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Polymorphisms in *ERBB4* and *TACR1* associated with dry mouth in clozapine-treated patients

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Abstract

Objective:

Sialorrhea is a common and uncomfortable adverse effect of clozapine, and its severity varies between patients. The aim of the study was to select broadly genes related to the regulation of salivation and study associations between sialorrhea and dry mouth and polymorphisms in the selected genes.

Methods:

The study population consists of 237 clozapine-treated patients, of which 172 were genotyped. Associations between sialorrhea and dry mouth with age, sex, BMI, smoking, clozapine dose, clozapine and noreclozapine serum levels, and other comedication were studied. Genetic associations were analyzed with linear and logistic regression models explaining sialorrhea and dry mouth with each SNP added separately to the model as coefficients.

Results:

Clozapine dose, clozapine or noreclozapine concentration and their ratio were not associated with sialorrhea or dryness of mouth. Valproate use ($p=0.013$) and use of other antipsychotics ($p=0.015$) combined with clozapine were associated with excessive salivation. No associations were found between studied polymorphisms and sialorrhea. In analyses explaining dry mouth with logistic regression with age and sex as coefficients, two proxy-SNPs were associated with dry mouth: *epidermal growth factor receptor 4 (ERBB4)* rs3942465 (adjusted $p=0.025$) and *tachykinin receptor 1 (TACR1)* rs58933792 (adjusted $p=0.029$).

Conclusion:

Use of valproate or antipsychotic polypharmacy may increase the risk of sialorrhea. Genetic variations in *ERBB4* and *TACR1* might contribute to experienced dryness of mouth among patients treated with clozapine.

Keywords: clozapine, adverse effects, sialorrhea, xerostomia, pharmacogenetics

Significant outcomes

1. The polymorphisms studied were not associated with sialorrhea, but two proxy-SNPs in *ERBB4* and *TACRI* genes were associated with dry mouth.
2. Use of valproate and antipsychotic polypharmacy increased the risk of sialorrhea

Limitations

1. Smaller effects may have been missed because of the small number of genotyped patients compared to a large number of polymorphisms, even after limiting the number of SNPs by minor allele frequency and using proxy SNPs representing multiple linked SNPs.
2. The amount of salivation and dryness of mouth was assessed with quite limited questions.
3. Patients who had stopped clozapine medication because of severe sialorrhea may have been selected out of the data because most of the patients had used clozapine for over a year.

Introduction

Clozapine is the most efficient antipsychotic drug for patients with treatment resistant schizophrenia (Lally, MacCabe 2015). It has fewer extrapyramidal adverse effects than previous antipsychotic drugs, but several other adverse effects limit its use. Hypersalivation is one of the most common adverse effects of clozapine. It is experienced by approximately 30 percent of patients, but the percentage varies in the literature. (Praharaj, Arora et al. 2006) The highest reported prevalence of sialorrhea is 91.8 percent of the clozapine-treated patients (Maher, Cunningham et al. 2016). Sialorrhea is negatively affecting patients' life. It causes discomfort and psychological complications, for example, embarrassment and low self-esteem. (Liang, Ho et al. 2010) One severe but rare possible impact of sialorrhea is the aspiration of saliva which can lead to aspiration pneumonia (Bird, Smith et al. 2011).

Sialorrhea is among the most common reasons for discontinuing clozapine (Legge, Hamshere et al. 2016). Dry mouth is also a common adverse effect and it is caused by the anticholinergic properties of clozapine (Kelly, Conley et al. 2003). Dry mouth is not known to be among the reasons for discontinuing clozapine, but it might be accompanied with other anticholinergic symptoms such as tachycardia, dizziness, and constipation which are common reasons for discontinuation. (Legge, Hamshere et al. 2016)

