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Association of nicotine metabolism and sex with relapse following varenicline and nicotine replacement therapy

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ABSTRACT

Nicotine is metabolized into cotinine and then into *trans*-3'-hydroxycotinine, mainly by cytochrome P450 2A6. Recent studies reported better effectiveness of varenicline in women and in nicotine normal metabolizers phenotypically determined by nicotine-metabolite ratio. Our objective was to study the influence of nicotine-metabolite ratio, *CYP2A6* genotype and sex on the response to nicotine replacement therapy and varenicline. Data were extracted from a longitudinal study which included smokers participating in a smoking cessation program. Response to treatment was defined by the absence of relapse when a set threshold of reduction in cigarettes per day relative to the week before the study was no more reached. The analysis considered total and partial reduction defined by a diminution of 100% and of 90% in cigarettes per day, respectively. The hazard ratio of relapsing was estimated in multivariate Cox regression models including the sex and the nicotine metabolism determined by the phenotype or by *CYP2A6* genotyping (rs1801272 and rs28399433). In the normal metabolizers determined by phenotyping and in women, the hazard ratio for relapsing was significantly lower with varenicline for a partial decrease (HR=0.33, 95%CI=[0.12-0.89] and HR=0.20, 95%CI=[0.04-0.91], respectively) and non-significantly lower for a total cessation (HR=0.45, 95%CI=[0.20-1.0] and HR=0.38, 95%CI=[0.14-1.0]). When compared to the normal metabolizers determined by phenotyping, the hazard ratio for a partial decrease was similar in the normal metabolizers determined by genotyping (HR=0.42, 95%CI=[0.18-0.94]) while it was significantly lower with varenicline for a total cessation (HR=0.50, 95%CI=[0.26-0.98]). Women and normal nicotine metabolizers may benefit more from varenicline over nicotine replacement therapy.

Keywords: Nicotine, varenicline, sex, genetics, nicotine metabolism

Public Significance Statements

This study confirms that varenicline has better effectiveness than nicotine replacement therapy in nicotine normal metabolizers determined by the nicotine-metabolite ratio, and in women. The nicotine normal metabolizers determined by CYP2A6 rs1801272 genotyping also have greater response to varenicline than to nicotine replacement therapy. The choice of the smoking cessation pharmacological treatment should take into account nicotine metabolism and sex to obtain better abstinence rate in smokers.

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Anaïs Glatard wrote manuscript, analyzed data.

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INTRODUCTION

Smokers willing to stop smoking may be helped by a pharmacotherapy such as nicotine replacement therapy (NRT), bupropion or varenicline. These three medications are used worldwide and several head-to-head effectiveness comparisons have been performed with mixed results. Varenicline has been associated with greater continuous abstinence rates than NRT after 52 weeks of follow-up (Aubin et al., 2008; Kralikova et al., 2013). A multicenter study including 47 smoking cessation services concluded in a small advantage of varenicline over NRT combinations (i.e. any formulations such as gums, spray and/or patches) although variations in population characteristics or clinical practice appear to influence the effectiveness of both therapies (Brose, West, & Stapleton, 2013). A randomized parallel clinical trial in 272 subjects reported equivalent success between varenicline and nicotine patches at 1, 6 and 12 months of follow-up (Heydari, Talischi, Tafti, & Masjedi, 2012), while a meta-analysis concluded that varenicline and combined NRT were equally effective in continuous or prolonged abstinence during at least 6 months (Cahill, Stevens, Perera, & Lancaster, 2013).

In humans, nicotine is metabolized into cotinine and then into *trans*-3'-hydroxycotinine, mainly by cytochrome P450 (CYP) 2A6 (Messina, Tyndale, & Sellers, 1997; Nakajima et al., 1996). The nicotine-metabolite ratio refers to the 3'-hydroxycotinine / cotinine ratio during smoking *ad libitum*, and is a marker of CYP2A6 activity. Interestingly, nicotine-metabolite ratio seems to be associated with 1-week abstinence, with nicotine slow metabolizers being more likely to achieve abstinence than normal metabolizers (odds ratio [OR] = 1.32, 95% confidence interval [CI] = 1.05, 1.67; P = .019) (Chenoweth et al., 2015). Furthermore, a recent double-blind placebo-controlled trial reported that varenicline had greater effectiveness than NRT in nicotine normal metabolizers as determined by the nicotine-metabolite ratio (Lerman et al., 2015).

