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Electrocardiogram Alterations Associated With Psychotropic Drug Use and CACNA1C Gene Variants in Three Independent Samples

Chiara Fabbri[1], Giuseppe Boriani[2], Igor Diemberger[3], Maria Giulia Filippi[4], Gloria Ravegnini[4], Patrizia Hrelia[4], Alessandro Minarini[5], Diego Albani[6], Gianluigi Forloni[6], Sabrina Angelini[4,*] and Alessandro Serretti[1,*]

[1]Department of Biomedical and NeuroMotor Sciences, University of Bologna, Bologna, Italy, [2]Department of Cardiology, Modena University Hospital, University of Modena and Reggio Emilia, Modena MO, Italy, [3]Department of Specialist, Diagnostic and Experimental Medicine, University of Bologna, Bologna, Italy, [4]Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy, [5]Department of Diagnostic, Clinical and Public Health Medicine, Section of Psychiatry, University of Modena and Reggio Emilia, Modena, Italy and [6]IRCCS - Institute for Pharmacological Research "Mario Negri", Milan, Italy

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Abstract: Several antipsychotics and antidepressants have been associated with QTc prolongation or other electrocardiogram (ECG) alterations, but their impact is still debated and other risk factors are known to affect QTc. We investigated the effect of antidepressants and antipsychotics on QTc and other ECG intervals/waves in three samples. Two discovery samples (cross-sectional sample n = 145 and prospective sample n = 68, naturalistic treatment) and a replication prospective sample (Clinical Antipsychotic Trials of Intervention Effectiveness, n = 515, randomized treatment) were analysed. In both prospective samples, baseline/follow-up changes in ECG parameters were analysed in relation to the number of psychotropic drugs stratified according to their known cardiovascular risk. In the cross-sectional sample, ECG parameters were compared among drugs with different risk profile. The possible effect of single nucleotide polymorphisms (SNPs) in the CACNA1C gene on QTc was also investigated. There was no evidence of mean QTc prolongation or increased risk of clinically relevant QTc prolongation (≥20 msec.) in association with psychotropic drugs stratified according to their known cardiovascular risk. The prescription of drugs with cardiovascular risk was less common in older individuals or individuals with cardiovascular comorbidities. Other factors (gender, baseline QTc, renal function) affected QTc. rs1006737 and SNPs in linkage disequilibrium with it modulated QTc duration/changes in all samples. An association between risk drugs and shorter RR interval or higher heart rate was found in all samples. A relevant effect of psychotropic drugs with cardiovascular risk on QTc duration was not observed. A number of factors other than psychotropic drugs may influence QTc. CACNA1C rs1006737 may modulate QTc in patients treated with psychotropic drugs.

A number of electrocardiogram (ECG) alterations have been associated with psychotropic drugs, but the most clinically relevant is QTc prolongation (i.e. prolongation of the interval representing electrical depolarization and repolarization of the ventricles). Indeed, QTc prolongation is a risk factor for serious adverse events, including torsade de pointes (TdP), a potential fatal cardiac arrhythmia [1]. Some antidepressant drugs (e.g. tricyclic antidepressants (TCAs) and citalopram [2]) and most antipsychotics have a potential to prolong QTc interval by inhibiting cardiac Na^+, Ca^{2+} and/or K^+ channels [3].

The most recent meta-analysis investigating the effects of antidepressant drugs on QTc showed that selective serotonin reuptake inhibitors (SSRIs) and TCAs appear to cause QTc prolongation by about 6 and 13 msec., respectively, as compared to placebo [2]. According to this meta-analysis, citalopram, escitalopram and sertraline were associated with greater prolongation than placebo, while fluoxetine and paroxetine were not. These results are based on randomized controlled trials (RTCs). Among antipsychotics, those associated with more QTc prolongation are sertindole, amisulpride, ziprasidone, iloperidone and risperidone (in decreasing order) [4]. Haloperidol has been associated with lower mean QTc prolongation, but cases of TdP and sudden death during treatment with haloperidol therapeutic doses have been reported [5]. Medications are not the only factors affecting QTc duration (and consequently TdP risk), as non-pharmacological variables are known to influence them, including gender, age, genetic factors, electrolyte disturbances, endocrine, metabolic and cardiovascular diseases [6–9]. Thus, the systematic evaluation of these factors is expected to be more effective in the prevention of arrhythmic events than the simple evaluation of QTc and/or of pharmacological risk factors. In recent years, several warnings were issued by regulatory authorities on the risk of ECG abnormalities among individuals exposed to psychotropic drugs. Nevertheless, there is the possibility that, in some cases, the risk of clinically significant QTc prolongation associated with psychotropic drugs has been overestimated. For example, the citalopram study that prompted safety warnings by regulatory authorities did not measure the proportion of individuals with QTc above a certain threshold, but it only reported a

