Research Article

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Pharmacogenomics

Dopamine gene variants in opioid addiction: comparison of dependent patients, nondependent users and healthy controls

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Aim: To determine whether specific dopaminergic system gene variants are associated with opioid dependence. **Patients & methods:** Subjects included 153 healthy controls, 163 opioid exposed, but not dependent and 281 opioid dependent. Genotypes of 90 variants in 13 genes were examined. **Results:** The most significant results were obtained for *DA β-hydroxylase* variants, rs2073837 and rs1611131, which were associated with protection from addiction ($q = 0.0172$, 0.0415, respectively) and the functional *TH* variant, rs2070762, was associated with more risk ($q = 0.0387$). The three variants also showed a combined effect that remained significant after correction for multiple testing ($p_{final} = 0.0039$). **Conclusion:** These data offer support that dopaminergic gene variants have a role in opioid dependence and warrant further study.

First draft submitted: 27 July 2017; Accepted for publication: 1 November 2017; Published online: 6 December 2017

Keywords: *DBH* • drug addiction • *TH*

Opioid addiction presents a major health and social problem worldwide. Heroin addiction is a complex, chronic and relapsing brain disease that is the product of environmental, drug-induced and genetic components. Some estimates have placed the genetic contribution of developing a drug addiction at 60% of the risk [1,2].

The current report is part of a larger ongoing project between this laboratory and our Dutch collaborators. In The Netherlands, the prevailing attitudes toward drug abuse and addiction are more open than in the USA, which made it possible to recruit and ascertain volunteer subjects who have self-exposed illicitly with short-acting opiates, primarily heroin, but who have never met the criteria of opioid dependence. The ability to conduct human molecular genetic studies to understand why some people may use heroin occasionally and never develop an addiction would be helpful both in primary and early intervention and helpful in making decision on pain medication for persons with chronic pain.

This study is a subset of our overall search for genes and gene variants that are involved in the vulnerability to develop opioid dependence or have protein products that are involved in its molecular neurobiology. We decided, *a priori*, to analyze candidate genes grouped by gene-related systems. In an earlier study, we focused on the gene system that is considered by many to be intricately involved in heroin addiction, the opioidergic system [3].

Here, we report on variants in the dopaminergic system, which plays an important role in the rewarding effects of drugs of abuse. It is generally accepted that heroin and other opioid receptor agonists increase dopamine (DA) levels indirectly by inhibiting GABAergic neurons and as a result a sustained synaptic level of DA in the ventral tegmental area can be observed [4]. Previous studies have found DA-related gene polymorphisms associated with various

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Table 1. Subject characteristics

Treatment group	Recruited	Caucasian by self-report	>70% Caucasian by AIMs	Excluded (DNA low quality)	Included in analysis	Mean age ± SD	Male (%)
HC	197	168	158	5	153	39.0 ± 10.4	56
NOD	198	171	166	3	163	40.1 ± 9.0	65
OD	400	289	285	4	281	43.4 ± 8.3	75

AIM: Ancestry informative marker; HC: Healthy control; NOD: Not-opioid dependent; OD: Opioid dependent; SD: Standard deviation.

addictions, including heroin dependence. *DRD2/ANKK1* SNPs were associated with heroin abuse/dependence in several studies [5–8]. Variants of *DRD1* have been reported to be associated with heroin dependence in African–Americans and Caucasians [7,9]. *CSNK1E* variants were reported to be associated with opioid dependence in subjects of European descent [6,10]. Among injecting heroin users, those with a TT genotype of *DBH* variant rs1611115 had a longer history of addiction and injected more heroin than those with TC or CC genotypes [11]. In addition, several *DBH* SNPs were found to be associated with the likelihood of relapse to nicotine [12]. Furthermore, *COMT* variants have been found nominally significantly associated with cocaine-induced paranoia in African–Americans [13]. A nicotine study examining DA metabolism genes found that men with a GG genotype for *DBH* SNP rs77905 were more likely to be persistent smokers if they smoked less than ten cigarettes per day [14].

