Original Article

# Variation of DNA Repair System Genes (*XRCC1*) rs25487 and (*RAD18*) rs373572 in Methamphetamine Abuse Cases

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## Abstract

**Background:** Methamphetamine abuse has significantly increased among the young Iraqi population in the past year. **Objectives:** The current study aimed to detect variations in the X-ray repair cross-complementing protein 1 (*XRCC1*) rs25487 and E3 ubiquitin-protein ligase (*RAD18*) rs373572 genes in cases of addiction. **Materials and Methods:** Blood samples and data collection: 1 mL of the whole blood sample was collected from each patient who attended the Psychiatric Center, Marjan Teaching Hospital, Hillah, Babylon Governorate, from February 2023 to May 2023. Then, the blood samples were transferred to the lab for deoxyribonucleic acid extraction and target sequence amplification. **Results:** The results indicated that the *XRCC1* genotyping distribution for AG was observed more, with the case group, than in the control group, and the GG genotype was found less in the case group than the control group, with significant differences [odds ratio (OR) = 4.649, $P = 0.0219$)]. The AA genotyping was not observed in the case group, but was found to a minimal extent in the control group with nonsignificant differences (OR = 0.4286, $P = 0.598$); the allele A was strongly associated with Meth in the case samples, demonstrating significant differences. The genotyping of the *RAD18* gene showed the Gln/Arg genotype was highly frequent in both cases and the control group, whereas Gln/Gln was more frequent in the case group compared with the control, with significant differences (OR = 8.400, $P = 0.0118$). The Arg/Arg genotype was not observed in the healthy control group with nonsignificant differences (OR = 12.87, $P = 0.098$). The allele frequency showed a non-significant distribution (OR = 1.060, $P = 0.835$). **Conclusion:** From these results, we can conclude that there were strong associations between XRCC1 and RAD18 and Meth addiction in the cases studied.

**Keywords:** Abuse cases, DNA repair systems, methamphetamine, variation

## Introduction

Methamphetamine (Meth) is a psychoactive medication that is characterized by significant uptake worldwide; according to the evidence provided by the EU, about 219 illegal generation loci were detected in 2015.[1] This drug was administered in low doses for treating hyperactivity in children and as a weight reduction agent earlier.[2-4]

There is limited evidence in the literature about the genotoxic properties of methamphetamine (MP); an *in vivo* study has found that MP induced DNA oxidation in brain cells, which plays a major role in the drug's neurodegenerative features.[5-8] Li *et al*.[9] reported that MP causes many genotoxic effects, including sister chromatid exchanges and micronuclei, in addition to DNA mutations in animal lab cells and bacteria; these effects may be generated by reactive oxygen species (ROS). Later, Johnson *et al*.[10] cited that MP forms comet-like patterns in specific areas in the brain rich in dopamine. The structurally related derivatives such as fenfluramine and amfepramone, that are weight loss agents, were observed to cause DNA damage in different ways.[11,12]

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MP also causes the generation of ROS and DNA oxidation, as evidenced by several studies.[5,7,8,13,14]

Studies reported four major ways of DNA repair: mismatch repair, base excision repair, double-strand break repair, and nucleotide excision repair, and those which conserve cells against oxidative DNA damage are main repair pathways like the base excision repair. The *XRCC1* is a vital enzyme in the base excision repair pathway;[15,16] thus, the polymorphisms of DNA repair system gene variations may be contributed to the progress in the Nero cell defects. E3 ubiquitin-protein ligase RAD18 is involved in the repair of post-replication DNA injury by UV light and the filling of a daughter strand during the replication of damaged DNA.

The current study helps detect indirect associations between DNA repair gene polymorphisms and addiction to methamphetamine. MT stimulates DNA injury by oxidation in addiction, that may be accumulated and cause severe complications and other diseases. Ours is the first report about the deleterious effects of addiction on DNA repair activities.

## Materials and Methods

### Sample collection

Twenty four samples were included in the current study. Cases were diagnosed by a psychiatric consultant at Hamurabi Medical College in the University of Babylon. Cases include those with Meth abuse for prolonged periods (1–2 years).

### Exclusion criteria

The exclusion criteria are as follows, smoking, cancer cases, diabetes mellitus type 1 and 2, hypertension and alcohol abuse cases.

### Blood samples and data collection

Approximately 1 mL of whole blood was collected from each case with written consent, according to the ethical approval of scientific research in Iraq, and blood samples were then transferred to the lab for DNA extraction and target sequence amplification. Cases were referred to Marjan Hospital City, Psychiatric Center during Feb–May 2023 and diagnosed by a specialist physician.

