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## QT length during methadone maintenance treatment: Gene x dose interaction

**Running title:** Gene Methadone dosage interaction in QT

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## ABSTRACT

Methadone is known to be a risk factor for sudden death by enlarging ECG QT corrected (QTc) interval. For other medical conditions, QTc lengthening has been described as the result of interactions between pharmacological treatments and genetic factors.

Former heroin dependent subjects under methadone maintenance treatment in remission for at last 3 months were recruited. We studied the association between QTc length (Bazett formula) and 126 SNPs located on 5 genes (*KCNE1*, *KCNQ1*, *KCNH2*, *NOS1AP* and *SCN5A*) previously associated with drug-induced QT prolongation. Both SNP-based and gene-based approaches were used, and we tested also the interaction of the top SNP with methadone dosage to predict the QTc length.

In our sample of 154 patients, current methadone daily dose was associated with QTc length ( $r_{\text{Pearson}} = 0.26$  ;  $p = 10^{-3}$ ). Only one SNP, rs11911509 on *KCNE1*, remained significantly associated with QT length after correction for multiple testing ( $p = 3.84 \times 10^{-4}$  ;  $p_{\text{corrected}} = 0.049$  ). Using a gene-based approach, *KCNE1* was also significantly associated with QTc length ( $p_{\text{empirical}} = 0.02$ ). We found a significant interaction between methadone dosage and rs11911509 minor allele count (allele A vs C ;  $p = 0.01$ ). Stratified analysis revealed that the correlation between QTc length and methadone dosage was restricted only to AA carriers of this top SNP.

Patients' genetic background should be taken into account in the case of clinically relevant QT enlargement during methadone maintenance treatment.

**Key words:** Methadone, rs11911509, *KCNE1*, QT prolongation.

## ABBREVIATIONS

ECG: Electrocardiograph

F<sub>ST</sub>: Fixation index

hERG: human Ether-A-Go-Go Related Gene

PCA: Principal components analysis

QTc: corrected QT interval

SNP: Single Nucleotide Polymorphism

TdP: Torsade de Pointe

## INTRODUCTION

Methadone is largely prescribed as maintenance treatment in patients suffering from opiate dependence around the world because it is considered as the most effective treatment in this indication [1]. Patients under methadone maintenance treatment have a lower heroin overdose mortality, lower probability of relapse, HIV infection and criminal activities, and higher functioning improvement compared to those that did not received treatment [2–7]. Moreover, Methadone appears to be more effective than other opiate maintenance treatments in retaining patients in cares [8].

Nevertheless, methadone may be responsible for severe cardiac side effects, especially QT interval prolongation in the ECG which can occur in up to 37 % of cases [9,10] and exposes to the occurrence of torsade de pointes (TdP) [11,9,12]. According to several studies, mortality associated with this adverse effect varies from 8 % to 11 % among Methadone users [13,14]. This QT prolongation can occur over a wide range of dosages including those recommended for maintenance treatment [14], although controversial, a dose-dependence relationship has been reported (Mayet, Gossop, Lintzeris, Markides, & Strang, 2011; Fanoë, Hvidt, Ege, & Jensen, 2007; Ehret et al., 2006; Krantz, Kutinsky, Robertson, & Mehler, 2003; Roy et al., 2012; Peles, Bodner, Kreek, Rados, & Adelson, 2007). It has also been reported that QT prolongation and TdP risk are more commonly reported in patients taking methadone for opioid dependence than for pain management [13].

One of the mechanisms involved in methadone-associated QT prolongation is the blockade in the human heart of the hERG (human Ether-A-Go-Go Related Gene, KCNH2) potassium channel that mediates the rapid repolarizing  $I_{Kr}$  current [21]. Although various opioids agonist are known to block the hERG channel, methadone appears to be among the most potent inhibitor of the hERG repolarizing current at therapeutic range [21]. This may explains why these adverse effects (QT prolongation and TdP) mainly affect patients under methadone compared to morphine, heroin or buprenorphine treatments [13,22].

