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Association of variants of prodynorphin promoter 68-bp repeats in Caucasians with opioid dependence diagnosis: Effect on age trajectory of heroin use

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Abstract

The dynorphin/kappa opioid receptor (Dyn/KOR) system is involved in reward processing and dysphoria/anhedonia. Exposure to mu-opioid receptor agonists such as heroin increases expression of the prodynorphin gene (*PDYN*) in the brain. In this study in a Caucasian cohort, we examined the association of the functional *PDYN* 68-bp repeat polymorphism with opioid use disorders. In this case-control study, 554 subjects with Caucasian ancestry (142 healthy controls, 153 opioid-exposed, but never opioid dependent, NOD and 259 with an opioid dependence diagnosis, OD) were examined for association of the *PDYN* 68-bp repeats with the diagnosis of opioid dependence (DSM-IV criteria), with a dimensional measure of heroin exposure (KMSK scale), and age trajectory parameters of heroin use (age of heroin first use, and age of onset of heaviest use). The *PDYN* 68-bp repeat genotype (classified as: “short-short” [SS], “long-long” [LL], and “short-long” [SL], based on the number of repeats) was not associated with categorical opioid dependence diagnoses. However, the LL genotype was associated with later age of first heroin use than the SS+SL genotype (19 versus 18 years; $p<0.01$). This was also confirmed by a significant positive correlation between the number of repeats and the age of first use of heroin, in volunteers

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Authors’ Contribution

MJK, JMvR and WvdB: original study concept; MR: sample preparation, data collection; JO: examined all statistical analyses; PB: ascertained study subjects. VY was in charge of genotyping; ERB carried out dimensional phenotypic analyses. All co-authors contributed to the content of the manuscript, provided critical reviews, and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Declarations of interest: none

with OD (Spearman r=0.16; p=0.01). This suggests that the functional *PDYN* 68-bp repeat genotype is associated with the age of first use of heroin in Caucasians diagnosed with opioid dependence.

Keywords

Opioid dependence; non-dependent opioid use; prodynorphin polymorphisms; VNTR; KMSK

Introduction

The kappa opioid receptor (KOR) and its cognate neuropeptides, the dynorphins, are involved in rewarding and aversive effects of drugs of abuse, as well as in dysphoria, anhedonia, and anxiety-like effects [1, 2]. Stress exposure and impulsivity can increase the risk of drug abuse, and reinstate extinguished drug-seeking in rodents [3].

In animal models, dynorphin A(1–17) and synthetic KOR agonists decrease basal and drug-induced increases in dopamine levels in nigrostriatal and mesolimbic–mesocortical systems [4, 5].

Previous human genetic studies revealed that gene polymorphisms of *PDYN* are associated with memory, emotional processing, drug addiction, and alcoholism [6, 7]. A 68-bp variable number tandem repeat (VNTR) located 1250 bp upstream of the transcription start site of the gene, was described in the 5' promoter region of *PDYN* [8]. This 68-bp repeat polymorphism (rs35286251) can be present in 1–5 copies, and each copy contains an activator protein-1 (AP-1) transcription complex binding site for c-Fos/c-Jun dimers [8, 9].

In vitro, *PDYN* expression studies using various constructs and cell lines reported divergent effects of the repeats [8–10]. The first *in vitro* reporter gene expression study in rodent NG108–15 cells showed that 1–2 copies of the repeat have lower phorbol-induced increases in expression of the reporter gene than 3–4 copies [8]. However, a more recent study, using an expression construct with a longer *PDYN* promoter sequence, and human neuroblastoma SK-NSH cells, showed an inverse relationship between number of repeats and promoter activity [9]. Thus, constructs with 1–2 repeats had higher caffeine-induced expression of the reporter gene than constructs with 3–4 repeats. There are different designations of the *PDYN* 68-bp repeat genotype [8, 9]. We have used a genotype designation based on the number of repeats; thus 1–2 copies are designated as SS (short/short) and 3–4 copies as LL (long/long) genotype [9, 11, 12].

The 68-bp repeat polymorphisms have been examined in several association studies with inconsistent results. Some studies showed that 3–4 repeats constitute a risk factor for SUD diagnoses, including cocaine/alcohol co-dependence and heroin dependence [11, 13–16]. Other studies reported no association with SUD diagnoses [8, 12, 17, 18].

In this study we examine for the first time whether there is an association of variants of the *PDYN* 68-bp tandem repeats with opioid dependence (OD) diagnoses in Caucasian volunteers from the Netherlands [19]. Different genetic mechanisms may underlie specific

stages of addictive diseases, including initiation and progression to maximal use [3]. Studies also show that age trajectory parameters of drug use (e.g., age of first use, or age of heaviest use) can be associated with specific genetic polymorphisms [20]. Therefore, we also examined whether age trajectory parameters of heroin use differed by genotype in these volunteers with OD diagnoses.

