The association between high and low levels of emotional reactivity (ER) as a temperamental trait and the dopamine D4 receptor (DRD4) exon III polymorphism in healthy men aged 18 to 27 (M = 21.03, SD = 2.23) was examined. ER, measured by the Formal Characteristics of Behavior–Temperament Inventory (FCB-TI), is defined as a tendency to react intensively to emotion-generating stimuli and is expressed in high emotional sensitivity and in low emotional endurance. Data analysis demonstrated a statistically significant difference in the distribution of genotypic frequencies between the low and high ER groups: \( \chi^2 = 4.88; \) df = 1; \( p = .027, \) odds ratio (OR) = 2.85, 95% confidence interval (CI) = 1.11–7.32. An insignificant difference in allele frequencies between the two groups was noted: \( \chi^2 = 7.47; \) df = 3; \( p = .058; \) OR = 2.9, 95% CI = 1.29–6.53. These findings suggest a role of the DRD4 exon III polymorphism in the modulation of ER as a temperamental trait. Due to the preliminary nature of our findings, replication is necessary.

According to Strelau’s Regulative Theory of Temperament (RTT), emotional reactivity (ER) is defined as a tendency to react intensively to emotion-generating stimuli and is expressed in high emotional sensitivity and in low emotional endurance. Data analysis demonstrated a statistically significant difference in the distribution of genotypic frequencies between the low and high ER groups: \( \chi^2 = 4.88; \) df = 1; \( p = .027, \) odds ratio (OR) = 2.85, 95% confidence interval (CI) = 1.11–7.32. An insignificant difference in allele frequencies between the two groups was noted: \( \chi^2 = 7.47; \) df = 3; \( p = .058; \) OR = 2.9, 95% CI = 1.29–6.53. These findings suggest a role of the DRD4 exon III polymorphism in the modulation of ER as a temperamental trait. Due to the preliminary nature of our findings, replication is necessary.
in the length of the third intracellular loop of the receptor (Jovanovic et al., 1999).

The DRD4 exon III polymorphism was associated with novelty seeking, a personality trait characterized by impulsive, exploratory, or sensation-seeking behavior (Benjamin et al., 1996; Ebstein et al., 1996). However, some replication studies have failed to confirm the finding (Gelernter et al., 1997; Jonsson et al., 1997; Vandenbergh et al., 1997). Similarly, the result of a meta-analysis performed by Schinka et al. (2002) was negative. This polymorphism has been associated with the intensity of reaction measured by means of the Toddler Temperament Scale (TTS) in 3-year-olds (De Luca et al., 2003). The aforementioned temperamental category refers to the energy with which the infants show their emotional states. Of interest is the fact that some data (Gendreau et al., 1998) show an important role for dopamine in the mediation of ER. Mutant mice lacking DRD4 demonstrated an increased reactivity in response to stress inducing stimuli compared with wild-type controls (Falzone et al., 2002).

The aim of our study was to examine the association between ER as a temperamental trait postulated in the Regulative Theory of Temperament, and polymorphism in the dopamine D4 receptor gene (DRD4). We followed a research scheme based on the comparison of allelic and genotypic frequencies in groups of subjects who had obtained extreme results on the ER scale.

Methods

The study sample was composed exclusively of males — 778 students of technical universities, aged 18 to 27 years (M = 21.03, SD = 2.23). The sample was homogenous with respect to the effects of age on ER (Zawadzki & Strelau, 1997). Each of the subjects completed the Polish version of the Formal Characteristics of Behavior-Temperament Inventory (FCB-TI) designed by Bogdan Zawadzki and Jan Strelau (Strelau & Zawadzki, 1993, 1995). The FCB-TI measures the following temperamental traits: briskness, perseveration, sensory sensitivity, ER, endurance and activity (20 items for each of the scales). Based on results obtained on the FCB-TI ER scale (possible range of results 0 to 20 points), a group of 112 males were distinguished — 51 of them with a low level of the assessed trait (results ranging from 0 to 1 point; M = 0.49, SD = 0.50) and 61 with a high level of the trait (results ranging from 15 to 20 points; M = 16.33, SD = 1.41). According to the FCB — TI norms, results ranging from 0 to 1 point are extremely low in men aged 15 to 29 years. The low ER group of subjects encompassed 6.56% of the study sample (i.e., 51 males). Adequately, we assigned the high ER group encompassing 6.56% of subjects with a high level of assessed trait, that is, 61 males with results ranging from 20 (extremely high) to 15 points inclusive.