The mechanisms behind clozapine-induced sialorrhea are multifactorial. Clozapine acts as an antagonist or agonist to many neurotransmitters that also play roles in the regulation of salivation. Salivary glands are controlled by the parasympathetic nervous system which releases acetylcholine to bind to muscarinic receptor 3 (CHRM3) and muscarinic receptor 1 (CHRM1) (Tobin, Giglio et al. 2009). Clozapine is an antagonist to CHRM1, muscarinic receptor 2 (CHRM2), CHRM3 and muscarinic receptor 5 (CHRM5) and alfa-2 adrenoreceptor (ADRA2A) but an agonist to muscarinic receptor 4 (CHRM4). Animal studies suggest that clozapine's antagonism to CHRM1 in acinar cells causes salivary secretion (Ekström et al 2010). One hypothesis is that ADRA2A antagonists increase salivary output by sympathetic stimulation. (Phillips, Szchadi et al. 2000). Another hypothesis is that clozapine's agonism to CHRM4 in salivary glands leads to increase in secretion. (Zorn et al. 1994). Clozapine's antimuscarinic effects at CHRM2 and CHRM3 may disturb the function of pharyngeal and esophageal smooth muscles leading to impaired swallowing of saliva. (Praharaj, Arora et al. 2006). Clozapine also has a high affinity, as an antagonist, for dopamine D4 receptors (DRD4). Dopamine D4 receptors are expressed in the prefrontal cortex and in the nucleus tractus solitarius and nucleus ambiguus which play prominent roles in different phases of swallowing. (Rajagopal, Sundaresan et al. 2014)

Substance P acts as a neurotransmitter in the parasympathetic nervous system and is a neuropeptide known to enhance the swallow response. (Jin, Sekizawa et al. 1994) Substance P binds to the tachykinin 1 receptor (TACR1) (Severini, Improta et al. 2002). In salivary glands, there are also epidermal growth factor receptors (ErbB) for epidermal growth factor (EGF). Clozapine affects ErbB kinases which leads to downregulating EGF. (Kobayashi, Iwakura et al. 2019) EGF has an important role in protecting the oral cavity. (Sarosiek, Bilski et al. 1988)

The activity of G-protein receptor kinases GRK2, GRK3, and GRK5 in cholinergic neurons increases sensitivity to muscarinic receptors (Daigle, Caron 2012). There has been

found an association between clozapine and expression of G-protein receptor kinases (Ahmed, Gurevich et al. 2008). Vasoactive intestinal peptide (VIP) increases salivary gland blood flow and protein secretion and, in rats, evokes fluid secretion. Also, a synergistic interaction between VIP and clozapine has been found. When rats were given a combination of clozapine and VIP, saliva secretion increased more than when given VIP and clozapine separately (Ekström, Godoy et al. 2014).

There have been some studies that have formerly found an association between clozapine-induced sialorrhea and genetic polymorphisms. A single nucleotide polymorphism (SNP) in the ADRA2A gene (rs1800544) has been reported to be associated with sialorrhea among patients treated with clozapine. Increased sialorrhea was associated with ADRA2A rs1800544 CC genotype in comparison with G-allele carriers (CG and GG genotypes). (Solismaa, Kampman et al. 2014). A 120-base pair duplication in DRD4 has also been reported to be associated with clozapine-induced sialorrhea. (Rajagopal, Sundaresan et al. 2014).

As the genetic background of clozapine-induced sialorrhea is still poorly known, it is necessary to further investigate possible genetic associations with sialorrhea and dry mouth by selecting a broad group of genes (*TACRI*, *VIL1*, *EKBBI*, *ERBB2*, *ERBB4*, *GRK2*, *GRK5*, *CHRM1*, *CHRM3*, *CHRM4*, *ADRA2A* and *DRD4*) related to salivary gland regulation. Proteins coded by selected genes have roles in the regulation of saliva secretion or swallowing or are present in salivary glands as explained above. The study explores the possible genetic mechanisms behind the variation in intensity of sialorrhea and dry mouth among patients treated with clozapine.

Material and methods

The study population consists of 237 clozapine treated patients who have diagnosis of schizophrenia, schizoaffective or other nonorganic and non-affective psychoses according to the International Classification of Diseases, 10th Revision (World Health Organization (WHO) 1993). All patients were over 18 years old, Caucasian, of Finnish origin. Written consent was collected from all the patients. The study was conducted in three hospital districts in Finland (Satakunta, Pirkanmaa and Southern Ostrobothnia). The Ethics Committee of Satakunta Hospital District approved the study. Characteristics of the patients are presented in Table 1.

Fasted blood samples for concentration measurements were collected in the morning, following a 12-hour interval post the evening dose. All patients were on a stable dosage of

clozapine. The concentrations of clozapine and norclozapine in serum were quantified through the application of liquid chromatography in a commercial laboratory.