Author for correspondence: Alessandro Serretti, Department of Biomedical and Neuromotor Sciences, University of Bologna, Viale Carlo Pepoli 5, 40123 Bologna, Italy (fax +39 051 521030, e-mail alessandro.serretti@unibo.it).
*These two authors jointly supervised the study.

mean QTc change from baseline to follow-up of 8.5 msec. with citalopram 20 mg/day, and 18.5 msec. with citalopram 60 mg/day [10]. No cases of citalopram-induced sudden cardiac death were reported among patients taking up to 60 mg/day of citalopram and free of risk factors for QTc prolongation and TdP [11]. Thus, it should be noted that a threshold of 5 msec. (as mean prolongation value) is generally used as the minimal difference for significance in population studies [5], but medications that prolong the mean QTc interval by more than 20 msec. are associated with increased risk of TdP (US Department of Health and Human Services). In terms of absolute QTc interval duration, values more than 500 msec. are considered at risk of TdP and this threshold has been suggested for drug discontinuation. A QTc of <450 msec. and 470 msec. for men and women, respectively, is generally deemed safe by most cardiologists as the cut-off before any intervention [5].

In addition to QTc interval prolongation, the use of antidepressant and antipsychotic drugs has been associated with several other ECG alterations, including QRS (that corresponds to the depolarization of the right and left ventricles) [12] and PR (the period that extends from the onset of atrial depolarization to the onset of ventricular depolarization) interval changes [13], heart rate modification [14,15] and morphological alterations of T and P waves [16,17]. These alterations have been much less studied than effects on QTc interval and they do not have established clinical significance.

As described, the prevalence and degree of QTc prolongation associated with psychotropic drugs are still debated. This study aimed to investigate changes in QTc duration in patients treated with antidepressants and/or antipsychotics to improve the current knowledge on the topic. Further, we evaluated whether other clinical factors and genetic polymorphisms in the CACNA1C (calcium channel, voltage-dependent, L-type, alpha-1C subunit) gene may be associated with QTc changes. It encodes for the ion permeating subunit of the alpha-1 subunit of cardiac L-type calcium channel (LTCC), which determines the main biophysical and pharmacological properties of the channel. The other reason for studying polymorphisms in this gene was that genetic variants of CACNA1C were associated with arrhythmic diseases (long QT syndrome and Brugada syndrome) [18]. Finally, CACNA1C has been also associated with different psychiatric disorders [19]; thus, variants in this gene are expected to show higher frequency in individuals with psychiatric disorders than in the general population. The possible association between psychotropic drugs and alterations in other ECG intervals/waves was investigated as secondary aim.

Materials and Methods

Samples.

Original samples. Samples were recruited among inpatients admitted at 'Ospedale Maggiore' in Bologna and outpatients under psychiatric care at 'Centri Ansia-Umore' of Bologna University. The inclusion criteria were as follows: (i) diagnosis of mood or psychotic disorder according to DSM-IV-R criteria; (ii) clinical indication for treatment with an antidepressant and/or antipsychotic drug; (iii) availability of at least one standard ECG recording; (vi) absence of unstable or severe medical conditions (e.g. recent acute myocardial infarction, acute or severe organ failure, cancer or severe chronic diseases); (v) absence of cognitive impairment or other conditions that would interfere with the ability to provide a valid informed consent; and (vi) pregnancy or breastfeeding. Polytherapy with psychotropic drugs or concomitant treatments for general medical conditions were not exclusion criteria, but they were considered in the analyses (see paragraph Statistical analysis and Data S1).

At the time of study entry, demographic characteristics, psychiatric and general medical diagnosis and current pharmacological treatments were recorded. A standard 12-lead ECG was prescribed to all patients at baseline as well as blood tests (including blood count, creatinine, sodium, potassium, magnesium and calcium plasma levels, transaminases). ECG and blood tests were repeated at discharge from hospital (inpatients) or after a change in treatment. ECG intervals as well as specific morphological alterations were evaluated independently by two cardiologists who were blind to genotypes and clinical information including drug prescription. Follow-up information was available in 68 patients (prospective sample). For 145 patients, no follow-up information was available (outpatients who did not provide the scheduled examinations or who had no clinical indications for follow-up examinations or dropped out from the study), so only baseline data were available (cross-sectional sample). A representation of the patients' selection process is provided in a flow chart in Figure S1.

All patients provided written informed consent after the explanation of the aims and procedures of the study. The protocol and the written informed consent were approved by the local ethics committee.