In the current study, we describe the results of an association study of 90 variants in 13 dopaminergic system genes. Including, genes involved in DA synthesis (*TH* and *DDC*), genes involved in DA biotransformation (*DBH* and *COMT)*, DA receptor genes, including both D1-like (*DRD1* and *DRD5*) and D2-like (*DRD2, DRD3* and *DRD4*), as well as the DA transporter (*SLC6A3*). Furthermore, several genes that regulate DA signaling (*ANKK1*, *CSNK1E* and *PPP1R1B*) were also included. We hypothesize that persons with opioid dependence have specific gene variants, including specific variants of the dopaminergic system which are significantly different than those in both healthy controls (HC) and exposed but not-dependent opioid users. Further, we propose that the exposed but not-dependent group may have different gene variants than HC but they are more likely in genes related to risk-taking and impulsivity.

Materials & methods

Subjects

Three groups of subjects were ascertained in The Netherlands, (n = 795; 30% females), as previously reported [15–17] (Table 1A). Briefly;

- HC meeting DSM-IV criteria of no history of alcohol or drug dependence;
- Nondependent opioid users (NOD) with a lifetime history of heroin or other nonprescribed opioids use without opioid dependence;
- Opioid dependent users (OD) meeting DSM-IV criteria for opioid dependence for at least 5 years and in methadone maintenance treatment or heroin-assisted treatment.

All participants were at least 25 years of age.

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 human molecular genetics study for all study groups. The genetics study was also approved by The Rockefeller University's Institutional Review Board. All subjects signed informed consent for the genetics research.

Socio-demographic & drug use assessment

Trained investigators performed extensive interviews of all subjects. Standard questionnaires were used for collection of age, gender and country of origin information. The DSM-IV was used for diagnosis of opioid dependence.

In addition, subjects were administered the Kreek–McHugh–Schluger–Kellogg (KMSK) scale [18], a rapid and quantitative instrument to assess a subject's exposure to opioids, cocaine, alcohol and nicotine. The KMSK scale assesses the frequency, amount and duration of exposure to each substance during a person's period of greatest use (lifetime score). In prior studies, the results of KMSK assessments were evaluated using receiver operator characteristics analysis for the optimal cut-point score for alcohol, cocaine and opiate dependence/addiction diagnoses using an ethnically diverse population [18].

Table 2. Dopaminergic system genes.

Symbol	Name	SNPs
ANKK1	Ankyrin repeat and kinase domain containing 1	4
COMT	Catechol-O-methyltransferase	13
CSNK1E	Casein kinase 1, epsilon	9
DBH	Dopamine β-hydroxylase	21
DDC	Dopa decarboxylase	17
DRD1	Dopamine receptor D1	3
DRD2	Dopamine receptor D2	16
DRD3	Dopamine receptor D3	14
DRD4	Dopamine receptor D4	3
DRD5	Dopamine receptor D5	3
PPP1R1B	Protein phosphatase 1, regulatory (inhibitor) subunit 1B (DARPP-32)	3
SLC6A3	Solute carrier family 6 (neurotransmitter transporter), member 3 (DAT)	10
TH	Tyrosine hydroxylase	2

Genotyping

Genomic DNA was isolated from the blood specimens of all subjects received from The Netherlands using standard techniques. The DNA was genotyped for 118 SNPs in 13 dopaminergic system genes (Table 2 & Supplementary Table 1) using a 1536-plex Illumina Golden Gate Custom Panel (GS0013101-OPA; Illumina, CA, USA), which is a modification of the 'addiction array' that has been previously described [10,19]. Data analysis was performed with BeadStudio v2.3.43 software (Illumina). Genotype data were visually inspected and filtered to include only SNPs with call rates >90%, a well-defined cluster separation and minor allele frequency (MAF) >0.05.