Materials and Methods

Subjects

This was a case-control study, with volunteer groups recruited in the Netherlands (n=795, 32% females, Table 1), as previously described [19, 21, 22].

Three groups were compared here:

1. Healthy controls (HC) without a history of any illicit opioid use and with no history of alcohol or drug dependence, according to DSM-IV criteria.
2. Volunteers self-exposed illicit opioids, but who did not have a DSM-IV lifetime opioid dependence diagnosis (NOD). These volunteers reported a lifetime history use of heroin or other non-prescribed opioids without ever reaching dependence.
3. Volunteers with opioid dependence diagnosis (OD) (DSM-IV criteria). They had been in methadone maintenance treatment (MMT), or in methadone maintenance with adjunctive heroin-assisted treatment (HAT) for at least 5 years [22].

The Central Committee on Research Involving Human Subjects in the Netherlands (protocol number P04.0156C) approved the study of heroin-assisted and methadone maintenance treatments, and the genetic study. The genetic study was also approved by the Rockefeller University Institutional Review Board. Subjects signed an informed consent for the study.

Diagnoses and Measurements

Diagnoses: The SUD section of the computerized structured Composite International Diagnostic Interview (CIDI Auto 2.0) was used to derive DSM-IV SUD diagnoses [21]. In this study, we focused on opioid dependence (OD) diagnoses.

Measurements: KMSK scales for maximal exposure to specific drugs (measuring alcohol, tobacco, cocaine, and heroin) [23, 24]. The KMSK scales characterize the period in a volunteer's life when use of a substance is the heaviest. Each KMSK scale measures maximal exposure to a specific substance, on an ordinal integer scale. A KMSK score=0 indicates that the participant had never used the substance of interest, and this ordinal score increases up to a maximum (13 for alcohol, tobacco, and heroin, and 16 for cocaine). KMSK scales have also been used as a dimensional phenotype in genetic association studies [12, 25]. A recent study re-validated KMSK scales for alcohol, cocaine and heroin with the respective DSM-IV dependence diagnosis [24], including determination of new optimal "cut-point" KMSK scores. The KMSK questionnaires for each substance also collected the age of first use and the age of onset of heaviest use (in whole years) examined here.

Assessment of Percentage of European Ancestry

Ethnicity was initially assigned based on self-reported family origin data, with 628 self-identified Caucasian volunteers [19]. Based on 155 ancestry informative markers (AIMs), the fraction of genetic affiliation of the individual was calculated using Structure v2.2 [26]. Each volunteer was "anchored" against 1051 samples from 51 worldwide populations in the Human Genome Diversity Cell Line Panel [27]. For the current study, the inclusion criteria was set at ≥70% European ancestry contribution estimate, to minimize population stratification, as described previously [19].

Genotyping of the 68-bp tandem repeat region in study subjects

Genomic DNA was extracted from blood cells using a salt-precipitation method. Genotyping of the 68-bp tandem repeat region was performed as described previously [11, 12]. In brief, 100 ng of genomic DNA was amplified by PCR using forward (5'- CTG TGT ATG GAG AGG CTG AGT −3') and reverse (5'- AGG CGG TTA GGT AGA GTT GTC −3') primers. PCR products were electrophoresed on a 2.0% agarose gel. *PDYN* genotypes were determined according to the size and number of PCR DNA fragments. Genotypes were then grouped as short/short "SS" (1,1; 1,2; 2,2 copies), short/long "SL" (1,3; 1,4; 2,3; 2,4 copies), and long/long "LL" (3,3; 3,4; 4,4 copies) repeat alleles.

Statistical analyses

Analyses were carried out with Graphpad Prism (V.7) and Plink v1.9 [28]. Demographic variables and KMSK scores were analyzed with t-test or Mann-Whitney tests, or with Fisher's test, as appropriate. Contingency analysis for OD diagnoses and *PDYN* genotype were carried out with Fisher's exact test. Age trajectory variables in OD volunteers (in whole years) were analyzed separately with Kaplan-Meier survival curves, using the Gehan-Breslow-Wilcoxon test, as we did not have an *a priori* hypothesis on whether curves would differ across early and late time points. In a follow-up examination in OD volunteers, the genotypic number of *PDYN* repeats (i.e., ranging from 2 to 8 repeats in this cohort) was examined for its Spearman correlation to age of first heroin use. For example, a volunteer with the homozygous 1/1 repeats would be counted as 2, and a volunteer with 4/4 repeats would be counted as 8 repeats. The alpha level of significance for the results was $p \leq 0.05$.

Missing data: If a measure was missing from a comparison, the data set for the volunteer was eliminated from analysis.

Results

Sample characteristics and demographics

There were 795 volunteers initially. After exclusion of those with >70% of Caucasian AIMS markers or poor DNA quality, 554 volunteers (382 males and 172 females) were included for analysis (Table 1). The mean age at ascertainment of the non-dependent controls (i.e., HC+NOD; see below) and the OD group at the time of ascertainment were significantly different: 39.5 ± 9.7 and 43.4 ± 10.4 years, respectively (Kruskal-Wallis test, $p<0.001$).