Compared to CYP2A6 genotyping, nicotine-metabolite ratio has the advantage of taking into account both genetic and environmental (eg. oestrogen) effects on CYP2A6 activity (Dempsey et al., 2004; Lerman et al., 2015; Malaiyandi, Goodz, Sellers, & Tyndale, 2006; Schnoll et al., 2009). CYP2A6 activity is indeed induced by estradiol, leading to increased nicotine metabolism (Higashi et al., 2007) and women are more likely to be normal metabolizers than slow metabolizers (Chenoweth et al., 2014; Lerman et al., 2015), especially when receiving estrogen-based hormonal therapy (Benowitz, Lessov-Schlaggar, Swan, & Jacob, 2006). The influence of sex on the effectiveness of smoking cessation treatment has been largely described. A meta-analysis of 14 studies comparing smoking cessation rate in men and women receiving nicotine patches reported a significantly lower rate of abstinence in women (Perkins & Scott, 2008). Men were also found to have a significantly better abstinence rate with the combination varenicline-bupropion than with varenicline alone, whereas women had a similar response to both treatments (Potter, 2014; Rose & Behm, 2014). It has therefore been suggested that women benefit more than men from varenicline alone and thus the addition of bupropion to varenicline would not improve the response in women (Gorelick, 2015; Rose & Behm, 2015). Interestingly, varenicline compared to nicotine patches doubled the odds of abstinence at the end of a 4-week treatment in an exploratory short-term double-blind randomized trial among women smokers (Gray et al., 2015). In a longer term, at 12 weeks, women had greater quit rates when receiving varenicline compared to NRT in a study involving almost 7000 smokers (Walker et al., 2016). Very recently a meta-analysis of 32 studies representing more than 14 000 smokers reported a greater efficacy in women taking varenicline compared to transdermal nicotine or bupropion. In men, no difference was shown between the three treatments (Smith et al., 2016).

Several single nucleotide polymorphisms (SNP) in the *CYP2A6* gene that affect enzyme activity have been characterized (Goodz & Tyndale, 2002; Mwenifumbo & Tyndale, 2007). One of the most studied allele is the rs1801272 (479T>A, Leu160His, *CYP2A6*2*) (Ray, Tyndale, & Lerman, 2009; Yamano,

Tatsuno, & Gonzalez, 1990). It leads to a predicted CYP2A6 activity between 40 and 50% of the normal activity, or less than 40% if two mutated alleles A are present (Malaiyandi et al., 2006). Thus, individuals carrying one or two *CYP2A6*2* alleles are considered slow nicotine metabolizers (Benowitz, Swan, Jacob, Lessov-Schlaggar, & Tyndale, 2006). Another common polymorphism, the rs28399433 (*CYP2A6*9*, -48T>G) located in the TATA box of the 5' flanking region of the *CYP2A6* gene has been identified. The activity of the mutated TATA was reduced by 55% when compared to the wild allele and was shown to reduce mRNA expression and enzyme activity. (Haberl et al., 2005; Pitarque et al., 2001).

The primary objective of our post-hoc analysis of data from usual clinical care was to explore the influence of nicotine metabolism determined by the nicotine-metabolite ratio and of sex on the response to NRT and varenicline. Based on previous clinical trials (Gray et al., 2015; Lerman et al., 2015; Perkins & Scott, 2008), we hypothesized that nicotine normal metabolizers and women would benefit more from varenicline. The second objective was to compare the influence of nicotine metabolism determined phenotypically by the nicotine-metabolite ratio and genetically by the *CYP2A6* rs1801272 and rs28399433 polymorphisms on the response to NRT and varenicline.

METHODS

Study design and participants

The original aim of this clinical and pharmacogenetic study was to examine the influence of smoking cessation on the activity of CYP1A2 isoform (Dobrinas, Cornuz, et al., 2011). For this purpose it included smokers from the general population wishing to participate in a smoking cessation program. This program offered a 3-month study period (5 visits every week from week 0 to week 4, 4 visits every 2 weeks from week 4 to week 12) comprising smoking-cessation counseling and pharmacological treatment prescription (combined nicotine replacement therapies: patches, gums and/or inhaler or varenicline) and a 6-month concluding visit. Details of the inclusion and exclusion criteria as well as clinical measures have been previously described (Dobrinas, Cornuz, et al., 2011). Blood sampling performed before the quit date was used to measure the nicotine-metabolite ratio. Abstinence was assessed during the follow-up by self-declaration and by measuring expired CO levels (Micro Smokerlyzer; Bedfont Scientific, Rochester, England). Abstinence was confirmed if CO level was less than 10 parts per million (ppm). The number of cigarettes smoked between two visits was also recorded. The study was approved by the ethics committee of the Lausanne University Medical School and by the Swiss Agency for Therapeutic Products (Swissmedic, Bern, Switzerland). Written informed consent was obtained from all participants.

Treatment

After a counseling session with a clinician, participants chose to receive either varenicline or combined nicotine replacement therapies (patches, gums and/or inhaler) in agreement with the clinician. NRT and varenicline were prescribed according to the manufacturers' information, to guidelines for smoking

cessation (The 2008 PHS Guideline Update Panel, 2008), and to patients' preferences. Main counter-indications included cardiovascular diseases (unstable angina pectoris, recent myocardial infarction) or skin disorders (eg. psoriasis, chronic dermatitis, urticaria) for NRT, and depression, past antidepressant treatments or other psychiatric diseases for varenicline. NRT formulations and dosing were chosen according to the nicotine dependence score measured by the Fagerström test for nicotine dependence (FTND) ranging from 0 to 10 (Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991). For instance, patients with a high dependence score (8 to 10) were prescribed with high concentration patches, combined with other NRT formulations (gums and/or inhaler). In patients with low dependence scores (0 to 3), one NRT formulation could be sufficient (low dose patches or gums/inhaler). NRT dosing was gradually decreased each month, for total treatment duration of 12 weeks. Varenicline was prescribed starting from one week before the quit date (0.5 mg once daily on days 1-3 and 0.5 mg twice daily on days 4-7), then continued with 1 mg twice daily for a total of 12 weeks. The pharmacological treatment was proposed for 12 weeks free-of-charge and treatments were delivered at each visit. Varenicline treatment but not combined NRT could be proposed for 12 supplementary weeks as recommended in the manufacturer's information.

