfamily have been

70 identified, including uracil-N glycosylase (UNG), single-strand-specific monofunctional uracil

71 DNA glycosylase 1 (SMUG1), methyl-binding domain glycosylase 4 (MBD4) and thymine

72 DNA glycosylase (TDG). In addition, uracil misincorporation into DNA can be prevented by the

73 hydrolysis of dUTP, a precursor that can be used by DNA polymerase instead of dTTP, to dUMP

74 and diphosphate (Barnes and Lindahl, 2004). This reaction is catalyzed by dUTPase, which in

75 humans is encoded by the *DUT* gene. Although there is no direct proof of an elevated uracil level

76 in the DNA of depressed patients, several reports indicate the presence of deficiencies in folate

77 (vitamin B_9) and cobalamin (vitamin B_{12}) (Papakostas et al., 2005; Reynolds, 2002; Beydoun et

78 al., 2010; Kim et al., 2008; Young, 2007; Araújo et al., 2015). These vitamins are important for

79 nucleotides biosynthesis, and their shortage may cause the balance between dTMP and dUMP to

80 shift towards the latter (Reynolds, 2014). Finally, oxidative stress may also cause the increased

81 presence of uracil in depressed patients' DNA (Endres et al., 2004; An et al., 2005).

82   The evidence suggests, then, that depression may be associated with impairment of

83 uracil metabolism, resulting in greater amounts of uracil being present in the DNA of those with

84 depression. Therefore, the present study examines the possible relationship between the

85 occurrence, onset, severity or treatment efficiency of rDD and the occurrence of three SNPs

86 located in uracil-processing genes: g.7245G>C (rs34259) of the *UNG*, c.-31A>G (rs3087404) of

87 the *SMUG1* and g.48638795G>T (rs4775748) of the *DUT*.

88

89 **Materials and methods**

90 *Study subjects and data collection*

91   A total of 585 people were randomly selected to participate in the study: 282 patients

92 diagnosed with DD and hospitalized at the Department of Adult Psychiatry of the Medical

93 University of Lodz (Poland), and 303 healthy controls. No replacement sampling was performed.

94 Detailed characteristics of the participants are presented in Table 1. All patients met the

95 diagnostic criteria for depressive episode and recurrent depressive disorder according to WHO

96 (WHO, 2015). The inclusion criteria were based on those outlined in the ICD-10 (F32.0–7.32.2,

97 F33.0–F33.8). Prior to the start of the experiment, a standardized Composite International

98 Diagnostic Interview (CIDI) was used to obtain a case history from each patient (Patten, 1997).

99 The severity of the disease symptoms was assessed by the 21-item Hamilton Depression Rating

100 Scale (HAM-D) (Hamilton, 1960). The intensity of the symptoms was measured in accordance

101 with Demyttenaere and De Fruyt (2003). Both the CIDI and HAM-D were performed by the

102 same psychiatrist: once before the patient was included in the study, and again after

103 antidepressant therapy with selective serotonin reuptake inhibitors (SSRIs). The exclusion

104 criteria included the presence of concurrent somatic diseases or axis I and II disorders, other than

105 depressive episodes, inflammatory or autoimmune disorders, central nervous system traumas,

106 familial prevalence of mental disorders other than recurrent depressive disorders or

107 unwillingness to give informed consent. A study group was formed of unrelated native residents

108 of central Poland.

109 Participation was voluntary, and the subjects were informed of the experiment's purpose and

110 assured of its voluntary nature. They were also informed that their personal data would be

111 confidential. All subjects gave their written consent to participate in this study. The protocol was

112 approved by the Bioethics Committee of the Medical University of Lodz (No. RNN/70/14/KE).

113

114 *Selection of single-nucleotide polymorphisms*

115 Selection of the polymorphisms was performed using the public domain of the database

116 for single nucleotide polymorphisms (dbSNP) of the National Center for Biotechnology

117 Information, available at http://www.ncbi.nlm.nih.gov/snp (Bethesda, MD, USA). Two criteria

118 were followed when selecting SNPs: a minor allele frequency (MAF) greater than 0.05

119 (population ID: HapMap-CEU), and a localization in either the coding or regulatory region of the

120 genes.

121

122 *DNA extraction*

123 Genomic DNA was isolated using the Blood Mini Kit (A&A Biotechnology, Gdynia,

124 Poland) from the venous blood of depressed patients before antidepressant therapy. The purity

125 and quantity of the DNA samples was determined by measuring their absorbance at 260 nm and

126 280 nm. To prevent multiple freezing and thawing, the blood was aliquoted as individual

127 samples and stored at -20°C.

128

Genotyping

130   TaqMan SNP Genotyping Assay (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and 2X Master Mix Takyon for Probe Assay – No ROX (Eurogentec, Liège, Belgium) were used to genotype selected polymorphisms. Reactions were performed and analyzed in the Mx3005P qPCR System and MxPro QPCR Software (Agilent Technologies, Santa Clara, CA, USA).

135

Statistical analysis

137 The collected data was analyzed in Statistica 12 (Statsoft, Tulsa, OK, USA), SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA), Resampling Stats Add-in for Excel v.4 (Arlington, VA, USA) and StudSize3.02 (CreoStat HB, Västra Frölunda, Sweden; used for power analysis). An unconditional multiple logistic regression model was used to calculate the association between case/control and each polymorphism. The results are shown as odds ratios (ORs) with 95% confidence interval (95% CI). ORs were adjusted for gender, due to the fact that women are twice more likely to suffer from depression than men (Kessler, 2004). In addition, the significant outcomes were further verified with the use of two approaches: the bootstrap-boosted multiple logistic regression (resampling with replacement, 10000 iterations) and the cross-validated logistic regression (that corresponds to *d*-jackknife technique) (control group was the modeled class). This was intended to overcome any possible bias related to relatively low sample size. The goodness of fit of logistic regression models pointing for a significant discrimination between controls and patients was estimated with Hosmer-Lemeshow test.