KMSK scores are summarized in Table 2. It may be noted that 57% of the healthy control subjects (HC) reported considerable alcohol exposure, but very low exposure to heroin or cocaine. Also, a substantial proportion of non-dependent opioid users (NOD) reported considerable cocaine exposure (i.e., 48%). The KMSK scores also indicated that 89% of the OD volunteers had high exposure to cocaine, and 79% also had high exposure to alcohol.

Contingency Analysis of *PDYN* 68-bp tandem repeat genotype with categorical diagnoses of opioid dependence:

Since the initial genetic association test (Fisher's exact test) did not show significant differences between HC and NOD, neither allelic (p=0.41) nor genotypic (p=0.13), they were combined into a "non-dependent" control group, to increase statistical power. Frequencies for the minor allele S (1–2 repeats) in the combined non-dependent control group (n=293), and in the OD group (n=261), were similar (i.e., 33.3% and 33.7%, respectively; Table 3). Although there was significant deviation in distribution of *PDYN* 68-bp genotypes from Hardy–Weinberg equilibrium (HWE) in the healthy control group alone (p=0.02, Pearson test), there was no significant deviation in distribution of the genotypes from HWE in the combined non-dependent control group (i.e., HC+NOD; p=0.21, Pearson test). The distribution frequencies of the PDYN 68-bp genotypes in MMT, HAT, and combined OD group (i.e., MMT+HAT) did not deviate from HWE (p-values 0.14, 0.91, and 0.34, respectively).

Fisher's exact test conducted for an L-recessive (i.e., LL versus SS+SL) or an L-dominant model (i.e., SS versus SL+LL) did not show significant association of the *PDYN* 68-bp repeat genotype with categorical OD dependence diagnosis (Table 3). A Fisher's exact test for the frequency of male and female volunteers with OD across genotype (i.e., SS+SL versus LL) was not significant in recessive or dominant models (not shown).

A recent study in this cohort also found that several other *PDYN* SNPs, including rs1997794 in the 5'-UTR, and rs2235749 in 3'-UTR, were not associated with categorical opioid dependence diagnoses [29]. Also, we found that the rs1997794 or rs2235749 SNPs were not associated with age of first use of heroin in these OD volunteers (not shown).

KMSK scores for heroin, cocaine, alcohol and tobacco across genotype, in OD volunteers

KMSK exposure scores were examined across genotype in the volunteers with OD (Table 4). When examined with Mann-Whitney tests, KMSK scores for heroin, cocaine, alcohol, and tobacco did not differ significantly by genotype (i.e., SS+SL versus LL).

Age of first use of heroin, in volunteers with OD, across genotype

Age of first use of heroin did not differ by gender, or whether a volunteer was in MMT or HAT (not shown). We examined the age of first heroin use with a survival analysis, across genotype (LL versus SS+SL), in volunteers with OD (i.e., MMT+HAT). This analysis indicated that OD volunteers with the LL genotype had a later age of heroin first use, compared with those with the SS+SL genotype (median 19 versus 18 years; Gehan-Breslow-Wilcoxon test p<0.01) (Fig.1A).

A Mann-Whitney test (U=6,407; p<0.01) detected a significantly later age of heroin first use in volunteers with OD with the LL genotype (mean=20.5 years; 95%CL: 19.5–21.6) compared to the SS+SL genotype (mean=18.9 years; 95%CL: 18.0–19.7) (Fig. 2). We also found that in the OD volunteers, the number of *PDYN* repeats (genotypic) was positively correlated with age of first use of heroin (Spearman r=0.16; p=0.01).

Age of onset of heaviest use of heroin, in volunteers with OD, across genotype.

We next examined the age of onset of heaviest use of heroin across genotypes. We again found that the volunteers with OD with the LL genotype had a later age of onset of heaviest use, compared to those with the SS+SL genotype (median 23 versus 20 years; Gehan-Breslow-Wilcoxon test p<0.04) (Fig. 1B).

There were no gender differences in the age of onset of heaviest use of heroin (not shown). However, we found that the age of onset of heaviest use of heroin was earlier in volunteers in MMT than in HAT (median 20 versus 24 years; Gehan-Breslow-Wilcoxon test p=0.0002). We therefore carried out a stratification of the above *PDYN* genotype effect in MMT (n=129) versus HAT (n=74) volunteers. After MMT and HAT stratification, there was no statistical significance of *PDYN* genotype, possibly because of reduced statistical power due to the lower “n” (not shown)”.

