RESEARCH ARTICLE

Polymorphism in the Serotonin Receptor 2a (HTR2A) Gene as Possible Predisposal Factor for Aggressive Traits

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Abstract

Aggressive manifestations and their consequences are a major issue of mankind, highlighting the need for understanding the contributory factors. Still, aggression-related genetic analyses have so far mainly been conducted on small population subsets such as individuals suffering from a certain psychiatric disorder or a narrow-range age cohort, but no data on the general population is yet available. In the present study, our aim was to identify polymorphisms in genes affecting neurobiological processes that might explain some of the inter-individual variation between aggression levels in the non-clinical Caucasian adult population. 55 single nucleotide polymorphisms (SNP) were simultaneously determined in 887 subjects who also filled out the self-report Buss-Perry Aggression Questionnaire (BPAQ). Single marker association analyses between genotypes and aggression scores indicated a significant role of rs7322347 located in the HTR2A gene encoding serotonin receptor 2a following Bonferroni correction for multiple testing (p = 0.0007) both for males and females. Taking the four BPAQ subscales individually, scores for Hostility, Anger and Physical Aggression showed significant association with rs7322347 T allele in themselves, while no association was found with Verbal Aggression. Of the subscales, relationship with rs7322347 was strongest in the case of Hostility, where statistical significance virtually equaled that observed with the whole BPAQ. In conclusion, this is the first study to our knowledge analyzing SNPs in a wide variety of genes in terms of aggression in a large sample-size non-clinical adult population, also describing a novel candidate polymorphism as predisposal to aggressive traits.

Introduction

Aggression, defined as any behavior intended to be destructive, lies at the root of numerous major ills of humanity ranging from verbal abuse through both interpersonal and self-directed violence to mass criminal acts. Consequences of aggression-driven acts pose an enormous burden on society and economics, rendering it important to understand the biological basis behind [1,2].
Increased levels of aggression are characteristic to patients with a variety of neurodegenerative and psychiatric disorders as well as to alcoholics and drug addicts [3–7], but can also often be observed among the normal human population, even conferring certain privileges to the aggressor under certain circumstances e.g. by means of social dominance [8,9]. From the evolutionary point of view, some degree of aggression is indeed necessary for gaining adequate fitness (through an improved access of food supplies and other resources) and reproductive success; however, these benefits are compensated for by an increased risk of injury and social isolation. Hence, optimal levels of aggression are presumably shaped by a fine balance between effects of positive and negative selection pressure, implying a strong genetic background next to the role of environment [10,11]. This assumption is further underpinned by the fact that aggression proved to be heritable in several twin studies, with an estimated genetic contribution to the risk of aggressiveness of above 40% [12–17].

Experimental evidence suggest that aggressive manifestations and the accompanying emotions (anger, anxiety, fear) can be strongly related to highly conserved brain regions, chiefly to the amygdala and its linked neural circuits, but also to the anterior cingulated cortex and the prefrontal cortex [18,19]. In terms of biochemistry, it is principally the monoaminergic neurotransmitter systems (e.g. dopamine, noradrenaline and serotonin pathways) that are believed to play a major role in aggressive behavior, though possible effects of sexual hormones, the hypothalamic-pituitary-adrenal (HPA) axis and blood sugar levels have also been implicated [20,21].

Great efforts have been made to decipher the possible genetic background behind predisposition to aggression, describing novel polymorphisms in a variety of genes with a role in neuropsychiatry, and also identifying promising candidates for aggressive behavior and the related mental states (impulsivity, hostility). However, most of these association studies were carried out in small samples, raising the possibility of committing statistical errors (Pavlov 2012). Besides, the vast majority of aggression-related genetic investigations either were based on comparisons between healthy individuals and patients suffering from personality disorders etc., or concentrated on restricted samples not representative of the general population (e.g. [22–28]). These factors render data evaluation challenging, and often lead to controversial results.

Our aim was to simultaneously examine the effect of a set of putatively functional single nucleotide polymorphisms (SNP) on aggressive tendencies of the general Hungarian adult population using a microarray system, with a principal focus on monoaminergic pathways and its close interactors. Selected SNPs are located in genes encoding monoaminergic neurotransmitter transporters and receptors, their associated proteins and other signal transduction molecules, enzymes involved in the biosynthesis or degradation of neurotransmitters, neurotrophic factors and regulators of circadian rhythm as well as of neuronal death, all with an implicated role in emotional responses and behavioral traits [20,29–32].

Materials and Methods

Individuals involved
Non-related individuals of Caucasian Hungarian origin without any known psychiatric disorder were recruited for this study on a voluntary basis at the Institute of Psychology, Eotvos Lorand University (Budapest). Buccal samples and self-filled out aggression questionnaires were obtained from 887 subjects (45.8% males and 54.2% females). The sample comprised of 495 psychology and law enforcement students studying in the Budapest area and 392 random volunteers recruited at academic institutions and events popularizing this survey. All participants belonged to the middle socioeconomic status. Mean age was 23.2 (±7.55) years within the range from 18 to 75 years. All participants gave written informed consent and the study was
approved by the Scientific and Research Ethics Committee of the Medical Research Council (“ETT TUKEB”—Ministry of Health, Medical Research Council, Budapest, H-1051 Hungary).

