Association between 5-hydroxytryptamine 1A receptor gene polymorphism and suicidal behavior

Suicidal behavior is highly correlated with many emotional disturbances and some psychiatric disorders. The biogenic amine, serotonin, is one of the most important neurotransmitter in the central nervous system believed to play a huge role in pathogenesis of some kind of mental disorders. Drugs targeting serotonin receptors like serotonin reuptake inhibitors (SSRIs) are useful in the present therapy of anxiety and depression. Recent studies have reported that genetic factors are associated with development of some psychiatric disorders. Serotonin receptor single nucleotide polymorphism (SNP) has emerged as the subject of controversial result in correlation with suicide attempt. Further studies should be performed to confirm the influence of allelic variation of serotonin receptor on elevated risk of auto-aggression behavior. The aim of our study was to examine the frequency and genotype distribution of C(-1019)G polymorphism of regulatory region 5-HT1A receptor in the group of 65 suicide attempters and 63 persons in the control group. Using allele specific amplification PCR (ASA-PCR), we found that allele G was higher in suicidal attempters. The genotype frequency was significantly different between hospitalized patients and control subjects. The most common intoxication causes were antidepressants (56.9%), analgesics (18.5%) and cardiologic drugs (10.8%). Our data support hypothesis which indicate role of the 5-HT1A C(-1019)G SNP polymorphism in elevated risk of suicidal attempt.

Zachowania samobójcze są związane z wieloma emocjonalnymi zaburzeniami i niektórymi chorobami psychicznymi. Serotonina jest jednym z najważniejszych neurotransmiterów ośrodkowego układu nerwowego. Uważa się, że odgrywa kluczową rolę w patogenezie wielu zaburzeń psychicznych. Selektywne inhibitory wychwytu serotoniny (SSRI), działające na receptory serotoniny wykazują zdolność działania przeciwdepresyjnego. Na poziomie molekularnym, depresja może być spowodowana niewłaściwym funkcjonowaniem przekaźników synaptycznych, takich jak serotoninina i noradrenalina. Uważa się, że polymorfizm genów kodujących receptor serotoninowy przekłada się na aktywność receptora 5-HT, a poprzez to na zdolność wiązania serotoniny, co w konsekwencji prowadzi do zaburzeń zachowania i występowania prób samobójczych. Celem naszych badań było określenie częstości występowania i rozkładu polymorfizmu C(-1019)G obszaru regulatorowego dla genu receptora serotoniny 5-HT1A u pacjentów hospitalizowanych z powodu popelnionych prób samobójczych. Badanie przeprowadzono na grupie 65 pacjentów i 63 osób z grupy kontrolnej. Używając metody ASA-PCR (allele specific amplification), obserwowano częstsze występowanie allelu G u pacjentów po próbie samobójczej. Stwierdzono również istotną statystycznie różnicę w rozkładzie genotypów pomiędzy hospitalizowanymi pacjentami a grupą kontrolną. Najczęstszymi przyczynami zatrucia było przedawkowanie leków p/depresyjnych (56,9%), leków p/bólowych (18,5%) i kardiologicznych (10,8%). Nasze badania potwierdzają tezę, iż polymorfizm C(-1019)G genu regulatorowego receptoru 5-HT1A ma związek ze zwiększonym występowaniem prób samobójczych.
Introduction
Suicidal behavior is a very important public health problem worldwide. Suicidal attempt is the considered action with potentially life-threatening effect. This kind of behavior is highly correlated with many emotional disturbances, such as: problematic family relationships, interpersonal conflict with family and friends, loss of a valued relationship, loss of a romantic relationship, death of a loved one, failure at school, socio-economic hardship, unemployment or financial problems and trouble with the law, or psychiatric disorders, such as: major depression, schizophrenia, bipolar disorder and anxiety. Statistically nine out of ten effective suicidal attempts are related to mood or other psychiatric disorders. Suicidal attempts are usually preceded by the use of alcohol.

The incidence of suicide behavior reaches two peaks: in group of people over the age of 65, who have lost a spouse through death or divorce and during mid-adolescent years, particularly between the ages of 15 and 19. The police data show that there were 4,621 successful suicidal attempts in Poland in the year 2005 (3,885 men and 736 women). The physicians need to detect the young populations at greatest risk from among the large number of children, adolescents and young - adults. Evidence and early studies suggest that genetic components greatly contribute in developing of psychiatric disorders [15, 24]. But in fact, the relationship of the underlying mechanisms for this psychiatric illness still remain poorly understood. Nowadays, the molecular biology tools give us possibility to identify the predisposition factors.

