Association between prodynorphin gene polymorphisms and opioid dependence susceptibility: a meta-analysis

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Abstract

Background: Prodynorphin (PDYN) gene polymorphisms have been linked with opioid dependence (OD) with conflicting outcomes, the aim of this study is to synthesize the existing evidence of the association between PDYN polymorphisms and OD susceptibility.

Methods: Four databases including PubMed, EMBASE, Web of Science, and Wanfang were retrieved for relevant studies before August, 2018. All identified studies were evaluated using predetermined inclusion and exclusion criteria. Summary odds ratio (OR) and 95% confidence interval (95%CI) were calculated to appraise the association. Statistical analysis was performed using RevMan 5.3 software.

Results: A total of seven case-control studies with 3129 cases and 3289 controls were recruited in the meta-analysis. For rs910080, rs1997794, rs1022563, and rs2235749 polymorphisms of PDYN gene, there were six, four, five, and four studies eventually included, respectively. The findings indicated that rs910080 polymorphism was significantly correlated with OD among Asian population under allelic model (A vs. G, OR = 1.30, 95% CI 1.04–1.62, P = 0.02, FDR = 0.05) and dominant model (AA+AG vs. GG, OR = 1.25, 95% CI 1.04–1.51, P = 0.02, FDR = 0.05). However, rs1022563, rs1997794 and rs2235749 polymorphisms did not appear to associate with OD susceptibility.

Conclusions: There existed a significant association between rs1022563 polymorphism and OD among Asian population. As the included studies were not adequate to guarantee a robust and convincing conclusion, future studies with larger sample size among more ethnicities are recommended.

Keywords: Prodynorphin, Opioid dependence, Polymorphism, Meta-analysis

Background

Opioid dependence (OD), mainly characterized by persistent drug-taking and drug-seeking behavior, is a chronic brain disorder that has seriously negative social and health consequences [1]. A range of environmental factors like severe stressors, family separation, divorce, as well as death in the family have been considered to be associated with OD for a long time [2]. However, researchers observe that only a few individuals that were exposed to drugs will finally develop specific addictions [3]. Thus, there are some factors beyond environmental factors that can contribute to OD.

Actually, early in 1990s, the isolation of genes that encoded opioid peptide precursors had opened an era of molecular and genetic investigations of OD [4]. Family-based studies in different countries and regions revealed the heritability estimates for substance dependence of twins ranged from 30 to 70% [5]. Over the past years, numerous genes and single-nucleotide polymorphisms have been reported to be contributors of OD [6, 7].

Prodynorphin (PDYN), also known as preprodynorphin, is the precursor of neoeendorphins and dynorphins [8]. The human PDYN gene is mapped to chromosome 20p13 [9]. PDYN gene knockout mice presented an increasingly explorative behavior in anxiety tests, which demonstrated the role of prodynorphin-derived peptides [10].

Since Zimprich et al. [11] initially reported the correlation between PDYN gene polymorphisms and OD,
several researchers have subsequently replicated with inconsistent results [12–18]. The lack of convergent findings might ascribe to weak statistical power to ascertain a significant effect, population structure or between-study heterogeneity. Thus, this meta-analysis was conducted to provide a more precise estimation on the relationship of PDYN gene polymorphisms and OD.

Methods
The procedures of the systematic review and meta-analysis were conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [19].

Search strategy of literature
A systematic search of potentially relevant literature was carried out in PubMed, Web of Science, EMBASE, and Wanfang databases. The following search string was employed: (diamorphine OR opioids OR narcotic OR opium OR opiate OR heroin OR “Heroin”[Mesh]) AND (PDYN OR prodynorphin) AND (“Polymorphism, Single Nucleotide” [Mesh] OR SNP OR mutation OR polymorphism OR variant). No restriction on language was imposed. The search period was set from inception to August, 2018.

Inclusion and exclusion criteria
Publications that were included in the present study had to satisfy the following criteria: (1) case-control studies looking into the association of PDYN gene polymorphisms and OD; (2) case participants with confirmed diagnosis of OD; (3) studies with sufficient genotype counts to estimate odds ratio (OR) as well as 95% confidence interval (95%CI). Accordingly, case-case studies, reviews, conference abstracts, as well as animal studies were excluded.