Phenotypic measure
The original 29-item version of the self-report Buss-Perry Aggression Questionnaire (BPAQ) [33] was used to assess aggressive tendencies. This instrument comprises four subscales: Verbal Aggression (5 items), Physical Aggression (9 items), Anger (7 items) and Hostility (8 items). Individual items are rated from one (‘extremely uncharacteristic of me’) to five (‘extremely characteristic of me’). Total score for aggression was calculated as the sum of ratings for all the items, with a possible range between 29 and 145. Hungarian version of the original English language questionnaire was obtained by the “forward-backward” translation method and was pilot tested prior to the present study [34].

Sample collection
Buccal cells were collected by gently scraping the inner cheek with cotton-tipped collection swabs. Genomic DNA preparation was performed by a traditional, salting-out procedure [35]. Briefly, collection swabs were incubated overnight in 450 μl cell lysis buffer (0.2 g/l Proteinase K, 0.1 M NaCl, 0.5% SDS, 0.01 M Tris buffer pH = 8.0) at 56°C, followed by RNase treatment at room temperature. Proteins were precipitated with saturated NaCl (6 M) and removed by centrifugation. DNA was precipitated with isopropanol, purified with 70% ethanol and resuspended in 100 μl of Tris-EDTA pH = 8.0 (containing 0.5 M EDTA). DNA concentrations were measured by a fluorometry based intercalation assay (AccuBlue Broad Range dsDNA Quantification Kit, Biotium). Concentration of samples analyzed in this study ranged between 15 and 200 ng/μl. Isolated DNA samples were kept at −20°C until used.

Marker selection
Common SNPs with a higher than 5% minor allele frequency (MAF) were selected from the dbSNP database of NCBI [36]. Priority was given to polymorphisms referred to in various association studies in connection with personality or mood disorders as well as aggression or impulsivity in psychiatric disorders, and to putative functional variants, either causing an amino acid change or with an implicated gene regulatory role.

Genotyping
Genotyping was performed in 384-well plates on an Open Array real-time PCR platform (Applied Biosystems) based on allele-specific, fluorescent (TaqMan) probes and pre-designed, validated primers immobilized to a solid surface obtained from the manufacturer. Approximately 100 ng DNA per sample was used in each measurement. DNA amplification was carried out in the GeneAmp PCR System 9700 (Applied Biosystems) according to the manufacturer’s instructions, using the master mix, containing each dNTP and AmpliTaq Gold DNA-polymerase, provided by the manufacturer. Endpoint detection of signal intensities of allele specific fluorescent dyes was conducted by the OpenArray NT Imager, and genotypes were called by the TaqMan Genotyper v1.2 software. Call rate for individual SNPs is shown in Table 1 (mean: 77.9%).