Clinical antipsychotic trials of intervention effectiveness sample. The Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study was a multi-centre, double-blind study that aimed to evaluate the relative effectiveness of a first-generation antipsychotic, perphenazine, compared with several second-generation antipsychotics (olanzapine, quetiapine, risperidone and ziprasidone), using a randomized design (phase 1). In phase 2, patients who stopped the first antipsychotic due to lack of efficacy were randomly assigned to receive either clozapine or an atypical antipsychotic (olanzapine, risperidone or quetiapine) different from the one taken in phase 1. Participants who stopped the first antipsychotic due to side-effects were randomly assigned to receive either ziprasidone or an atypical medication different from the phase 1 medication. If the phase 2 study drug was discontinued, individuals could enter phase 3, in which clinicians helped individuals to select an open-label treatment based on individuals' experiences in phases 1 and 2. Concomitant medications (including psychotropic drugs) were permitted throughout the trial, except for additional antipsychotic agents, and this was taken into account in the analyses (see paragraph Statistical analysis and Data S1). Further information about CATIE (including inclusion and exclusion criteria) was reported elsewhere [20] and in Figure S1.

For the purpose of the present study, all the three phases were considered. Indeed, standard ECG and blood tests (including blood count, creatinine, sodium, potassium, calcium) were recorded at baseline and at the end of each phase. For patients who lacked information of interest at the end of phase 1, those recorded at the end of phase 2 or 3 were considered. Using this method, 515 patients had both baseline and follow-up data referred to ECG and blood tests. A representation of the patients' selection process is provided in Figure S1.

Outcome under investigation. The primary outcome was the evaluation of the effect of psychotropic drugs on QTc interval and its possible modulation by CACNA1C polymorphisms. This outcome was investigated through the classification of psychotropic drugs according to their risk of inducing QTc prolongation (high–moderate

risk, low risk and no risk according to updated popular guidelines [21], see Table S1 for an overview). This classification was chosen because this study primarily aimed to test the applicability of previously reported pharmacological risk factors for QTc prolongation across naturalistic clinical settings (i.e. settings reflecting real clinical practice). QTc was calculated according to Bazett's formula ($QTc = QT/\sqrt{RR}$) [22]). Bazett's formula is the most popular in clinical practice, research and education, although its performance is different as heart rate varies [23]. Several measures of QTc duration were considered: 1) QTc per cent change between baseline and follow-up and QTc change ≥20 msec. in the two prospective samples; and 2) absolute QTc value and QTc >median or >450 msec. in the cross-sectional sample. QTc change ≥20 msec. and QTc >450 msec. were chosen because of their clinical significance [21,24]. The measures described in 1) and 2) were evaluated for association with a) change in the number of medications with moderate–high risk, low risk and the total number of medications with cardiovascular risk between baseline and follow-up in the two prospective samples both considering the whole sample and only individuals who were drug-free at baseline; b) the number of medications with moderate–high risk, low risk and the total number of medications with cardiovascular risk in the cross-sectional sample; and c) monotherapy (only one drug with cardiovascular risk) *versus* polytherapy (combinations of two or more drugs with cardiovascular risk) in all samples. Indeed, combination of psychotropic drugs was not an exclusion criterion in any of the analysed samples, but only in CATIE antipsychotic combinations were not allowed. The number of drugs with cardiovascular risk considered for analyses a) and b) was dichotomized (≤0 and >0).

The secondary outcome of the present study was the evaluation of the effect of psychotropic drugs on other ECG intervals (PR, QRS, RR (the interval between successive R waves) and QR (that corresponds to the first phase of ventricular depolarization)) and morphological alterations of T, ST and R waves. The effect of CACNA1C polymorphisms was not considered when investigating secondary outcome. Psychotropic drugs were classified according to the criteria described above. The following measures were considered when we investigated secondary outcome: (i) per cent change in the reported ECG intervals in both prospective samples and new observation of a morphological alteration in the reported waves in the original prospective sample; and (ii) duration of the reported intervals and observation of morphological alterations in the cross-sectional sample. The measures described in (i) and (ii) were evaluated for association with (a) change in number of medications with moderate–high risk, low risk and change in total number of medications with cardiovascular risk between baseline and follow-up in the two prospective samples; and (b) the number of medications with moderate–high risk, low risk and the total number of medications in the cross-sectional sample. The number of drugs with cardiovascular risk considered for analyses (a) and (b) was dichotomized as described above.

Single nucleotide polymorphisms selection and genotyping. The complete list of genotyped polymorphisms and their main characteristics is reported in Table S2. In the original samples, genotyping was performed by real-time PCR using Taqman® Assay (Thermo Fisher Scientific, Waltham, MA USA 02451) as recommended by the manufacturer. Details about single nucleotide polymorphisms (SNPs) selection and quality control are reported in Data S1.

In CATIE, 738 participants were genotyped by Perlegen Sciences using the Affymetrix 500K and Perlegen's custom 164K chip [25]. As the SNPs of interest were not available in the original data set, chromosome 12 was imputed using the Haplotype Reference Consortium (HRC version 1) panel as reference. Standard pre- and post-imputation quality control was performed and the SNPs of interest were extracted. Details are provided in Data S1.