Serotonin (also called 5-hydroxytryptamine; 5-HT), the biogenic amine, which was discovered in the late 1940s., is one of the most important neurotransmitter in the central nervous system believed to play a huge role in pathogenesis of some kind of mental disorders [25]. The development of serotoninergic neurons is regulated by serotonin itself. The disturbance in serotonergic development may permanently change the brain's function and behavior and lead to depression, aggression, anxiety, irregular appetite and even pain sensation. The serotonin imbalance may occur for a number of reasons. In the serotonin biosynthesis pathway, the human tryptophan hydrodrolases (two variants: TPH1 and TPH2), which are the rate-limiting enzymes, add the hydroxyl group to tryptophan's benzene ring, which are the rate-limiting enzymes, add a hydroxyl group to tryptophan's benzene ring forming serotonine. Some well known polymorphism of TPH1 and TPH2 (e.g. in intron 7 in TPH1) genes are considered as responsible for depression [7], bipolar disorder [1], aggression [13], alcoholism and suicidal behavior [17]. But other researchers [4-6, 8-14] noted positive correlations with suicidal behavior [11], mood disorders [20] and attention-deficit hyperactivity disorder [23], generalized anxiety disorder [3, 19].

The second possibility in the 5-hydroxytryptamine imbalance is the polymorphism of the promoter region of the coding gene of the serotonin transporter. The serotonin transporter is a protein, which allows neurons, platelets and other cells to accumulate the 5-hydroxytryptamine, regulating in this way serotonin concentration in a gap or synapse. There are many studies which report association or lack of association between some mental illness and polymorphism in serotonin transporter genes [2, 4-6, 8, 14]. It also should be noticed, that unsettled interaction between 5-HT and multiple serotonin receptors, located in the central and peripheral nervous system as well as in a number of non-neuronal tissues, has been implicated in the etiology of some psychiatric disorders. According to IUPHAR Subcommittee for the Classification and Nomenclature of Serotonin Receptors, 5-HT receptors are divided in thirteen subtypes. One of them, G-protein linked receptor 5HT1A which modulate cyclic AMP is considered to be relevant to anxiety disorders. Therefore drugs targeting serotonin receptors such as selective serotonin reuptake inhibitors (SSRIs) are used in treatment of depression and other anxiety disorders [9].

The aim of the study was to examine the frequency and genotype distribution of C(-1019)G polymorphism of regulatory region 5HT1A receptor in the group of 65 suicide attempters and 63 persons in the control group. We took into the consideration the role of C(-1019)G polymorphism in prevalence of suicide attempt in correlation with some clinical data.

Materials and methods
Subjects
This study was conducted at the Regional Center of Toxicology, Jan Bo¿y Hospital in Lublin. The subjects were 65 patients with mean age 28.66 ± 11.56 years (23 males with mean age 29.17 ± 10.49 years and 42 females with mean age 28.4 ± 12.22 years), admitted to the hospital ward due to suicidal attempt. All participants had no previous history suggesting psychiatric illness and were not under the influence of alcohol. Demographic data, medical histories and laboratory data were collected for each patient. All patients were unrelated and of Polish descent. The diagnostic assessment was performed without knowledge of genotype data. There were 63 patients, without previous history of psychiatric illness, qualified to the control group with mean age 65.22 ± 8.13 years (54 males with mean age 65.76 ± 8.05 years and 9 females with mean age 62.00 ± 8.38 years).

DNA extraction
For genotyping, high-molecular weight DNA was isolated from whole blood with the use of GenElute Blood Genomic DNA Kit (Sigma, St. Louis, USA). The quantity and purity of nucleic acid was measured by spectrophotometric method.

Detection of the C(-1019)G 5-HT1A promoter polymorphism
The genotype of the C(-1019)G 5-HT1A promoter polymorphism was determined with the use the allele-specific PCR method, because of impossibility to find any restriction endonuclease to cleave amplified PCR product in desired site (position -1019). The oligonucleotides sequences for the human 5-HT1A promoter region are: variant C sense -1021/-1014 5' - AAAGAGAAGGAAAGGAG - 3'; variant G sense -1021/-1014 5' - AAAGAGAAGGAAAGGAG - 3'. The amplification primers used in this study were based on previously described sequences [21] with our modification. The forward primer was common for both variants: For 5HT1A 5' - GGCTGGAATTTGATGATTAGAAGC - 3', while the reverse primers were: Rev5HT1A_C 5' - AAAGAGAAGGAAAGGAGGACCCGCGGTCCTC - 3' and Rev5HT1A_G 5' - AAAGAGAAGGAAAGGAGGACCGCGGTCCTC - 3' for variant C and variant G, respectively. The PCR amplification was performed in two sets. The final 50 µl volume of PCR reaction mixture contained 25 µl of RED Taq Ready Mix PCR Reaction Mix (Sigma, St. Louis, USA), 10 µl 100 - 300 ng of genomic DNA, 13 µl of H2O, 1 µl 10 pmol forward primer For5HT1A and 1 µl 10 pmol reverse primer Rev5HT1A_C or Rev5HT1A_G for variant C or G, respectively. After the initial denaturation step for 5 min. at 95°C, 35 cycles of denaturing at 95°C for 30 sec., annealing at 59.5°C for 40 sec. and extension of 72°C for 50 sec were performed, followed by a final extension step of 72°C for 5 min. To confirm the presence or absence of amplified fragment of the expected (161bp) size, each of the PCR products were size - fractionated by 2% agarose electrophoresis and visualized by staining with ethidium bromide (figure 1). The amplification product for C/C homozygotes was observed only with variant C sense -1021/ -1014 5' - AAGAAGAAGGAAAGGAG - 3'. The amplification product for G/G homozygotes was observed only with variant G sense -1021/ -1014 5' - AAGAAGAAGGAAAGGAG - 3'. The perfect hybrid with the template DNA (figure 2).