Medication-induced QT interval prolongation implies the interaction of several non-genetic and genetics risk factors [23–25]. Among identified genetic risk factors, DNA variants on *KCNE1* [26], *KCNQ1* [27], *KCNH2* [27], *NOS1AP* [28] and *SCN5A* [27] have been shown to predispose to QT prolongation in case of drug exposure. Moreover, we previously observed a significant association of a *KCNH2* single nucleotide variant with increased QT length in methadone-treated patients [29].

The aim of the current study was to test the association of additional candidate genes with corrected QT (QTc) duration among an extended population of methadone users, and to focus on a possible gene x methadone dosage interaction in this association.

## METHODS

### Subjects

Two hundred and sixteen subjects aged over 18 years, with lifetime opiates dependence in total remission for more than 3 months were recruited in outpatient treatment program. The main objective of this study was to describe gene x environment interactions regarding the dose of methadone prescribed, but also to identify gene x environment interactions regarding QTc enlargement was only a secondary objective.

Steady state was defined as being on methadone maintenance program with a stable dose, and regular follow up for at least three months. Furthermore, subjects with persistent use of intravenous route of administration for any kind of substances were excluded, as well as those with recent history of drug overdose (during the last three months).

Trained interviewers asked patients about their lifetime use of opiates, previous maintenance treatments (methadone and buprenorphine) and other substances use. The Mini International Neuropsychiatric Interview (MINI) [30,31] was used to assess current and lifetime dependence or abuse criteria for opiates and other substances of abuse according to the DSM IV TR classification [32]

Concomitantly prescribed medications were also recorded and any described QT prolongation was checked in each patient according to the CredibleMeds.org database (accessed on 06.03th.2016). In this database, medications were gradually classified as with conditional, possible, or confirmed risk of TdP (due to QT lengthening). Every concomitant medication was flanked with a score from 1 to 3 proportional to its risk category, and treatments that were not listed in the database have a 0 score. Finally, we computed for each patient a “Drugs lengthening QT score” by summing the scores of his medications.

No patient reported previous history of syncope, TdP or Long QT syndrome upon enrolment. All subjects signed a free and informed consent and received complete description of the study. The study was approved by the Ethic Committee of Paris, Ile-de-France (CPP VI, May 19<sup>th</sup> 2008) and was registered to the clinicaltrials.gov website (NCT00894452).

### Electrocardiographs (ECGs)

ECGs were recorded at rest using a 12 leads digital apparatus in the morning before the daily methadone intake in order to be in steady state trough condition. ECGs were blindly analysed by two trained cardiologists. According to their ECGs, we did not include patients with: poor ECG quality, conditions that may lengthen QT interval such as bradycardia or bundle branch block, or for which QT interval calculation was impossible. The QTc interval was then calculated using Bazett's formula and was considered as borderline or prolonged if it exceeded 430 ms for men and 450 ms for women. If ECG abnormalities were detected, a senior cardiologist then followed those patients.

## Single Nucleotide Polymorphisms (SNPs) genotyping

Genomic DNA was extracted from EDTA-treated peripheral blood samples and genotyped on the Illumina Infinium PsychArray24 v1.1 BeadChip, containing 571 054 SNPs.

### SNPs selection and genetic quality control:

We selected *KCNE1*, *KCNQ1*, *KCNH2*, *NOS1AP* and *SCN5A* genes according to previously published studies that found association of variants on these genes with drug-induced QT interval prolongation [28,26,27]. We thus retrieved SNPs that overlap with their genomic coordinates according to the GH37p13 version of the Human genome (version used in the PsychArray). We finally retained only those that passed quality control selection with the following parameters: Minor Allele Frequency  $\geq 0.1$ , Genotyping rate  $\geq 0.05$ , and no significant deviation from Hardy Weinberg Equilibrium ( $p$ -value  $< 0.001$ ).

The per-individual quality control consisted of three steps. First, we removed subjects for which we suspect inbreeding or DNA sample contamination according to their identity by descent calculation and retained only subjects with PI HAT between 0.2 and 0.8. Second, we searched for sex discordances between genetic and reported sex, and third we excluded outliers with excessive missing genotyping ( $> 0.03$ ) and heterozygosity ( $> \pm 2.5$  SD) rates [33].