Discussion

We found that a functional *PDYN* polymorphism in a Caucasian sample with OD diagnosis was associated with the age trajectory of the use of heroin, principally the age of first use. Intriguingly, this *PDYN* polymorphism was not significant in the contingency analysis for the categorical OD diagnosis. The latter result replicates some earlier studies [8, 17, 18], showing lack of association of the *PDYN* VNTR polymorphism with the diagnosis of OD in Caucasians. Also, heroin KMSK scores, which are dimensional measures of maximal exposure to the heroin, did not differ by *PDYN* genotype in these volunteers with OD.

Investigation of the genetic basis for the initiation of drug use, and also of the onset of heaviest use (or onset of a diagnosed SUD), has received recent attention [20, 30]. For example, some studies have identified genetic markers of early age of first use of alcohol and other drugs [31, 32]. To our knowledge, this is the first report of a functional polymorphism of the *PDYN* gene that is associated with the age trajectory of heroin use in persons diagnosed with OD. We have also recently reported that the *PDYN* 68-bp “LL” genotype is associated with later age of first use of cannabis in African-American males [12]. This suggests that this functional *PDYN* polymorphism may affect age trajectory for more than one type of drug of abuse.

Endogenous activation of KOR by dynorphins can result in aversion/dysphoria and anhedonia, and can also underlie relapse-like effects and escalation of drug self-exposure in animal models [33, 34]. Furthermore, the KOR / dynorphin system modulates basic behavioral functions, including impulsivity, in animal models, and this could affect specific age trajectory phenotypes in OD (e.g., age of first heroin use) [35, 36].

Limitations and methodological considerations:

As is common in the SUD field, volunteers in the OD group also had considerable exposure to other substances, as characterized with KMSK scales. However, there were no significant differences in KMSK scores for other drugs (tobacco/nicotine, alcohol, or cocaine) in the OD volunteers, based on LL versus SL+SS genotypes. Therefore, it is unlikely that the *PDYN* genotype effect on age trajectory of heroin use was due primarily to different exposure levels to these other substances.

In the overall OD group (i.e., MMT+HAT), we also found that age of onset of heaviest use of heroin was later in the LL versus the SL+SS genotype. However, the age of onset of heaviest use of heroin differed in the MMT versus the HAT group. The underlying clinical study could not use a randomized assignment to MMT or HAT, for practical and ethical reasons [37]. Therefore it is not surprising that some endophenotypic differences of this kind were observed in MMT versus HAT. Follow-up studies with a larger number of volunteers would therefore be needed to confirm the association of *PDYN* VNTR genotype with age of onset of heaviest use of heroin.

There are a number of naturally-occurring haplotypes in the 3 kb cis-regulatory region of *PDYN*, including the 68 bp repeats, and they may have differential impact on gene expression across cell types and brain regions [10]. The interaction of genetic variants of *PDYN* could also differ across human populations. Further studies with greater statistical power are warranted to corroborate the results, and to assess the clinical significance of these findings.

Conclusions: This study reports the first genetic association of the functional repeat genotype in the promoter region of the *PDYN* gene with the age trajectory of heroin use in Caucasians with OD diagnoses. Our results suggest that this functional *PDYN* polymorphism may be associated with age trajectory of heroin use, and the SS+SL genotype may be a risk factor for relatively early-onset opioid use disorders.

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Abbreviations:

HAT	heroin-assisted treatment, added to methadone maintenance
HC	Healthy controls
KMSK	Kreek-McHugh-Schluger-Kellogg scale of maximum exposure to specific drugs
L	“Long” 68-base PDYN pair repeats: 3 or 4 copies
S	“Short” 68-base PDYN pair repeats: 1 or 2 copies

MMT	methadone maintenance treatment
NOD	Non-dependent opioid users
OD	Opioid dependence diagnosis (by DSM-IV criteria)
SUD	substance use disorders
PDYN	Prodynorphin gene
VNTR	variable number tandem repeats

REFERENCES

[1]. Butelman ER, Yuferov V, Kreek MJ, kappa-opioid receptor/dynorphin system: genetic and pharmacotherapeutic implications for addiction, Trends in neurosciences 35 (2012) 587–596. [PubMed: 22709632]

[2]. Chavkin C, Koob GF, Dynorphin, Dysphoria, and Dependence: the Stress of Addiction, Neuropsychopharmacol. 41 (2016) 373–374.