Statistical analysis
Statistica 6.0 for Windows was applied to all statistical procedures. The genotype and allele distributions were analyzed using the Pearson Chi-square test. The p-value < 0.05 was considered as statistically significant.

Results
A total number of 65 patients after suicidal attempt and 63 persons of control...
The use of allele specific PCR method revealed the frequency of 5-HT1A receptor gene C(-1019)G polymorphism, which is summarized in table I. Analyzing the genotype distribution, only 6 of the 65 (9.2%) were C-homozygote in suicidal group, while this type of homozygote reached 27% in control group. The genotype frequency (chi square=7.98; p=0.02) was significantly different between hospitalized patients and control subjects, indicating an association between the C(-1019)G polymorphism and suicidal attempt. The allele frequency (chi square=1.52; p=0.22) was not significantly different between hospitalized patients and control subjects, although allele G was higher in suicidal attempters group (table I). We observed that the genotype distribution was within Hardy-Weinberg equilibrium only in control group.

The clinical data of study group is presented in table II and III. The most common causes of intoxications, in order of frequency, were: antidepressants (56.9%), analgesics (18.5%) and cardiologic drugs (10.8%). Psychiatric examination of suicidal patients revealed serious psychiatric disorders in 23% and immediate hospitalization was recommended to them. The number of patients who had been suggested to undergo ambulatory treatment supervised by psychiatrist or psychologist was 43%. It is worthy to notice, that over 1/3 patients after suicidal attempt refused the psychiatric consultation or were discharged from hospital on their own demand.

Discussion
Allelic variations in 5-HT1A receptor gene has been suspected to play a role in predisposition to aggression, differences in pharmacokinetic response and modulation of depression-related personality traits [16,21]. There are controversial data regarding the correlation between suicide attempt and the 5HT1A receptor polymorphism published so far [10,12,27]. 5HT1A C(-1019)G SNP polymorphism is located in gene regulatory region that bind transcription factor NUDR (nuclear deformed epidermal auto-regulatory factor). According to described functional model and published statistical data G allele derepresses 5HT1A autoreceptor expression and reduce serotonergic neurotransmission and in this way predisposing to suicide [12]. Our data support hypothesis that the 5-HT1A C(-1019)G SNP polymorphism, demonstrate the association with the suicide attempt. We exclude for this study patients with previous history suggesting psychiatric disorder and those who were under the influence of alcohol. Suicide, by its nature, is an effect of heterogeneous implications which are often enhanced by alcohol consumption. By avoiding this “enhancer”, we tried to select group where genetic influence may be observe. Recently published data show no evidence for significant association between 5-HT1A C(-1019)G polymorphism and history of suicide attempts in alcoholic patients [10]. Obtained result in this way are more consistent with research were suicidal behavior indicate a role of G-allele.
in connection with high exposure to traumatic and/or stressful life events [27]. Because of its role in serotonergic system, not only C(-1019)G polymorphism of 5-HT1A was the subject of studies, but also Gly272Asp polymorphism show lack of association with suicide. On the other hand Gly272Asp polymorphism show effects on the clinical response to fluvoxamine [18,22]. Not only 5-HT1A receptor genes but also 1B, 1F, and 2A were the subject of investigations between the polymorphisms in the 5-HT and correlation with suicidal behavior. None of the tested receptors polymorphism appears to be associated with suicidal behavior [26].

Conclusions
Our result do not indicate the deciding effect of one SNP in whole serotonergic system as the major cause of suicide but, as the part of broad studies, is trying to answer some open question which still remain. Although our statistical significant data may help to distinguish this gene polymorphism which lead to higher risk for suicide and personality disorders. Further studies should be performed to better characterize multiple mechanism and to create genetic panel test for early detection of elevated suicide attempt risk.

References