Statistical analysis. Associations were investigated using linear or logistic regression models as appropriate. Covariates were selected for each outcome considering the impact of possible confounders. Heterogeneity between patients receiving or not risk drugs was also taken into account. In CATIE, ancestry-informative principal components were determined using Eigensoft [26,27] and they were used as covariates in all genetic analyses to account for different ancestry components. The use of non-psychotropic medications that can induce QTc prolongation was also considered. Further details are reported in Data S1.

In the two original samples, the possible impact of genetic polymorphisms on QTc duration or change was investigated as well as the possible interaction between polymorphisms and risk medications in determining the investigated phenotypes. In the latter case, a regression model including an interaction term, calculated as number of medications with cardiovascular risk (cross-sectional sample) or change in the number of medications with cardiovascular risk (prospective samples) × genotype, was included. Regression models included the same covariates used in the clinical analyses. Both dominant and recessive models were investigated.

In CATIE, the same method was applied but only SNPs showing evidence of association with outcome in at least one original sample or in linkage disequilibrium (LD) with them ($R^2 \geq 0.30$) were investigated.

Power and significance threshold. Associations were considered significant when they were replicated in the same direction in at least two of the analysed samples with p value < 0.05.

For continuous outcome, considering an alpha value of 0.05, the present study had a power of 0.80 to identify effect sizes of 0.07, 0.15 and 0.02 in the original cross-sectional, original prospective and CATIE samples, respectively. For dichotomous outcome, considering an alpha value of 0.05, the present study had a power of 0.80 to identify an OR of 1.85, 2.55 and 1.37 in the original cross-sectional, original prospective and CATIE samples, respectively. Power estimation was performed using G*Power 3.1 [28].

Results

The clinical demographic characteristics of the three samples are reported in Table S3. The variables with an impact on ECG parameters or that were heterogeneous between patients who received or not drugs with cardiovascular risk are summarized in Table S4. These variables included gender, age, GFR (Glomerular Filtration Rate), baseline QTc duration, calcium or magnesium plasma levels, cardiovascular comorbidities, ethnic group. These variables were used as covariates in the corresponding regression models.

QTc interval: clinical findings.

No individual had a QTc interval >500 msec., except one patient from the CATIE study. This was a 43-year-old, White female who had a baseline QTc = 423 msec.; at follow-up, she had QTc = 511 msec. and she switched from olanzapine to ziprasidone, while she did not take other medications with known cardiovascular risk.

In all the analysed samples, individuals who increased the number of medications with cardiovascular risk (prospective samples) or were taking medications with cardiovascular risk (cross-sectional sample) had no evidence of mean QTc increase or longer QTc interval, but the opposite was observed

(table 1). Indeed, in the original prospective sample, patients who increased the number of medications with moderate–high cardiovascular risk had a lower mean QTc per cent change compared with patients who did not change the number of medications with moderate–high risk ($p = 0.001$, Figure S2). The same association was found in CATIE when considering the variation in the total number of drugs with cardiovascular risk ($p = 0.01$, Figure S2). The same effect was observed when considering the risk of QTc increase ≥20 msec. in CATIE ($p = 0.02$, OR = 0.63, 95% CI = 0.43–0.93; Figure S3); a similar trend was found in the original prospective sample ($p = 0.07$; Figure S3). In the cross-sectional sample, patients who were not taking drugs with cardiovascular risk showed higher probability of longer QTc duration than the median value (380 msec.) compared with patients who were treated with these drugs ($p = 0.03$, OR = 0.44, 95% CI = 0.20–0.93). The same finding was observed when including only patients who were drug-free at baseline in the original prospective sample but not in CATIE (Table S5). When we compared patients treated with combinations of drugs with cardiovascular risk compared with patients treated with a single drug (Table S6), we found a trend of longer QTc duration in patients treated with polytherapies compared with monotherapy in the cross-sectional sample ($p = 0.07$).

QTc interval: pharmacogenetic findings.