Quality assessment
The quality of eligible studies was evaluated separately by two investigators (CW and MM) according to Newcastle-Ottawa Scale (NOS). A 'star system' was used to judge the quality of each study on the basis of selection, comparability, as well as exposure. The NOS ranges from zero to nine stars. Studies with five or more stars indicated a high quality.

Data extraction
The following data were extracted independently by two investigators (CW and MM) from each qualified article: author, year of publication, country, ethnicity and gender of participants, sample size, and genotype distribution. Once encountering discrepancies, two authors rechecked the original articles until an agreement was achieved.

Results

Statistical analysis
Hardy-Weinberg Equilibrium (HWE) [20] of control subjects was calculated. The strength of association of PDYN gene polymorphisms and OD was presented as OR and 95%CI in allelic model (M vs. W), homozygous model (MM vs. WW), heterozygous model (MW vs. WW), dominant model (MM + MW vs. WW), as well as recessive model (MM vs. MW + WW). To evaluate the heterogeneity across included studies, Q-statistical test alongside I² test were utilized. $P < 0.1$ and $I^2 > 50\%$ suggested the heterogeneity was not significant, and the fixed-effect model could be applied, otherwise the random-effects model should be employed. Subgroup-analysis by ethnicity was performed to test if there was an ethnicity-specific effect. For multiple testing corrections, the Benjamini-Hochberg method was applied to control false discovery rate (FDR) [21]. Forest plots were made by RevMan 5.3 software. Funnel plots were exploited to assess the publication bias.

Main characteristics
The general characteristics of included studies were displayed in Table 1. A total of seven case-control studies [12–18] with 3129 cases and 3289 controls were included in the meta-analysis. Of the included studies, six [12–16, 18] were published in English and one [17] was in Chinese. It should be noted that Clarke et al.’s study [15] published in 2012 consisted of two different cohorts. The sample size of an individual cohort ranged from 119 to 1030 for cases, and 90 to 668 for controls. For rs910080 polymorphism, Hashemi et al.’s study [14] was not in HWE. When it came to rs1022563 polymorphism, three studies [16–18] were out of HWE. As to rs2235749 polymorphism, three studies [14, 16, 17] were not in HWE. According to NOS, each study received five or more stars (Table 2).
Results of meta-analysis
The correlations of PDYN gene polymorphisms (rs910080, rs1997794, rs1022563, and rs2235749) and OD vulnerability were summarized in Table 3.

Data synthesis for rs910080 was available on six studies \([12–17]\) with seven independent cohorts including 3092 cases and 3136 controls. Due to considerable heterogeneity, random-effect model was employed with the exception of heterozygous model. The pooled data indicated a null correlation between rs910080 polymorphism and OD susceptibility under five genetic models. Subgroup analysis stratified by ethnicity suggested a significant association between rs910080 polymorphism and OD across Asian population in allelic model (A vs. G, \(\text{OR} = 1.30, 95\% \text{CI} 1.04–1.62, P = 0.02, \text{FDR} = 0.05\)) and dominant models (AA+AG vs. GG, OR = 1.25, 95% CI 1.04–1.51, \(P = 0.02, \text{FDR} = 0.05\)). While no correlation was detected under other models.

As for rs1997794, four studies \([12, 13, 15, 18]\) with five cohorts involving 2942 cases and 2605 controls went into final data synthesis. Due to heterogeneity between studies was significant, the random-effect model was applied. The result indicated that rs1997794 polymorphism did not significantly associate with OD in five genetic models. Subgroup analysis stratified by ethnicity also yielded a null association.

In respect of rs1022563, five studies with six cohorts containing 2378 cases and 2400 controls satisfied the eventual data combination. Because of huge heterogeneity, random-effect model was utilized. Rs1022563 polymorphism appeared to have no significant correlation with OD in five genetic models. Subgroup analysis by ethnicity as well indicated a non-significant association.