Assessment of percentage of European ancestry

Ethnicity was initially assigned based on self-reported family origin data with 628 self-identified Caucasian subjects as previously reported [3]. Based on 155 ancestry informative markers, the fraction of genetic affiliation of an individual in each of seven clusters was calculated with *STRUCTURE* v2.2 [20]. Each subject was anchored against 1051 samples from 51 worldwide populations represented in the Human Genome Diversity Cell Line Panel, as described [21]. For the current study, the inclusion criteria was set to 70% or greater European ancestry contribution estimate to minimize population stratification.

Statistical analysis

We followed the recommendation to test for deviations from Hardy–Weinberg equilibrium (HWE) in control individuals [22]. Using Plink v1.9 [23] we computed p-values for deviation from HWE with mid-p adjustment [24] and obtained Bonferroni-corrected p-values for the 90 SNPs as $pBon = 1 - (1 - p)^{90}$. The smallest corrected value was $pBon = 0.1314$. The fact that genotype frequencies of controls conform to HWE provides a surrogate of genetic association studies quality in terms of design and conduct [22]; thus, the clear absence of significant deviations from HWE serves as our control for population stratification. Pairwise linkage disequilibrium (LD) was estimated using Haploview v4.2 [25].

Initially, a standard genetic association test was performed for comparisons of HC versus NOD and NOD versus OD. If no significant differences are found for one or both comparisons, the compared groups can be combined to increase power. Two types of analyses for case/control association were performed for each SNP separately, and combination effect of multiple SNPs.

Analysis (1): individual SNP association analysis was performed using the maxstat program [26]. For each of the 90 SNPs, the basic test statistic was the maximum of chi-squares, recessive and dominant allele action. Such maximum tests have been shown to have superior power [17]. Based on 40,000 permutation replicates, an empirical significance level was computed, p_0 = nominal p-value for the given SNP. To control for the false discovery rate, a q-value was calculated for each p-value [27,28].

Analysis (2): the sumstat program [26] was used to look for any combined effect from more than one SNP. SNPs were ranked by their resulting test statistics and sequential sums were computed, such that s_2 = sum of largest two test statistics, s_3 = sum of largest three test statistics and so on. Each sum had an associated significance level, p_{sum}.

Generally, these sum-specific significance levels tend to drop as more and more test statistics are added to the sums, and after a minimum significance level, p_{min}, they tend to increase again. The number of test statistics in the sum furnishing p_{min} is regarded as the number of SNPs potentially contributing to disease in a combined manner. To allow for testing of multiple sums, p_{min} is taken as a new test statistic whose significance level, p_{final}, is corrected for testing multiple SNPs and sums. If a single SNPs p-value is less than the p_{final} ($p < p_{final}$) there is evidently no validity in considering sums of test statistics as the best single SNP is more significant than any sum.

Results

Sample characteristics

Subjects with <70% estimated proportion of European ancestry based on 155 ancestry informative markers were excluded from the analyses. Twelve subjects were removed due to the low quantity and/or poor quality of DNA. The remaining 597 subjects were included in the association analyses (Table 1A).

Of the 118 SNPs genotyped, 21 SNPs were excluded based on MAF <0.05 and seven SNPs were excluded due to call rate <90%. Genotypes of 90 SNPs from 13 genes were analyzed for association with nondependent opioid use (NOD) and opioid dependence (OD; Table 2 & Supplementary Table 1). None of the SNPs significantly violated HWE in the control group (HC). Among the OD subjects, there was a strong deviation from HWE for *DBH* SNP rs2073837 (more CT genotypes and fewer CC genotypes than expected) but this deviation may be interpreted as evidence for association. Further, LD analysis of all SNPs, in the HC sample, revealed 21 SNP pairs that were in strong LD, $r^2 > 0.8$ (Supplementary Figure 1). LD analysis of the *DBH* gene (Figure 1) shows there are three main LD blocks. The third LD block includes SNPs rs1611131 and rs2073837, which are in strong LD ($r^2 = 0.85$).