150   The descriptive data presented in figures and tables are shown as means ± SD or medians with interquartile ranges. Normality of the studied group was assessed via the Shapiro–Wilk test, homogeneity of variance was verified with Brown-Forsythe test, and then, accordingly, either unpaired Student's t test or Mann-Whitney *U* test was used. In some bivariate and multivariate analyses we used the approach of resampling with replacement (the bootstrap-boosted versions of the tests, 10000 iterations) to make sure that the revealed differences were not detected by a pure chance. The effect of the studied SNPs on the age of the onset of depression was evaluated in two different ways. The first approach used age as a continuous

158 variable, while the second employed a cut-off at 35 years, an age which might be regarded as the

159 transition between young adult to middle-aged adult.

160

161 **Results**

162 *Single-nucleotide polymorphisms of genes encoding proteins involved in the removal of uracil*

163 *from DNA and the risk of recurrent depressive disorder*

164   Table 2 presents the distribution of the genotypes and alleles of the studied

165 polymorphisms in both depressed patients and controls. The distribution of all SNPs was in

166 agreement with Hardy-Weinberg equilibrium. Only one of the studied SNPs modulated the risk

167 of depression occurrence: while the G/G genotype and G allele of *UNG* g.7245G>C (rs34259)

168 had a protective effect, the G/C genotype and C allele increased the odds ratio of the disease

169 incidence.

170

171 *Gene-gene interactions and the risk of recurrent depression disorder*

172   Regarding the effect of combined genotypes, it was found that the T/T-A/A-G/C (*DUT*

173 (rs4775748), *SMUG1* c.-31A>G (rs3087404) and *UNG* (rs34259) carriers had an increased risk of

174 depression, whereas the T/G-A/G-G/G carriers had a reduced risk (Table 3).

175

176 *Single-nucleotide polymorphisms of genes encoding proteins involved in the removal of uracil*

177 *from DNA and the age of the first episode of recurrent depressive disorder*

178   To evaluate whether the studied SNPs could have had an impact on the onset of the

179 disease the patients were divided according to genotype and their age of onset was compared

180 (Fig. 1A-C). In the case of *SMUG* c.-31A>G(rs3087404), it was found that carriers of the A/A

181 genotype underwent their first episode significantly later than the G/G carriers. Similarly, in case

182 of *UNG* g.7245G>C (rs34259), patients with a G/G genotype had a greater age of onset than the

183 heterozygotes. When the patients were stratified into the two groups based on age, it was found

184 that the UNG g.7245G>C (rs34259) affected the disease occurrence only in patients with early

185 onset depression, i.e. in those under 35 years of age (Table 4). However, no statistically

186 significant difference was found between patients with early and late onset DD (Table S1).

187

188 *Single-nucleotide polymorphisms of genes encoding proteins involved in the removal of uracil*

189 *from DNA and the severity of recurrent depressive disorder or treatment effectiveness*

190   No significant difference was found between patients with different genotypes of the

191 studied genes with regard to HAM-D (Fig. 2A-C), different treatment efficiency or HAM-D

192 score after treatment (Fig. 2D-F and 2G-I). Effectiveness of the therapy was calculated using the

193 following formula:

194 $$TE = \frac{(HAM\text{-}D_0 - HAM\text{-}D_E) \times 100\%}{HAM\text{-}D_0};$$

195 where TE is treatment effectiveness, $HAM\text{-}D_0$ is the score obtained by the patient before therapy

196 and $HAM\text{-}D_E$ is score achieved after therapy.

197

198

199 **Discussion**

200   To our best knowledge, this is the first study to demonstrate that SNPs for uracil-

201 processing genes modulate the risk of depression. The mutagenic potential of uracil depends on

202 its origin in DNA. Uracil is introduced into DNA either through its misincorporation by DNA

203 polymerase during replication, or by the deamination of cytosine (Kavli et al., 2002), which has

204 been estimated to occur 100-500 times per cell per day (Frederico et al., 1990; Lindahl, 1993). In

205 the first case, the lesion is processed by BER, and if not repaired correctly, may lead to the

206 production of an apurinic/apyrimidinic (AP) site or a DNA strand break (Dianov et al., 1991;

207 Sousa et al., 2007). In addition, the replacement of thymine by uracil in a protein binding

208 sequence may affect the affinity of these proteins to the DNA, resulting in cytotoxic properties

209 (Verri et al., 1990). In the second case, a C:G pair becomes a 100 per cent mutagenic U:G

210 mismatch, which has been attributed to the fact that DNA polymerases cannot distinguish

211 between U and T in the template (Visnes et al., 2009). Unless it is repaired, it yields C:G and

212 U:A pairs after replication, resulting in a transition from C:G to T:A (Sousa et al., 2007).

213   Uracil derivatives may also possess a mutagenic character arising from oxidative stress.

214 For example, 5-hydroxymethyluracil (5-hmeU) can be formed by the oxidation of a methyl

215 group in thymine or deamination of 5-hydroxymethylcytosine, which is formed from 5-

216 methylocytosine (5mC) by its oxidation: a common modification of cytosine present in human

217 cells which is thought to be responsible for the epigenetic regulation of gene expression

218 (Boorstein et al., 2001). In the light of evidence that depression may be associated with

219   disturbances in uracil metabolism, and our own previous research showing the impairment of

220   DNA repair in patients with depression, the present study evaluates whether the presence of

221   SNPs within the genes of two DNA glycosylases and that of dUTPase affect the occurrence of

222   the disease.