Statistical analysis
Statistical analyses were performed by the SPSS 22.0 (SPSS Inc.) software. Allele and genotype frequency distributions were determined by the $\chi^2$ test. Independent samples t-test was used to assess gender differences, and relationship with age was tested by Pearson correlation. Genetic
| SNP     | Gene   | N    | MM      | Mm      | mm      | HWE*   | Call rate |
|---------|--------|------|---------|---------|---------|--------|-----------|
| rs1048101 | ADRA1A | 763  | 218     | 384     | 161     | 21,1%  | 0.945     | 86%       |
| rs3808585 | ADRA1A | 722  | 396     | 277     | 49      | 6,8%   | 0.998     | 81%       |
| rs2236554 | ADRA1D | 757  | 293     | 346     | 118     | 15,6%  | 0.641     | 85%       |
| rs553668  | ADRA2A | 692  | 519     | 158     | 15      | 2,2%   | 0.770     | 78%       |
| rs11030104 | BDNF   | 702  | 393     | 264     | 45      | 6,4%   | 0.997     | 79%       |
| rs2049045 | BDNF   | 690  | 419     | 241     | 30      | 4,3%   | 0.820     | 78%       |
| rs6265    | BDNF   | 601  | 362     | 212     | 27      | 4,5%   | 0.847     | 68%       |
| rs7103411 | BDNF   | 715  | 393     | 276     | 46      | 6,4%   | 0.966     | 81%       |
| rs7094179 | CDNF   | 687  | 305     | 302     | 80      | 11,6%  | 0.924     | 77%       |
| rs7900873 | CDNF   | 696  | 384     | 273     | 39      | 5,6%   | 0.573     | 78%       |
| rs1051730 | CHRNA3 | 753  | 320     | 346     | 118     | 15,6%  | 0.943     | 85%       |
| rs16969968 | CHRNA5 | 663  | 279     | 307     | 77      | 11,6%  | 0.866     | 75%       |
| rs4680    | COMT   | 603  | 177     | 295     | 131     | 21,7%  | 0.927     | 68%       |
| rs135745  | CSNK1E | 718  | 187     | 375     | 156     | 21,7%  | 0.460     | 81%       |
| rs1997644 | CSNK1E | 688  | 176     | 364     | 148     | 21,5%  | 0.291     | 78%       |
| rs1611115 | DBH    | 761  | 443     | 283     | 35      | 4,6%   | 0.482     | 86%       |
| rs6271    | DBH    | 780  | 657     | 116     | 7       | 0,9%   | 0.759     | 88%       |
| rs4532    | DRD1   | 761  | 286     | 357     | 118     | 15,5%  | 0.931     | 86%       |
| rs6277    | DRD2   | 579  | 169     | 284     | 126     | 21,8%  | 0.948     | 65%       |
| rs1800497 | DRD2   | 605  | 399     | 192     | 14      | 2,3%   | 0.261     | 68%       |
| rs1079597 | DRD2   | 608  | 443     | 158     | 7       | 1,2%   | 0.226     | 69%       |
| rs1800498 | DRD2   | 595  | 215     | 280     | 100     | 16,8%  | 0.862     | 67%       |
| rs2134655 | DRD3   | 760  | 410     | 295     | 55      | 7,2%   | 0.981     | 86%       |
| rs3732790 | DRD3   | 734  | 243     | 365     | 69      | 9,2%   | 0.857     | 83%       |
| rs6280    | DRD3   | 749  | 354     | 326     | 69      | 9,2%   | 0.887     | 84%       |
| rs963468  | DRD3   | 736  | 246     | 364     | 126     | 17,1%  | 0.909     | 83%       |
| rs11246226 | DRD4   | 685  | 173     | 347     | 165     | 24,1%  | 0.941     | 77%       |
| rs3758653 | DRD4   | 714  | 468     | 208     | 20      | 2,8%   | 0.923     | 80%       |
| rs916455  | DRD4   | 702  | 644     | 56      | 2       | 0,3%   | 0.803     | 79%       |
| rs936460  | DRD4   | 697  | 344     | 284     | 69      | 9,9%   | 0.655     | 79%       |
| rs3733829 | EGLN2  | 683  | 263     | 321     | 99      | 14,5%  | 0.998     | 77%       |
| rs222843  | GABARAP| 683  | 307     | 293     | 83      | 12,2%  | 0.601     | 77%       |
| rs11111   | GDNF   | 719  | 540     | 160     | 19      | 2,6%   | 0.241     | 81%       |
| rs1549250 | GDNF   | 710  | 231     | 353     | 126     | 17,7%  | 0.907     | 80%       |
| rs1981844 | GDNF   | 576  | 320     | 223     | 33      | 5,7%   | 0.771     | 65%       |
| rs2910702 | GDNF   | 705  | 387     | 269     | 49      | 7,0%   | 0.971     | 79%       |
| rs2973041 | GDNF   | 695  | 492     | 182     | 21      | 3,0%   | 0.710     | 78%       |
| rs2973050 | GDNF   | 582  | 242     | 275     | 65      | 11,2%  | 0.608     | 66%       |
| rs3096140 | GDNF   | 671  | 320     | 287     | 64      | 9,5%   | 1.000     | 76%       |
| rs3812047 | GDNF   | 679  | 521     | 144     | 14      | 2,1%   | 0.559     | 77%       |
| rs6925    | HTR1A  | 607  | 167     | 289     | 151     | 24,9%  | 0.510     | 68%       |
| rs1228814 | HTR1B  | 599  | 432     | 153     | 14      | 2,3%   | 0.995     | 68%       |
| rs130058  | HTR1B  | 595  | 330     | 232     | 33      | 5,5%   | 0.642     | 67%       |
| rs13212041 | HTR1B | 606  | 376     | 209     | 21      | 3,5%   | 0.467     | 68%       |
| rs11568817 | HTR1B | 600  | 187     | 292     | 121     | 20,2%  | 0.937     | 68%       |

(Continued)
associations were tested by one way analysis of covariance (ANCOVA) assuming a dominant model of inheritance with sex and age as covariates. Bonferroni correction for multiple testing was applied for the total number of SNPs in this study when assessing relationship between BPAQ scores and individual SNPs (the corrected level of significance was $p = 0.05 / 55 = 0.0009$). In all other cases, $p < 0.05$ values were regarded as significant. Effect of prior associations in males and females was analyzed by two-way ANCOVA with age as covariate. All tests were two-tailed.

Lewontin’s $D'$ and $r^2$ values of linkage disequilibrium were calculated using HaploView 4.2. [37]. Haplotypes were determined by the PHASE software [38,39].

### Results

#### Reliability of the markers analyzed

Internal consistency of the self-report BPAQ was assessed by Chronbach’s alpha, which had a value of 0.895 for total scores ensuring reliability of the study. Coefficients for Verbal Aggression, Physical Aggression, Anger and Hostility were 0.640, 0.842, 0.831 and 0.792, respectively. Alleles of all the SNPs studied were in Hardy-Weinberg equilibrium (Table 1).