Measuring of salivation was carried out by using the Liverpool University Neuroleptic Side Effect Scale (LUNSERS) (Day, Wood et al 1995) where patients rate the severity of 51 different side effects from 0-4 (0 = not at all, 1 = very little, 2 = a little, 3 = quite a lot and 4 = very much).

The salivation was analyzed in three ways. First, the answer of LUNSERS symptom number 37 (over-wet drooling mouth) was divided into two different variables: 'excessive salivation' and 'non-excessive salivation'. Secondly, patients using tricyclic antidepressants (TCA) for excessive salivation were added to the 'excessive salivation' group even if they did not report sialorrhea in the questionnaire. Of the 32 patients using TCA, two were excluded because their indication for this medication was not sialorrhea.

LUNSERS symptom number 6 (dry mouth) was also divided into two groups (patients who did not have dryness of the mouth at all (0) vs. those who had from very little to very much dryness of mouth (1-4)) and analyzed as a dichotomous variable. Number and percentage of patients reporting excessive salivation or dry mouth are presented in Table 1.

Genotyping and imputation

Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp DNA Blood Midikit and an automated biorobot M48 extraction (Qiagen, Hilden, Germany). Samples were genotyped using an Illumina Infinium HumanCoreExome-12 DNA Analysis Beadchip, version 1.0., according to the manufacturer's instructions at Helmholtz Zentrum, München, Germany. The following quality control filters were applied: GenCall score < 0.15 , GenTrain score less than 0.20, sample and an SNP call rate less than 0.95, Hardy-Weinberg equation P value less than $\times 10^{-6}$, excess heterozygosity, cryptic relatedness ($\pi\text{-hat} > 0.2$), gender check, and multidimensional scaling. After quality control, genotype samples and 531,983 SNPs were available for 172 patients.

Imputation was performed in two stages: haplotype phasing was done using SHAPEIT v2 and genotype imputation using IMPUTE2 v. 2.3.2 and 1000 Genomes Phase I integrated variant set haplotypes as a reference. SNPs with $\text{info} \geq 0.3$ were considered well imputed.

A total of 9039 polymorphisms (SNPs including insertions and deletions) from the selected genes (17 *ADRA2A*, 32 *CHRM1*, 1592 *CHRM3*, 6 *CHRM4*, 36 *DRD4*, 580 *ERBB1*,

57 *ERBB2*, 4879 *ERBB4*, 21 *GRK2*, 901 *GRK5*, 365 *IP3*, 463 *NK1*, 73 *NTS*, and 17 *VIP*) were available after genotyping and imputation for analyses.

Statistical methods

To assess the distribution of continuous variables, Kolmogorov-Smirnov test was applied. Exploratory two-variable comparisons between sialorrhea or dry mouth and age, sex, BMI, smoking, clozapine and norclozapine concentrations, clozapine/norclozapine ratio, use of other medications (antidepressants, other antipsychotics, mood stabilizers, benzodiazepines, and blood pressure medication) were analyzed with t-test, or Mann-Whitney U test if the variable was not normally distributed, and Pearson's chi-squared test. For the analyses, all antipsychotic doses were converted to chlorpromazine equivalents (Aronson JK 2009).

Genetic associations were analyzed with multiple linear and logistic regression models explaining sialorrhea and dry mouth with each SNP tested separately in the model as coefficients and including other coefficients selected after the exploratory analyses or otherwise hypothesized to be linked with sialorrhea or dry mouth. P values were adjusted using the false discovery rate (FDR) method for multiple testing correction. Due to the high number of SNPs related to the number of patients genotyped, a clumping of SNPs was performed by using one proxy SNP for SNPs that had a linkage disequilibrium of $r^2 > 0.2$ with a window of one million base pairs (500k base pairs downstream and 500k pairs upstream). Selection of the proxy SNP was made favoring the lowest p-value in the logistic and linear regression models and the lowest minor allele frequency (MAF). SNPs with a MAF below 0.1 were excluded. Best fitting models were selected. The combined effect of multiple proxy SNPs with an FDR-adjusted $p < 0.05$ was analyzed by forming a genetic risk score by multiplying the dosage of the SNP by the estimate in the previous regression models and furthermore summing the estimate-weighted dosages of the SNPs.

Results

Age, sex, regular smoking, and BMI were not associated with sialorrhea or dryness of mouth in clozapine-treated patients.