If needed and with the approval of a clinician, a switch of treatment was allowed during the study. As the aim of the present study was to compare the effectiveness on smoking cessation of these two treatments, data from subjects who switched from one treatment group to another were excluded from the analysis (Figure 1).

Nicotine-metabolite ratio

Cotinine and 3'-hydroxycotinine plasma levels were simultaneously measured by an ultra performance liquid chromatography-tandem mass spectrometry method while participants were smoking *ad libitum*

at the beginning of the study (Dobrinas, Choong, et al., 2011). Subjects were phenotypically considered slow metabolizers if nicotine-metabolite ratio was inferior to 0.26 and normal metabolizers if nicotine-metabolite ratio was higher or equal to 0.26 as previously described (Schnoll et al., 2009). The cut-off was set to the originally determined value of 0.26 in (Schnoll et al., 2009).

Genotyping

CYP2A6 SNPs were obtained using the CardioMetaboChip, a custom Illumina iSelect genotyping array designed to test DNA variation of over 200,000 SNPs from regions identified by large scale meta-analyses of genomewide association studies for metabolic and cardiovascular traits. 3642 customized SNPs covering pharmacokinetic genes were added in the Cardiometabochip (Lubomirov, Csajka, & Telenti, 2007; Lubomirov et al., 2010), among which 8 SNPs from the *CYP2A6* gene (rs5031016, rs8192730, rs1809810, rs1801272, rs28399453, rs28399454, rs28399433, rs2892625). Four SNPs were excluded from analysis due to a very low minor allele frequencies (MAF<0.005) as reported in European population (rs5031016, rs8192730, rs1809810, rs28399454) (Flicek et al., 2014). The rs28399453 polymorphism is not reported to influence *CYP2A6* activity and was thus excluded from analysis. Quality control excluded samples from the analysis if sex was inconsistent with genetic data from X-linked markers, genotype call rate less than 0.96 or Gene Call score less than 0.15. The SNP rs2892625 was excluded from analysis due to a Gene Call score < 0.15. The two polymorphisms rs1801272 and rs28399433 were in Hardy Weinberg Equilibrium (p=0.68 and p=0.65, respectively) and were used for determining *CYP2A6* genotype. In the sample analysis, individuals were homozygous non-mutated (TT) or heterozygous (TA) for the SNP rs1801272 and individuals were homozygous non-mutated (TT) or heterozygous (TG) for the SNP rs28399433. There were no homozygous mutated for any of the SNPs in the sample analysis. Subjects were classified as slow metabolizers if they were heterozygous for the

SNPs rs1801272 or rs28399433, or for both SNPs according to results previously published (Benowitz, Swan, et al., 2006; Haberl et al., 2005; Pitarque et al., 2001). The homozygous non-mutated for both SNPs were defined as normal metabolizers. GenomeStudio Data Analysis Software was used to export results generated by Illumina CardioMetaboChip.

Statistical analysis

In this work, total smoking cessation and partial smoking reduction is used to evaluate response to nicotine replacement therapy or varenicline. Response to treatment was defined by the absence of relapse (i.e. when the reduction in cigarettes per day (CPD) consumption relative to the week before the study was not reached anymore).

- *Exploratory analysis for selection of thresholds*

An exploratory analysis was first conducted to find the most appropriate threshold in CPD reduction defining partial smoking reduction. The week before the study and at each visit, participants self-reported the number of cigarettes per day or over the period between two visits. For non-daily smokers, the number of cigarettes smoked over the period between 2 visits was divided by the number of days of the period to calculate CPD. Overall, there was a concordance between the CPD and the CO levels recorded (r^2=0.49, $p < 0.01$). For each subject and each visit the reduction in the number of CPD relative to the number of CPD the week before the study was calculated. A binary variable was defined as follows: status=0 if the threshold of CPD reduction was reached, status=1 if the threshold was not reached anymore (i.e relapse). By varying the potential thresholds between 50% and 100% in the reduction of CPD, 51 variables status were coded for each subject at each visit. Then, for each threshold of reduction and each subject, a time-to-event variable was determined as the duration from the first

visit when the threshold was reached to the time when the threshold was not reached anymore, or to the end of follow-up. Consequently, data from the visits during which the threshold was not already reached and participants who never reached the threshold were excluded from analysis (Figure 1). Fifty-one hazard-ratios (HR) representing the risk of relapse (for each one of 51 potential thresholds) were estimated to assess the effect of treatment (varenicline vs. nicotine replacement therapy) by adjusting for nicotine-metabolite ratio (slow metabolizers vs. normal metabolizers) and sex (men vs. women) in fitting a Cox proportional hazard regression model.