#### Potential confounders

Gender differences on the BPAQ scale were evaluated by Independent samples t-test. Males presented significantly higher scores than females (68.52±17.14 compared to 64.49±15.09; $p<0.001$). Relationship between BPAQ scores and age was tested by Pearson correlation coefficient and was found to be significant ($p = 0.008$). Thus, both gender and age were used as covariates in all association analyses.

#### Significant association of the HTR2A rs7322347 T/A intronic SNP with aggression

Table 2 summarizes results of phenotypic data as a function of each SNP analyzed. Association with aggression reached nominal level of significance $p<0.05$ in the case of two SNPs, rs916455 located in the promoter region of the DRD4 gene and rs7322347 in intron 2 of...
Table 2. Association of the 55 polymorphisms studied with aggression levels.

| SNP       | Gene   | Aggression (total score) | p^2   |
|-----------|--------|--------------------------|-------|
|           |        | MM          | Mm     | mm     |
| 1.        | rs1048101 | ADRA1A       | 66.66  | 66.50  | 66.46  | 0.9684 |
| 2.        | rs3808585  | ADRA1A       | 66.15  | 68.19  | 65.93  | 0.2294 |
| 3.        | rs2236554  | ADRA1D       | 65.31  | 67.18  | 68.29  | 0.0840 |
| 4.        | rs533668   | ADRA2A       | 66.52  | 66.61  | 70.47  | 0.8682 |
| 5.        | rs11030104 | BDNF         | 66.56  | 66.60  | 67.73  | 0.8735 |
| 6.        | rs2049045  | BDNF         | 66.34  | 67.15  | 66.73  | 0.5703 |
| 7.        | rs6265     | BDNF         | 66.94  | 66.80  | 65.98  | 0.9220 |
| 8.        | rs7103411  | BDNF         | 65.55  | 66.53  | 67.34  | 0.9163 |
| 9.        | rs7094179  | CDNF         | 65.81  | 66.46  | 68.32  | 0.6485 |
| 10.       | rs7900873  | CDNF         | 67.03  | 66.49  | 64.68  | 0.3912 |
| 11.       | rs1051730  | CHRNA3       | 67.53  | 65.51  | 66.58  | 0.1190 |
| 12.       | rs16969968 | CHRNA5       | 67.45  | 65.77  | 66.61  | 0.2138 |
| 13.       | rs4680     | COMT         | 67.07  | 66.58  | 67.62  | 0.8569 |
| 14.       | rs135745   | CSNK1E       | 65.99  | 66.63  | 66.24  | 0.7121 |
| 15.       | rs1997644  | CSNK1E       | 66.83  | 66.31  | 65.68  | 0.7781 |
| 16.       | rs1611115  | DBH          | 65.74  | 67.36  | 70.89  | 0.0941 |
| 17.       | rs6271     | DBH          | 66.59  | 66.68  | 61.00  | 0.8731 |
| 18.       | rs4532     | DRD1         | 66.55  | 65.94  | 67.91  | 0.9000 |
| 19.       | rs6277     | DRD2         | 66.81  | 67.06  | 66.39  | 0.9148 |
| 20.       | rs1800497  | DRD2         | 66.72  | 67.70  | 61.29  | 0.7106 |
| 21.       | rs1079597  | DRD2         | 67.20  | 66.38  | 57.95  | 0.4397 |
| 22.       | rs1800498  | DRD2         | 67.07  | 66.46  | 67.34  | 0.7979 |
| 23.       | rs2134655  | DRD3         | 65.71  | 67.81  | 66.12  | 0.1250 |
| 24.       | rs3732790  | DRD3         | 67.03  | 66.42  | 65.90  | 0.5267 |
| 25.       | rs6280     | DRD3         | 66.98  | 66.60  | 64.46  | 0.4667 |
| 26.       | rs963468   | DRD3         | 67.25  | 67.01  | 65.06  | 0.5779 |
| 27.       | rs11246226 | DRD4         | 67.31  | 66.33  | 66.41  | 0.4831 |
| 28.       | rs3758653  | DRD4         | 66.51  | 66.48  | 69.93  | 0.9091 |
| 29.       | rs916455   | DRD4         | 66.93  | 62.92  | 46.67  | 0.0275 |
| 30.       | rs936460   | DRD4         | 66.60  | 66.39  | 67.70  | 0.9890 |
| 31.       | rs3733829  | EGLN2        | 66.96  | 66.76  | 65.38  | 0.6238 |
| 32.       | rs222843   | GABARAP      | 66.50  | 66.09  | 68.77  | 0.9562 |
| 33.       | rs11111    | GDNF         | 66.56  | 65.90  | 73.85  | 0.9972 |
| 34.       | rs1549250  | GDNF         | 66.75  | 65.38  | 70.34  | 0.8604 |
| 35.       | rs1981844  | GDNF         | 66.48  | 66.85  | 72.22  | 0.4727 |
| 36.       | rs2910702  | GDNF         | 66.27  | 66.40  | 68.79  | 0.5293 |
| 37.       | rs2973041  | GDNF         | 66.68  | 66.24  | 71.95  | 0.9268 |
| 38.       | rs2973050  | GDNF         | 66.30  | 66.49  | 68.89  | 0.5259 |
| 39.       | rs3096140  | GDNF         | 65.79  | 66.97  | 68.52  | 0.1457 |
| 40.       | rs3812047  | GDNF         | 66.45  | 67.46  | 70.87  | 0.3422 |
| 41.       | rs6925     | HTR1A        | 66.55  | 67.63  | 65.63  | 0.9441 |
| 42.       | rs1228814  | HTR1B        | 67.20  | 66.47  | 63.95  | 0.5336 |
| 43.       | rs130058   | HTR1B        | 67.22  | 65.55  | 70.74  | 0.3419 |
| 44.       | rs13212041 | HTR1B        | 67.09  | 66.21  | 66.87  | 0.5259 |
| 45.       | rs11568817 | HTR1B        | 68.70  | 65.76  | 67.12  | 0.0605 |