Association analysis testing each SNP individually (1)

Comparison of HC versus NOD

Basic association analysis testing for each of the SNPs one at a time found no significant difference (after correction for multiple testing) between HC versus NOD (see Supplementary Table 4). In an earlier report with a separate set of genes, significant differences were found between HC versus NOD and therefore they were not combined [3]. However, for the current study in the absence of significant differences, we combined the HC and NOD groups for comparison to the group of OD.

Comparison of NOD versus OD

Individual SNP analysis for the comparison of NOD versus OD (Table 3A) showed three SNPs with nominally significant association using a recessive model of inheritance, *DBH* SNPs rs2073837 and rs1611131 and *TH* SNP, rs2070762 ($p_0 = 0.0009$, $p_0 = 0.0039$ and $p_0 = 0.0062$, respectively). The signal from rs2073837 remained significant after correction (q = 0.0474; OR = 0.26; 95% CI: 0.11–0.58).

Comparison of HC versus OD

Individual SNP analysis for the comparison of HC versus OD (Table 3B) showed the same three SNPs with nominally significant association using a recessive model of inheritance, *DBH* SNPs rs2073837 and rs1611131 and *TH* SNP, rs2070762 ($p_0 = 0.0010$, $p_0 = 0.0031$ and $p_0 = 0.0044$, respectively). Again, the signal from rs2073837 remained significant after correction (q = 0.0419; OR = 0.24; 95% CI: 0.11–0.54).

Comparison of (HC + NOD) versus OD

Since we found now significant difference (after multiple testing correction) when we compared HC versus NOD we combined the groups and compared the larger group to OD. Again, the same three SNPs, rs2073837 (*DBH)*, rs2070762 (*TH*) and rs1611131 (*DBH)*, were found to be significant using a recessive model of inheritance (Table 3C). After correction, all three signals remained significant (q = 0.0172, OR = 0.25; 95% CI: 0.12–0.52; q = 0.0387, OR = 2.12; 95% CI: 1.43–3.14; and q = 0.0415, OR = 0.29; 95% CI: 0.14–0.59, respectively).

Association analysis testing the combined action of multiple SNPs (2)

Forming sums of the test statistics revealed that there is a combined effect of the top three SNPs (rs2073837, rs1611131 and rs2070762) for the comparisons, HC versus OD and (HC + NOD) versus OD.