The SNPs with evidence of association with QTc change or duration in at least one sample are reported in table 2, while an overview of all findings is reported in Table S7 for the original samples and Table S8 for CATIE. rs10848635 and rs2283326 showed an effect on QTc in both original samples, but with no consistency in the direction of the effect. rs1006737 had a consistent effect in the two original samples (table 2). In the prospective sample, minor homozygotes (AA) showed lower mean QTc per cent variation than carriers of the major allele (G) ($p = 0.01$) between baseline and follow-up. In addition, AA homozygotes did not show QTc prolongation when one drug with moderate–high risk was added while G allele carriers did ($p = 0.002$; Figure S4). In the cross-sectional sample, AA homozygotes showed lower mean QTc duration (msec.) than G allele carriers when at least one medication with moderate–high risk was prescribed ($p = 0.045$; Figure S4). In CATIE, four SNPs in LD with rs1006737 were associated with QTc increase in patients who received the prescription of drugs with cardiovascular risk. The rs1006737 A allele was found in LD with rs2007044 G allele, rs2239048 A allele, rs11062188 C allele and rs2239049 G allele, and carriers of these alleles showed a lower QTc per cent increase when the number of medications with cardiovascular risk was increased ($p = 0.01$). These findings are shown in Figure S4. Interestingly, rs2239048 was also associated with the risk of QTc prolongation ≥20 msec. in CATIE ($E = -1.69$, S.E. = 0.50, $z = -3.36$, $p = 0.0008$) and the protective A allele showed OR = 0.18 (95% CI = 0.07–0.49). rs2007044 G allele showed a trend of protective effect towards the risk of QTc prolongation ≥20 msec. ($E = 0.78$, S.E. = 0.45,

Table 1.
Association among moderate- to high-risk, low-risk and total risk medications (meds) and QTc in the analysed samples. Results are referred to the models corrected for covariates (see Table S4 for further information).

Sample – outcome	Moderate- to high-risk meds	Low-risk meds	Total risk meds
Prospective – QTc % change	$E = -0.14$, S.E. = 0.04, $t = -3.58$, $p = 0.001$	$E = -0.0005$, S.E. = 0.03, $t = -0.02$, $p = 0.99$	$E = -0.04$, S.E. = 0.03, $t = -1.39$, $p = 0.17$
Prospective – QTc change ≥20 msec.	$E = -11.40$, S.E. = 6.19, $z = -1.84$, $p = 0.07$	$E = 0.39$, S.E. = 0.73, $z = 0.54$, $p = 0.59$	$E = -0.22$, S.E. = 0.14, $z = -1.64$, $p = 0.11$
Cross-sectional – QTc duration	$E = -6.52$, S.E. = 6.90, $t = -0.95$, $p = 0.35$	$E = -4.70$, S.E. = 6.80, $t = -0.69$, $p = 0.49$	$E = -12.52$, S.E. = 6.81, $t = -1.84$, $p = 0.07$
Cross-sectional – QTc >median (380 msec.)	$E = -0.53$, S.E. = 0.40, $z = -1.31$, $p = 0.19$	$E = -0.26$, S.E. = 0.39, $z = -0.67$, $p = 0.50$	$E = -0.83$, S.E. = 0.39, $z = -2.12$, $p = 0.03$
CATIE – QTc % change	$E = 0.009$, S.E. = 0.005, $t = 1.62$, $p = 0.11$	$E = -0.005$, S.E. = 0.005, $t = -0.99$, $p = 0.32$	$E = -0.01$, S.E. = 0.005, $z = -2.48$, $p = 0.01$
CATIE – QTc change ≥20 msec.	$E = 0.21$, S.E. = 0.21, $z = 0.99$, $p = 0.32$	$E = -0.30$, S.E. = 0.19, $z = -1.56$, $p = 0.12$	$E = -0.46$, S.E. = 0.20, $z = -2.34$, $p = 0.02$

Meds, medications; CATIE, Clinical Antipsychotic Trials of Intervention Effectiveness.

Table 2.
Genetic association with $p < 0.05$ in the prospective original sample (A), cross-sectional sample (B) and CATIE (C).