(Continued)
Corresponding statistical values for these were \([F = 4.878, p = 0.0275, \eta^2 = 0.007, \text{power} = 0.597]\) and \([F = 11.617, p = 0.0007, \eta^2 = 0.015, \text{power} = 0.926]\), respectively. In order to reduce the likelihood of a type I error, Bonferroni adjustment on the target alpha level was performed to correct for multiple testing. Effect of the rs7322347 polymorphism remained significant after Bonferroni-correction, labeled by an asterisk in Table 2. Individuals homozygous for the wild type allele (T) of rs7322347 had significantly higher aggression scores (69.21±17.00) compared to those carrying at least one minor allele (A) of this polymorphism (65.34±15.69). The corresponding Cohen’s d effect size for rs7322347 was \(d = 0.24\).

In order to gain a more detailed insight into the nature of the observed association, post hoc analyses were performed testing for possible relationship between rs7322347 and each of the four individual BPAQ subscales (Fig. 1). With the exception of Verbal Aggression, where mean scores did not differ in non-carriers compared to carriers of allele A (15.09±3.47 vs. 14.65±3.24; \(p = 0.1076\)), scores of all subscales showed significant association with rs7322347. Differences in mean scores between those homozygous for rs7322347 T and those with at least one
copy of rs7322347 A was most remarkable in the case of Hostility (18.41±5.55 vs. 17.05±5.48), with statistical difference between groups virtually equaling that observed with the overall BPAQ scale \(F = 11.535, p = 0.0007, \eta^2 = 0.015, \text{power} = 0.924\). Mean scores for both Physical Aggression and Anger were also higher in the absence of rs7322347 A than in its presence (18.86±7.08 vs. 17.80±6.63) \(F = 7.419, p = 0.0066, \eta^2 = 0.010, \text{power} = 0.776\) and (16.91±5.67 vs. 15.89±5.46) \(F = 5.858, p = 0.0157, \eta^2 = 0.008, \text{power} = 0.676\), respectively.

**Effect of the HTR2A rs7322347 polymorphism on male and female aggression**

As significant gender effect was observed in the BPAQ scores, male vs. female differences were also tested in terms of rs7322347 genotype and aggression using two-way ANCOVA with age as covariate. Although interaction between gender and aggression scores was highly significant \(F = 10.991, p = 0.0010, \eta^2 = 0.014, \text{power} = 0.912\), no gene-sex interaction was found \(p = 0.8834\). Both males and females carrying the minor (A) allele of rs7322347 showed lower levels of aggression (Fig. 2).