HR and the 95%CI are presented in Figure S1. With increasing threshold, the number of subjects included in each analysis decreased and the number of relapses increased. From 90% to 100% of CPD decrease, the 95%CI of the HR was the smallest, stable, and mostly below the value of 1. Two thresholds of reduction: the first one considering total abstinence: diminution of 100%, and the second one considering a partial reduction of smoking: diminution of 90%, were therefore selected for further analysis.

- *Relapse analysis for a total cessation and a partial reduction of CPD*

Participants were considered as relapser if their reduction in CPD consumption became lower than the predefined threshold and as no relapser if they maintained the predefined threshold of CPD reduction during the overall period of follow-up. In the overall sample, Chi-squared tests evaluated the distributions of treatment group, nicotine metabolism (phenotypically determined by the nicotine-metabolite ratio) and sex among the relapsers and non relapsers as well as the distribution of the normal metabolizers and slow metabolizers among men and women. The cumulative probability of maintaining the selected threshold of CPD reduction by treatment group in the overall sample analysis was assessed using Kaplan-Meier survival analyses with subsequent Log-Rank tests.

Multivariate Cox proportional hazards regression models were carried out to obtain the HR of relapse and its 95%CI for the treatment (varenicline vs. NRT) in the overall sample and in subgroups: normal metabolizers, slow metabolizers, men and women. The analyses were adjusted for nicotine-metabolite ratio, nicotine-metabolite ratio-by-treatment interaction, sex, age of onset for smoking and the number of CPD usually smoked in the overall sample. In normal and slow metabolizers, the analyses were adjusted for sex, age of onset for smoking and the number of CPD usually smoked. In men and women, the adjustment was made by the nicotine-metabolite ratio, the nicotine-metabolite ratio-by-treatment interaction, the age of onset for smoking and the number of CPD usually smoked. All statistical analyses were performed using the R software (v. 3.1.2, http://www.r-project.org). Results were considered statistically significant if $p \leq 0.05$ (two-tailed). Finally, with regards to the Cox regression, proportional hazard assumption was verified using the Grambsch Therneau test and results were satisfactory (Grambsch & Therneau, 1994).

- *Influence of the genetically determined nicotine metabolism on the treatment response*

To investigate the effect of nicotine metabolisation effect on risk of relapse, the association of nicotine metabolism genetically determined (normal and slow metabolizers) versus the relapse status (relapser and no relapser) was first assessed using Chi-squared test. Then, the sample was stratified between normal metabolizers and slow metabolizers to obtain the HR of relapse and its 95% CI for the treatment (varenicline vs. NRT) in two subgroups using Cox proportional hazards regression models by adjusting for sex, age of onset for smoking and the number of CPD usually smoked.

RESULTS

Study participants and analysed sample

211 smokers were recruited for a smoking cessation study and 194 subjects fulfilled the inclusion criteria. 153 and 164 participants were analyzed for the 100% (total cessation) and for the 90% (partial decrease) of CPD decrease, respectively (Figure 1).

Participants did not differ in any of the examined variables except in the age of onset for smoking with a lower age in the varenicline compared to the NRT group (Table 1). In addition, in the analysis of a 90% of CPD decrease, the number of usually smoked CPD was significantly smaller in the NRT group than in the varenicline group (n=20 CPD and n=24 CPD respectively, p=0.03), but there was no significant difference in the number of smoked CPD the week before the study. Separate data between male and female are shown in Table S1. Of note, the number of usually smoked cigarettes per day is significantly different between NRT and varenicline in males but not in females, which was taken into account in the statistical analysis. Although compliance cannot be ascertained, all participants were asked about the actual intake of varenicline or nicotine patches, gums or inhalers at each visit.

When phenotypically determined, women were more likely to be normal metabolizers than slow metabolizers in the analyses for a 100% and a 90% of CPD decrease (Table 2, p=0.03 and p=0.01, respectively).

Relapse analysis for a total cessation and a partial reduction of CPD

For the decrease of 100% and 90%, relapse was significantly associated with the treatment (Table 3, p=0.04 and p=0.02, respectively), NRT associated with more cases of relapse. The Kaplan-Meier estimate of the probability of maintaining the threshold was significantly higher for varenicline when compared

to NRT for a 90% of CPD decrease (Figure 2B, p=0.014). The estimate was also higher for varenicline but not significant for a 100% of CPD decrease (Figure 2A, p=0.054).

The multivariate Cox regression predicted in the overall sample that participants treated with varenicline had a significant lower risk of relapse for a 90% of CPD decrease (Table 4, HR=0.34, 95%CI=[0.13-0.90]) and a non-significant lower risk of relapse for a 100% of CPD decrease (HR=0.49, 95%CI=[0.22-1.07]). In the nicotine normal metabolizers determined by phenotyping, varenicline was significantly better for a partial decrease (HR=0.33, 95%CI=[0.12-0.89]) and non-significantly better (HR=0.45, 95%CI=[0.20-1.0]) for a total cessation. In women, the risk of relapse with varenicline was significantly lower for a partial decrease (HR=0.20, 95%CI=[0.04-0.91]) but non-significantly lower for a total cessation (HR=0.38, 95%CI=[0.14-1.0]). No treatment was found significantly better in slow metabolizers determined by phenotyping for a total cessation and a partial decrease (HR=0.70, 95%CI=[0.28-1.75] and HR=0.48, 95%CI=[0.14-1.7], respectively) and in men, for a total cessation and a partial decrease (HR=0.70, 95%CI=[0.20-2.5] and HR=0.55, 95%CI=[0.15-2.0], respectively).