DNA collection and PCR programs: DNA was isolated from whole blood using a Favorgen extraction kit for frozen blood. The oligo primers of the **XRCC1** target sequence were used for PCR-CTPP: **F1**: tcc ctgcgc cgc tgc agt ttc t **r1:** tgg cgt gtgagg cct tac ctc c **f2** tcg gcg gct gccctc cca **r2** agc cct ctg tga cct cccagg c. The amplicon sizes are as follows: G and A allele have 447 and 222 bp, respectively, and the common band has 630 bp.[17] The RAD18 Arg302Gln (rs373572) oligos: F1: 5'-ata ccc atc acc cat ctt c and r1, gtc ttctct ata ttt tcg att tct t to produce 146 pb of the Gln allele, f2, tta aca gct gct gaa atagtt cg and R2, ctg aaa tag ccc att aac ata ca to produce 106 bp of Arg allele and common band has 206 bp, The products were visualized by electrophoresis (1% agarose gel, 100 V, 25 mA for 50 min) and imaged using photo-documentation equipment with ethidium bromide staining.

### Ethical approval

Blood samples were obtained from each of the cases with written consent, according to the ethical approval of scientific research in Iraq, and data collection was performed.

## Results

This study aimed to detect variations in DNA repair enzyme system genes in methamphetamine addiction cases among young Iraqis, the mean age of the cases was 26.43 ± 1.08 years, and the age of the controls was 33.1 ± 2.07 years, with insignificant differences ($t = 2.60$, $P = 0.012$), and about 25% of the case samples were female [Table 1]. The incidence of meth abuse was recorded at 35% in a study population in Vancouver, Canada.[18] In another study, about 60.5% of women began MA uptake before their 18th birthday.[19] There were several differences between women and men; for instance, men were more likely to initiate MA uptake earlier than women, and this agrees with other previous reports in America,[20,21] but did not deal with a study in Taiwan,[22] and America.[23]

The electrophoresis patterns of XRCC1 and RAD18 are shown in Figure 1 in XRCC1 three bands (AG, AA, and GG) were observed, and two bands in RAD18 (Gln/Arg, Arg/Arg and Gln/ Gln).

The genotyping distribution found that AG was more frequent in cases (83.33%) than in the control group (48.27%) and the GG was less frequent in cases (16.66%) than in the control group (44.82%), with significant differences (OR = 4.649, $P = 0.0219$). The AA genotype was not observed in the case group, but was found in a less proportion in the healthy group (10.34%), with non-significant differences (OR =0.4286, $P = 0.598$); the allele A was strongly associated with Meth case samples with significant differences [Table 2].

**Table 1: Study subject characterization (age and sex)**

| Subjects | Case | Control | *P* |
|---|---|---|---|
| Age | 26.43 ± 1.08 | 33.1 ± 2.07 | $P = 0.012$ |
| *Sex* | | | |
| Male | 75% | 80% | $P = 0.3968$ |
| Female | 25% | 20% | |

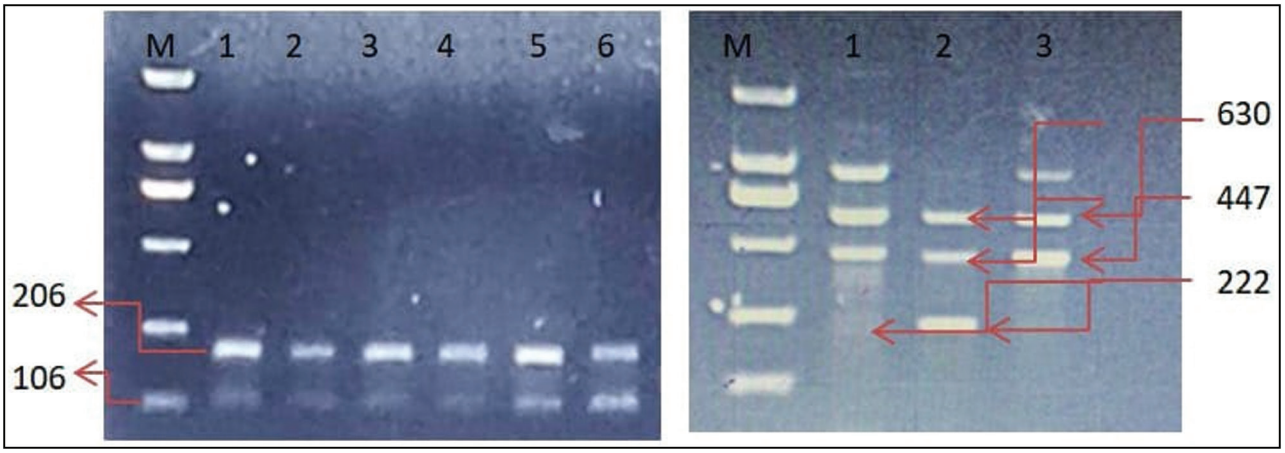


**Figure 1:** The XRCC1 and RAD18 genotyping patterns of study groups, M DNA marker, lanes 1 to 6 (left) RAD18 gene genotyping, lanes 1 to 3 (right), 100 V, 20 mA, 1% agarose and 0.5X TBE