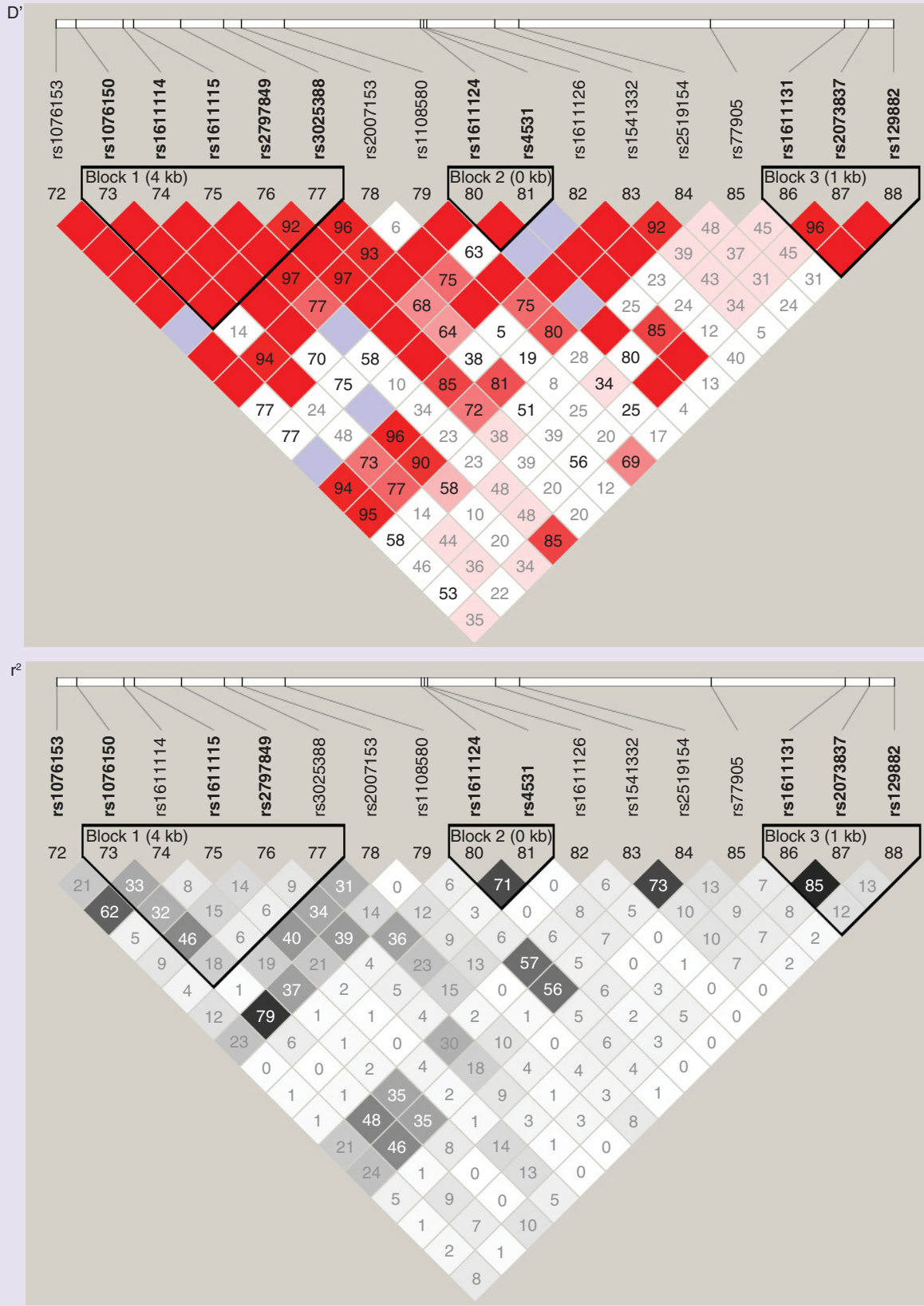

Figure 1. Pairwise linkage dsequilibrium for *DA β-hydroxylase*. The pairwise correlation between single nucleotide polymorphisms measured in D' (red) and r^2 (black). The values shown are (×100) in each box. The color scheme indicates the magnitude of the value where the darker the color indicates the greater linkage dsequilibrium. When the value is equal to 1.0 the box is empty. For colour figures please see: https://www.futuremedicine.com/doi/suppl/10.2217/pgs-2017-0134

Table 3. Significant associations from single SNP analysis: maxstat results (stat. code = 18).

SNP	Gene	Chr	Position	Alleles	Location	MAF	Test	Stat	p_0	$q^{\pm}$	OR (95% CI)
NOD versus OD											
rs2073837†	*DBH*	9	133657805	C/T	Intron	0.3	Rec	11.88	0.0009	**0.0474**	0.26 (0.11–0.58)
rs1611131†	*DBH*	9	133657064	A/G	Intron	0.29	Rec	9.35	0.0039	0.0978	0.29 (0.13–0.63)
rs2070762	*TH*	11	2165104	T/C	Intron	0.49	Rec	9.25	0.0062	0.1038	2.07 (1.27–3.42)
HC versus OD											
rs2073837†	*DBH*	9	133657805	C/T	Intron	0.3	Rec	12.91	0.001	**0.0419**	0.24 (0.11–0.54)
rs1611131†	*DBH*	9	133657064	A/G	Intron	0.29	Rec	10.27	0.0031	0.1866	0.29 (0.13–0.63)
rs2070762	*TH*	11	2165104	T/C	Intron	0.49	Rec	9.59	0.0044	0.2437	2.08 (1.27–3.42)
(HC + NOD) versus OD											
rs2073837†	*DBH*	9	133657805	C/T	Intron	0.3	Rec	16.96	0.0001	**0.0172**	0.25 (0.12–0.52)
rs2070762	*TH*	11	2165104	T/C	Intron	0.49	Rec	14.28	0.0004	**0.0387**	2.12 (1.43–3.14)
rs1611131†	*DBH*	9	133657064	A/G	Intron	0.29	Rec	13.51	0.0007	**0.0415**	0.29 (0.14–0.59)