Comparison of the influence of the nicotine metabolism determined either phenotypically or genetically, on treatment response

The frequency of slow metabolizers was found to be much lower than the normal metabolizers when classifying participants according to *CYP2A6* genotype as compared to the classification based on phenotype (Table 1).These differences could probably be explained by the fact that classification based on phenotyping includes environmental factors, while genotyping was based on two SNPs only, whereas other mutations are possibly contributing to a slow metabolizer phenotype.

For a partial decrease, the multivariate Cox regression predicted a similar significant lower risk of relapse with varenicline in the normal metabolizers determined by genotyping when compared to the normal metabolizers determined by phenotyping (Table 4, HR=0.42, 95%CI=[0.18-0.94] and HR=0.33, 95%CI=[0.12-0.89], respectively). But for a total cessation, the lower risk of relapse with varenicline in the normal metabolizers determined by genotyping was significant (HR=0.50, 95%CI=[0.26-0.98]) however it was non-significant in the normal metabolizers determined by phenotyping (HR=0.45, 95%CI=[0.20-1.0]). Concerning the slow metabolisers no treatment was found significantly better in the case of a genotype-based and phenotype-based determination (HR=0.81, 95%CI=[0.06-10.2] and HR=0.48, 95%CI=[0.14-1.7], respectively) for a partial decrease and a total cessation (HR=1.05, 95%CI=[0.22-5.1] and HR=0.70, 95%CI=[0.28-1.75], respectively).

DISCUSSION

It has recently been shown that nicotine normal metabolizers (phenotypically determined by the nicotine-metabolite ratio) have better quit rates with varenicline compared to nicotine patches (Lerman et al., 2015). To our knowledge, the present study is the first to replicate this finding in usual clinical care data, using both phenotyping and genotyping tests. Varenicline response was superior to NRT in phenotype-based normal metabolizers in the case of a partial reduction. The finding that women smokers have higher response with varenicline over NRT for a partial reduction are in accordance with other recent findings (Gorelick, 2015; Gray et al., 2015; Rose & Behm, 2015). The influence of sex in the effectiveness of the smoking cessation agent is now well acknowledged (McKee & Weinberger, 2015; Weinberger, Smith, Kaufman, & McKee, 2014). Because *CYP2A6* mRNA is induced by estradiol (Higashi et al., 2007), women and especially premenopausal women, may metabolize nicotine and cotinine faster than men (Benowitz, Lessov-Schlaggar, et al., 2006). Supposedly, with nicotine being rapidly metabolized in women, the pharmacological effect of NRT on withdrawal symptoms is lower, which is in agreement with the reported lower success rate with nicotine patches (Perkins & Scott, 2008). No difference was observed in treatment success in slow metabolizers and in men.

Of note, in normal metabolizers genetically determined by the rs1801272 and rs28399433 mutations, the multivariate Cox regression model showed similar response to treatment for a partial CPD decrease compared to the same subgroup phenotypically determined. For a total abstinence, response to treatment was similar in normal metabolizers genetically or phenotypically determined, except a lack of statistical power in the analysis to observe a significant result in the phenotype-based normal metabolisers. The similarity of the result could be explained, at least in part, by the demonstrated influence of these mutations on *CYP2A6* activity (Haberl et al., 2005; Malaiyandi et al., 2006; Pitarque et al., 2001). On the other hand, it can be assumed that the prediction of *CYP2A6* activity based on

nicotine-metabolite ratio is more accurate, as it integrates both genetic factors (taking into account all genetic variations and not only one mutation) and environmental factors (e.g. induction by estrogens).

In the overall sample analyzed, the probability of maintaining a diminution of 90% of smoked cigarettes per day over 6 months was higher in the varenicline group than in the NRT group, while a trend was found when using a total abstinence as threshold. These results are not in agreement with the equivalent efficacy of varenicline and combined NRT reported in a meta-analysis (Cahill et al., 2013) comparing both treatments. It has to be stressed that the sample size in the present study was limited and the results should therefore be replicated. However, a special emphasis should be put on the selection of outcomes, which differ between the present and the meta-analysis (Cahill et al., 2013): time during which a threshold of total cessation or partial reduction in CPD is maintained vs. continuous or prolonged abstinence at least 6 months from the start of the treatment, respectively.