### Statistical analysis

Our study is an ancillary objective of a larger project, the "Methadose" project, which the main objective is to predict the steady dose for methadone maintenance treatment according to genetic and clinical factors. Thus, we did not perform an *a priori* power calculation for our main objective, which is testing the association between candidate SNPs and QTc length.

Associations between QTc interval and dichotomic clinical variables were explored with the Wilcoxon-Mann-Whitney U test. For continuous variables, we used the Pearson or the Spearman correlation coefficients, as appropriate.

Genetic associations were tested with linear regression model taking the QTc length as the dependent variable. The same regression was used to test for interaction between the top SNP and methadone dosage, by including also clinical variables associated with QTc length with a 10% entry  $p$ -value. We chose Cohen's  $f^2$  [34] method for the calculation of the genetic association effect size. This method allows the evaluation of a selected variable effect size within a multivariate regression model.

As a supplementary quality check, the association tests with both clinical variables and the regressions were performed again without two subjects with extreme methadone dosage (above 200 mg per day) to ensure that the association was not only driven by those two outliers.

Gene-based analysis was performed according to Plink reported method [35] with the following parameters ( $r^2=0.5$ ,  $p=0.2$  and max SNP number = 5). Empirical  $p$ -value was obtained after 10 000 permutations.

All tests were performed with a two-tailed hypothesis and an alpha risk of 5%. Corrections for multiple testing were applied using the Bonferroni method. Analyses were achieved with Plink v1.90b4.10 [35,36] and R version 3.2 [37].

### Population stratification managing

Ethnic diversity in our sample was taken into account by three means. First, Principal components analysis (PCA) was used to correct for population stratification as a covariate in the linear regression model. Principal components were calculated using the whole genotype data available in the PsychArray chip after pruning markers that are in linkage disequilibrium ( $r^2 > 0.2$ ), that are located in sex chromosomes, and those that mapped to the hot spot region of the major histocompatibility complex.

Second, QTc length as well as association with SNPs and genes were compared between ethnic subgroups observed in our population. To define those groups, the 154 subjects retained for the analysis were merged with 1000 Genomes populations to calculate principal components of the whole sample (1000 Genomes & our cohort). The ethnicity of our subjects was then visually determined (Figure S1). One hundred and forty three subjects (92.9 %) mapped to European population of 1000 Genomes samples, while the rest of the sample mapped to Africans population.

Third, we also calculated the fixation index ( $F_{ST}$ ) for all variants on the PsychArray chip that passed quality control filters according to *Weir and Cockerham* method [38]. The  $F_{ST}$  was then used to assess the impact of population divergence on the variants especially those retained for the analysis. This analysis was conducted with Plink software.

## RESULTS

After selection for available variables of interest, genotyping quality control, 154 individuals were retained for subsequent analysis (Figure 1).

### Population characteristics

In our cohort, the average QTc length was  $415.2 \pm 2.5$  ms (range : 350 to 575 ms). Thirty-eight (24.7 %) patients have prolonged or borderline QTc interval (Table I). Patients were predominately men (76.6 % ; sex ratio 3.3), and the QTc interval was significantly longer in women than in men ( $422.4$  ms vs  $412.9$  ms respectively;  $W = 1600$  ;  $p = 0.03$ ). Subjects were aged between 20 and 64 years with a mean of 40.9 years ( $\pm 0.7$ ) and a median of 42 years. Age was not significantly correlated with QTc length ( $r_{\text{Pearson}} = 0.04$  ;  $p = 0.62$ )

The methadone dosage varied from 7 to 320 mg per day (mean :  $63.8$  mg per day  $\pm 3.3$  mg per day, median : 60 mg per day). QTc length and methadone dosage were significantly correlated ( $r_{\text{Pearson}} = 0.26$  ;  $p = 1 \times 10^{-3}$ ), and we calculated that each additional 10 mg per day of methadone increases the QTc length by 1.8 ms (IC<sub>95%</sub> [0.7 ms – 3 ms]).

We observed that two subjects (1.3 %) were stabilized with a daily dose of more than 200 mg/day of methadone, and even after removing them from the analysis the correlation between QTc and methadone dosage remained significant ( $r_{\text{Pearson}} = 0.18$  ;  $p = 0.02$ ).