Potential factors relating to the mouth dryness

Clozapine dose, clozapine or norclozapine concentration and clozapine to norclozapine ratio were not associated with dryness of mouth. There was a trend of dose of selective serotone reuptake inhibitor (SSRI) medication being associated with dryness of the mouth: mean doses were 8.17 ± 16.0 fluoxetine equivalent mg in patients who did not report

dryness of mouth vs 13.4 ± 20.3 mg fluoxetine equivalent mg in those who did (Mann-Whitney $U = 4055$, $p = 0.055$).

Potential factors relating to the sialorrhea

Clozapine dose, clozapine or norclozapine concentration and clozapine to norclozapine ratio were not associated with excessive salivation.

Chi square test statistics of comparisons between use of different additional medications and excessive salivation are presented in Table 2. Patients using mood stabilizers suffered from excessive salivation more commonly than the group that did not use mood stabilizers. In more detailed comparisons, the association seems to be related particularly to patients on valproate medication reporting excessive salivation whereas other mood stabilizers were not associated with sialorrhea. Patients who used other antipsychotics in addition to clozapine also had increased salivation. The higher dose of other antipsychotic drug was associated with excessive salivation (Mann-Whitney $U = 5157$, $p = 0.009$). Doses of antipsychotics other than clozapine are presented in Figure 1 in non-excessive and excessive salivation groups. There was a trend of benzodiazepine users having more salivation compared to those who do not use benzodiazepines when the use of tricyclic antidepressants was included in the 'excessive salivation' variable. Beta-blockers and blood pressure medication were not associated with excessive salivation or dryness of the mouth.

Potential roles of genetic variations in the development of dry mouth following clozapine therapy

After clumping, the number of polymorphisms decreased from 9039 to a total of 97 proxy SNPs. In linear and logistic regression analyses explaining sialorrhea with various coefficients and the proxy SNPs, no proxy SNP was associated with sialorrhea. In analyses explaining dry mouth with logistic regression with age and sex as coefficients, two proxy-SNPs were associated with dry mouth: ERBB4 rs3942465 (FDR-adjusted $p = 0.025$) and TACR1 rs58933792 (FDR-adjusted $p = 0.029$). A combined effect of these SNPs in a logistic regression model is presented in Table 3. When regular smoking was added to the model, the SNPs associations were not statistically significant, a trend of association remained for ERBB4 rs3942465 (FDR-adjusted $p = 0.051$), although regular smoking was not associated with dry mouth. SNPs for which ERBB4 rs3942564 and TACR1 rs58933792 were used as proxies and had an r^2 over 0.2 are presented in Supplementary Table 1.

Discussion

The focus of this study was to find possible associations with sialorrhea, dry mouth and the studied polymorphisms in the selected genes related to the regulation of salivation. No associations between sialorrhea and studied polymorphisms were found. Clozapine dose, clozapine or norclozapine concentration and clozapine to norclozapine ratio did not associated with sialorrhea or dryness of mouth. The use of valproate or additional antipsychotic medication with clozapine was associated with increased sialorrhea.

Proxy-SNPs in *ERBB4* (rs3942465) and *TACR1* (rs58933792) were associated with dry mouth. *ERBB4* (rs3942465) was a proxy SNP for 676 SNPs in *ERBB4* and *TACR1* rs58933792 for 153 SNPs. Both proxy-SNPs are in the intron region of their respective genes. No highly correlated ($r^2 > 0.8$) SNPs were in linkage disequilibrium with the proxy-SNPs. Two of the lesser correlated ($r^2 < 0.8$) SNPs in *TACR1* (rs17010664 and rs72824221) are located in 3'-UTR and the rest are located in the intron region. Also, one deletion (chr2.213404504) in the promoter region of *ERBB4* was linked to the proxy SNP. There are no previous publications of the above-mentioned SNPs or the deletion. In this study, the *TACR1* gene was studied because clozapine affects the parasympathetic nervous system and substance P is a neurotransmitter in the parasympathetic nervous system. The hypothesis is that activity of substance P could cause sialorrhea by reducing swallow response and causing accumulation of saliva. Dysphagia is known as a rare but severe adverse effect of clozapine (Crouse, Alastanos et al. 2018). *ERBB4* is receptor-tyrosine-receptor kinase. *ErbB4* kinases might be molecular targets of clozapine (Kobayashi, Iwakura et al. 2019). EGF has an important role in protecting the oral cavity and there are EGF receptors in salivary glands. Nevertheless, it is still unclear what is the role of EGF in the regulation of saliva, thus the mechanism by which the deletion in *ERBB4* would cause dryness of mouth remains unknown. Formatting...