**Linkage disequilibrium (LD) and haplotype analyses within the HTR2A gene**

Taken that four other SNPs than rs7322347 (rs6311 C/T, rs6313 G/A, rs6314 G/A and rs7984966 T/C) within the HTR2A gene were also genotyped in this study, LD and haplotype analyses were performed as well to explore possible further contribution of loci in nearby regions to higher aggression levels. The associating polymorphism rs7322347 was found to be in complete linkage disequilibrium \((D’ = 1)\) with rs6314 located 1069 bp upstream from rs7322347 (Fig. 3), due to the fact that allele A of rs6314 could only be observed in subjects also carrying rs7322347 A and that all individuals homozygous for rs6314 A were homozygous for rs7322347 A as well. However, this was accompanied by a relatively low \(r^2\) value as there was a marked difference in MAFs for these two SNPs (8.8% for rs6314 vs. 44.2% for rs7322347). The polymorphism rs7322347 was in strong LD with rs7984966 as well (chromosomal distance: 19343 bp), although to a lesser extent than with rs6314. In addition, prominently high LD was also observed between rs6313 and rs6311 spaced 1538 bp apart, where in the majority of cases allele A of rs6313 was linked to rs6311 T (662/665 chromosomes; 99.6%) and allele G of rs6313 to rs6311 C (850/853 chromosomes; 99.7%) (Fig. 3).
One-way ANCOVAs were applied on the overall BPAQ scale scores with 2-SNP haplotypes (comprising rs7322347 and each of the other four HTR2A variants genotyped) as the grouping variable and gender and age as covariates (Table 3). In a dominant model (haplotypes containing only major alleles of the constituting SNPs), haplotypes rs6314/rs7322347 and rs7322347/rs7984966 showed a significant effect \[ F = 11.128, p = 0.0009, \eta^2 = 0.014, \text{power} = 0.915 \] and \[ F = 7.352, p = 0.0068, \eta^2 = 0.009, \text{power} = 0.773 \] respectively, while no significant differences in the mean scores of aggression were observed with regard to the other two haplotypes analyzed \( (p = 0.1875 \text{ and } p = 0.1232, \text{respectively}) \). Subjects homozygous for haplotype rs6314 G/rs7322347 T had higher aggression scores as compared to the rest of the population (69.05±17.07 vs. 65.33±15.77). Similarly, individuals carrying haplotype rs7322347 T/rs7984966 T on both chromosomes presented with higher mean BPAQ scores than those with other haplotype combinations (68.83±17.19 vs. 65.64±15.85). Haplotype-wise analyses also indicated significant association of haplotype rs6314/rs7322347, but to a lesser extent than in the dominant model \( [F = 3.205, p = 0.0408, \eta^2 = 0.004, \text{power} = 0.614 ] \) (Table 4).

![Fig 3. Linkage disequilibrium patterns between SNPs studies within the HTR2A gene. A: Lewontin's D' (%) and B: r2 (%) values of linkage disequilibrium between each SNP pairs, as determined by HaploView (version 4.2.). Higher values and darker colors indicate stronger LD between loci pairs. Red square indicates 100% LD.](https://example.com/figure3)

**Table 3. Association of rs7322347 comprising 2-SNP within-HTR2A haplotypes with aggression scores.**

| Haplotype                     | Aggression score | \( p \)   |
|-------------------------------|------------------|----------|
| HH                            | 69.05±17.07      | 0.0009   |
| Hh                            | 65.33±15.77      |          |
| rs6314/rs7322347              |                   |          |
| rs7322347/rs7984966           | 68.83±17.19      | 0.0068   |
| rs7322347/rs6313              | 66.23±15.89      | 0.1875   |
| rs7322347/rs6311              | 66.19±15.92      | 0.1232   |

H: Haplotype containing major alleles of the constituting SNPs; 

h: haplotype containing minor allele of at least of the two constituting SNPs 

Significant associations are indicated by bold, italics.

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In this study, we examined possible contribution of 55 SNPs to aggressive tendencies measured by the BPAQ in the general adult Hungarian population [33,34]. Only two of these SNPs showed association reaching nominal significance, and merely rs7322347 of the HTR2A gene retained significant effect after Bonferroni adjustment. These findings underpin the long-suspected key role of the serotonin neurotransmitter system in aggression and the related disorders [40,41]. There is convergent evidence that low or impaired serotonergic function underlies aggression and impulsivity [42–44]. As within the central nervous system (CNS) serotonin is synthesized solely in neurons of the raphe nuclei innervating virtually the entire neuraxis, this neurotransmitter is believed to exert a global effect on the brain with a holistically general role, even though local specialized functions are achieved by a variety of receptors [45,46]. It has been proposed that the principal role of serotonin might be the withdrawal from dangerous and aversive situations; consequently, serotonergic hypofunction could lead to impaired avoidance of undesirable stimuli, which in turn could provoke aggressive responses [47]. Strong experimental evidence supports this concept. The inverse correlation of aggression, impulsivity and antisocial behavior with serotonin metabolite 5-hydroxyindoleacetic acid levels in the cerebrospinal fluid was already known decades ago [40,48–50]. Later on, numerous studies confirmed these early observations regarding the relationship between dysregulation of the serotonergic system and aggressive-impulsive traits both in human and animals [51–54]. Behavioral functions of serotonin and also the effect of drugs influencing serotonergic mechanisms shows a marked conservation even between evolutionarily remote species [55]. This enables utilization of animal models for different types of aggression, e.g. affective (or defensive) and predatory (referred to as impulsive and premiated in humans, respectively) [56]. Data especially on rodents and felines provide valuable insight into underlying molecular mechanisms, shedding light for example on the interplay of proinflammatory cytokines and serotonin receptors in defensive rage and also on differential modulation