[3]. Kreek MJ, Nielsen DA, Butelman ER, LaForge KS, Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction, Nature neuroscience 8 (2005) 1450–1457. [PubMed: 16251987]

[4]. Di Chiara G, Imperato A, Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats., The Journal of pharmacology and experimental therapeutics 244 (1988) 1067–1080. [PubMed: 2855239]

[5]. Zhang Y, Butelman ER, Schlussman SD, Ho A, Kreek MJ, Effect of the endogenous kappa opioid agonist dynorphin A(1–17) on cocaine-evoked increases in striatal dopamine levels and cocaine-induced place preference in C57BL/6J mice, Psychopharmacology 172 (2004) 422–429. [PubMed: 14712335]

[6]. Kreek MJ, Bart G, Lilly C, LaForge KS, Nielsen DA, Pharmacogenetics and human molecular genetics of opiate and cocaine addictions and their treatments, Pharmacological reviews 57 (2005) 1–26. [PubMed: 15734726]

[7]. Levran O, Yuferov V, Kreek MJ, The genetics of the opioid system and specific drug addictions, Human genetics 131 (2012) 823–842. [PubMed: 22547174]

[8]. Zimprich A, Kraus J, Woltje M, Mayer P, Rauch E, Hollt V, An allelic variation in the human prodynorphin gene promoter alters stimulus-induced expression, J. Neurochem 74 (2000) 472–477. [PubMed: 10646497]

[9]. Rouault M, Nielsen DA, Ho A, Kreek MJ, Yuferov V, Cell-specific effects of variants of the 68-base pair tandem repeat on prodynorphin gene promoter activity, Addict. Biol 16 (2011) 334–346. [PubMed: 20731629]

[10]. Babbitt CC, Silverman JS, Haygood R, Reininga JM, Rockman MV, Wray GA, Multiple Functional Variants in cis Modulate PDYN Expression, Mol. Biol. Evol 27 (2010) 465–479. [PubMed: 19910384]

[11]. Williams TJ, LaForge KS, Gordon D, Bart G, Kellogg S, Ott J, Kreek MJ, Prodynorphin gene promoter repeat associated with cocaine/alcohol codependence, Addict. Biol 12 (2007) 496–502. [PubMed: 17559549]

[12]. Yuferov V, Butelman ER, Kreek MJ, Gender-specific association of functional prodynorphin 68 bp repeats with cannabis exposure in an African American cohort, Neuropsychiatr. Dis. Treat 14 (2018) 1025–1034. [PubMed: 29713172]

[13]. Dahl JP, Weller AE, Kampman KM, Oslin DW, Lohoff FW, Ferraro TN, O'Brien CP, Berrettini WH, Confirmation of the association between a polymorphism in the promoter region of the prodynorphin gene and cocaine dependence, Am. J. Med. Genet. B Neuropsychiatr. Genet 139B (2005) 106–108. [PubMed: 16184603]

[14]. Wei SG, Zhu YS, Lai JH, Xue HX, Chai ZQ, Li SB, Association between heroin dependence and prodynorphin gene polymorphisms, Brain research bulletin 85 (2011) 238–242. [PubMed: 21382455]

[15]. Yuanyuan J, Rui S, Hua T, Jingjing C, Cuola D, Yuhui S, Shuguang W, Genetic association analyses and meta-analysis of Dynorphin-Kappa Opioid system potential functional variants with heroin dependence, Neuroscience letters 685 (2018) 75–82. [PubMed: 30138645]

[16]. Saify K, Saadat I, Saadat M, Association between VNTR polymorphism in promoter region of prodynorphin (PDYN) gene and heroin dependence, Psychiatry research 219 (2014) 690–692. [PubMed: 25048760]

[17]. Ray R, Doyle GA, Crowley JJ, Buono RJ, Oslin DW, Patkar AA, Mannelli P, DeMaria PA Jr., O'Brien CP, Berrettini WH, A functional prodynorphin promoter polymorphism and opioid dependence, Psychiatric genetics 15 (2005) 295–298. [PubMed: 16314761]

[18]. Nikoshkov A, Drakenberg K, Wang X, Horvath MC, Keller E, Hurd YL, Opioid neuropeptide genotypes in relation to heroin abuse: dopamine tone contributes to reversed mesolimbic proenkephalin expression, Proc. Natl. Acad. Sci. USA 105 (2008) 786–791. [PubMed: 18184800]

[19]. Randesi M, van den Brink W, Levran O, Yuferov V, Blanken P, van Ree JM, Ott J, Kreek MJ, Dopamine gene variants in opioid addiction: comparison of dependent patients, nondependent users and healthy controls, Pharmacogenomics 19 (2018) 95–104. [PubMed: 29210332]

[20]. Peng S, Jiang H, Du J, Lin S, Pan S, Yu S, Zhao M, Methadone Dosage and Plasma Levels, SNPs of OPRM1 Gene and Age of First Drug Use Were Associated With Outcomes of Methadone Maintenance Treatment, Frontiers in genetics 9 (2018) 450. [PubMed: 30420869]

[21]. Zaaijer ER, Bruijel J, Blanken P, Hendriks V, Koeter MW, Kreek MJ, Booij J, Goudriaan AE, van Ree JM, van den Brink W, Personality as a risk factor for illicit opioid use and a protective factor for illicit opioid dependence, Drug Alcohol Depend. 145 (2014) 101–105. [PubMed: 25454407]