SNP	Genetic model	Model	Results
(A)			
rs10848635	Dominant	SNP × tot risk meds	$E = -0.14$, S.E. = 0.05, $t = -2.59$, $p = 0.01$
rs1006737	Dominant	Non-interaction model	$E = -0.08$, S.E. = 0.03, $t = -2.65$, $p = 0.01$
		SNP × mod- to high-risk meds	$E = -0.19$, S.E. = 0.06, $t = -3.32$, $p = 0.0016$
rs1016388	Dominant	SNP × tot risk meds	$E = -0.16$, S.E. = 0.05, $t = -3.11$, $p = 0.003$
rs11615998	Recessive	Non-interaction model	$E = 0.12$, S.E. = 0.05, $t = 2.50$, $p = 0.02$
rs2283326	Recessive	Non-interaction model	$E = 0.05$, S.E. = 0.02, $t = 2.28$, $p = 0.03$
rs215976	Recessive	Non-interaction model	$E = 0.06$, S.E. = 0.02, $t = 2.58$, $p = 0.01$
(B)			
rs1006737	Dominant	SNP × mod- to high-risk meds	$E = -46.63$, S.E. = 22.97, $t = -2.03$, $p = 0.045$
rs2283326	Dominant	SNP × low-risk meds	$E = -59.19$, S.E. = 27.01, $t = -2.19$, $p = 0.03$
rs10848635	Recessive	Non-interaction model	$E = 15.37$, S.E. = 7.53, $t = 2.04$, $p = 0.04$
rs11062296	Recessive	Non-interaction model	$E = 21.46$, S.E. = 10.01, $t = 2.14$, $p = 0.03$
rs1034936	Recessive	SNP × mod- to high-risk meds	$E = -29.52$, S.E. = 14.44, $t = -2.04$, $p = 0.04$
(C)			
rs1034936	Dominant	SNP × tot risk meds	$E = 2.65e\text{-}02$, S.E. = 1.30e-02, $t = 2.04$, $p = 0.04$
rs2007044	Recessive	SNP × tot risk meds	$E = -2.32e\text{-}02$, S.E. = 1.15e-02, $t = -2.02$, $p = 0.04$
rs2239048, rs11062188, rs2239049	Recessive	SNP × low-risk meds	$E = -0.03$, S.E. = 0.01, $t = -2.53$, $p = 0.01$
rs1016388	Recessive	SNP × tot risk meds	$E = -2.34e\text{-}02$, S.E. = 1.10e-02, $t = -2.13$, $p = 0.03$
rs2283326	Recessive	SNP × mod- to high-risk meds	$E = 3.07e\text{-}02$, S.E. = 1.11e-02, $t = 2.78$, $p = 0.006$
		SNP × tot risk meds	$E = 2.57e\text{-}02$, S.E. = 1.04e-02, $t = 2.46$, $p = 0.01$
rs10848677	Recessive	SNP × mod- to high-risk meds	$E = 0.02$, S.E. = 0.01, $t = 2.08$, $p = 0.038$

CATIE, Clinical Antipsychotic Trials of Intervention Effectiveness; SNPs, single nucleotide polymorphisms.

$z = -1.74$, $p = 0.08$). The possible interactive effect rs1006737 × medications towards the risk of QTc prolongation ≥20 msec. was not evaluable in the original prospective sample, due to the small sample size, while there was only a slight trend of association in the cross-sectional sample ($p = 0.11$).

rs2283326 and rs1034936 were associated with QTc in a consistent way in CATIE and in the original cross-sectional sample. For both SNPs, minor allele carriers showed a protective effect (table 2).

Finally, the effect of rs1016388 on QTc was not consistent between CATIE and the original prospective sample (table 2).

Other ECG intervals.
The most robust finding was the association between the prescription of medications with high–moderate risk and the decrease in RR interval duration or increase in heart rate that was found in all samples (Table S9). CATIE patients who received at least one additional medication with moderate–high risk compared with baseline had a heart rate increase of 7.82 ± 25.56% compared with baseline value.

No other association was replicated at least in two samples. An overview of all findings is reported in Table S9.

Discussion

Overview of findings.
In the three analysed samples, no evidence of QTc prolongation or longer QTc duration was found in patients treated with psychotropic drugs with previously reported cardiovascular risk. On the contrary, patients who received the prescription of at least one additional drug with cardiovascular risk compared with baseline showed lower QTc mean prolongation and lower risk of QTc prolongation ≥20 msec. at follow-up in the prospective original sample and in CATIE (table 1). In the cross-sectional sample, higher QTc mean values were found in individuals who were not treated with drugs with cardiovascular risk, in line with findings in the two prospective samples (table 1). These results can be explained taking into account other factors affecting QTc duration. In the original prospective sample, patients who did not receive the prescription of drugs with moderate–high cardiovascular risk were older ($p = 0.04$) and they had higher prevalence of cardiovascular diseases ($p = 0.025$). Non-significant trends in the same direction were found in the cross-sectional sample while in CATIE, the prescription of risk drugs was different across ethnic groups (European, African American and other ethnic groups, $p = 0.002$) and Europeans showed higher QTc duration at baseline ($p = 0.03$) according to previous findings that supported interethnic differences in QTc duration [29]. Further, other non-pharmacological factors were found to modulate QTc change or duration (gender, baseline QTc duration and renal function). Only the combination of more than one drug with cardiovascular risk showed a trend of association with longer mean QTc duration ($p = 0.07$ in the cross-sectional sample, Table S6). We suggest that the lower prescription of psychotropic drugs with cardiovascular risk in individuals with pre-existing risk factors for QTc prolongation, including the ones reported above, is partly responsible for the obtained results, probably in the context of clinicians' awareness of