223   The first of the studied polymorphisms, *DUT* g.48346598G>T (rs4775748), is located in

224   the downstream region of the gene encoding dUTPase, and is known to be significantly

225   associated with blood DNA uracil concentration (Chanson et al., 2009); more precisely, G/G

226   genotype carriers had lower levels of uracil in blood DNA than the other two genotypes. The

227   authors speculate that the location of the SNP in the 3'-untranslated region causes it to modify

228   the secondary structure of mRNA, thus influencing the stability or affinity of small RNAs to the

229   mRNA. In contrast, the present study did not find any association between this polymorphism

230   and the occurrence, the onset, the severity or the treatment efficiency of depression. However,

231   the analysis of combined genotypes revealed that the heterozygote of the *DUT* SNP combined

232   with heterozygote of *SMUG* SNP and G/G genotype of the *UNG* SNP decreased the incidence of

233   depression, while the T/T homozygote of the *DUT* SNP combined with A/A genotype of the

234   *SMUG* SNP and heterozygote variant of the *UNG* SNP increased it (Table 4).

235   The second studied SNP, *SMUG1* c.-31A>G(rs3087404), is located in a promoter

236   region of the gene encoding SMUG1. Although its full name – single-strand-specific

237   monofunctional uracil DNA glycosylase 1 – could indicate that this glycosylase is specific to

238   uracil in single-stranded DNA, in reality, it removes this base from both single- and double-

239   stranded DNA in the context of U:A and U:G pairs (Masaoka et al., 2003; Pettersen et al., 2007).

240   In addition, it recognizes uracil derivatives that have been formed by oxidation, i.e. 5-hmeU, 5-

241   formylouracil (foU) and 5-hydoxyuracil (hmU) (Boorstein et al., 2001; Masaoka et al., 2003).

242   SMUG1 cannot be considered only as a "backup" for UNG2, since its knock out results in an

243   increased number of mutations in mouse embryonic fibroblast (MEFs) cell line (An et al., 2005).

244   Moreover, SMUG1 was found to be involved in the metabolism of RNA, particularly ribosomal

245   RNA quality control (Jobert et al., 2013).

246   The SNP was associated with age-related macular degeneration (AMD), a

247   neurodegenerative disease causing blurred or no vision, in a Polish population (Synowiec et al.,

248   2014). The polymorphism is believed to be located in the regulatory region, and so may have an

249   impact on mRNA stability, degradation and gene expression (Nadeau, 2002; Synowiec et al.,

250 2014). However, no such association between the SNP and the occurrence of AMD was found in

251 a study conducted on Iranian patients (Bonyadi et al., 2017). Similarly, no statistically significant

252 difference was observed between patients and controls with regard to the distribution of alleles

253 and genotypes of this polymorphism in the present study, and the presence of the SNP did not

254 affect the severity of depression symptoms or the effectiveness of antidepressant treatment (Fig.

255 2B,E,H); however, carriers with a G/G genotype reported a significantly earlier first episode than

256 those with A/A (Fig. 1B).

257   The last studied polymorphism, *UNG* g.7245G>C (rs34259), is located in the

258 downstream region of the gene encoding two forms of UNG: a mitochondrial form (UNG1) and

259 a nuclear one (UNG2). Both variants share a common catalytic domain, but their *N*-terminal

260 sequences vary, resulting in different subcellular targeting (Nilsen et al., 1997). Like SMUG1,

261 UNGs remove uracil from both single- and double-stranded DNA (Kavli et al., 2002). Moreover,

262 UNGs recognize and excise 5-fluorouracil (5-FU) as well as oxidized pyrimidines, i.e.

263 isodialuric acid, hmU and alloxan (Dizdaroglu et al., 1996; Krokan et al., 1997). Although *UNG*-

264 deficient mice are viable, they accumulate uracil in their DNA, and the mutation rate of their

265 cells is five-times greater (Andersen et al., 2005b; Nilsen et al., 2000; An et al., 2005).

266 Interestingly, they develop lymphoid hyperplasia and B-cell lymphomas, indicating that the lack

267 of UNG disrupts the adaptive immunity (Andersen et al., 2005a; Nilsen et al., 2003).

268   Analogically to *DUT* g.48346598G>T (rs4775748), the *UNG* SNP (rs34259) has also

269 been found to modulate the amount of uracil in blood DNA (Chanson et al., 2009). However,

270 while the effect of the *DUT* SNP best fitted a recessive model, i.e. the heterozygotes did not

271 differ from homozygous wild-type individuals, the *UNG* SNP displayed a more additive effect,

272 with G/G genotype carriers having the lowest concentration of uracil in blood DNA, the

273 heterozygotes a moderate level and the C/C genotype carriers with the highest. Chanson et al

274 speculate that variant C may modify the secondary structure of the mRNA, thus decreasing the

275 level of UNG synthesis. Accordingly, in the present study, the G/G genotype and allele G

276 decreased the risk of depression occurrence, while the G/C genotype and allele C increased it;

277 however, no association was found between the occurrence of C/C genotype and the disease,

278 which may be due to the small number of individuals carrying this genotype. Nevertheless, this

279 polymorphism may also affect the onset of depression: G/G carriers demonstrated significantly

280 later onset of the disease than the heterozygotes (Fig. 1C). In addition, the polymorphism was

281 found to affect the occurrence of depression only in patients that had their first episode before 35

282 years of age, while no association was found in patients with the first episode at or after 35 years

283 of age (Table 3). This could indicate that the G/G genotype has a protective effect against the

284 occurrence of depression in early life. As with the other SNPs included in the study, no evidence

285 was found that the *UNG* SNP could affect the severity of the symptoms and the treatment

286 outcome (Fig. 2C,F,I).