### Table 4. Haplotype-wise analysis of rs7322347 and each of the other HTR2A SNPs studied.

|                        | N    | Haplotype frequency | Aggression score ± Standard Error | p     |
|------------------------|------|--------------------|-----------------------------------|-------|
| rs6314G-rs7322347T     | 862  | 0.56               | 67.28±16.53                       | 0.041 |
| rs6314G-rs7322347A     | 547  | 0.35               | 65.78±16.04                       |       |
| rs6314A-rs7322347A     | 135  | 0.09               | 64.46±15.28                       |       |
| rs6314A-rs7322347T     | 0    | 0                  | -                                 |       |
| rs7322347T-rs7984966T   | 809  | 0.52               | 67.34±16.54                       | 0.115 |
| rs7322347A-rs7984966C   | 347  | 0.22               | 65.45±15.63                       |       |
| rs7322347A-rs7984966T   | 335  | 0.22               | 65.59±16.18                       |       |
| rs7322347T-rs7984966C   | 53   | 0.03               | 66.37±16.38                       |       |
| rs7322347T-rs6313G      | 592  | 0.38               | 67.02±16.50                       | 0.072 |
| rs7322347A-rs6313A      | 405  | 0.26               | 65.88±16.40                       |       |
| rs7322347A-rs6313G      | 277  | 0.18               | 64.99±15.14                       |       |
| rs7322347T-rs6313A      | 270  | 0.17               | 67.83±16.60                       |       |
| rs7322347T-rs6311C      | 590  | 0.38               | 67.07±16.48                       | 0.074 |
| rs7322347A-rs6311T      | 403  | 0.26               | 65.92±16.42                       |       |
| rs7322347A-rs6311C      | 279  | 0.18               | 64.94±15.10                       |       |
| rs7322347T-rs6311T      | 272  | 0.18               | 67.74±16.66                       |       |

Significant p value is indicated by bold, italics.

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### Discussion

In this study, we examined possible contribution of 55 SNPs to aggressive tendencies measured by the BPAQ in the general adult Hungarian population [33,34]. Only two of these SNPs showed association reaching nominal significance, and merely rs7322347 of the HTR2A gene retained significant effect after Bonferroni adjustment. These findings underpin the long-suspected key role of the serotonin neurotransmitter system in aggression and the related disorders [40,41]. There is convergent evidence that low or impaired serotonergic function underlies aggression and impulsivity [42–44]. As within the central nervous system (CNS) serotonin is synthesized solely in neurons of the raphe nuclei innervating virtually the entire neuraxis, this neurotransmitter is believed to exert a global effect on the brain with a holistically general role, even though local specialized functions are achieved by a variety of receptors [45,46]. It has been proposed that the principal role of serotonin might be the withdrawal from dangerous and aversive situations; consequently, serotonergic hypofunction could lead to impaired avoidance of undesirable stimuli, which in turn could provoke aggressive responses [47]. Strong experimental evidence supports this concept. The inverse correlation of aggression, impulsivity and antisocial behavior with serotonin metabolite 5-hydroxyindoleacetic acid levels in the cerebrospinal fluid was already known decades ago [40,48–50]. Later on, numerous studies confirmed these early observations regarding the relationship between dysregulation of the serotonergic system and aggressive-impulsive traits both in human and animals [51–54]. Behavioral functions of serotonin and also the effect of drugs influencing serotonergic mechanisms shows a marked conservation even between evolutionarily remote species [55]. This enables utilization of animal models for different types of aggression, e.g. affective (or defensive) and predatory (referred to as impulsive and premiated in humans, respectively) [56]. Data especially on rodents and felines provide valuable insight into underlying molecular mechanisms, shedding light for example on the interplay of proinflammatory cytokines and serotonin receptors in defensive rage and also on differential modulation...
of aggression by distinct types of serotonin receptors [57–59]. Administration of selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, citalopram or paroxetine usually reduces aggression [60–69], though contradictory results have also been reported, especially in juvenile humans and animals [70–72]. Reduced levels of serotonin caused by depletion of its precursor tryptophan have been linked to aggressive behavior [73–76], and disrupted function of enzymes involved in serotonin metabolism, such as tryptophan hydroxylase or monoamine oxidase, are also related to aggressive traits [77–79]. Observations on the link between life history of aggression and platelet serotonin content as well as platelet serotonin receptor and transporter binding further underpin the constantly growing body of evidence referring to a close relationship between the serotonergic system and aggression [80–82].

Serotonin (5-hydroxytryptamine) receptor 2a, encoded by the gene HTR2A, is a G-protein coupled excitatory receptor exerting its influence through the activation of secondary messengers phospholipase C and D [83]. Among others it is expressed in high levels on pyramidal cells of the prefrontal cortex, where it is ideally positioned to modulate both cognitive functions such as working memory or executive control and also emotions through dynamic interactions with the amygdala [84,85]. Serotonin receptors are also distributed along the midbrain periaqueductal grey (PAG) and the hypothalamus [56], brain areas that both have a direct connection with the prefrontal cortex and amygdala and long have been proved to control components of aggression including vocalization [86,87]. In accordance, mice with inherited aberrations in development and function of serotonergic neurons in the CNS exhibit increased levels of aggression which can be ameliorated by SSRIs [88]. Functional polymorphisms of the HTR2A gene are thus expected to influence neuronal networks regulating all the above mentioned features, providing a physiological basis for associations between HTR2A genetic variations and different mental states. During the last decade, several groups investigated SNPs of the HTR2A gene in connection with psychiatric and personality disorders [89–95]. Noteworthy observations have been made with regard to a number of variations located mainly in the promoter or the coding region; however, though scarce, literature data also indicate that intronic variant rs7322347 might as well be of interest from behavioral aspects, as it showed marked association with the combined subtype of childhood attention-deficit hyperactivity disorder (ADHD) and with suicide attempt in females subjected to physical assault in younger age [96,97].

