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DRD2 ?141C Ins/Del Genotypes and Dopamine Profiles in Amphetamine Use Disorder

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Abstract

General Background: Substance use disorder arises from complex interactions between neurobiological mechanisms and genetic susceptibility, particularly within dopaminergic pathways. **Specific Background:** Variants in the ANKK1/DRD2 region, including the −141C Ins/Del polymorphism, have been linked to altered dopamine receptor regulation, which is central to reward processing in amphetamine use disorder (AUD). **Knowledge Gap:** Evidence remains limited regarding how this polymorphism relates to dopamine receptor concentrations among treated amphetamine users in Middle Eastern populations. **Aims:** This study examined demographic patterns of amphetamine use and evaluated the distribution of −141C Ins/Del genotypes and associated dopamine concentrations in individuals with AUD in Basra, Iraq. **Results:** The Ins/Ins genotype predominated among addicts, while the Ins/Del genotype was associated with markedly higher dopamine receptor concentrations compared with Ins/Ins carriers. Significant differences were observed between addicts and controls in both genotype frequencies and dopamine levels. **Novelty:** This work provides region-specific molecular evidence linking −141C Ins/Del heterozygosity to distinct dopaminergic profiles in AUD. **Implications:** The findings support the relevance of DRD2 regulatory variants in understanding neurobiological heterogeneity in amphetamine addiction and may inform future genetic screening and personalized intervention strategies.

Highlights:

- Ins/Del genotype shows the highest dopamine receptor concentration among amphetamine users.
- Clear genotype distribution differences distinguish addicts from non-addicted controls.
- Findings add molecular evidence from an underrepresented regional population.

Keywords:

DRD2 −141C Ins/Del; Dopamine Receptor; Amphetamine Use Disorder; Genetic Polymorphism; Basra Population

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Introduction

Drug addiction depends on both biological and psychological factors. The negative effects of drug addiction range from the initial motives for use (such pain relief) to the powerful psychoactive changes of chronic use[1]. Epidemiological data 2017 indicates that 271 million people (representing 5.5% of the world's population) were involved in the use of illicit alcohol, while the number of drug users was 35.6 million. Cannabis (marijuana) was the most prevalent, with 188 million users, followed by opioids, which were used by 53 million people (a 56% increase over previous estimates). Reports of drug-related illnesses and deaths occurred over a three-month period. . Dopamine (DA) is a monomeric neurotransmitter, partially synthesized in the neurite outgrowth, and consequently in the ventral tegmentum, the less visible part of the substantia nigra. The structure of this neurotransmitter is further modified in dopaminergic neurotransmitters . It plays a pivotal role in regulating motor, motivational, and emotional functions. The biological effects of dopamine are mediated through five types of receptors (D1, D2, D3, D4, and D5. These receptors work together to play a crucial role in regulating motivation and the reward circuitry, and they also contribute to goal-directed behaviors in addictions, such as drug addiction . Studies have shown that this receptor defect, associated with addiction disorders, contributes to variations in dopamine levels. Experimental evidence demonstrates the role of these receptors in regulating different cell types and their neurological and psychiatric effects . The dopamine receptor gene, type D2, is of vital importance in many neurological and psychiatric disorders. including schizophrenia, Parkinson's disease, and substance use disorders. Polymorphisms in this gene present several challenges, most notably the high cost and the inability to detect all clinically and functionally significant genetic variants simultaneously. These variants are distributed across the coding region, including Val96Ala (rs6275), Leu141Leu (rs6277), Val154Ile (rs1800498), Pro310Ser (rs6278), and Ser311Cys (rs1801028); and in the non-coding regions, including TaqIA (rs1800497), A-241G (rs1799978), and 141C Ins/Del (rs1799732 The TaqIA (rs1800497) polymorphism, located in the Ankyrin Repeat and Kinase Domain Containing1 (ANKK1 gene), is considered one of the most extensively studied genetic variations in the context of addiction and behavioral disorders . These two forms express the two identical proteins (the short form of the dopamine D2 receptor and the long form of the dopamine D2 receptor) through connection to exon 6, which consists of 87 base pairs. The gene encodes the dopamine transporter D2, which contains 443 amino acids, and the second form contains 443 amino acids .These two forms arise from a single gene through the splicing and cutting of different parts of the genetic code. The locus is located in the receptor region (postsynaptic), while the locus is located in the transmitter region (presynaptic). The function of D2L is to receive the signal, while D2S monitors and regulates the amount of neurotransmitter released . rs1799732) is a polygenic indel (one cytosine base insertion/deletion) in the regulatory region of the dopamine receptor gene DRD2This variant is associated with several (such as TaqIA polymorphism in the ANKK1 gene) pleiotropic substances with substance use disorders, partly attributed to its effect on supplementation or dopamine D2 receptor function in certain areas of the 99 (such as the body) . It is a single cytosine (C) base polymorphism/deletion in a non-large encoding region of the dopamine D2 receptor (DRD2) gene, which plays a crucial role in regulating its receptors, thus partially and directly influencing the risk of addiction and related behaviors. As noted: the presence or absence of a cytosine (C) base at a specific motivating locus may affect the responsiveness of the DRD2 gene, its specific location, and the dopamine receptor level . Amphetamine derived from alpha-methylphenethylamine(ADHD) is a potent central nervous system stimulant used to treat narcolepsy, obesity, and attention deficit hyperactivity disorder . Deoxyephedrine (AMPH), a psychostimulant and central nervous system stimulant, has devastating effects on the body and system, causing severe neurological and physical consequences. Through experiencing a state of increased physical activity, monitoring heart rate, blood pressure, and body temperature, in addition to visual stimuli . Amphetamine stimulates the brain by affecting neurotransmitter systems such as dopamine, serotonin, and norepinephrine, leading to a feeling of euphoria, but it is a highly addictive substance . Therefore, this study aimed to assess the prevalence of addictive behavior, through standardized measurements at the first level (primary level), among drug users undergoing treatment within a rehabilitation center in Basra.. This was achieved through a multi-use analysis of the Ins/Del effect on dopamine concentration.