287 Our findings are especially interesting in the light of our earlier papers reporting

288 impairment of DNA damage repair and those by other teams suggesting disturbances of uracil

289 metabolism in the course of depression. As mentioned in the Introduction, folate and cobalamin

290 deficiencies may play a role in the etiology of the disease (Papakostas et al., 2005; Reynolds,

291 2002; Beydoun et al., 2010; Kim et al., 2008; Young, 2007; Araújo et al., 2015). In the brain,

292 both of these vitamins play a crucial role as cofactors in the synthesis of neurotransmitters,

293 hormones, myelin and membrane phospholipids, as well as in epigenetic regulation (Hughes et

294 al., 2013; Reynolds, 2006). Interestingly, they are also important for the biosynthesis of nuclear

295 acids, particularly the formation of dTMP from dUMP. Thus, a deficiency of the vitamins causes

296 a shifting of the balance between these two nucleoside monophosphates towards dUMP resulting

297 in the incorporation of uracil into DNA (Reynolds, 2014). This, together with the impairment of

298 DNA damage repair discovered by our team, that can be, at least partly, attributed to the

299 presence of specific SNP variants, may lead to the introduction of AP sites or DNA strand breaks

300 and apoptosis of neurons, especially in the hippocampus (Young et al. 2007; Czarny et al.,

301 2017a). In addition, folate deficiencies are also known to affect mtDNA, causing *inter alia* an

302 increased number of deletions and thus the induction of oxidative stress (Chou and Huang, 2009;

303 Chou et al., 2007; Kronenberg et al., 2011). This suggests that the impairment of uracil

304 metabolism may contribute to the dysfunction of mitochondria and the increased level of

305 oxidative stress observed in depression (Araújo et al., 2015). Our results indicating that the *UNG*

306 variant is associated with the decreased level of uracil in blood DNA, and a lower risk of disease

307 development, further support this hypothesis. On the other hand, the results suggest that the

308 studied SNPs did not influence neither the effectiveness of antidepressant therapy nor the

309 severity of depression before and after treatment. In contrast, it was proven that folate deficiency

310 may be associated with a bad response to therapy (Papakostas et al., 2005; Reynolds, 2002).

311 Thus, we speculate that the impairment of uracil removal from DNA may be an adjunctive factor

312   to the shortage of folate, which speeds up the development of depression, but affects neither the

313   severity nor the treatment of the disease. However, those assumptions must be taken with

314   caution, since the level of uracil or folate was not measured.

315   The study does have some limitations. While the sample size is relatively small, similar

316   sample sizes have been used in recently published studies concerning polymorphic variability

317   (Joung et al., 2017; Peitl et al., 2017). Moreover, due to ethnic homogeneity of the studied

318   population, results obtained by our team cannot be extrapolated to the world population.

319   Furthermore, as no expression analysis was performed of the studied genes on the mRNA or on

320   the protein level, it is not certain that the presence of the studied SNPs causes changes in the

321   expression of those genes in patients with depression.

322

323   **Conclusion**

324   The functional polymorphism located in *UNG* modulated the occurrence and the onset

325   of depression, and the *SMUG1* SNP affected the time of the first episode. Our results,

326   particularly the evidence of the protective effect of the *UNG* SNP variant in patients with the

327   early-onset depression and the fact that this variant is associated with a low level of uracil,

328   suggest that disturbances of uracil removal from DNA may be an additional factor, apart from

329   folate shortage, to hasten the development of the disease. This study further supports the

330   hypothesis that DNA damage and the impairment of DNA repair are important for the

331   pathophysiology of depression. Further research, including the analysis of levels of uracil

332   incorporation into DNA of depressed patients, is needed to elucidate the potential casual-effect

333   mechanisms.