When it came to rs2235749, four studies \([12, 14, 16, 17]\) with 1273 cases and 1285 controls carried out among Asian population were analyzed. OR and 95%CI were estimated by the random-effect approach due to significant heterogeneity. The pooled results indicated a null association between rs2235749 polymorphism and OD susceptibility.

Sensitivity analysis and publication bias
After studies that deviated from HWE were excluded, the corresponding OR did not reverse. Consequently, they were not removed from our meta-analysis. Sensitivity analysis confirmed the stability of the outcomes, where exclusion of individual dataset did not significantly change the overall effects. Furthermore, visual inspection of funnel plots did not identify substantial asymmetry for all the four loci under any genetic model.

Discussion
OD affects the lives of millions of people worldwide and imposes a heavy burden on society \([22]\). Hence, seeking new strategies to prevent and treat OD is necessary. Investigation into the etiology of OD has focused on multiple areas. Genetic factors and environmental factors are both considered as contributors to OD. As a genetic...
### Table 1 Characteristics of included studies

| Study                | Year  | Country | Ethnicity | Gender | Sample size | OD (%)          | Control (%)       | HWE (P-value) |
|---------------------|-------|---------|-----------|--------|-------------|-----------------|------------------|---------------|
| Rs910080 (A/G)      |       |         |           |        |             |                 |                  |               |
| Yuanyuan et al. 2018 | China | Asian   | Both      |        | 541/566     | 14.3            | 85.7             | 22.2          |
| Nagaya et al. 2018  | Malaysia | Asian | Male      |        | 459/543     | 16.1            | 83.9             | 34.9          |
| Hashemi et al. 2018 | Iran  | Asian   | Both      |        | 216/219     | 43.1            | 56.9             | 19.9          |
| Clarke et al. I 2012 | USA   | Caucasian | Both    |        | 1030/644    | 25.0            | 75.0             | 6.0           |
| Clarke et al. II 2012 | USA   | African  | Both     |        | 330/663     | 46.8            | 53.2             | 23.0          |
| Wei et al. 2011     | China | Asian   | Both      |        | 304/301     | 21.9            | 78.1             | 4.9           |
| Jia et al. 2010     | China | Asian   | Both      |        | 212/200     | 23.6            | 76.4             | 3.3           |
| Rs1997794(G/A)      |       |         |           |        |             |                 |                  |               |
| Yuanyuan et al. 2018 | China | Asian   | Both      |        | 541/566     | 16.4            | 83.6             | 2.0           |
| Nagaya et al. 2018  | Malaysia | Asian | Male      |        | 459/543     | 48.2            | 51.8             | 22.7          |
| Clarke et al. I 2012 | USA   | Caucasian | Both    |        | 1037/652    | 35.0            | 65.0             | 11.7          |
| Clarke et al. II 2012 | USA   | African  | Both     |        | 336/668     | 28.3            | 71.7             | 8.6           |
| Clarke et al. 2009  | China | Asian   | Female    |        | 119/176     | 19.3            | 80.7             | 3.4           |
| Rs1022563(C/T)      |       |         |           |        |             |                 |                  |               |
| Nagaya et al. 2018  | Malaysia | Asian | Male      |        | 459/543     | 17.9            | 82.1             | 3.9           |
| Clarke et al. I 2012 | USA   | Caucasian | Both    |        | 948/644     | 13.5            | 86.5             | 14.8          |
| Clarke et al. II 2012 | USA   | African  | Both     |        | 336/660     | 21.6            | 78.4             | 5.1           |
| Wei et al. 2011     | China | Asian   | Both      |        | 304/300     | 16.3            | 83.7             | 3.9           |
| Jia et al. 2010     | China | Asian   | Both      |        | 212/90      | 19.1            | 80.9             | 4.7           |
| Clarke et al. 2009  | China | Asian   | Female    |        | 119/163     | 17.6            | 82.4             | 5.9           |
| Rs2235749(T/C)      |       |         |           |        |             |                 |                  |               |
| Yuanyuan et al. 2018 | China | Asian   | Both      |        | 541/566     | 15.2            | 84.8             | 2.4           |
| Hashemi et al. 2018 | Iran  | Asian   | Both      |        | 216/219     | 38.2            | 61.8             | 7.9           |
| Wei et al. 2011     | China | Asian   | Both      |        | 304/300     | 21.9            | 78.1             | 5.3           |
| Jia et al. 2010     | China | Asian   | Both      |        | 212/200     | 18.6            | 81.4             | 4.7           |