Materials and Methods

2.1. Sample Collection

Eighty blood samples were collected from AMPH addicts and 20 blood samples from a control group at the Basra Addiction Rehabilitation Center in Al-Fayhaa Teaching Hospital, Basra Governorate, Iraq, between October 2, 2024, and October 2, 2025. The ages of the participants ranged from 14 to 45 years and older. Five milliliters of venous blood were drawn from each addict and the control group (non-addicts). The samples were transferred to a new 3-milliliter gel tube and another new 2-milliliter EDTA (ethylenediaminetetraacetic acid) tube. Samples containing 3 ml of blood in gel were left at room temperature for 15 minutes to coagulate, then centrifuged at 3000 rpm for 10 minutes. The serum was then separated and transferred to new, single-use Eppendorf tubes, which were stored in a freezer at -20°C until the concentrations of dopamine, opioid, and serotonin receptors could be measured to study protein expression. Samples containing 2 ml of anticoagulant were stored at -20°C for use in DNA (deoxyribonucleic acid) extraction to study mutations in dopamine, opioid, and serotonin receptors . After coordination between the College of Education of Al-Qurna and the Basra Health Department regarding the collection of samples and the personal information that includes (age, marital status, residence, work, educational attainment, weight, substance used, last dose used, duration of

2.2. Molecular Diagnosis

1. DNA Extraction 2.2

DNA was extracted from blood samples of AMP-addicted and non-addicted individuals using a dedicated extraction kit from the Taiwanese company Geneaid, following the manufacturer's protocol. The concentration and purity of the DNA were

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measured using a Nanodrop spectrophotometer. The DNA was then stored at -20°C until its use in subsequent steps

2.2.2. Nested Polymerase Chain Reaction:

The first-round PCR reaction was performed in a total volume of 25 microliters, consisting of Promega GoTaq® G2 Green Master Mix (12.5 µL), primers (1 µL each), nuclease-free water (4.5 µL), and DNA template (6 µL). The second-round PCR reaction was similar to the first-round reaction with a few exceptions. These exceptions included performing two reactions per sample (one using wild-type primers and the other using mutant primers), using 3 µL of the first-round PCR product as the template, and using 7.5 µL of nuclease-free water. The PCR cycling conditions were as follows: an initial denaturation at 94°C for 2 minutes, followed by 35 cycles (for PCR1) or 15 cycles (for PCR2) of denaturation at 94°C for 1 minute, annealing at 65°C (for PCR1) or 63°C (for PCR2) for 1 minute, and extension at 72°C for 2 minutes, concluding with a final extension at 72°C for 5 minutes. The PCR products were analyzed by electrophoresis on a 1% (weight/volume) agarose gel and visualized using a UV transilluminator.