[22]. Blanken P, van den Brink W, Hendriks VM, Huijsman IA, Klous MG, Rook EJ, Wakelin JS, Barendrecht C, Beijnen JH, van Ree JM, Heroin-assisted treatment in the Netherlands: History, findings, and international context, European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 20 Suppl 2 (2010) S105–158. [PubMed: 20362236]

[23]. Kellogg SH, McHugh PF, Bell K, Schluger JH, Schluger RP, LaForge KS, Ho A, Kreek MJ, The Kreek-McHugh-Schluger-Kellogg scale: a new, rapid method for quantifying substance abuse and its possible applications, Drug Alcohol Depend. 69 (2003) 137–150. [PubMed: 12609695]

[24]. Butelman ER, Chen CY, Fry RS, Kimani R, Levran O, Ott J, Correa da Rosa J, Kreek MJ, Re-evaluation of the KMSK scales, rapid dimensional measures of self-exposure to specific drugs: Gender-specific features, Drug & Alcohol Dependence 190 (2018) 179–187. [PubMed: 30041093]

[25]. Crystal HA, Hamon S, Randesi M, Cook J, Anastos K, Lazar J, Liu C, Pearce L, Golub E, Valcour V, Weber KM, Holman S, Ho A, Kreek MJ, A C17T polymorphism in the mu opiate receptor is associated with quantitative measures of drug use in African American women, Addict. Biol 17 (2012) 181–191. [PubMed: 21070507]

[26]. Pritchard JK, Stephens M, Donnelly P, Inference of population structure using multilocus genotype data, Genetics 155 (2000) 945–959. [PubMed: 10835412]

[27]. Ducci F, Roy A, Shen PH, Yuan Q, Yuan NP, Hodgkinson CA, Goldman LR, Goldman D, Association of substance use disorders with childhood trauma but not African genetic heritage in an African American cohort, The American journal of psychiatry 166 (2009) 1031–1040. [PubMed: 19605534]

[28]. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC, PLINK: a tool set for whole-genome association and population-based linkage analyses, American journal of human genetics 81 (2007) 559–575. [PubMed: 17701901]

[29]. Randesi M, van den Brink W, Levran O, Blanken P, Butelman ER, Yuferov V, da Rosa JC, Ott J, van Ree JM, Kreek MJ, Variants of opioid system genes are associated with non-dependent opioid use and heroin dependence, Drug Alcohol Depend. 168 (2016) 164–169. [PubMed: 27664554]

[30]. Richmond-Rakerd LS, Slutske WS, Lynskey MT, Agrawal A, Madden PA, Bucholz KK, Heath AC, Statham DJ, Martin NG, Age at First Use and Later Substance Use Disorder: Shared Genetic and Environmental Pathways for Nicotine, Alcohol, and Cannabis, Journal of abnormal psychology 125 (2016) 946–959. [PubMed: 27537477]

[31]. Sartor CE, Lynskey MT, Bucholz KK, Madden PA, Martin NG, Heath AC, Timing of first alcohol use and alcohol dependence: evidence of common genetic influences, Addiction (Abingdon, England) 104 (2009) 1512–1518.

[32]. Agrawal A, Sartor CE, Lynskey MT, Grant JD, Pergadia ML, Grucza R, Bucholz KK, Nelson EC, Madden PA, Martin NG, Heath AC, Evidence for an interaction between age at first drink and genetic influences on DSM-IV alcohol dependence symptoms, Alcoholism, clinical and experimental research 33 (2009) 2047–2056.

[33]. Schlosburg JE, Whitfield TW Jr., Park PE, Crawford EF, George O, Vendruscolo LF, Koob GF, Long-term antagonism of kappa opioid receptors prevents escalation of and increased motivation for heroin intake, J. Neurosci 33 (2013) 19384–19392. [PubMed: 24305833]

[34]. Beardsley PM, Howard JL, Shelton KL, Carroll FI, Differential effects of the novel kappa opioid receptor antagonist, JDTic, on reinstatement of cocaine-seeking induced by footshock stressors vs cocaine primes and its antidepressant-like effects in rats, Psychopharmacology 183 (2005) 118–126. [PubMed: 16184376]

[35]. Funk D, Tamadon S, Coen K, Fletcher PJ, Le AD, Kappa opioid receptors mediate yohimbine-induced increases in impulsivity in the 5-choice serial reaction time task, Behavioural brain research 359 (2019) 258–265. [PubMed: 30414973]

[36]. Walker BM, Kissler JL, Dissociable Effects of Kappa-Opioid Receptor Activation on Impulsive Phenotypes in Wistar Rats, Neuropsychopharmacol. 38 (2013) 2278–2285.

[37]. Blanken P, Hendriks VM, van Ree JM, van den Brink W, Outcome of long-term heroin-assisted treatment offered to chronic, treatment-resistant heroin addicts in the Netherlands, Addiction (Abingdon, England) 105 (2010) 300–308.