Interestingly, according to our findings the missense polymorphism rs6314 is in complete LD with rs7322347, and the haplotype defined by these two SNPs has a similarly high impact on aggression levels as rs7322347 alone, despite the great difference observed between their MAFs. This might reflect that a complex background lies behind the robust association observed in the case of rs7322347, possibly consisting of several minor factors. Intrinsically, marked physiological effect of a single genetic variation with a MAF nearing 50% is generally improbable, simply based on the consideration that the spread of newly arisen alleles with functional relevance is most probably controlled by either positive or negative selection, hardly allowing quasi equal allele frequencies to evolve. Although in the present case it is plausible that a fine evolutionary balance has been struck between avoiding fights thus injury and gaining access to better resources, it cannot be excluded that other, linked polymorphic loci also contribute to the overall observed effect, even though similarly high D’ values as seen for rs6314 are unlikely for any such sites. Indeed, full linkage disequilibrium can only be expected when no crossing over event between the linked loci has yet occurred, which is mainly characteristic to the situation when at least one of the polymorphic sites is evolutionarily young. It is, though, noteworthy that immensely strong LD has been identified elsewhere within the HTR2A gene as well (between rs6311 and rs6313), both in this study and before [98–100].

As the linked polymorphism rs6314 causes a histidine to tyrosine change, thus the substitution of a basic amino acid residue to an uncharged one, this SNP could potentially affect both
protein structure and function [101]. In vitro studies implicate that its rare allele causes slower receptor response, decreased activation levels of phospholipases C and D, reduced calcium ion mobilization and thus a general hypofunctioning of the whole signaling cascade [102,103]. Recent findings imply that rs6314 also interferes with adequate splicing of pre-mRNA, with defective transcript forms triggering the RNA surveillance machinery, leading to a lower expression of the variant allele both on RNA and protein level [104].

Another possible explanation for the observed relationship between rs7322347 and aggression lies in gene regulation. Over the last few years, growing number of disease-associating polymorphisms in intergenic and intronic regions identified especially in GWA studies, combined with the fact that the more complex an organism is, the larger proportion of its genome will consist of non-coding sequences, has drawn attention of the scientific community towards the significance of expression regulation. By now, light has been thrown on several molecular mechanisms modifying gene expression, mostly with the involvement of non-coding sequences. Polymorphic intronic sites can lead to splicing efficiency bias or modified pre-mRNA stability, or they might affect long-distance gene regulation, for instance as part of an enhancer or an insulator, or through the RNAi pathway. In fact, according to the miRBase registry, T allele of rs7322347 disrupts a potential miRNA binding site [105,106]. It has recently been demonstrated by our group that differences in transcriptional regulation caused by a miRNA binding site disrupting SNP can indeed contribute to elevated aggression levels [107]. Though functional relevance of intronic miRNA target sites is obscure, recent evidence suggests that at least in plants miRNA interaction with intronic sequences is indeed involved in gene regulation processes [108]. In addition, expression quantitative trait loci (eQTL) data (http://genenetwork.nl/bloodeqtlbrowser) indicate that minor allele (A) of rs7322347 negatively affects (Z-score: -8.06) transcription of the ESD gene located 34 kb downstream of HTR2A [109]. ESD encodes esterase D, a poorly characterized protein with a suggested role in the recycling of sialic acids and also in detoxification [110,111]. Thus, it would be intriguing to explore possible interaction of ESD with neurobiological aspects and behavioral traits, especially as it is expressed all across the brain in considerable amounts according to AceView and TiGER databases [112,113].

In conclusion, this study adds on the growing evidence that the serotoninergic system greatly influences aggressive tendencies. To our best knowledge, this is the first report demonstrating a direct relationship between the HTR2A gene and aggression. However, confirmation of the present findings by independent replication would inevitably be necessary before drawing
any further conclusions from these results. Functional studies should also be performed in order to explore the exact biochemical background of the association described, and to elicit possible contribution of rs7322347 to psychiatric and personality disorders. By no means forgetting about the significance of environmental exposure, our findings will hopefully provide help to elucidate the genetic basis behind increased predisposition to aggression.

Author Contributions
Conceived and designed the experiments: ZR MSS. Performed the experiments: ZE AS. Analyzed the data: TN ZN. Wrote the paper: ZB ZR MSS.

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