mutant	PCR	Primer	Sequence	Product size	Reference
-141	PCR 1	Reverse (R1)	5 TGAAGCTGGACAGCTCTGC -3	276 bp	[15]
		Forward (F1)	5 ACTGGCGAGCAGACGGTGA -3		
	PCR 2	F2:D2 -141 WT	5 AACCCCTCCTACCCGTTCC -3	bp151	
		R2: D2 PR RV	5TGAAGCTGGACAGCCTCTGC-3		
		F2: D2 -141 MUT	5 AACCCCTCCTACCCGTTCA -3	151 bp	
		R2: D2 PR RV	5TGAAGCTGGACAGCCTCTGC-3		

Figure 1. Table (2-7) Prefixes used in dopamine receptors

2.2.3. Dopamine Concentrati

Dopamine concentrations in the serum of AMP addicts and non-addicts were determined using the Human Dopamine ELISA Kit (Chemical Degradation Technology Laboratory) according to the manufacturer's instructions.

2. 2. 4. Statistical Analysis

Statistical analyses were performed using SPSS version 25. Data were analyzed using either the Chi-square test or one-way ANOVA. The differences were considered statistically significant when p was less than 0.01.on

Results and Discussion

A. Result

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3.1. Demographic data of the AMPH addict

3.1.1. Demographic Data of AMPH-Addicted Individuals by Age Group

The current results show that the 25-34 age group had the highest rate of amphetamine use, at 31.83%. Conversely, the 45+ age group had the lowest rate, at 18.8%. The 14-24 age group had a rate of 26.32%, and the 35-44 age group had a rate of 23.1%. Highly significant differences were observed between the age groups, with statistical significance ($p < 0.0001$). (Table 2).

Age group	No.	%
14 - 24	105	26.32
25 - 34	127	31.83
35 - 44	92	23.1
45 <	75	18.8
Total	399	100
Chi-square value = 4.827, p value = 0.0001		

Figure 2. **Table (2): Percentage of amphetamine addicts over the previous year prior to the current study, divided by age group**

3 .1.2. Demographic data of AMPH addicts distributed by occupation 3

Figure 1 shows the percentage of amphetamine addicts, with the highest percentage recorded among the "workers" category at 86.5%, while the lowest percentage was recorded among the "employees" category at 6.3%, and among the "students" category at 7.3%. Despite the difference in percentages, statistical analysis revealed statistically significant differences ($p<0.05$).

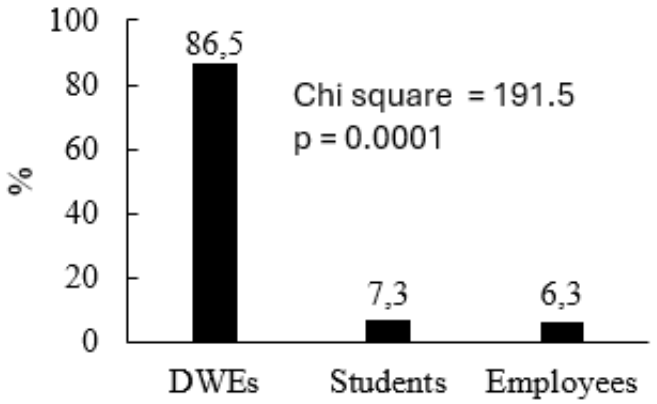

Figure 3. **Percentage of AMPH addicts distributed by occupation**

3 .1.3. Demographic data of AMPH addicts distributed by residence 3

The current results show that the highest rate of amphetamine addiction was recorded in various areas of Basra city center, reaching 22.3%, while Al-Sadiq district recorded the lowest rate at 2.8%. The rate of amphetamine addiction in the remaining residential areas of Basra Governorate ranged between 18.7% and 3.0%. Accordingly, the statistical results showed highly significant differences 0.0001) when analyzed (Figure 2).