334

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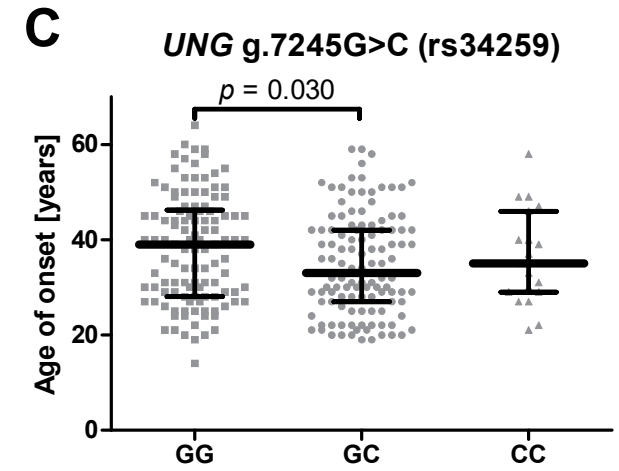
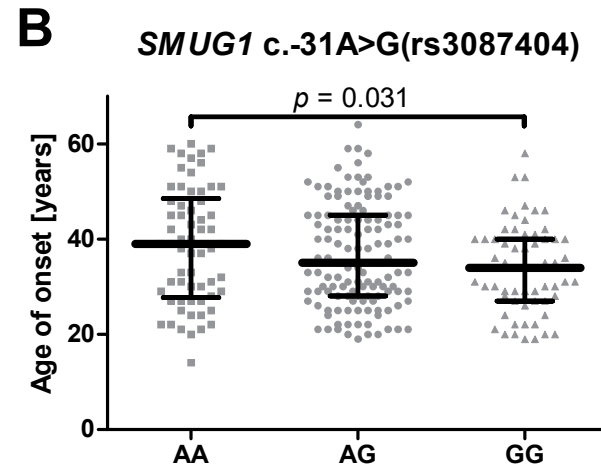
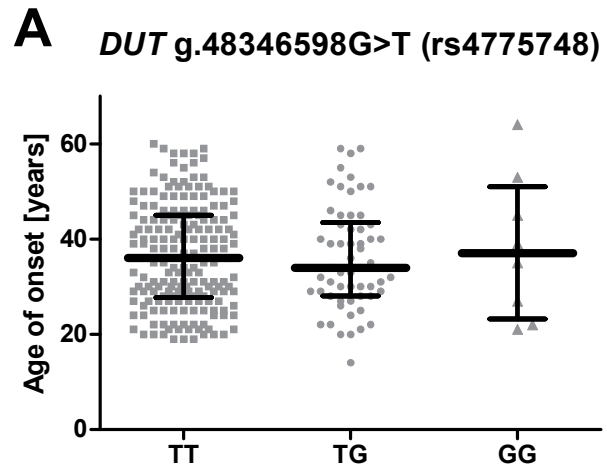
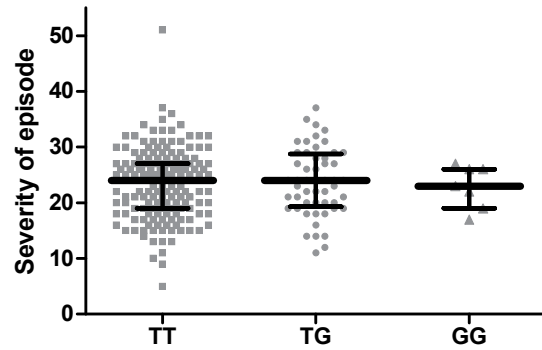
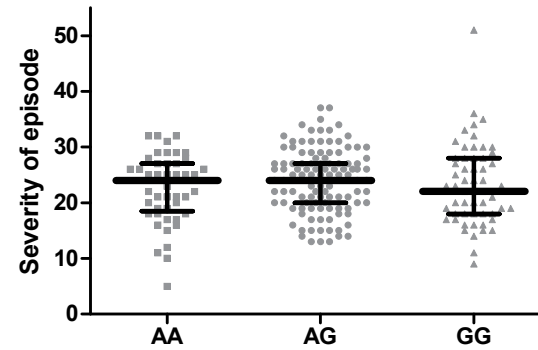
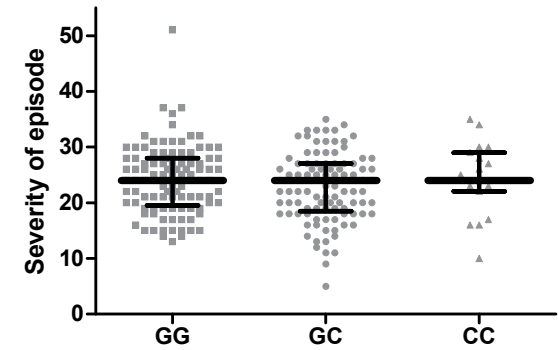
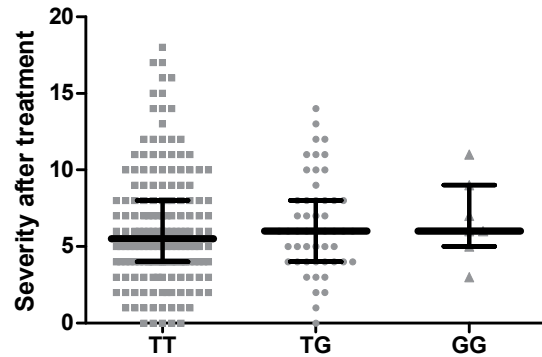
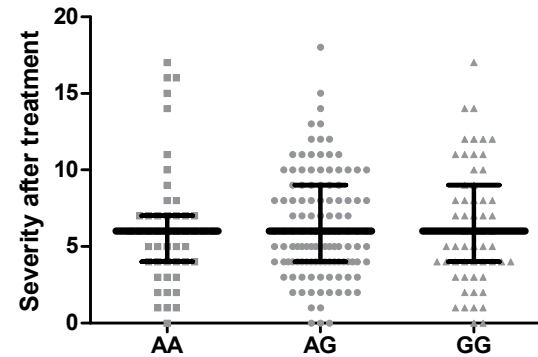
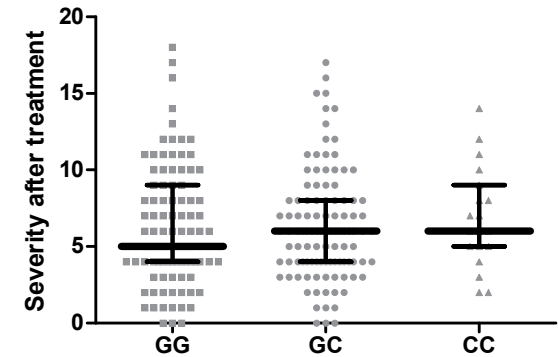
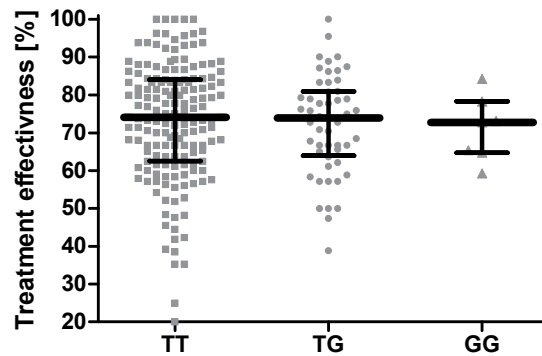
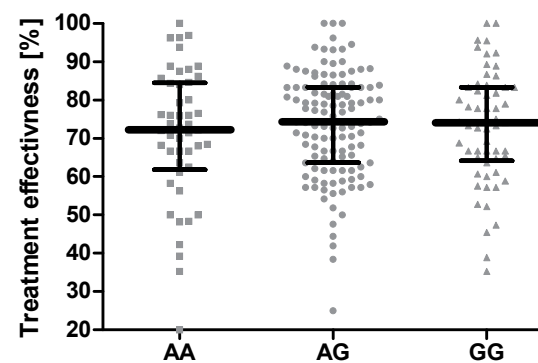
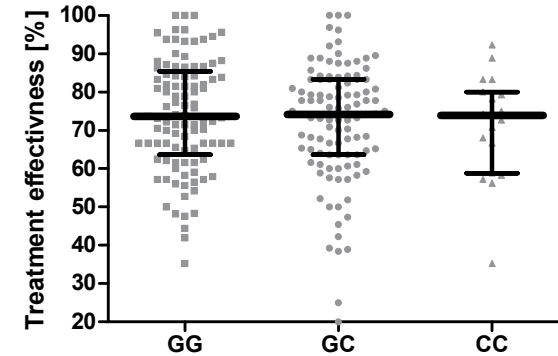
Impact of single-nucleotide polymorphisms localized in uracil-processing genes on age of depression onset.