The difference between these two subgroups with regard to ER was statistically significant (t = -76.14, df = 110, p < .001).

In the end the study sample comprised of 99 males selected on the basis of the ER assessment. The remaining subjects (13 males) had finished studying or moved out before the procedure of genotyping had begun.

DRD4 Polymorphism Genotyping

Genotype analysis was performed by the Laboratory of DNA Sequencing and Oligonucleotide Synthesis at the Polish Academy of Science Institute of Biochemistry and Biophysics.

The procedure of the subjects’ genotyping was conducted with the use of the polymerase chain reaction (PCR) method. Genomic DNA was extracted from buccal epithelial cells taken from each of the selected subjects. In the process of the DRD4 gene amplification, the following primers described by Lichter et al. (1993) were used: DRD4_4_U (5’-GCGACTACGTTGCTTACTGG-3’) and DRD4_4_L (5’-AGGACCCCT-CATGGCCTTG-3’). Hot-start PCR was used. To this end, two reaction mixtures were prepared (Mix-1 and Mix-2). Mix-1 contained 40 ng DNA, 0.5 µl 10 mM dNTP mix, 10 pmol of each primer, 1.5 µl 25M MgCl2,1.25 µl 10 × PCR buffer with (NH4)2SO4 (Fermentas, Fermentas International, Inc.), 5 µl 5 M betaine (Sigma, Sigma–Aldrich Co., USA; in a total volume of 12.5 µl). Mix-2 contained 1.25 U Taq polymerase (Fermentas), 1.25 µl 10 × PCR buffer with (NH4)2SO4 (Fermentas; in a total volume of 12.5µl). Mix-1 was subjected to a preliminary denaturation for 5 minutes at 95ºC. The two mixtures were subsequently combined on ice in a 1:1 ratio and the PCR reaction carried out under the following conditions: introductory cycle for 10 minutes at 95ºC; two incubation cycles of 30 seconds at 95ºC, 30 seconds at 65ºC, and 60 seconds at 72°C; 10 incubation cycles of 30 seconds at 95ºC, 30 seconds at 65ºC, and 60 seconds at 72°C (the annealing temperature was lowered by 1ºC after each cycle); 30 incubation cycles of 30 seconds at 95ºC, 30 seconds at 65ºC, and 60 seconds at 72°C; and then the final cycle of 30 minutes at 72°C. After completing the amplification, the products of each reaction were resolved by 3% agarose gel electrophoresis and visualized by ethidium bromide staining.

Statistical Analyses

The association between the polymorphism in the DRD4 gene and individual differences with regard to ER was analyzed by means of the SPSS for Windows statistical program (SPSS Inc., 1999). Pearson’s chi-square test was applied as a significance test for qualitative variables. The subjects were categorized on the basis of the level of the said temperamental trait as well as their DRD4 genotype identified.
through molecular analysis. The genotypes were segregated according to the presence or lack of the 7-repeat allele (a dominant mode of inheritance with respect to this allele was assumed).

The ARLEQUIN package (Schneider et al., 2000) was used in order to test the compliance between the distribution of genotypic frequencies and the Hardy–Weinberg equilibrium.

**Results**

The DNA of 9 subjects was unable to be amplified, and they were subsequently excluded from further analyses. The final analysis was conducted on a sample comprising 90 individuals: 44 of them were identified as low ER (M = 0.49, SD = 0.51), 46 as high ER (M = 16.17, SD = 1.31).