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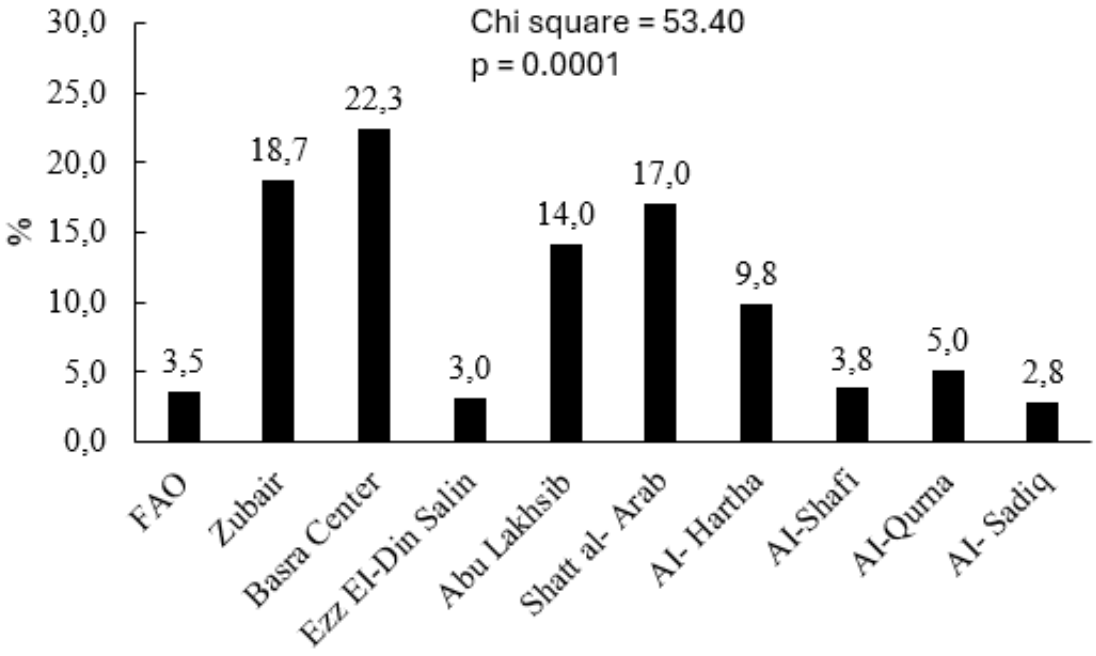

Figure 4. **The percentage of AMPH addicts distributed by residence**

3.1.4 Detection of the -141 C mutation in the dopamine receptor gene

The (-141C) polymorphism adjacent to the dopamine receptor D2 was genotyped using Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR). The study comprised 80 samples from individuals with amphetamine use disorder (AMPH) and 20 samples from non-addicted controls (Figure 3, partial results) The results revealed the presence of a single polymorphism pattern in the amphetamine-dependent group (Figure 3D) compared to the control group (Figure 3B). A heterozygous (insertion-deletion) genotype was identified in samples 2, 3, and 5.