FDR adjustment assumed 90 SNPs and 3 group comparisons. q , false discovery rate (FDR) associated p-value, with FDR set to 0.05.
†SNPs in strong linkage disequilibrium ($r^2 = 0.85$).
Chr: Chromosome; HC: Healthy control; MAF: Minor allele frequency; NOD: Nondependent opioid user; OD: Opioid dependent; OR: Odds ratio; p_0: Nominal p-value; ; Rec: Recessive; Stat: Test statistic.

Table 4. Significant associations–combined effect of multiple SNPs: *sumstat* results (stat. code = 18).

Rank	SNP	Gene	Chr	Position	Alleles	Location	Stat	Sum	p_0	p_{Stat}	p_{Sum}	
HC versus OD												
1	rs2073837	*DBH*	9	133657805	C/T	Intron	12.91	12.91	0.001	**0.0486**	0.0486	
2	rs1611131	*DBH*	9	133657064	A/G	Intron	10.27	23.18	0.0031	0.1866	0.0406	
3	rs2070762	*TH*	11	2165104	T/C	Intron	9.59	32.77	0.0044	0.2437	**0.0319**	(p_{min})
												$p_{final} = 0.0595$
(HC + NOD) versus OD												
1	rs2073837	*DBH*	9	133657805	C/T	Intron	16.96	16.96	0.0001	**0.0048**	0.0059	
2	rs2070762	*TH*	11	2165104	T/C	Intron	14.28	31.24	0.0004	**0.0234**	0.0027	
3	rs1611131	*DBH*	9	133657064	A/G	Intron	13.51	44.75	0.0007	**0.0346**	**0.0016**	(p_{min})
												$p_{final} = \mathbf{0.0039}$

Chr: Chromosome; HC: Healthy control; NOD: Nondependent opioid user; OD: Opioid dependent; p_0: Nominal p-value; p_{final}: p_{min} corrected for testing multiple SNP and sum; p_{min}: Smallest p_{sum} for a given comparison; p_{sum}: Sum-specific significance level; p_{stat}: p-value corrected for testing multiple SNPs and two models of inheritance per SNP; Stat: Test statistic; Sum: Sum of largest n test statistics.

Comparison of HC versus OD

The sum of the largest three test statistics furnished a $p_{min} = 0.0319$ (Table 4A). Taking p_{min} as a new test statistic and calculating $p_{final} = 0.0595$, which is corrected for testing multiple SNPs and sums. Since p_{final} > p-value for the best individual SNP result ($p = 0.0486$), in this case, calculating sums did not seem to contribute more that the best SNP does by itself.

Comparison of (HC + NOD) versus OD

The sum of the largest three test statistics furnished a $p_{min} = 0.0016$ (Table 4B), which is highly significant with a $p_{final} = 0.0039$, corrected for testing multiple SNPs and sums. Since p_{final} < p-value for the best individual SNP

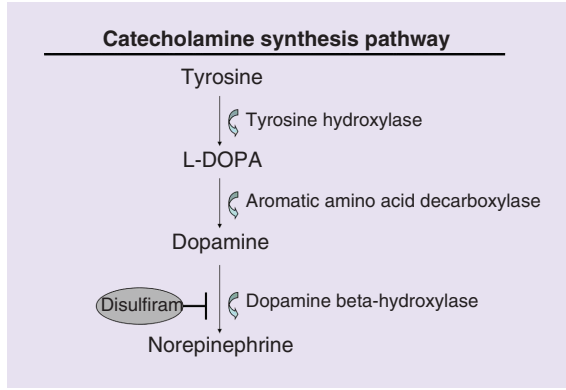

Figure 2. Catecholamine synthesis pathway.