In the present study, the analyses considered an absolute abstinence (reduction of 100%) as well as a partial reduction in CPD (90% reduction). Both thresholds gave essentially similar results. It should be mentioned that reduction in CPD consumption (instead of cessation) does not cancel health risks: a significantly higher risk of dying, especially from ischaemic heart disease and lung cancer, was observed in men and women smoking 1 to 4 cigarettes per day compared to never-smokers (Bjartveit & Tverdal, 2005). Thus, changes in CO and cotinine plasma levels consistently showed smaller reduction than CPD (Stead & Lancaster, 2007) due to the phenomenon of oversmoking: smokers involuntarily increase the number and depth of inhalations from the remaining cigarettes to obtain the necessary nicotine quantity (Sadowski, Clair, & Cornuz, 2015). This phenomenon is also observed in the present study: for the analysis of 90% of CPD decrease when compared to the NRT group, the number of usually smoked CPD is significantly lower in the varenicline group while there is no difference in cotinine and 3'-hydroxycotinine plasma levels. On the other hand, consumption reduction seems not to discourage

smokers unmotivated to a project of total abstinence and can even encourage them when it is supported by NRT (Hughes & Carpenter, 2006; Stead & Lancaster, 2007). Gradual reduction before the quit day appears to be associated with equivalent smoking cessation rates compared with abrupt cessation (Lindson-Hawley, Aveyard, & Hughes, 2012). Sustained reduction was defined as reduction in 50% or more of the baseline reported cigarettes consumption in some industry sponsored trials (Moore et al., 2009). In this work, when the reduction in cigarette consumption was below 90% of baseline, more subjects reached this objective, the number of relapses was lower and Cox regressions provided a very large HR 95%CI. We hypothesize that decreasing the cigarettes consumption by less than 90% of baseline value is a more feasible objective, leading to the difference between treatment responses being smaller with a large HR 95%CI.

Several limitations of the present study must be discussed. Firstly, this clinical and pharmacogenetic study was not designed for the purpose of the present evaluation. However the valuable comparison of varenicline and NRT effectiveness by nicotine metabolism and sex obtained with clinical trials previously published deserved an investigation in the natural context of a smoking cessation program as proposed in tobacco consultation. Secondly the analysis used a unique longitudinal dataset with rather small sample size per treatment group and no corrections for multiple testing were performed. Analyses should therefore be repeated in a larger cohort to confirm the influence of sex and nicotine metabolism on the response to varenicline and nicotine replacement therapy. However, the present findings are in agreement with a recently published study (Lerman et al., 2015), and it is remarkable that similar results were obtained when examining the influence of CYP2A6 activity based both on phenotyping and genotyping methods. Thirdly, this study was not randomized and it is not known whether the choice of the treatment by the participant could have an influence on the results. However the significant difference in age of onset for smoking and in number of usually smoked cigarettes per day between the

varenicline and the NRT group has been taking into account in the multivariate analysis by the correction with these two variables.

In summary, treatment of nicotine dependence is very challenging and can be hampered by the lack of motivation, environmental factors or ineffective pharmacological treatment (Fiore & Baker, 2011). In the present work, we showed that nicotine normal metabolizers are more likely to benefit from varenicline over NRT in usual clinical care data. Women who are reported to achieve a lower abstinence rate with NRT than men may have better success in smoking cessation with varenicline treatment. Future studies should also address the question on whether normal metabolizers and women would equally benefit, instead of varenicline, from higher nicotine doses. Because of the scarcity of existing data, our results contribute valuably to the extensive process of tailoring smoking cessation strategy.

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Table 1: Description of the population included in the analysis of a 100% and a 90% of CPD decrease

Characteristic	Analysis for a 100% of CPD decrease					Analysis for a 90% of CPD decrease				
	NRT (N=82)		Varenicline (N=71)		p	NRT (N=87)		Varenicline (N=77)		p
	N	%	N	%		N	%	N	%	
Men	43	52	33	46	0.57[2]	43	49	37	48	0.9[2]
Nicotine NM based on NMR	52	63	43	61	0.8[2]	56	64	47	61	0.8[2]
Nicotine NM based on genotyping	77	94	66	93	1[2]	76	87	65	84	0.75[2]
Treatment duration[4]					0.27[2]					0.21[2]
Less than or equal to 2 months	36	44	24	34		40	46	27	35	
More than 2 months	45	55	46	65		46	53	49	64	
Subjects with smokers at home	20	24	19	27	0.9[2]	20	23	20	26	0.8[2]
Ethnicities					0.9[3]					0.9[3]
Caucasian	79	97	68	97		84	97	74	97	
African/African-American	2	2	1	1		2	2	1	1	
Arabic	0	0	1	1		0	0	1	1	
Others	1	1	1	1		1	1	1	1	
	Mean	**SD**	**Mean**	**SD**		**Mean**	**SD**	**Mean**	**SD**	
Age, years	41	11	38	10	0.1[1]	41	11	39	11	0.2[1]
Cotinine before the quit date, ng/ml	271	140	264	127	0.95[1]	269	138	262	124	0.96[1]
3'-hydroxycotinine before the quit date, ng/ml	87	50	89	51	0.69[1]	87	50	88	50	0.76[1]
Age of onset for smoking, years	19	7	16	3	0.009[1]	19	7	17	4	0.02[1]
Number of usually smoked CPD	20	8	22	10	0.07[1]	20	8	24	11	0.03[1]
Number of smoked CPD during the	19	8	20	10	0.63[1]	19	8	21	11	0.37[1]

week before the study										
Number of previous quit attempts	1.6	0.9	1.7	0.8	0.6[1]	1.6	0.9	1.7	0.8	0.7[1]
FTND[6]	5	2.1	5[5]	2.0[5]	0.8[1]	5	2.2	5[5]	2.0[5]	0.7[1]

CPD: Cigarettes Per Day, NRT: Nicotine Replacement Therapy, NM: Normal Metabolizer, NMR: Nicotine-metabolite Ratio, FTND: Fagerström Test for Nicotine Dependence

[1]: Wilcoxon rank sum test

[2]: Chi-squared test

[3]: Fisher exact test

[4]: For both analyses, the treatment duration was unknown for one subject in the NRT group and one subject in the varenicline group.