Clozapine has both anticholinergic and cholinergic effects. This explains why clozapine can cause both dry mouth and sialorrhea. (Ignjatović Ristić, Cohen et al. 2018) The percentage of patients on clozapine medication having dry mouth varies from 0% (Tollefson, Birkett et al. 2001) to 20% (Kelly, Conley et al. 2003). Previously it has been found that patients with high clozapine concentration in plasma have more sialorrhea (Schoretsanitis, Kuzin et al. 2021) and it is commonly managed by lowering the dose (Stroup, Gray 2018). In this study, surprisingly, a similar association was not found; sialorrhea occurred regardless of the dosage and concentrations.

Clozapine users who also used mood stabilizers suffered more from excessive salivation.

Previously it has been found that lithium, one of the mood stabilizers, causes both sialorrhea and dry mouth. (Alexopoulos, Streim et al. 2004) The higher dose of other antipsychotic drugs, in patients who use other antipsychotics in addition to clozapine, was associated with excessive salivation. The reason for this is unclear because sialorrhea is generally known to be associated only with clozapine use and not with other antipsychotics (Stroup, Gray 2018).

The strength of the study is that salivation-related genes and their associations with clozapine-induced sialorrhea have not been examined as broadly formerly. This is also the first study suggesting that genotypes can affect the dryness of the mouth during clozapine treatment. The SNP in the *ADRA2A* gene (rs1800544) that was previously found to be associated with sialorrhea was not associated with sialorrhea in this study (Solismaa, Kampman et al. 2014). . Limitations of the study include that the sample size was limited and quite restrictive in relation to the number of polymorphisms. It is possible that smaller effects were missed because of the data's small size when examining large numbers of genes even though the number of polymorphisms was reduced with MAFF elimination and clumping. This is possibly the reason that the single SNP *ADRA2A* rs1800544 was not associated with sialorrhea in this study. Another limitation is that the in-depth mechanism of found proxy-SNPs and effect on for example mRNA expression was not assessed. Another limitation is that the amount of salivation was asked with only two questions in LUNSERS. Most of the examined patients had used clozapine for over a year. Therefore, some patients who had to stop the medication early because of difficult sialorrhea, have probably been selected out of the data.

In conclusion, this study suggests that genetic variations in *ERBB4* and *TACR1* genes contribute to experienced dryness of mouth in clozapine-treated patients. Use of valproate and antipsychotic polypharmacy was associated with sialorrhea, but dose or concentration of clozapine was not.

Author contributions

Olli Kampman and Niko Seppälä contributed to planning and designing the original study and data collection. Niko Seppälä and Merja Viikki participated locally in acquisition of data. Hanna Puolakka, Anssi Solismaa and Olli Kampman were responsible for planning the present study and forming the present research hypothesis. Leo-Pekka Lyytikäinen, Nina Mononen and Terho Lehtimäki participated in planning and performing the laboratory analyses and genotyping and provided the methodology for laboratory analyses. Hanna Puolakka, Anssi Solismaa and Leo-Pekka Lyytikäinen conducted the statistical analyses. Hanna Puolakka, Anssi Solismaa, and Olli Kampman contributed to the interpretation of the

data. Hanna Puolakka, Anssi Solismaa and Olli Kampman drafted the article. Olli Kampman, Merja Viikki, Nina Mononen, Niko Seppälä, Terho Lehtimäki, Leo-Pekka Lyytikäinen revised the article. All authors have reviewed and approved the article.

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Statement of interest

Niko Seppälä has given lectures and worked as a Clinical Advisor at viatris, Finland. Niko Seppälä is an Advisory Board Member at Viatris, Italy.