the cardiovascular risks associated with some psychotropic drugs. Previous population studies investigating the effect of antidepressants and antipsychotics on QTc interval duration are useful to interpret the present findings as they reflect real clinical practice settings. A longitudinal study including 8222 individuals investigated QTc duration, changes and abnormal prolongation in association with psychotropic drug use, and it reported shorter mean QTc duration in users of several psychotropic drugs previously associated with QTc prolongation as compared with non-users (e.g. perphenazine and phenothiazines as a group, quetiapine, sulpiride and clozapine [30]). A cross-sectional population study that included 2411 patients did not find associations between the use of haloperidol, citalopram or escitalopram in addition to antipsychotic drugs, and QTc prolongation, whereas the use of two or more antipsychotic drugs was positively associated with QTc prolongation [31]. The same study reported that only 1% of patients treated with risk psychotropic drugs showed a QTc >500 msec. and 3% had a QTc exceeding 480 msec. Another interesting study, examining severe cardiac adverse drug reactions in 169,278 psychiatric inpatients treated with antidepressants [32], reported QTc interval prolongation above 500 msec. in 18 cases (0.01%) associated with TCAs prescription; alternative explanations for the QTc interval prolongation or pre-existing risk factors were suspected in 11 cases. On the other hand, a study based on the examination of electronic health records of 38,397 patients receiving antidepressant drugs demonstrated an effect of amitriptyline, citalopram and escitalopram on QTc interval whereas no effect of the TCA nortriptyline was observed [33]. Other similar inconsistencies can be found in naturalistic studies. For example, a study on a sample of 1006 institutionalized schizophrenic patients on long-term antipsychotic treatment found longer QTc intervals in patients treated with clozapine compared to risperidone and typical antipsychotics; in particular, risperidone, perphenazine, chlorpromazine and haloperidol had no significant effect on QTc prolongation [34]. The result was confirmed after correction for cardiovascular disease, while metabolic alterations, including obesity and diabetes, that are associated with clozapine, were not considered, but they could have influenced the findings [8]. Thus, taking into account the results of previous naturalistic studies and the present findings, the prevalence of significant QTc prolongation and severe cardiac adverse events in association with risk psychotropic drugs is probably low. Inconsistent findings have been reported about which drugs may be responsible of QTc prolongation or cardiac adverse events. Overall, it is known that several demographic and clinical factors affect QTc duration [8] and they may have more relevance together in influencing QTc than psychotropic drugs in real clinical settings.

Genetic factors are also known to affect QTc interval duration. Candidate gene studies investigated mainly polymorphisms in genes coding for ion channels involved in myocardiocyte depolarization and repolarization, particularly subunits of potassium channels [35], sodium (SCN5A) ion channels [36,37] and calcium channels (CACNA1C [18,38–40]. Other studies were focused on genes coding for enzymes involved in drug pharmacokinetics (e.g. CYP450 isoenzymes) [41,42] and the nitric oxide synthase 1 adaptor protein gene (NOS1AP) that affects cardiac repolarization [9]. The present study investigated the association between 13 polymorphisms in the CACNA1C gene and QTc duration; possible interactions between polymorphisms and prescription of drugs with cardiovascular risk were considered. rs1006737 A allele was associated with a protective effect towards QTc prolongation in patients treated with psychotropic drugs with moderate–high cardiovascular risk in the original perspective sample. The same allele was associated with lower mean QTc duration in patients treated with these drugs in the cross-sectional sample. In CATIE, four alleles in LD with the rs1006737 A allele (rs2007044 G, rs2239048 A, rs11062188 C and rs2239049 G) were found to have a protective effect towards QTc prolongation, in line with the findings in the original prospective sample. The rs1006737 variant is located in an intronic region, and it has been associated with increased risk of several psychiatric disorders [19]. Interestingly, recently a case report suggested the hypothesis that this polymorphism may confer susceptibility to both Brugada syndrome and bipolar disorder [43].

In regard to secondary outcome, the present study found an association between psychotropic drugs with cardiovascular risk and reduced/shorter RR interval in the two original samples and higher heart rate in CATIE. Noradrenergic antidepressants, inhibiting noradrenaline reuptake, were reported to increase heart rate [15], an effect that is common to several psychotropic drugs through different combination of α-adrenergic and muscarinic antagonist mechanisms. Indeed, TCAs, first-generation (e.g. phenothiazines) and second-generation (e.g. clozapine, olanzapine, quetiapine, risperidone, paliperidone) antipsychotics, have been associated with increased heart rate or tachycardia [44–47]. A growing body of epidemiological and clinical evidence has shown that high resting heart rate even within the accepted normal range is independently associated with increased risk of cardiovascular mortality and all-mortality causes [48,49]. Thus, high resting heart rate should not be overlooked in patients treated with psychotropic drugs, particularly because pharmacological heart rate control can be easily obtained.

Strengths and limitations.