Fifty-three patients (34.4 %) had a prescription of at least one other medication with a known TdP risk. Those patients had nominally longer QTc length but without statistically significant difference (with vs. without, 419.3 ms vs 413 ms;  $W = 2409$ ;  $p = 0.31$ ).

We did not find significant difference in QTc length mean between Caucasian and African subjects (415.4 ms vs 411.7 ms;  $W = 813$ ;  $p = 0.86$ ). Additional population characteristics and variables associations with QTc length are shown in (Table I).

### Genetic associations

Of the 579 selected SNPs, 126 passed quality control (Figure S2). Fourteen SNPs were nominally significantly associated with QTc length ( $p < 0.05$ ), but only one (rs11911509 on *KCNE1*) remained significant after Bonferroni multiple testing correction ( $p = 3.84 \times 10^{-4}$ ;  $p_{\text{corrected}} = 0.049$ , Cohen  $f^2$  effect size = 0.131) (Table II). According to our adjusted model after further correction for ethnicity, the QTc length increases by 13.2 ms ( $\pm 3.6$  ms) for each additional minor allele (A vs C) of the rs11911509 SNP.

<Table II here>

The distribution of this SNP, rs11911509, was not different in the two ethnic groups observed in our population, defined as patients with European or African ancestry according to 1000 Genomes database ( $\chi^2 = 0.96$  ;  $df = 2$  ;  $p = 0.62$ ). Moreover, the eleven *KCNE1* SNPs were not impacted by population structure as all the maximum  $F_{ST}$  over *KCNE1* SNPs was of 0.12, especially the  $F_{ST}$  of the rs11911509 SNP which was of zero.

Overall comparison of QTc length found a significant difference with respect to rs11911509 SNP genotype ( $F = 6.46$ ,  $Df = 2$ ,  $p = 2.1 \times 10^{-3}$ ) (Figure S4). The average QTc length was significantly longer in AA than in AC+CC carriers (AA vs AC+CC : 430.4 ms ( $\pm 7.2$  ms) vs 412.2 ms ( $\pm 2.6$  ms) ;  $W = 2119$  ;  $p = 0.01$ ) (Figure 2). We also performed the analysis again after removing the 2 subjects with methadone dosage above 200 mg per day, one is AA carrier and the other is CC carrier. The overall comparison of QTc lengths across genotypes was still significant ( $F = 4.94$  ;  $Df = 2$  ;  $p = 8.3 \times 10^{-3}$ ) (Figure S5). Doing so, the mean QTc length was also significantly longer among AA carriers (AA vs AC+CC : 424.4 ms ( $\pm 4$  ms) vs 412.2 ms ( $\pm 2.6$  ms);  $W = 1970$  ;  $p = 0.03$ ).

The gene-based approach found a significant association of *KCNE1* with the QTc length using ethnic PCA dimensions as a covariate (number of used SNPs = 5,  $p_{\text{empirical}} = 0.02$ ) (Table S1).

### Methadone dosage x rs 11911509 SNP interaction



To test for interaction, we built a regression model with QTc length as dependant variable. As independent variables we included: (1) the methadone dosage, (2) the rs 11911509 SNP minor allele count, (3) their interaction, (4) independent variables that were associated with QTc length in univariate tests with an entry *p-value* below 0.1 (gender and antipsychotics co prescription), and (5) principal components to correct for ethnic stratification.

We found a significant interaction between methadone dosage and the number of rs11911509 SNP minor allele A ( $\beta = 0.18 \pm 0.07$ ;  $p = 0.01$ ) to predict QTc length. This model explains 15 % of the QTc length variance. The comparison of this model with the regression model that did not include the interaction found a significant improvement of the second model (15% vs 12% of QTc explained variance;  $F = 6.6$  ;  $p = 0.01$ ). Furthermore, The correlation between methadone dosage and QTc length was only significant among AA carriers ( $r_{pearson} = 0.65$  ;  $p = 4.6 \times 10^{-4}$ ) (Figure 3).

## DISCUSSION

The main finding of this study is the association of the *KCNE1* gene with the QTc length among French methadone users. The association was found both in gene-based approach and at an individual SNP level (rs11911509). Interestingly though, we have found a significant interaction between the rs11911509 genotype & the methadone dosage that could explain a non-negligible variance of the QTc length among patients at steady state of methadone maintenance treatment.