OD opioid dependence, M mutant allele, W wild allele, HWE Hardy-Weinberg Equilibrium

### Table 2 Quality of included studies

| Item/Study               | Nagaya et al. 2018 [13] | Hashemi et al. 2018 [14] | Clarke et al. 2012 [15] | Wei et al. 2011 [16] | Jia et al. 2010 [17] | Clarke et al. 2009 [18] | Yuanyuan et al. 2018 [12] |
|-------------------------|------------------------|--------------------------|-------------------------|----------------------|------------------------|--------------------------|-------------------------|
| Adequate definition of cases | ★                     | ★                       | ★                       | ★                    | ★                     | ★                        | ★                       |
| Representativeness of cases | ☆                    | ☆                       | ☆                       | ☆                    | ☆                     | ☆                        | ☆                       |
| Selection of control subjects | ☆                   | ☆                       | ☆                       | ☆                    | ☆                     | ☆                        | ☆                       |
| Definition of control subjects | ★                   | ★                       | ★                       | ★                    | ★                     | ★                        | ★                       |
| Control for important factor or additional factor | ★☆                  | ★☆                       | ★☆                      | ★☆                   | ☆☆                    | ☆☆                       | ☆☆                      |
| Exposure assessment | ★                      | ★                       | ★                       | ★                    | ★                     | ★                        | ★                       |
| Same method of ascertainment for all subjects | ★                    | ★                       | ★                       | ★                    | ★                     | ★                        | ★                       |
| Non-response rate | ★                      | ★                       | ★                       | ★                    | ★                     | ★                        | ★                       |

★, a star given; ☆, a star not given
Table 3 Association between prodynorphin gene polymorphisms and opioid dependence