The limitations of the study have to be considered. Firstly, the limited size of the original samples probably influenced the power of detecting significant effects. It should be noted that this was the reason why we used multiple samples (including CATIE, n = 515) to test our hypotheses. Secondly, samples were heterogeneous in terms of study design (cross-sectional and longitudinal) as well as clinical demographic characteristics. On the other hand, this could be seen as strength as findings are expected to be reproducible in different clinical contexts and reflect a real clinical scenario. Similarly, mild cardiac diseases such as non-severe cardiac valvulopathies, mild hypertensive heart disease or past myocardial infarction

with conserved ventricular function were not considered as exclusion criteria, and this may be interpreted as a limitation as well as a reflection of real clinical world. Thirdly, the effect of single drugs or classes of drugs was not investigated due to sample size issues. To the best of our knowledge, this is the first study that investigated ECG alterations in association with psychotropic drugs classified as having moderate–high, low and no cardiovascular risk according to previous findings. This classification may seem a simplification that does not take into account the different pharmacodynamic profiles of psychotropic drugs, but it was a strategy to study the cardiac effects of these drugs in real clinical settings. Thus, this study was an attempt to verify relatively established research findings (included in clinical guidelines) in real clinical settings. We had to choose *a priori* to refer to one classification of psychotropic drugs' cardiovascular risk, resulting in potential bias; thus, we decided to refer to widespread clinical guidelines and not to new and poorly replicated findings. Despite its popularity in clinical practice, research and education, Bazett's formula shows a different performance depending on heart rate [23]. Indeed, Bazett's formula produces higher QTc values above 60 bpm (beats per minute) compared with other formula and generally lower values below 60 bpm. Despite this limitation, we decided to use this formula because of its widespread use and consequently higher comparability of results with other studies. Interestingly, our results showed lower mean QTc change or duration in patients who increased the number of medications with cardiovascular risk, and in this group, an increase in heart rate was demonstrated. Thus, the lower risk of QTc prolongation (prospective samples) or shorter QTc duration (cross-sectional sample) cannot be a consequence of heart rate variation. Finally, other genetic and non-genetic factors have probably contributed to the observed changes in QTc and other ECG variables.

Conclusions

The present study found no evidence of QTc interval prolongation in patients treated with psychotropic drugs with moderate–high or low cardiovascular risk compared with patients not treated with these drugs. On the contrary, patients who were not treated with these drugs showed higher QTc mean prolongation and higher risk of QTc prolongation ≥20 msec. in the prospective original sample and in CATIE, probably as a result of non-pharmacological factors (such as age, concomitant cardiovascular diseases and race). These results suggest that popular classifications of psychotropic drugs' cardiovascular risk (that are often based on randomized trials) do not necessarily reflect what is observed in naturalistic clinical settings. CACNA1C rs1006737 SNP and SNPs in LD with it may influence the risk of QTc prolongation in patients treated with psychotropic drugs with cardiovascular risk. The effect of medications with cardiovascular risk on heart rate was confirmed in all the analysed samples, suggesting that a careful monitoring of heart rate and treatment of high resting heart rate should be considered.

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Disclosures

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Flow chart summarizing inclusion/exclusion criteria for the analyzed samples.

Figure S2. QTc mean percent change in patients who did not change the number of prescribed drugs with cardiovascular risk (drug change = 0) and those who increased the number of drugs with cardiovascular risk (drug change = 1) compared to baseline in the original prospective sample and in CATIE

Figure S3. Number of subjects showing or not a QTc increase ≥20 msec. in CATIE (A.) and in the original prospective sample (B.) depending on the increase in the number of medications with cardiovascular risk

Figure S4. rs1006737 effect on QTc in the two original samples (A). Effects of SNPs in LD with rs1006737 (rs2007044 and rs2239048 (in complete LD with rs11062188 and rs2239049)) on QTc change in CATIE (B). rs1006737 A allele was found in LD with rs2007044 G allele, rs2239048 A allele, rs11062188 C allele and rs2239049 G allele.

Table S1. Classification of psychotropic drugs according to their cardiovascular risk.

Table S2. Description of the SNPs genotyped in the original samples (A) and those analyzed in the CATIE sample for replication (B).

Table S3. Clinical-demographic characteristics of the investigated samples.

Table S4. Summary of the covariates included in regression models in the original prospective sample (A), cross-sectional sample (B) and CATIE (C).

Table S5. Association among the absolute number of total, moderate-high risk and low risk drugs and QTc in patients who were drug-free at baseline in the prospective original sample (n = 31) and in the CATIE (n = 112).

Table S6. Comparison of QTc duration in patients treated with only one medication with cardiovascular risk *versus* patients treated with combinations of risk drugs in the original prospective sample, cross-sectional sample and in the CATIE.

Table S7. Association between CACNA1C polymorphisms and QTc change and duration in the prospective (A.1 and A.2) and in the cross-sectional (B.1 and B.2) sample.

Table S8. Associations between SNPs and QTc change in CATIE (A: dominant model; B: recessive model).

Table S9. Association among moderate-high risk, low risk and total risk medications (meds) and ECG parameters other than QTc.

Data S1. Materials and methods.