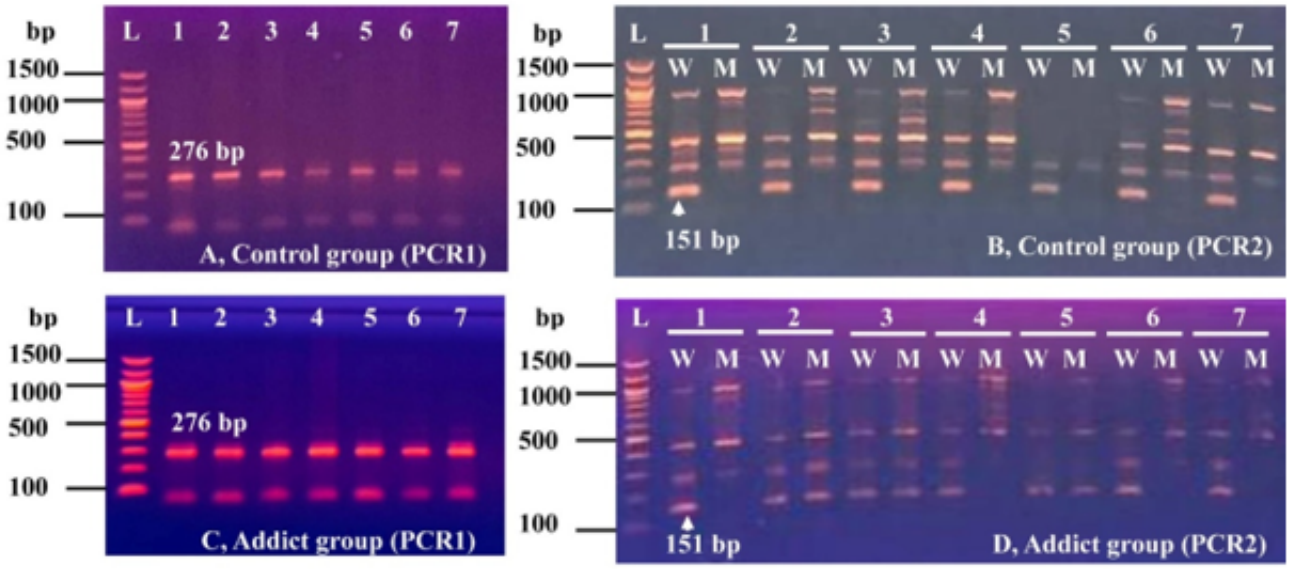

Figure 5. **shows the use of agarose gel electrophoresis to detect the C141-carbon polymorphism in AMPH addicts. A and B represent control samples, while C and D represent addicts**

3. 1. 5. Genetic patterns resulting from the -141C mutation

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Table 3 shows the control and AMP addict groups. The control group, with the wild-type Ins-Ins genotype, recorded the highest percentage at 100%, comprising 20 samples. Neither the zygosity variant Ins-Del nor the mutational variant Del-Del recorded any percentage (0%). As for the AMP addict samples, the wild-type Ins-Ins genotype recorded the highest percentage at 81.25%, comprising 65 out of 80 samples. The Del-Del mutation had the lowest prevalence at 0% (0 out of 80 samples), while the Ins-Del zygosity variant had the highest prevalence at 18.75% (15 out of 80 samples). Therefore, statistical analysis (Table 3) showed significant differences (P<0.001) between the control group and the AMP addicts regarding the -141C mutation (Table 2).

Samples	Total No.	Wild type		Mutant type				Total
		Ins-Ins		Ins-Del		Del-Del		
		NO.	%	NO.	%	NO.	%	
Non addicts	20	20	100	0	0	0	0	100
Addicts	80	65	81.25	15	18.75	0	0	100
Total	100	85	85	15	15	0	0	100
Chi-square value = 80.18, p value = 0.0001								

Figure 6. **Table (3) Percentage of -141C mutation in the samples of the current study**

3.1.6. Effect of a mutation at position -141 C of the dopamine receptor's excitatory region

The current study revealed clear differences in receptor concentration between groups carrying different genotypes. The receptor concentration in the control group (Ins/Ins) was 290 ng/ml, while amphetamine addicts carrying the Ins/Del genotype recorded the highest dopamine receptor concentration, averaging 433 ng/ml. In contrast, amphetamine addicts carrying the Ins/Ins genotype recorded a significantly lower receptor concentration, averaging 339 ng/ml. Statistical analysis of the results showed significant differences in dopamine receptor concentrations between the groups, suggesting a potential effect of polymorphisms on DRD2 receptor regulation in amphetamine addicts, as these differences were statistically significant (Figure 4).

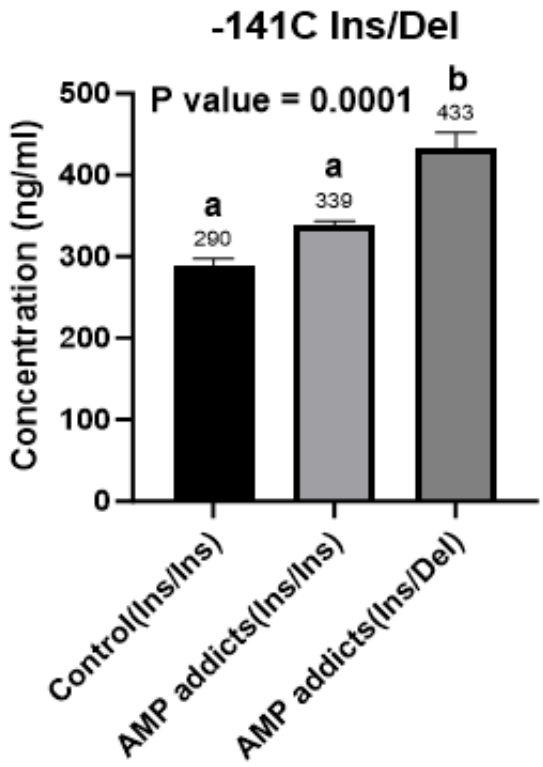

Figure 7. **shows the effect of a mutation at position - 141 C of the promoter region of the dopamine receptor D2**