In our patient cohort, the median methadone daily dose was slightly higher than the usually prescribed dose in France (60 mg per day vs 51.3 mg per day) [39], but lower than in other non French studies [16–18] in which the median daily dose is commonly higher than 70 mg/day. The association between methadone daily dose and QTc prolongation is still debated. Genetic factors might account for the previously reported discrepancies between studies [15–20]. Here, we observed that the association between methadone dose and QTc length could be genotype dependent. Indeed, it was only significant among rs11911509 AA carriers. We conducted a previous study [29] that did not find a significant association between QT length and other *KCNE1* SNPs, but this could be due to lack of power, or because SNPs that were previously tested, i.e., *KCNE1* rs1805127, rs1805128 and rs2236609 are not implicated in the interaction of the methadone molecule and the KCNE1 Potassium voltage-gated channel protein. Those SNPs were not in linkage disequilibrium with the significant SNPs rs11911509 that we identified in the current study ( $r^2$ : 0.027 and 0.017 respectively). Furthermore, we herein tested a larger sample of SNPs (126 SNPs versus 3 SNPs in Hajj & al.) and recruited additional patients (154 subjects versus 82 subjects in Hajj & al.) [29], which allowed us to perform a gene-based approach that was not available in our previous methodology.

The rs11911509 SNP that we identified here is an intronic variant of the *KCNE1* gene with no known effect according to the Ensembl database [40], and no phenotype has been associated with this SNP to our knowledge. Nevertheless, according to the 1000 Genomes database (accessed via Ensembl), this variant is in a high linkage disequilibrium ( $r^2 \geq 0.8$ ) among European populations with several other SNPs (rs4817657, rs4816488, rs4817658, rs35983014, rs9984007, rs11701051, rs62211545, rs8128386, rs8131017 and rs11702790) that were previously associated with phenotypes involving QT prolongation. Thus, it may act as a proxy for other variations that are associated with lengthened

repolarization.

The *KCNE1* gene encodes for a transmembrane domain protein that combines with another protein coded by the *KNCQ1* gene to form a Potassium voltage-gated channel, also referred to as Minimal K<sup>+</sup> channel (MinK), with a slow activation compared to the homomeric KCNQ1 channels [41] and KCNH2 (hERG) channels. Mutations in *KCNE1* can cause Long QT syndrome [42] and is associated with an increased risk of atrial fibrillation [43]. Most of these mutations were associated with a reduction of the slowly activating potassium current [44], mechanism that partly explains QT prolongation [45]. But it is interesting to notice that some studies also involve *KCNE1* mutations in dysfunctions of the hERG potassium channel that mediates the rapid repolarizing current [46,47]. As a reminder, it's well established that methadone QT prolongation involves a potent blockade of the hERG potassium channel [21]. The hypothesis of a double disruption that may lead to an additive effect on QT lengthening can be proposed [48].

In our cohort, we found that women had a longer QTc interval than men, which is consistent with previous studies showing that women were more prone to drug-induced QTc prolongation. Some authors suggest that a greater polypharmacy and/or intrinsic factors linked to gender may explain such propensity [49]. To explore a potential polypharmacy effect, we used as proxy variables the methadone dosage and the Drugs lengthening QT score, and none of them was associated with sex (data not shown). Furthermore, neither allelic nor genotypic distributions of the rs11911509 SNP were different between genders. This suggests that the observed difference in QTc length between sexes may be explained by other not explored factors.

Association of antipsychotics co prescription with the QTc length did not reach significance in our cohort, but this prescription remains a risk factor of interest. Indeed, many studies pointed out the potential for antipsychotics to prolong the QTc interval [50], particularly in case of methadone maintenance treatment [51]. The lack of association that we observed may be due to insufficient statistical power to detect this effect.

A major strength of this study is that patients were included at steady state of methadone maintenance treatment, which excludes the confounding effect of methadone dosage fluctuations. Moreover, the drugs lengthening QT score allows us to weigh the propensity of each concomitantly prescribed medication to lengthen the QT. This method was previously proposed in a pharmacogenomics GWAS of QT interval [52].