Results are presented as scatter dot plots, horizontal lines represent median, and whiskers denote interquartile range.

Impact of single-nucleotide polymorphisms localized in uracil-processing genes on severity of the episode before and after therapy, and on treatment effectiveness.

(A-C) Severity of current episode according to 21-item Hamilton Depression Rating Scale (HAM-D). (D-F) Severity after treatment according to HAM-D. (G-I) Treatment effectiveness expressed as percentage of HAM-D decline after treatment. Results are present as scatter dot plots, horizontal lines represent median, and whiskers denote interquartile range. In all cases $p > 0.05$.

The detailed characteristic of patients which were qualified the study.

Depression severity (HAM-D range of scores)	Percentage of patients before treatment	Percentage of patients after treatment
None (0-7)	0.41%	68.00%*
Mild (8-16)	13.11%	30.67% *
Moderate (17-23)	34.43%	1.33%*
Severe (≥24)	52.05%	0%*
Mean age of patients ± SD		49.34 ± 10.32#
Mean age of controls ± SD		51.39 ± 13.37
Gender (male/female) of patients		146/136
Gender (male/female) of controls		155/148&
Duration of disease from the first episode		Percentage of patients
0-10 years		52.00%
11-20 years		19.56%
21-30 years		17.78%
31-40 years		9.78%
≥ 41 years		0.89%
Number of episodes		Percentage of patients
1		13.78%
2		31.11%
3		32.00%
4		18.22%
5		4.44%
6		0.44%

1   Significance of comparisons estimated with the Yates-corrected chi^2 test or the Fisher exact test

2   &P=0.881 vs. patients; *P<0.0001; #P=0.225 vs. controls

Association between the studied single-nucleotide polymorphism and depression.

Table presents a distribution of genotypes and alleles of *DUT* g.48346598G>T (rs4775748), *SMUG1* c.-31A>G(rs3087404) and *UNG* g.7245G>C (rs34259) single-nucleotide polymorphisms, and OR with 95 % CI in groups of patients with rDD and controls without mental disorders.

Genotype /Allele	Control (n = 303)		Depression (n = 282)		Crude OR (95% CI)	p	Adjusted OR (95% CI)*	p
	Number	Frequency	Number	Frequency				
DUT g.48346598G>T (rs4775748)								
T/T	205	0.677	207	0.734	1.319 (0.923-1.886)	0.128	1.319 (0.922-1.885)	0.129
T/G	89	0.294	67	0.238	0.749 (0.518-1.084)	0.126	0.749 (0.518-1.084)	0.126
G/G	9	0.030	8	0.028	0.954 (0.363-2.507)	0.924	0.956 (0.364-2.515)	0.928
				$\chi^2 = 2.420; p = 0.298$				
T	499	0.823	480	0.854	1.250 (0.915-1.706)	0.160	1.249 (0.915-1.705)	0.162
G	107	0.177	82	0.146	0.800 (0.586-1.092)	0.160	0.801 (0.586-1.093)	0.162
SMUG1 c.-31A>G(rs3087404)								
A/A	66	0.218	71	0.252	1.220 (0.832-1.790)	0.309	1.220 (0.831-1.790)	0.310
A/G	168	0.554	145	0.514	0.851 (0.614-1.179)	0.331	0.851 (0.614-1.179)	0.331
G/G	69	0.228	66	0.234	1.025 (0.697-1.508)	0.899	1.026 (0.697-1.511)	0.896
				$\chi^2 = 1.187; p = 0.552$				
A	300	0.495	287	0.509	1.077 (0.843-1.358)	0.579	1.070 (0.842-1.358)	0.582
G	306	0.505	277	0.491	0.935 (0.736-1.187)	0.579	0.935 (0.736-1.187)	0.582
UNG g.7245G>C (rs34259)								
G/G	178	0.587	134	0.475	**0.636 (0.458-0.882)*** **[b]0.641 (0.462-0.888)** **[cv]0.636 (0.458-0.882)**	**0.007** **0.008** **0.007**	**0.636 (0.459-0.882)*** **[b]0.642 (0.464-0.888)** **[cv]0.636 (0.458-0.882)**	**0.007** **0.008** **0.007**
G/C	107	0.353	127	0.450	**1.480 (1.061-2.064)**** **[b]1.478 (1.060-2.061)** **[cv]1.480 (1.061-2.063)**	**0.021** **0.021** **0.021**	**1.479 (1.059-2.065)**** **[b]1.476 (1.062-2.060)** **[cv]1.479 (1.060-2.064)**	**0.021** **0.020** **0.021**
C/C	18	0.059	21	0.074	1.274 (0.664-2.444)	0.466	1.276 (0.665-2.449)	0.464
				$\chi^2 = 7.401; p = 0.025$				
G	463	0.764	394	0.702	**0.716 (0.552-0.928)[#]** **[b]0.627 (0.485-0.811)** **[cv]0.710 (0.546-0.925)**	**0.012** **0.0004** **0.011**	**0.715 (0.551-0.929)[#]** **[b]0.631 (0.488-0.807)** **[cv]0.711 (0.546-0.926)**	**0.012** **0.0003** **0.011**
C	143	0.236	168	0.298	**1.385 (1.062-1.806)[##]** **[b]1.561 (1.210-2.015)**	**0.016** **0.001**	**1.384 (1.062-1.806)[##]** **[b]1.560 (1.211-2.015)**	**0.016** **0.001**

[cv]1.385 (1.063-1.805)	0.016	[cv]1.385 (1.062-1.805)	0.016

1   * OR adjusted for sex; the superscript [b] means the bootstrap-boosted OR (resampling with replacement, 10000 iterations); [cv] means the cross-
2   validated OR. Statistical power (1-β) for significant comparisons given for the ranges: [*] 0.782-0.791, [**]0.647-0.654, [#]0.353-0.361 and [##]0.426-
3   0.435. $P_{Hosmer-Lemeshow}$= 0.296-0.969 for all significant comparisons.
4   *p* < 0.05 along with corresponding ORs are in bold

Association between the combined genotypes of the studied single-nucleotide polymorphism and depression.