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(DRD2) on dopamine concentration in amphetamine addicts

B. Discussion

The high prevalence of addiction in the 25-34 age group (31.83%) may be attributed to pressures related to social participation, independence, and unemployment, leading to drug use. The National Center (2025) highlighted this, noting that the prevalence of these substances was highest among 18-25 year olds (39%) and is projected to reach 26 years and older (23.9%). However, the 14-24 age group, many of whom have struggled with drug addiction since childhood, has a significantly higher prevalence (26.32%) due to drug exposure, peer pressure, and a lack of behavioral and psychological support. This has also been demonstrated in previous studies . In Thailand, 39% of the 15-24 year old population was found to use illicit substances, such as amphetamines. In our current case, the percentage of amphetamine users in the 35-44 age group is 23.1% .

This may indicate greater stability in new living arrangements, higher health awareness, and other factors that reduce drug tolerance . The study indicated that the 35-68 age group had the lowest percentage of addicts compared to younger age groups, at 22%. The lack of statistically significant differences in AMPH addiction between age groups suggests that age is not the sole influencing factor, and that other factors (such as social media promotion and unemployment) may be more important, as indicated in the findings of . The study confirmed that there were no statistically significant differences between age (AMPH) and degree of addiction among individuals addicted to the mother.

A recent study showed that the highest prevalence of vegetable deficiency was among manual laborers, reaching 86.5%, while central processing assistants or education staff showed a high prevalence rate (7.3%). This includes recent data from the study (Helander & Sparing 2025). Analysis of 23,900 urine and visible fluid tests in workplaces revealed that over 20% of construction workers responded to vitamin use, resulting in significantly lower performance levels. While some of the findings were based on prescriptions, the results confirm that physically demanding occupations are associated with disproportionately high rates of amphetamine use. These findings highlight the need for targeted workplace interventions, new harm assessments, and strategies to address the reasons. The lower rates are attributed to the influence of preventive and supervisory workplace policies, which help reduce usage through education and continuous monitoring. This is supported by the findings of the (Federal Recovery-Ready study et al. 2023). Meanwhile, the percentage of AMP addiction among students, which was 7.3%, stems from a complex interaction between individual adolescent traits (such as impulsivity and psychological issues) and socio-environmental factors (such as peer pressure and easy access to substances), in addition to economic and political conditions that either increase the likelihood of use or reduce protective measures. This has been indicated by . Despite the differences in the percentage values above .

The current results indicated that the highest rate of AMPH addiction among residents was in Basrah Center, reaching 22.3%. In contrast, the Al-Sadiq district recorded the lowest rate of AMPH addiction at 2.8%. The addiction percentages for AMPH in the remaining residential areas of Basrah Governorate ranged between 19.7% and 4.0%. Consequently, the statistical analysis showed significant differences ($p < 0.05$) when the results were analyzed statistically (Figure2). This is attributed to the higher availability and easier access to drugs in the city center, particularly among university students and the unemployed, as noted by (Alharbi et al., 2022).

Our data revealed that amphetamine-addicted individuals carrying the Ins/Del genotype exhibited the highest concentration of D2 dopamine receptors. This genotype is often considered a risk factor for addiction. An explanation for this is neural adaptation: chronic amphetamine use floods the brain with dopamine. After exceeding 290 ng/ml in the non-addicted group (CC), the highest dopamine receptor concentration was observed, while AMPH addicts with the Ins/Del heterozygous gene had the highest average dopamine receptor concentration, at 433 ng/ml. Conversely, concentrations were significantly lower in AMPH addicts, including those with the Ins/Ins gene, at 339 ng/ml. These statistical analyses revealed clear differences in dopamine receptor concentration between the two groups, suggesting a significant influence of ANKK1 gene polymorphisms on DRD2. Regarding the ANKK1/DRD2 gene, our data indicate that AMH addicts with Ins/Del show heterozygous zygote have a higher concentration of dopamine D2 receptors. This is a risk factor for addiction. The explanation lies in genetic adaptation. Chronic amphetamine addicts become overwhelmed with dopamine. As a result, they may attempt to compensate by increasing the number of D2 receptor variants to balance. These findings are consistent with several recent neuroimaging studies . that showed a 22% to 5% concordance rate in D2 receptor availability among chronic steroid users after a period of abstinence, suggesting a strong compensatory mechanism (effect size, d. 0.85).

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