| Genetic model | Association | FDR | Effect model | Heterogeneity |
|---------------|-------------|-----|--------------|---------------|
|               | OR          | 95% CI | P-value | I² (%) | P-value |
| Rs910080      |             |       |         |       |         |
| Overall       |             |       |         |       |         |
| A vs. G       | 1.16        | 0.94–1.41 | 0.16   | 0.28   | R       | 81      | <0.01  |
| AA vs. GG     | 1.53        | 0.83–2.84 | 0.17   | 0.28   | R       | 84      | <0.01  |
| AG vs. GG     | 1.06        | 0.95–1.19 | 0.32   | 0.32   | F       | 32      | 0.18   |
| AA + AG vs. GG| 1.12        | 0.93–1.34 | 0.24   | 0.30   | R       | 63      | 0.01   |
| AA vs. AG + GG| 1.52        | 0.85–2.70 | 0.16   | 0.28   | R       | 83      | <0.01  |
| Asian         |             |       |         |       |         |
| A vs. G       | 1.30        | 1.04–1.62 | 0.02   | 0.05   | R       | 70      | 0.01   |
| AA vs. GG     | 2.21        | 0.96–5.09 | 0.06   | 0.07   | R       | 76      | <0.01  |
| AG vs. GG     | 1.17        | 1.01–1.36 | 0.04   | 0.07   | F       | 7       | 0.37   |
| AA + AG vs. GG| 1.25        | 1.04–1.51 | 0.02   | 0.05   | F       | 40      | 0.15   |
| AA vs. AG + GG| 2.13        | 0.94–4.83 | 0.07   | 0.07   | R       | 76      | <0.01  |
| Rs1997794     |             |       |         |       |         |
| Overall       |             |       |         |       |         |
| G vs. A       | 1.02        | 0.85–1.22 | 0.87   | 0.94   | R       | 72      | 0.01   |
| GG vs. AA     | 0.98        | 0.68–1.43 | 0.94   | 0.94   | R       | 58      | 0.05   |
| GA vs. AA     | 1.06        | 0.94–1.20 | 0.36   | 0.94   | F       | 20      | 0.29   |
| GG + GA vs. AA| 1.07        | 0.89–1.30 | 0.46   | 0.94   | R       | 55      | 0.06   |
| GG vs. GA + AA| 0.95        | 0.69–1.31 | 0.77   | 0.94   | R       | 52      | 0.08   |
| Asian         |             |       |         |       |         |
| G vs. A       | 1.06        | 0.76–1.46 | 0.74   | 0.74   | R       | 78      | 0.01   |
| GG vs. AA     | 0.90        | 0.65–1.23 | 0.50   | 0.63   | F       | 49      | 0.14   |
| GA vs. AA     | 1.10        | 0.91–1.32 | 0.34   | 0.63   | R       | 51      | 0.13   |
| GG + GA vs. AA| 1.16        | 0.82–1.63 | 0.41   | 0.63   | R       | 68      | 0.04   |
| GG vs. GA + AA| 0.89        | 0.68–1.17 | 0.41   | 0.63   | F       | 39      | 0.20   |
| Rs1022563     |             |       |         |       |         |
| Overall       |             |       |         |       |         |
| C vs. T       | 0.85        | 0.62–1.17 | 0.32   | 0.40   | R       | 88      | <0.01  |
| CC vs. TT     | 1.29        | 0.57–2.89 | 0.54   | 0.54   | R       | 75      | <0.01  |
| CT vs. TT     | 0.66        | 0.44–1.00 | 0.05   | 0.25   | R       | 89      | <0.01  |
| CC + CT vs. TT| 0.71        | 0.47–1.07 | 0.11   | 0.28   | R       | 89      | <0.01  |
| CC vs. CT + TT| 1.45        | 0.79–2.65 | 0.23   | 0.38   | R       | 57      | 0.04   |
| Asian         |             |       |         |       |         |
| C vs. T       | 0.80        | 0.47–1.37 | 0.42   | 0.50   | R       | 91      | <0.01  |
| CC vs. TT     | 1.71        | 0.36–8.07 | 0.50   | 0.50   | R       | 83      | <0.01  |
| CT vs. TT     | 0.54        | 0.26–1.13 | 0.10   | 0.35   | R       | 92      | <0.01  |
| CC + CT vs. TT| 0.60        | 0.29–1.25 | 0.17   | 0.35   | R       | 93      | <0.01  |
| CC vs. CT + TT| 2.04        | 0.66–6.27 | 0.21   | 0.35   | R       | 69      | 0.02   |
| Rs2235749     |             |       |         |       |         |
| T vs. C       | 1.04        | 0.74–1.46 | 0.83   | 0.83   | R       | 83      | <0.01  |
| TT vs. CC     | 1.34        | 0.42–4.25 | 0.62   | 0.83   | R       | 85      | <0.01  |
| TC vs. CC     | 1.09        | 0.92–1.30 | 0.32   | 0.83   | F       | 2       | 0.38   |
factor, PDYN gene has received more and more attention [23].

Several studies across different populations have been performed on the association of PDYN gene polymorphisms and OD vulnerability, while it is quite regrettable that no convincing evidence is available. This inconsistency could be interpreted by several factors, including severity of diagnosis, limited sample size, inadequate power, ethnic heterogeneity, population stratification, as well as large phenotype range. The present study, with pooled data from Asian (Malaysia, Iran, and China), Caucasian (European American), and African (African American) populations, indicated that rs910080 polymorphism was significantly correlated with OD among Asian population. However, rs1022563, rs1997794 and rs2235749 polymorphisms did not appear to associate with OD susceptibility.