result ($p = 0.0048$), sum statistics suggest that these three SNPs (rs2073837, rs2070762 and rs1611131) appear to contribute jointly to disease association. As noted earlier, the two *DBH* SNPs, rs2073837 and rs1611131 were found to be in strong LD; therefore, the signal detected is most likely coming from just one of the SNPs.

Genotype frequencies of *DBH* SNPs, rs2073837 and rs1611131, and the *TH* SNP rs2070762 for the treatment groups (HC, NOD and OD) are shown in Supplementary Table 2.

Discussion

In this study, we have tested 90 SNPs in 13 dopaminergic system genes for association with nondependence opioid use and heroin dependence. We found two *DBH* SNPs in strong LD, rs2073837 and rs1611131, to be associated with protection from OD, and one *TH* SNP, rs2070762, to be associated with a risk or vulnerability to develop OD. The three SNPs have not been previously reported to be associated with OD. The *DBH* variant rs1611131 has been reported to be linked to dependence symptoms of cocaine users, with carriers of the G allele having more dependence symptoms [29]. This is in contrast to the current report which found that persons with the variant allele for rs1611131 should be protected from OD.

However, comparing the two studies is difficult since the earlier report was looking at DA genes effect on dependence symptoms among cocaine users and not opioid users as in the current study.

DBH has 12 exons and spans 23 kb and there are two known splice variants [30]. Since the *DBH* SNPs are in intronic regions they may be involved in splicing processes with formation of new *DBH* variants or to be targets for miRNA activity, possibly leading to the production of an enzyme with reduced or no activity.

DBH converts DA to norepinephrine, thereby playing a direct role in determining the ratio of DA to norepinephrine in noradrenergic neurons (Figure 2). It is known that plasma DBH activity varies widely between individuals [31]. Another SNP in *DBH*, rs1611115 (1021C/T), that was not found to be significant in the current study, is functional and located in the 5′ flanking region of the gene, has been shown to account for '35–50%' of levels of plasma DBH activity in populations of European origin [32]. Several other functional polymorphisms in the *DBH* gene such as rs2519152 and rs6271 have been reported by different investigators. Association between *DBH* SNPs and plasma DBH activity, as well as cocaine-induced paranoia were reported [31,33].

TH is the rate-limiting enzyme in the synthesis of catecholamines; including DA, noradrenaline and adrenaline. TH converts tyrosine to L-DOPA that is in turn converted to DA by DOPA decarboxylase. Several variants in *TH* have been tested for association with affective disorders, such as bipolar disorder and schizophrenia. A polymorphic four base repeat in *TH*, that was not included in this study, was found associated with schizophrenia in Japanese females, but not males [34]. The functional intronic *TH* SNP rs2070762, identified in this study, was previously found to confer risk of hypertension in a northern Chinese Han population [35]. SNP rs2070762 was also found nominally associated with cocaine dependence in a Spanish sample [36]. The current study is the first to report an association of *TH* rs2070762-C allele with the vulnerability to develop opiate dependence.

As stated above, our findings indicate that persons having the *DBH* variant alleles for SNPs rs2073837 and rs1611131 would be more 'protected' from the vulnerability of developing OD than someone having the wild-type alleles. Further, the findings indicate persons with the variant allele for the *TH* SNP rs2070762 would have more risk or be more vulnerable to the development of OD. Mechanistically, one can envision how this might occur, tyrosine hydroxylase converts L-tyrosine into L-DOPA, precursor to DA, a variant *TH* would result in a less

active enzyme and in turn less free DA therefore more vulnerable to addiction. By the same reasoning, since DBH catalyzes the conversion of DA to noradrenaline, a variant *DBH* would result in higher levels of free DA therefore less vulnerable to development of addiction. Of course this scenario can only occur when two assumptions are made; the specific gene variant is functional and results in reduced levels of the gene product and that increasing free DA levels would result in a decrease in the vulnerability to develop an addiction.