Table 1 presents distributions of allelic frequencies for the DRD4 exon III polymorphism in groups distinguished on the basis of ER assessment.

After excluding 5- and 8-repeat alleles from the analysis (due to their extremely low frequency), statistically insignificant differences in allele frequencies between the two groups were found: $\chi^2 = 7.47; df = 3; p = .058$; odds ratio (OR) = 2.9, 95% confidence interval (CI) = 1.29–6.53.

Table 2 presents distributions of genotypic frequencies for DRD4 exon III polymorphism in groups selected on the basis of the ER assessment. The DRD4 polymorphism frequencies were consistent with the Hardy–Weinberg equilibrium in both groups, that is individuals characterized by low levels of ER ($p = .133$) and in the group of subjects with a high level of this trait ($p = .078$).

A statistically significant difference in the distribution of genotypic frequencies between the two groups was identified: $\chi^2 = 4.88; df = 1; p = .027, OR = 2.85, 95\% CI = 1.11–7.32$.

**Discussion**

This study concerned the association between high and low levels of ER as a temperamental trait and dopamine D4 receptor (DRD4) exon III polymorphism in healthy men aged 18 to 27 years. Results demonstrated a statistically significant association between the DRD4 genotypes and ER. The results of research hitherto regarding associations between DRD4 exon III polymorphism and temperamental (personality) traits, published in a variety of articles and partially summed up by Benjamin et al. (2002), are highly inconsistent. In studies resembling this one, it also appeared difficult to achieve congruous results. Ekelund et al. (1999) submitted proof for the existence of discrepant allelic frequencies in groups of subjects who scored high and those who scored low on the Novelty Seeking scale. Nevertheless, in a study reported by Vandenbergh et al. (1997), no association of this kind was found. The study reported by De Luca et al. (2003) offers significant support for the results obtained here. De Luca demonstrated that children in whose genotypes the 7-repeat allele was found scored lower on the intensity of reaction dimension compared to those in whose genotypes the allele in question was not identified. Intensity of reaction, assessed by means of the TTS, refers to the amount of energy a child uses to express his or her emotions. It seems that this dimension, related to Thomas and Chess’s (1977) concept, resembles the understanding of ER offered by Strelau (1998). Of interest though, as demonstrated by Hornowska (2003), ER measured by the FCB-TI negatively correlates with novelty seeking. Therefore, the results of our study seem to be consistent with findings concerning the association of novelty seeking with 7-repeat allele of the DRD4 gene.

However, this study has certain limitations. In comparison with projects employing an analogous research scheme (Ekelund et al., 1999; Vandenbergh...
et al., 1997), this study sample was small (in addition, the group of subjects from which we selected on the basis of ER measurement was smaller) and was comprised exclusively of young males. Gender has been regarded as a potential moderator of the association between personality traits and DRD4 polymorphism. In a recent study, Becker et al. (2005) demonstrated an association between novelty seeking and DRD4 polymorphism in males, but not in females.

Another flaw in the project stems from the use of the method of extremes. As demonstrated by some authors (Sirota et al., 1999), the application of this approach with regard to genetic analyses may be problematic. A nonlinear association between gene and personality trait which is related to the heterosis effect can not be detected (Comings & MacMurray, 2000). The results of the study by Van Tol et al. (1992) suggest that heterosis plays a role in the pheno-
typic effect of DRD4 gene.

In conclusion, a statistically significant difference in the distribution of genotypic frequencies between the low and high ER groups has been demonstrated, but only a trend toward association between allele frequencies and the trait of ER (difference in allele frequencies between the two groups was statistically insignificant) was noted.

The results obtained contribute a new element to studies of the biological foundations of personality, and especially the role of dopamine in the development of temperamental traits. Nevertheless, due to the aforementioned limitations of the study and preliminary nature of the findings, the demonstrated association between the DRD4 gene polymorphism and ER requires further, more exhaustive research.

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