**Table 2: The XRCC1 genotyping frequency in the study groups (MA cases and control)**

| Genotyping XRCC1(rs25487) | Cases (%) | Healthy (%) | OR (CI 95%) | *P* |
|---|---|---|---|---|
| AG | 20 (83.33) | 14 (48.27) | 4.6429 (1.2497 to 17.2485) | 0.0219* |
| GG | 4 (16.66) | 13 (44.82) | 0.4286 (0.0184 to 9.9957) | 0.5980 |
| AA | 0 | 3 (10.34) | RG | |
| A | 0.583 | 0.333 | 2.8780 (1.6156 to 5.1271) | 0.0003* |
| G | 0.416 | 0.666 | | |

*refer to significant differences between groups

## Discussion

To the best of our knowledge, no previous reports have investigated the role of XRCC1 and RAD18 in methamphetamine addiction; thus, the current study aimed to detect the indirect association between DNA repair gene polymorphisms and MT addiction based on the role of MT in stimulating DNA injury by oxidation in addiction, which may be accumulated and cause severe complications and other diseases, Ours is the first report about the deleterious effects of addiction on DNA repair activities.

In an early study, Thompson *et al.*[24] found that the XRCC1 enzyme is a vital component of the base excision repair (BER) pathway; this gene was first isolated from mammalian cells and was found to stimulate cell sensitivity to ionizing radiation. The *XRCC1* gene mutation might lead to cancer risk because of impairing the interaction of *XRCC1* with other proteins and stimulating changes in DNA repair activity;[25,26] in other studies, these mutations may induce carcinogenesis in different locations, including head and neck, lung, esophagus, and breast.[27,28]

The association of XRCC1 with Meth abuse indicates the significance of oxidative stress in the addiction cases. The XRCC1 is involved in some repair processes in addition to base excision repair, such as the mismatch, single-strand break, and double-strand breaks. The DNA injury repair may be constructed by ROS, ionizing radiation, and alkylating molecules.[29,30] Common lesions in DNA are repaired by interactions of XRCC1 with other DNA proteins such as DNA ligase and DNA polymerase-*β*.,[31] poly ADP-ribose polymerase; there were more than 300 mutations (SNPs) recorded in the XRCC1 gene.[32]

The genotyping of the RAD18 gene is clarified in Table 3. The Gln/Arg genotype was more frequent in both the case and control groups (93.33%, 62.5%) respectively, while Gln/Gln was more frequent in the cases than control (25%, 6.66%), with significant differences (OR = 8.400, *P* = 0.0118). The Arg/Arg genotype was not observed in the healthy group, with non-significant differences (OR = 12.87, *P* = 0.098). The allele frequency showed a non-significant distribution (OR = 1.060, *P* = 0.835). The association of RAD with addiction was not documented in previous literature, and the association between RAD18 rs373572 and addiction is first recorded in the present study. The role of RAD18 genotyping in methamphetamine abuse may be associated with the mutation or SNPs induced by UV light in the directly related addiction genes, which are repaired by RAD18 such as the role of mutated RAD18 in different diseases.[33-36]

## Conclusion

The association between DNA repair gene system polymorphisms, including XRCC1 and RAD18, with

**Table 3: The RAD18 genotyping distribution in the study groups (MA cases and control)**

| Genotyping RAD18 rs373572 | Cases | Healthy | OR (CI95%) | *P* |
|---|---|---|---|---|
| Gln/Gln | 6 (25%) | 2 (6.66%) | 8.4000 (1.6042 to 43.9835) | 0.0118 |
| Gln/Arg | 15 (62.5%) | 28 (93.33%) | | |
| Arg/Arg | 3 (12.5%) | 0 | 12.8710 (0.6237 to 265.6265) | 0.0981 |
| Gln | 0.55 | 0.53 | 1.0608 (0.6072 to 1.8531) | 0.8357 |
| Arg | 0.45 | 0.46 | | |

methamphetamine addiction in some Iraqi cases, according to our acknowledgment, has been first reported in this study. The results concluded a strong association between XRCC1, RAD18, and methamphetamine addiction. Further investigations should be conducted to gather more details and information about other contributing factors in this relationship.

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## Conflicts of interest
There are no conflicts of interest.

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