PDYN gene codes ligand of opioid receptor. Functional variant in PDYN gene hampers the downstream signaling pathway and affects the overall biological networks, thus implicating in the pathophysiology of OD. Dynorphins are a cluster of posttranslational products of PDYN gene. They bind to all three types of opiate receptors, but exhibit considerable affinity to kappa opioid receptor [24]. Products of PDYN gene have been reported to inhibit neurotransmission and take part in mood regulation, stress responses [25], reward [26], and motor functions. Yuferov et al. [27] reported the mRNA of PDYN was rich in brain areas that were associated with drug-taking and drug-withdrawal. Wang et al. [28] found that acute intermittent morphine administration could raise PDYN and kappa opioid receptor mRNA level in the brains of rat models. Soleczi et al. [29] observed that chronic exposure to heroin could lead to an increased PDYN mRNA in the nucleus accumbens shell and core of rats. All this evidence suggested that PDYN gene played a crucial role in the occurrence and development of OD.

Heterogeneity is a crucial issue when elucidating the outcomes of a meta-analysis. We attempted to minimize this issue by developing strict criteria for study inclusion, information extraction, as well as data analysis. Considerable heterogeneity between studies still existed despite subgroup analysis was stratified. Several potential reasons might give rise to the presence of heterogeneity. For demographics, age of individual study ranges greatly. For gender, although majority studies are mixed genders, some are focusing on only men or women. For genetic background, included studies are multi-national and many ethnic groups were represented within them. For environmental factor, participants from both developing countries and developed countries were incorporated into the present study.

### Study limitations

Although the current study has collected available data on the association between PDYN gene polymorphisms and OD predisposition, several limitations could not be neglected. First, the present outcomes were based on unadjusted estimates. Because raw data for confounding factors like age [30], gender [15], socioeconomic status [31] of the participants were unavailable, we could not perform the corresponding stratification analysis. Second, the number of participants of each included study was limited, thus lacking enough statistic power to guarantee the association. Third, we only investigated the role of four independent polymorphisms in PDYN gene. However, finding susceptibility polymorphisms and genes was a difficult task owing to the intricate mode of inheritance and multiple polymorphisms in various genes contributing to increased disease risk. OD, without exception, is a multifactor disorder determined by the synthetic effects and interactions of a series of polymorphisms, genes, and environmental factors. These interactions might conceal or enlarge the function of the included polymorphisms. Lastly, only seven articles from four databanks were searched for this study, potentially relevant studies published in other databanks might be missed.

### Conclusion

Taken together, the current study indicated the rs910080 polymorphism was significantly correlated with OD among Asian population. However, rs1022563, rs1997794 and rs2235749 polymorphisms did not appear to associate with OD susceptibility. Due to limitations demonstrated above, the associations between PDYN gene polymorphisms and the risk of OD could not be entirely concluded. As the included studies were not adequate to guarantee a robust and convincing conclusion, studies with larger sample size among more populations are recommended.

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**Table 3** Association between prodynorphin gene polymorphisms and opioid dependence (Continued)

| Genetic model          | Association OR (95% CI) | P-value | FDR  | Effect model | Heterogeneity | I² (%) | P-value |
|------------------------|------------------------|---------|------|--------------|---------------|--------|---------|
| TT + TC vs. CC         | 1.05 (0.78–1.41)       | 0.75    | 0.83 | R            | 64            | 0.02   |         |
| TT vs. TC + CC         | 1.34 (0.43–4.16)       | 0.61    | 0.83 | R            | 85            | <0.01  |         |

**Note:** SNP single nucleotide polymorphism, OR odds ratio, CI confidence interval, F fix-effect model, R random-effect model, FDR adjusted P-value for false discovery rate.
Abbreviations
Cls: Confidence interval; HWE: Hardy-Weinberg equilibrium; NOS: Newcastle-Ottawa Scale; OD: Opioid dependence; ORs: Odds ratios; PDYN: prodynorphin

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Authors’ contributions
RL designed the study. CW and MM were responsible for collection of data and performing the statistical analysis and manuscript preparation. CW and WL were responsible for checking the data. All authors were responsible for drafting the manuscript, read and approved the final version.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author Ru-qin Luo, ruqinluo@126.com.

Ethics approval and consent to participate
from the corresponding author Ru-qin Luo, ruqinluo@126.com.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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