HIGHLIGHTS

- The functional *PDYN* 68-base pair polymorphism affects transcription levels of the gene
- We studied heroin use in Caucasians diagnosed with opioid dependence
- *PDYN* genotype “SS+SL” was associated with earlier age trajectory of heroin use

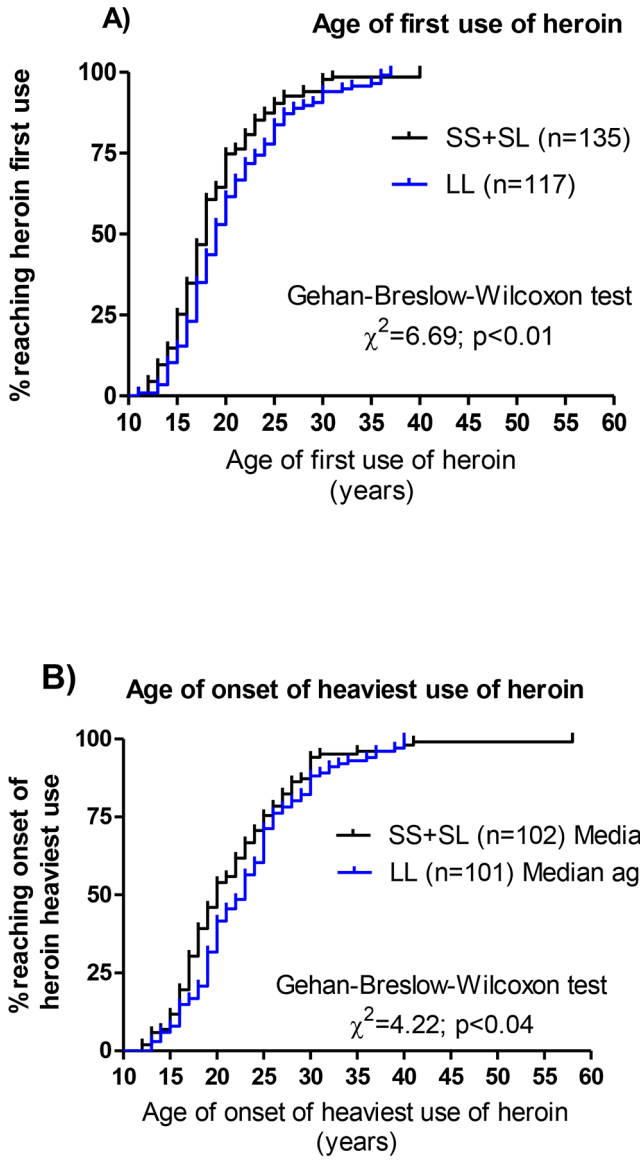

Figure 1:

A) Kaplan-Meier survival curves for age of first use of heroin, and B) for age of onset of heaviest use of heroin, in volunteers with OD diagnoses, comparing *PDYN* genotype. The curves in each panel compare volunteers with *PDYN* LL versus the SS+SL genotype. Ages were entered in whole years.