Hanna Puolakka, Anssi Solismaa, Leo-Pekka Lyytikäinen, Merja Viikki, Nina Mononen, Terho Lehtimäki and Olli Kampman declare no competing interests.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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Table 1. Demographics and clinical characteristics of the study participants

Sex, n (%)	
Men	136 (57.4 %)
Women	101 (42.6 %)
Age, mean \pm sd years	43.5 \pm 10.9
BMI, mean \pm sd kg/m ²	29.8 \pm 6.38
Clozapine dose, mean \pm sd mg	403 \pm 152
Antipsychotic medication	
Clozapine monotherapy, n (%)	155 (65.4 %)
Antipsychotic polypharmacy, n (%)	82 (34.6 %)
Regular smoking, n (%)	89 (50.6 %)
Reported excessive salivation, n (%)	108 (46.4 %)
Reported excessive salivation and/or TCA in use, n (%)	122 (52.8 %)
Reported dry mouth, n (%)	84 (36.1 %)

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Table 2. Comparisons between salivation groups (no excessive salivation vs. excessive salivation) and different other medications used in addition to clozapine

Drug groups	χ^2	p
All mood stabilizers vs. no mood stabilizers	6.82	0.009¹
Valproate vs. other mood stabilizers or no mood stabilizers	6.20	0.013²
Antipsychotic polypharmacy vs. clozapine monotherapy	5.90	0.015³
Benzodiazepine vs. no benzodiazepine	3.30	0.069
Beta blockers vs. no beta blockers	0.25	0.61
Blood pressure medication vs. no blood pressure medication	0.38	0.54
SSRI vs. no SSRI	0.065	0.80
SNRI vs. no SNRI	0.13	0.72

¹ Patients on mood stabilizers had a higher risk of excessive salivation than those not on mood stabilizers

² Valproate users were more likely to experience excessive salivation compared to those on other mood stabilizers or none

³ Antipsychotic polypharmacy increased the risk of excessive salivation compared to using clozapine alone

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Table 3. Logistic regression model explaining dry mouth (no dryness of mouth vs. at least very little dryness of the mouth) with the following coefficients: age, sex, and score formed of ERBB4 rs3942465 and TACR1 rs58933792 SNPs

	B	SE	z	p	OR (95% CI)
Age	-0.0063	0.017	-0.38	0.71	0.99 (0.32-1.26)
Sex	-0.24	0.37	-0.65	0.52	0.79 (0.38-1.62)
SNP score*	2.09	0.45	4.66	<0.001	8.12 (3.37-19.56)

* Estimate-weighted genetic risk score of two SNPs: ERBB4 rs3942465 and TACR1 rs58933792

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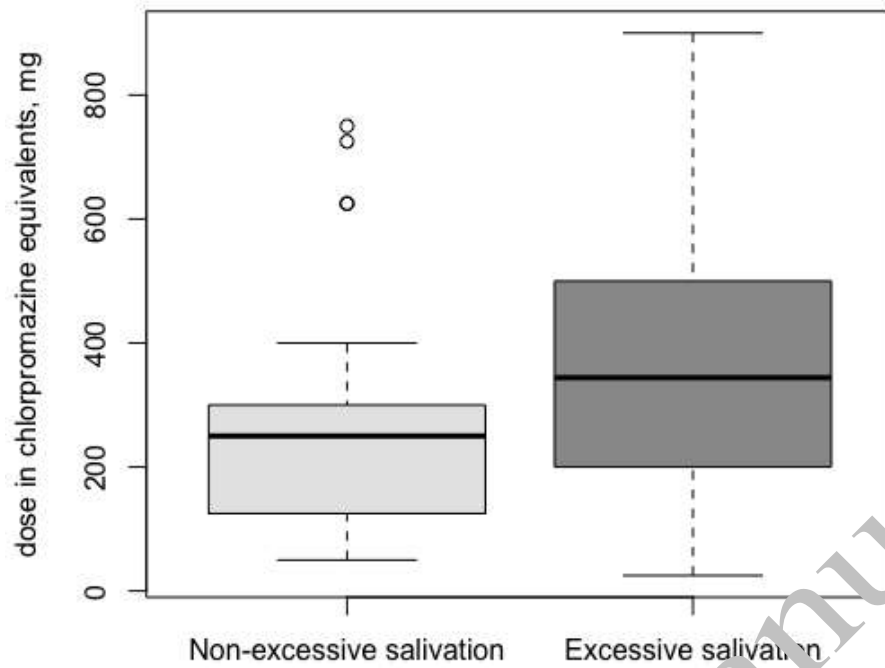


Figure 1. Doses of other antipsychotic drugs than clozapine in two groups: non-excessive salivation and excessive salivation

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