Although we found no variants with significant differences that survived correction for multiple testing in their genotype frequencies between HC and NOD subjects with these genes and their variants, recently we reported differences for several variants in genes of the opioid system [3].

There are some important limitations for the current study that should be considered when interpreting the findings. Drug use assessment using the KMSK (Supplementary Table 3) revealed that among the NOD subjects 33% reported heavy cocaine use, 6% reported regular opioid use and 68% reported alcohol use, all of which would be classified as dependence by KMSK criteria. In addition, the KMSK confirmed that the HC subjects were not using cocaine or heroin, but 31% did report excess alcohol use consistent with dependence by the KMSK criteria. The KMSK also indicated that 76% of the OD subjects were abusing cocaine and 70% were abusing alcohol. Regrettably, the numbers of subjects in the groups were too small to allow statistical analysis for concomitant cocaine, alcohol or cocaine with alcohol. It is of interest that the genes identified in the current study are well known to be coding for enzymes involved in reward pathways for cocaine and alcohol. The high percentage of subjects with polydrug use could very well be confounding the results presented.

The deviation from HWE in the OD sample for *DBH* SNP rs2073837 can be interpreted as evidence for association. However, one must consider the possibility that this deviation is the result of population stratification. We tested for deviations from HWE in control individuals, where no deviation was found. The fact that genotype frequencies of controls conform to HWE provides a surrogate of genetic association studies quality in terms of design and conduct [22]; thus, the clear absence of significant deviations from HWE served as our control for population stratification.

Our current study provides the first evidence that *DBH* SNPs, rs2073837 and rs1611131 and *TH* SNP rs2070762 play a role in opioid dependence. The relatively small number of subjects in the current study may have limited detection of additional significant differences between groups. Further studies with greater statistical power are warranted to corroborate the results and to assess the clinical significance of the findings.

Summary points

- Dopaminergic system plays an important role in the rewarding effects of drugs of abuse.
- Subjects include exposed yet not addicted opioid group, an understudied group.
- Genotypes of 90 variants in 13 genes were examined.
- The most significant results were obtained for DA β-hydroxylase variants, rs2073837 and rs1611131, which were associated with protection from addiction ($q = 0.0172$, 0.0415, respectively) and the functional *TH* variant, rs2070762, was associated with more risk ($q = 0.0387$). The three variants also showed a combined effect that remained significant after correction for multiple testing ($pfinal = 0.0039$).
- This study provides the first evidence that *DBH* SNPs, rs2073837 and rs1611131 and *TH* SNP rs2070762 play a role in opioid dependence.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: https://www.futuremedicine.com/doi/suppl/10.2217/pgs-2017-0134

Author contributions

MJ Kreek, JM van Ree and W van den Brink performed original study concept and design. MJ Kreek oversaw all aspects of the study as a principal investigator. M Randesi performed sample preparation, data collection, analysis and interpretation and drafted the manuscript. J Ott performed all statistical analyses. O Levran performed array design, SNP selection, data analysis and interpretation. V Yuferov assisted with data interpretation. P Blanken ascertained study subjects. All co-authors contributed to the content, provided critical reviews and approved the final version of the manuscript.

Acknowledgements

We would like to thank and acknowledge the contribution of P-H Shen, D Goldman and C Zhao for genotyping and structure analysis.

Financial & competing interests disclosure

This study was supported by grants from the Dr Miriam and Sheldon G Adelson Medical Research Foundation, NIDA grant P50-DA005130 (MJ Kreek), a special supplement to R01-DA012848 (MJ Kreek) and project grant 31470070 from the Natural Science Foundation of China (J Ott:), and a grant from The Netherlands Ministry of Health, Welfare and Sports. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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