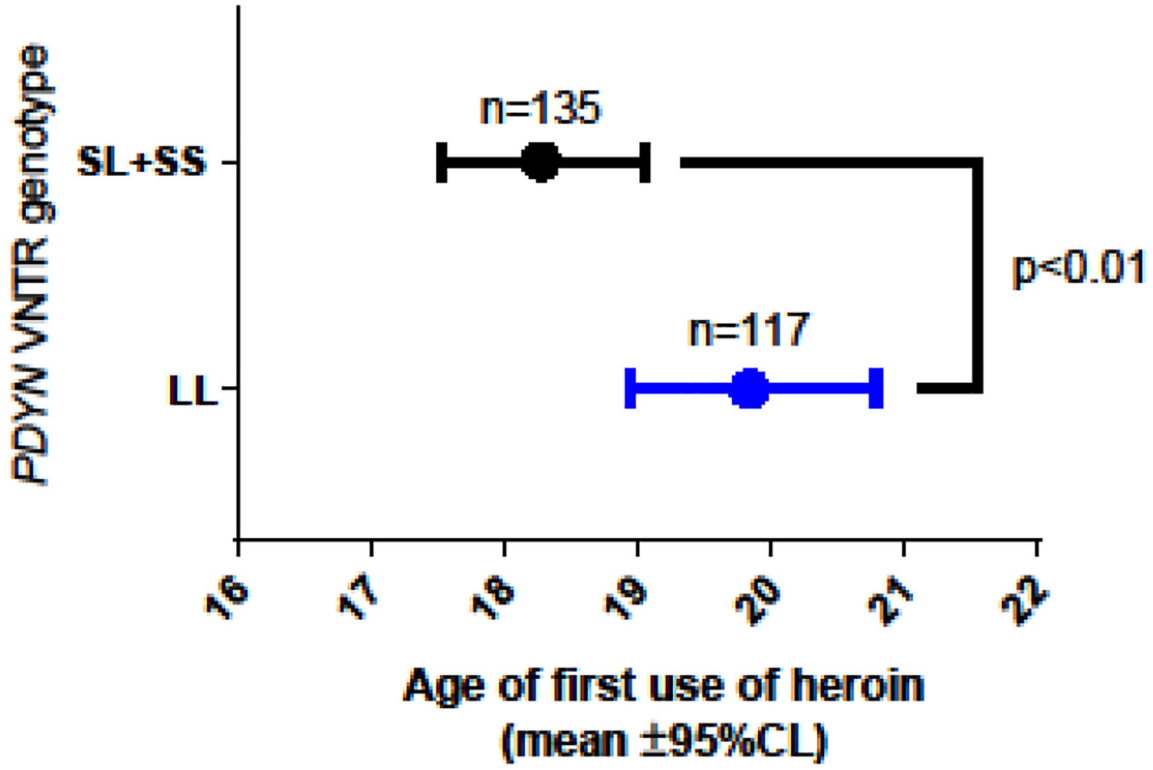

Figure 2:
Ages of first use of heroin in volunteers with OD diagnosis, depending on *PDYN* genotype. Data were analyzed with a Mann-Whitney test (U=6,407; p<0.01).

Table 1

Demographics of the Netherland cohort included in the study

Characteristics	Controls (HC)	Controls (NOD)	OD (MMT+HAT)	Total	*P*
Total	142	153	259	554	
Male	86 (61%)	100 (65%)	196 (76%)	382 (69%)	
Female	56 (39%)	53 (35%)	63 (24%)	172 (31%)	
Age, years (±SD)	39.0±10.4	40.1±9.0	43.4±10.4		<0.001
Treatment groups					
MMT					
Male	-		85 (64%)	132	
Female	-		47 (36%)		
HAT					
Male	-		111 (87%)	127	
Female	-		16 (13%)		

HC, healthy controls; NOD, non-dependent opioid users; OD-MMT, opioid dependent in methadone maintenance treatment; OD-HAT, opioid dependent in methadone maintenance with adjunctive heroin-assisted treatment

Table 2.

KMSK Scores: maximal exposure to specific drugs

Treatment group		KMSK scale			
		Alcohol	**Nicotine**	**Cocaine**	**Heroin**
OD n= 261 total	Range	1–13	0–13	0–16	0–13
(*PDYN* genotyped)	Median (IQR)	12 (10–13)	10 (9–11)	12 (11–14)	9 (9–10)
	Subjects over cut-point *	n=206 (79%)	N/A **	n=234 (89%)	n=254 (97%)
NOD n= 153 total	Range	6–13	0–13	0–16	0–12
(*PDYN* genotyped)	Median (IQR)	11 (10–13)	11 (9–13)	8 (6–11)	5 (4–6.5)
	Subjects over cut-point	n=122 (78%)	N/A	n=74 (48%)	n=56 (37%)
HC n= 142 total	Range	3–13	0–13	0–10	0–2
(*PDYN* genotyped)	Median (IQR)	10 (9–11)	8 (0–10)	0 (0–0)	0 (0–0)
	Subjects over cut-point	n=81 (57%)	N/A	0	0

* Cutpoints for respective DSM-IV diagnosis, based on new determination (Butelman et al., 2018)

** Cutpoint for nicotine not available

Table 3.

Fisher's exact test analyses of association of the *PDYN* 68-bp repeat grouped genotype with opioid dependence

PDYN VNTR	Alelle1	Alelle2	Test	Model	OD	HC+NOD	Chi-square	DF	P-value
68 bp repeats	S	L	Genotypic		33/110/118	37/121/135	0.0465	2	0.9770
68 bp rpt	S	L	Allelic		176/346	195/391	0.024	1	0.8769
68 bp rpt	S	L	Dominant	SS+SLvsLL	143/118	158/135	0.0416	1	0.8385
68 bp rpt	S	L	Recessive	SS vs SL+LL	33/228	37/256	0.000031	1	0.9956

Table 4:

KMSK scores for different substances in OD volunteers, by genotype

Genotype		**Alcohol KMSK** ***scale range: 0–13***	**Nicotine KMSK** ***scale range: 0–13***	**Cocaine KMSK** ***scale range: 0–16***	**Heroin KMSK** ***scale range: 0–13***
LL	n	117	118	118	118
	score range	1–13	0–13	0–16	0*–13
	Median (IQR)	12 (11–13)	10 (9–11)	13 (11–15)	10 (9–13)
SS+SL	N	135	143	143	143
	score range	1–13	0–13	0–16	0–13*
	Median (IQR)	12 (10–13)	10 (9–11)	12 (10–14)	10 (9–13)
Mann-Whitney test significance (p)		0.096	0.22	0.099	0.65

*A very small number of the OD volunteers (n=3) had heroin KMSK scores=0 because their opioid use was not of heroin, but of other opioid compounds; IQR – interquartile range.