It is important to note, however, that our study has also some limitations. First, the QTc interval was not prospectively assessed. Second, additional transient risk factors associated with prolonged cardiac repolarization such as potassium and magnesium serum concentrations were not available. Third, two subjects had a daily methadone dosage up to 200 mg, which can drive the associations. Nevertheless, after removing these individuals, the correlation between QTc length and Methadone remained significant. Moreover, none of those two individuals was influential (*Cook distance* < 1) when we performed a diagnosis of our genetic association models (data not shown). Fourth, we had two ethnic populations in our sample. However, we used Eigenstrat method to control for population stratification, which seems sufficient enough to control for type-I error rate inflation especially for SNPs with low  $F_{ST}$  on both SNP-based and Gene-based analysis [53]. Furthermore, we found that the effect size of genetic association of rs11911509 SNP with QTc length does not vary much when the analysis was restricted to Caucasian ( $n = 142$  ; *Cohen  $f^2$  effect size* = 0.135 vs 0.131 on

the whole sample), but then the association is not statistically significant, probably due to statistical power reduction. Finally, we did not perform an *a priori* power calculation for the genetic association, however, the achieved power for the presented results on 154 subjects was of 80 % ( $\alpha$  risk of  $4 \times 10^{-4}$ , and *Cohen's*  $f^2$  effect size of 1.131).

## CONCLUSION

In conclusion, we herein highlighted a gene x environment interaction explaining part of the variance of QT length during methadone maintenance treatment. Our clinical message is that among the rs11911509 SNP AA genotype carriers, where the QTc lengthening after methadone-increased dosage is more significant, QTc monitoring should be mandatory.

As this side effect may have potentially life-threatening consequences, and because methadone remains the most prescribed treatment for patients suffering from opiates dependence, further studies including *KCNE1* genotyping are needed to better explore the implication of the MinK channel in the pathogenesis of this side effect.

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## CONFLICT OF INTEREST

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Authors have no conflict of interest to declare with respect to the study topic

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## TABLES

**Table I:** Demographic and clinical variables and their association with QTc length

	Whole cohort N = 154	Ranges (min – max)	Association with QTc length (continuous variable) <i>p-value</i>	Normal QTc interval (n=116)	Borderline or prolonged QTc interval (n=38)
<b>Sex (men)</b>	76.6 % (118)	-	<b>0.03</b>	71.6 % (83)	92.1 % (35)
<b>Mean Age years (± SEM)</b>	40.9 (± 0.7)	20 – 64	0.62	41 (± 0.8)	40.6 (± 1.4)
<b>Methadone dosage mg (± SEM)</b>	63.8 (± 3.3)	7 – 320	<b>0.02</b>	61 (± 3.8)	72.3 (± 6.8)
<b>Co prescribed medications %(n)</b>					
Antidepressants	21.4 % (33)		0.45	21.6 % (25)	21.1 % (8)
Antipsychotics	22.1 % (34)		0.06	17.2 % (20)	36.8 % (14)
Benzodiazepines	34.4 % (53)	-	0.64	34.5 % (40)	34.2 % (13)
Mood stabilizers	3.9 % (6)		0.53	2.6 % (3)	7.9 % (3)
Other treatments that lengthen QTc #	9.7 % (15)		0.87	9.5 % (11)	10.5 % (4)
<b>Drugs lengthening QT score (± SEM)</b>	0.9 (± 0.1)	0 - 8	0.28	0.8 (± 0.1)	1.1 (± 0.3)
<b>Current alcohol intake %(n/N*)</b>	62.1 % (90/145)	-	0.39	62.4 % (68/109)	61.1 % (22/36)
<b>Current cocaine intake %(n/N*)</b>	13.2 % (12/91)	-	0.12	13.7 % (10/73)	11.1 % (2/18)
<b>Positive HIV serology %(n/N*)</b>	9.6 % (9/94)	-	0.32	9.5 % (7/74)	10 % (2/20)
<b>Positive HCV serology %(n/N*)</b>	52.7 % (48/91)	-	1	50.7 % (37/73)	61.1 % (11/18)