[5]: Missing data for one individual

[6]: Fagerström Test for Nicotine Dependence score ranged from 0 to 10.

Table 2: Distribution between nicotine metabolism and sex

	Analysis for a 100% of CPD decrease			Analysis for a 90% of CPD decrease		
Sex	**NM[1]**	**SM[1]**	**p[2]**	**NM[1]**	**SM[1]**	**p[2]**
Men	40	36	0.03	42	38	0.01
Women	55	22		61	23	

CPD: Cigarettes Per Day, NM: Normal Metabolizer, SM: Slow Metabolizer

[1] phenotypically determined by the nicotine-metabolite ratio

[2] Chi-squared test

Table 3: Distribution of study participants according to the binary variable: no relapse (status = 0) and relapse (status = 1), treatment, sex, nicotine metabolism phenotypically determined and nicotine metabolism genetically determined.

	Analysis for a 100% of CPD decrease					Analysis for a 90% of CPD decrease				
Variables	**No relapse**		**Relapse**		**p**	**No relapse**		**Relapse**		**p**
	N	%	N	%		N	%	N	%	
Treatment					0.04[1]					0.02[1]
NRT	47	57	35	43		61	70	26	30	
Varenicline	53	75	18	25		67	87	10	13	
Sex					0.34[1]					0.69[1]
Men	53	70	23	30		64	80	16	20	
Women	47	61	30	39		64	76	20	24	
Nicotine metabolism based on phenotype[2]					0.84[1]					0.46[1]
NM	61	64	34	36		78	76	25	24	
SM	39	67	19	33		50	82	11	18	
Nicotine metabolism based on genotype[3]					1[4]					1[1]
NM	86	66	45	34		110	78	31	22	
SM	14	64	8	36		18	78	5	22	

CPD: Cigarettes Per Day, NRT: Nicotine Replacement Therapy, NM: Normal Metabolizer, SM: Slow Metabolizer

[1] Chi-squared test

[2] Phenotypically determined by the nicotine metabolic ratio (slow metabolizer if nicotine-metabolite ratio < 0.26 and normal metabolizer if nicotine-metabolite ratio >=0.26)

[3] Genetically determined by the genotyping of *CYP2A6* rs1801272 and rs28399433.

[4] Fisher exact test

Table 4: Adjusted hazard ratios of relapse for a 100% and a 90% of CPD decrease in the overall sample and in subgroups: normal metabolizers, slow metabolizers, men and women.