Table presents a distribution of combined genotypes of *DUT* rs4775748, *SMUG* rs3087404 and *UNG* rs34259 single-nucleotide polymorphisms, and OR with 95 % CI in groups of patients with rDD and controls without mental disorders.

Combined genotype	Control (n = 303)		Depression (n = 282)		Crude OR (95% CI)	p	Adjusted OR (95% CI)	p
	Number	Frequency	Number	Frequency				
DUT (rs4775748), *SMUG1* c.-31A>G (rs3087404) and *UNG* (rs34259)								
T/T-A/A-G/G	37	0.122	27	0.096	0.761 (0.450-1.287)	0.308	0.758 (0.448-1.283)	0.303
					3.402 (1.423-8.133)$^{0.892}$	**0.006**	**3.401 (1.422-8.134)$^{0.875}$**	**0.006**
T/T-A/A-G/C	7	0.023	21	0.074	^{b}**3.411 (1.425-8.161)**	**0.006**	^{b}**3.410 (1.424-8.163)**	**0.006**
					^{cv}**3.402 (1.423-8.133)**	**0.006**	^{cv}**3.401 (1.422-8.134)**	**0.006**
T/T-A/A-C/C	2	0.007	4	0.014	2.165 (0.394-11.915)	0.374	2.166 (0.394-11.921)	0.374
T/T-A/G-G/G	58	0.191	52	0.184	0.955 (0.630-1.447)	0.828	0.956 (0.631-1.449)	0.833
T/T-A/G-G/C	46	0.152	49	0.174	1.175 (0.757-1.824)	0.472	1.174 (0.756-1.822)	0.476
T/T-A/G-C/C	3	0.010	5	0.018	1.805 (0.427-7.623)	0.422	1.806 (0.428-7.627)	0.421
T/T-G/G-G/G	29	0.096	24	0.085	0.879 (0.499-1.549)	0.655	0.880 (0.499-1.552)	0.659
T/T-G/G-G/C	20	0.066	19	0.067	1.022 (0.534-1.958)	0.947	1.024 (0.534-1.961)	0.944
T/T-G/G-C/C	3	0.010	6	0.021	2.174 (0.538-8.776)	0.275	2.172 (0.538-8.769)	0.276
T/G-A/A-G/G	7	0.023	7	0.025	1.076 (0.373-3.108)	0.892	1.083 (0.374-3.136)	0.883
T/G-A/A-G/C	8	0.026	10	0.035	1.356 (0.527-3.485)	0.528	1.352 (0.525-3.479)	0.532
T/G-A/A-C/C	3	0.010	1	0.004	0.356 (0.037-3.441)	0.372	0.358 (0.037-3.463)	0.375
					0.492 (0.265-0.915)$^{0.767}$	**0.025**	**0.491 (0.264-0.914)$^{0.765}$**	**0.025**
T/G-A/G-G/G	33	0.109	16	0.057	^{b}**0.496 (0.267-0.922)**	**0.027**	^{b}**0.495 (0.266-0.921)**	**0.026**
					^{cv}**0.492 (0.265-0.915)**	**0.025**	^{cv}**0.491 (0.264-0.914)**	**0.025**
T/G-A/G-G/C	19	0.053	16	0.057	0.899 (0.453-1.785)	0.761	0.898 (0.452-1.784)	0.759
T/G-A/G-C/C	4	0.013	1	0.004	0.266 (0.030-2.394)	0.238	0.267 (0.030-2.401)	0.238
T/G-G/G-G/G	9	0.030	6	0.021	0.710 (0.250-2.021)	0.521	0.712 (0.250-2.028)	0.525
T/G-G/G-G/C	4	0.013	8	0.028	2.182 (0.650-7.329)	0.207	2.189 (0.652-7.356)	0.205
T/G-G/G-C/C	2	0.007	2	0.007	1.075 (0.150-7.683)	0.943	1.069 (0.149-7.651)	0.947
G/G-A/A-G/G	2	0.007	0	0.000	-	-	-	-
G/G-A/A-G/C	0	0.000	0	0.000	-	-	-	-
G/G-A/A-C/C	0	0.000	1	0.004	-	-	-	-
G/G-A/G-G/G	1	0.003	2	0.007	2.157 (0.195-23.921)	0.531	2.150 (0.194-23.852)	0.533
G/G-A/G-G/C	3	0.010	4	0.014	1.439 (0.319-6.486)	0.636	1.437 (0.319-6.478)	0.637
G/G-A/G-C/C	1	0.003	0	0.000	-	-	-	-
G/G-G/G-G/G	2	0.007	0	0.000	-	-	-	-
G/G-G/G-G/C	0	0.000	0	0.000	-	-	-	-
G/G-G/G-C/C	0	0.000	1	0.004	-	-	-	-

1   * OR adjusted for sex; the superscript ^{b} means the bootstrap-boosted OR (resampling with replacement, 10000 iterations); ^{cv} means the cross-
2   validated OR. Statistical power (1-β) for significant comparisons given in superscripts.
3   $p < 0.05$ along with corresponding ORs are in bold

Association between the studied single-nucleotide polymorphism and the onset of depression.

Table presents a distribution of genotypes and alleles of *DUT* rs4775748, *SMUG* rs3087404 and *UNG* rs34259 single-nucleotide polymorphisms, and OR with 95 % CI in group of patients with rDD that had their first episode before 35 years of age (marked as early onset depression) or after 35 years of age (marked as late onset depression), when compared to the control group without mental disorders.