SEM : standard error of the mean

# : Medications that lengthen QTc other than antidepressants and antipsychotics.

\* : Data not available in all subjects

\*\* : Fisher's Exact Test

N : number of subjects with available data



**Table II:** Variants associated with QTc interval (nominal  $p < 5\%$  )

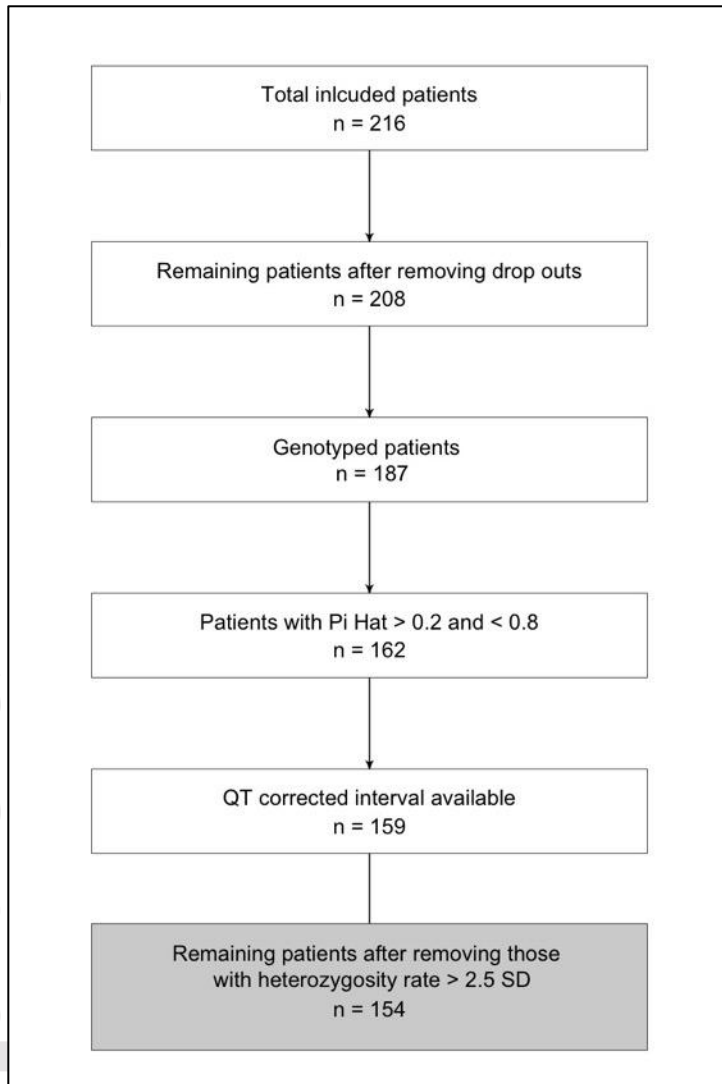
SNP	Locus	Chr.	Gene related position	Position (Build Gh37)	Minor/Major Allele	MAF	n	BETA (ms)	SE (ms)	$p$	$p_{corrected}$
rs11911509	KCNE1	21	intron variant	35841740	[A/C]	0.41	154	13.17	3.6	$3.83 \times 10^{-4}$	<b>0.049</b>
rs727957	KCNE1	21	intron variant downstream gene variant	35880072	[A/C]	0.17	154	13.67	5	$7.13 \times 10^{-3}$	0.91
rs3807375	KCNH2	7	intron variant non coding transcript variant	150667210	[A/G]	0.49	154	10	3.7	$7.72 \times 10^{-3}$	0.98
rs6599228	SCN5A	3	intron variant non coding transcript variant	38662961	[G/A]	0.44	153	-10.48	3.9	$7.86 \times 10^{-3}$	1
rs1337062	NOS1AP	1	intron variant NMD transcript variant	162063023	[G/A]	0.33	154	10.72	4	$8.17 \times 10^{-3}$	1
rs4657154	NOS1AP	1	intron variant NMD transcript variant	162108119	[A/G]	0.28	154	10.4	4.2	$1.41 \times 10^{-2}$	1
rs234868	KCNQ1	11	intron variant downstream gene variant non coding transcript variant	2859055	[G/A]	0.40	154	9.744	4	$1.51 \times 10^{-2}$	1
rs10157390	NOS1AP	1	intron variant NMD transcript variant	162247296	[C/A]	0.43	154	8.572	3.7	$2.25 \times 10^{-2}$	1
rs2070359	KCNE1	21	intron variant downstream gene variant	35826776	[A/G]	0.2	154	-10.47	4.7	$2.68 \times 10^{-2}$	1
rs347300	NOS1AP	1	intron variant NMD transcript variant	162299823	[G/A]	0.33	154	8.272	3.8	$3.19 \times 10^{-2}$	1
rs3807370	KCNH2	7	intron variant non coding transcript variant	150673314	[A/G]	0.37	154	-8.394	3.9	$3.23 \times 10^{-2}$	1
rs2106467	KCNQ1	11	intron variant non coding transcript variant	2635797	[A/C]	0.42	154	7.88	3.8	$3.81 \times 10^{-2}$	1
rs7528328	NOS1AP	1	intron variant NMD transcript variant	162247779	[G/A]	0.17	154	10.03	5	$4.71 \times 10^{-2}$	1

MAF : Minor allele frequency

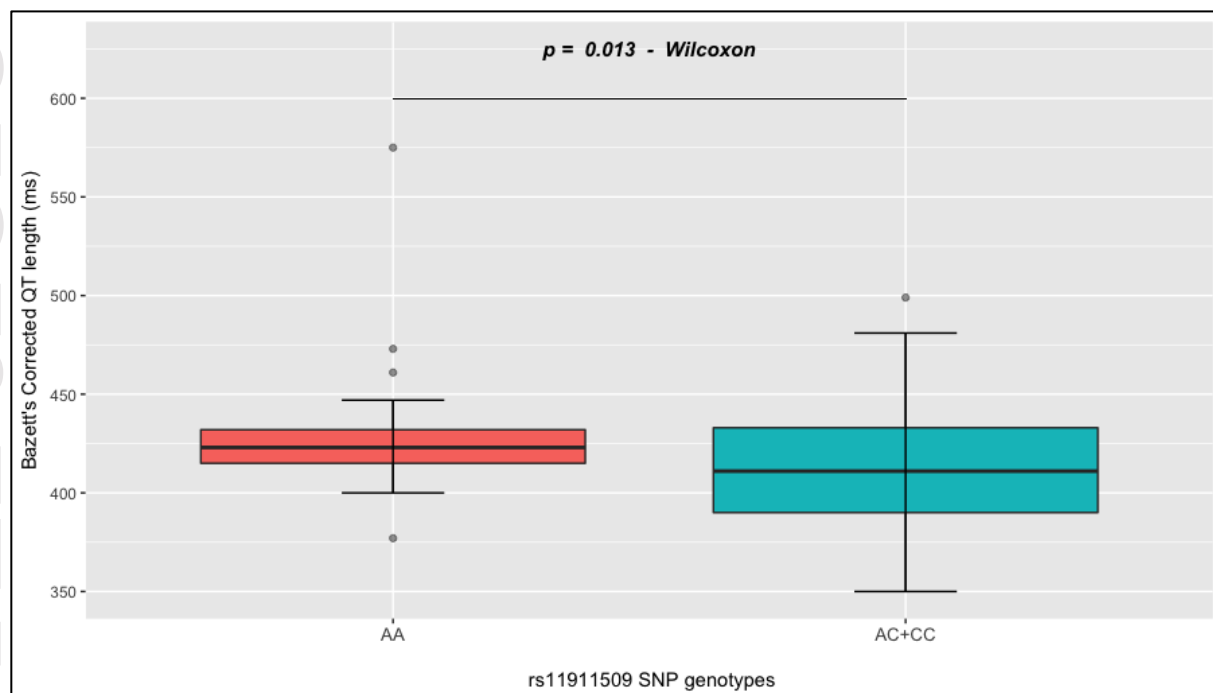
NMD : Nonsense-mediated decay

## FIGURES

Figure 1: Flowchart of patients' selection



**Figure 2** : Comparison of Bazett corrected QT length between AA and AC+CC carriers of the rs11911509 SNP genotypes (*KCNE1* gene)



**Figure 3** : correlations of Bazett corrected QT length with methadone dosage according to rs11911509 SNP genotypes.