Genotype/Allele	Control (n = 303) N (Freq.)	Early onset depression (n = 127) N (Freq.)	Crude OR (95% CI)	p	Adjusted OR (95% CI)	p	Late onset depression (n = 125) N (Freq.)	Crude OR (95% CI)	p	Adjusted OR (95% CI)	p
DUT g.48346598G>T (rs4775748)											
T/T	205 (0.68)	92 (0.72)	1.26 (0.80-1.99)	0.328	1.25 (0.79-1.30)	0.333	94 (0.75)	1.45 (0.90-2.32)	0.123	1.45 (0.90-2.33)	0.123
T/G	89 (0.29)	31 (0.24)	0.78 (0.48-1.25)	0.296	0.77 (0.48-1.24)	0.291	27 (0.22)	0.66 (0.41-1.08)	0.101	0.66 (0.41-1.09)	0.104
G/G	9 (0.03)	4 (0.03)	1.06 (0.32-3.52)	0.921	1.10 (0.33-3.66)	0.875	4 (0.03)	1.08 (0.33-3.57)	0.900	1.05 (0.32-3.49)	0.933
$\chi^2 = 1.096; p = 0.578$							$\chi^2 = 2.709; p = 0.258$				
T	499 (0.82)	215 (0.85)	1.18 (0.79-1.76)	0.415	1.17 (0.79-1.75)	0.430	215 (0.86)	1.31 (0.87-1.98)	0.197	1.31 (0.87-1.98)	0.193
G	107 (0.18)	39 (0.15)	0.85 (0.57-1.26)	0.415	0.85 (0.57-1.27)	0.430	35 (0.14)	0.76 (0.51-1.15)	0.197	0.76 (0.50-1.15)	0.193
SMUG1 c.-31A>G(rs3087404)											
A/A	66 (0.22)	26 (0.20)	0.92 (0.55-1.54)	0.763	0.91 (0.55-1.52)	0.731	36 (0.29)	1.45 (0.90-2.33)	0.122	1.46 (0.91-2.35)	0.118
A/G	168 (0.55)	66 (0.52)	0.87 (0.57-1.32)	0.509	0.87 (0.57-1.31)	0.502	63 (0.50)	0.82 (0.54-1.24)	0.341	0.82 (0.54-1.24)	0.349
G/G	69 (0.23)	35 (0.28)	1.29 (0.80-2.07)	0.291	1.31 (0.81-2.10)	0.266	26 (0.21)	0.89 (0.54-1.48)	0.655	0.88 (0.53-1.47)	0.629
$\chi^2 = 1.118; p = 0.572$							$\chi^2 = 2.401; p = 0.301$				
A	300 (0.49)	118 (0.46)	0.87 (0.64-1.19)	0.393	0.86 (0.63-1.18)	0.360	135 (0.54)	1.22 (0.89-1.66)	0.213	1.22 (0.90-1.67)	0.201
G	306 (0.50)	136 (0.54)	1.14 (0.84-1.56)	0.393	1.16 (0.85-1.57)	0.360	115 (0.46)	0.82 (0.60-1.12)	0.213	0.82 (0.60-1.11)	0.201
UNG g.7245G>C (rs34259)											
G/G	178 (0.59)	51 (0.40)	**0.47 (0.31-0.72)*** **[b]0.47 (0.31-0.72)** **[cv]0.47 (0.31-0.72)**	**<0.001** **<0.001** **<0.001**	**0.47 (0.31-0.72)*** **[b]0.47 (0.31-0.72)** **[cv]0.47 (0.31-0.72)**	**<0.001** **<0.001** **0.001**	63 (0.50)	0.71 (0.47-1.08)	0.114	0.70 (0.46-1.07)	0.102
G/C	107 (0.35)	65 (0.52)	**1.92 (1.26-2.92)**** **[b]1.91 (1.26-2.91)** **[cv]1.92 (1.26-2.92)**	**0.002** **0.002** **0.002**	**1.92 (1.27-2.91)**** **[b]1.91 (1.27-2.91)** **[cv]1.91 (1.25-2.91)**	**0.002** **0.002** **0.003**	53 (0.42)	1.35 (0.88-2.06)	0.169	1.37 (0.89-2.10)	0.147
C/C	18 (0.06)	10 (0.08)	1.35 (0.6-3.02)	0.460	1.37 (0.61-3.05)	0.445	9 (0.07)	1.23 (0.54-2.81)	0.627	1.21 (0.53-2.79)	0.646
$\boldsymbol{\chi^2 = 12.490; p = 0.002}$							$\chi^2 = 2.506; p = 0.286$				
G	463 (0.76)	167 (0.66)	**0.59 (0.43-0.82)#** **[b]0.55 (0.40-0.75)** **[cv]0.58 (0.42-0.81)**	**0.001** **0.0001** **0.001**	**0.59 (0.42-0.82)#** **[b]0.54 (0.39-0.74)** **[cv]0.58 (0.42-0.81)**	**0.001** **0.0001** **0.001**	179 (0.72)	0.78 (0.56-1.09)	0.142	0.77 (0.55-1.08)	0.133

			1.63 (1.18-2.24)[##]	0.003	1.65 (1.20-2.24)[##]	0.002					
C	143 (0.24)	85 (0.34)	[b]1.46 (1.06-2.01)	0.02	[b]1.51 (1.09-2.08)	0.01	71 (0.28)	1.28 (0.92-1.79)	0.142	1.29 (0.92-1.80)	0.133
			[cv]1.66 (1.19-2.31)	0.003	[cv]1.66 (1.19-2.31)	0.003					

2   * OR adjusted for sex; the superscript ^{b} means the bootstrap-boosted OR (resampling with replacement, 10000 iterations); ^{cv} means the cross-
3   validated OR. Statistical power (1-β) for significant comparisons given for the ranges: *0.970-0.971, **0.916-0.921, #0.831-0.832 and ##0.576-
4   0.597. $P_{\text{Hosmer-Lemeshow}}$= 0.152-0.999 for all significant comparisons.
5   $p < 0.05$ along with corresponding ORs are in bold