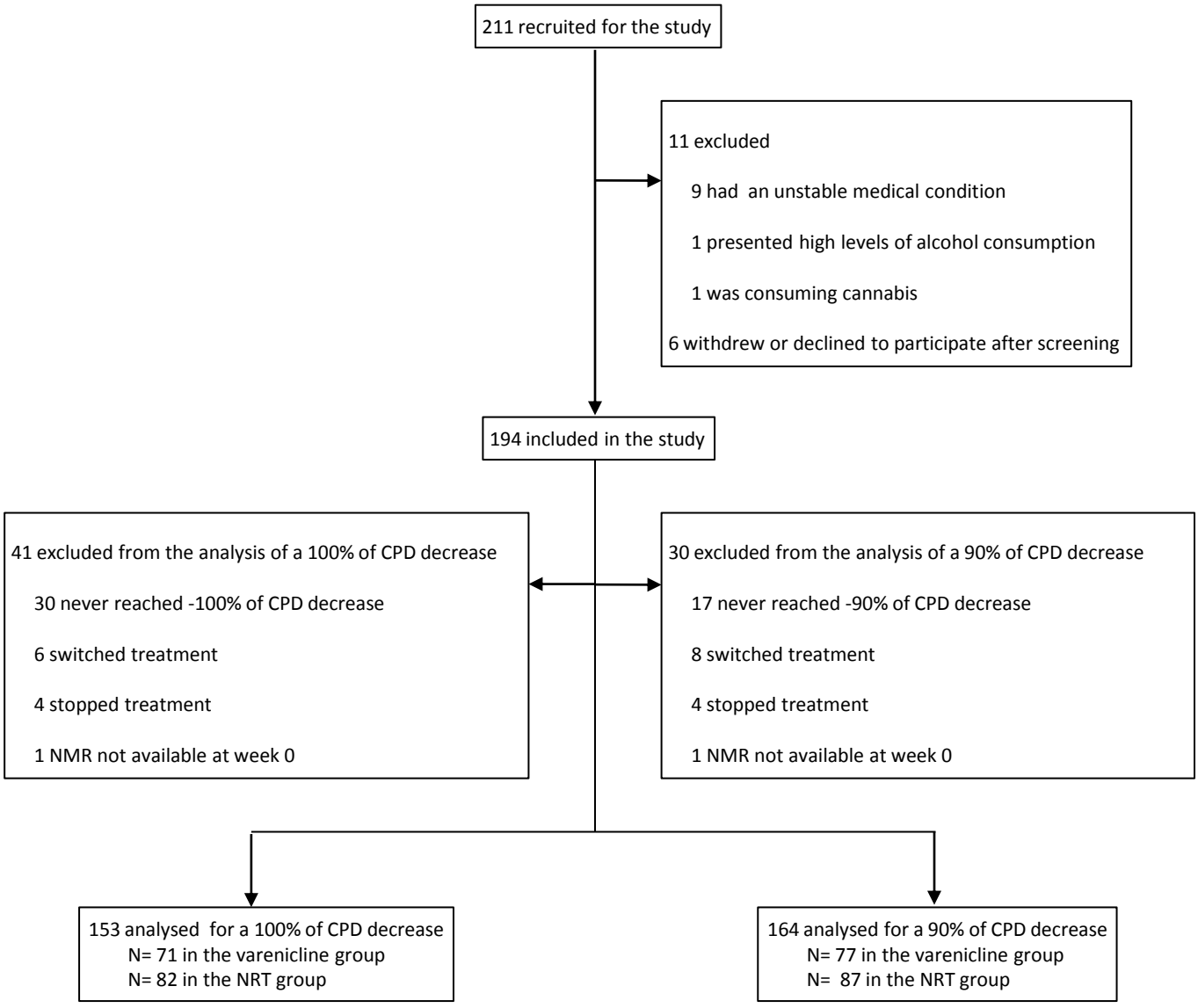
	Analysis for a 100% of CPD decrease			Analysis for a 90% of CPD decrease		
Analysis groups	**N**	**Hazard Ratio**	**95%CI**	**N**	**Hazard Ratio**	**95%CI**
In overall sample						
Treatment (V vs. NRT)	71 vs. 82	0.49	0.22-1.07	77 vs. 87	0.34	0.13-0.90
In NM (phenotyping)[1]						
Treatment (V vs. NRT)	43 vs. 52	0.45	0.20-1.0	47 vs. 56	0.33	0.12-0.89
In SM (phenotyping)[1]						
Treatment (V vs. NRT)	28 vs. 30	0.70	0.28-1.75	30 vs. 31	0.48	0.14-1.7
In NM (genotyping)[2]						
Treatment (V vs. NRT)	59 vs. 72	0.50	0.26-0.98	65 vs. 76	0.42	0.18-0.94
In SM (genotyping)[2]						
Treatment (V vs. NRT)	12 vs. 10	1.05	0.22-5.1	12 vs. 11	0.81	0.06-10.2
In men						
Treatment (V vs. NRT)	33 vs. 43	0.70	0.20-2.5	37 vs. 43	0.55	0.15-2.0
In women						
Treatment (V vs. NRT)	38 vs. 39	0.38	0.14-1.0	40 vs. 44	0.20	0.04-0.91

CPD: Cigarettes Per Day, V: Varenicline, NRT: Nicotine Replacement Therapy, SM: Slow Metabolizer, NM: Normal Metabolizer

1. Phenotypically determined by the nicotine-metabolite ratio (NMR) (SM if NMR < 0.26 and NM if NMR >=0.26)

2. Genetically determined by the CYP2A6 rs1801272 andrs28399433 genotyping.

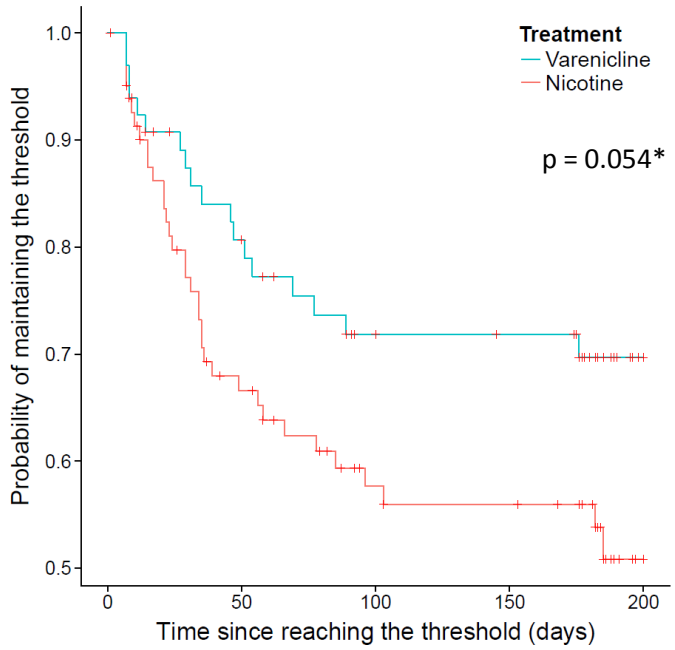
Figure 1: Flow chart for selection of patients



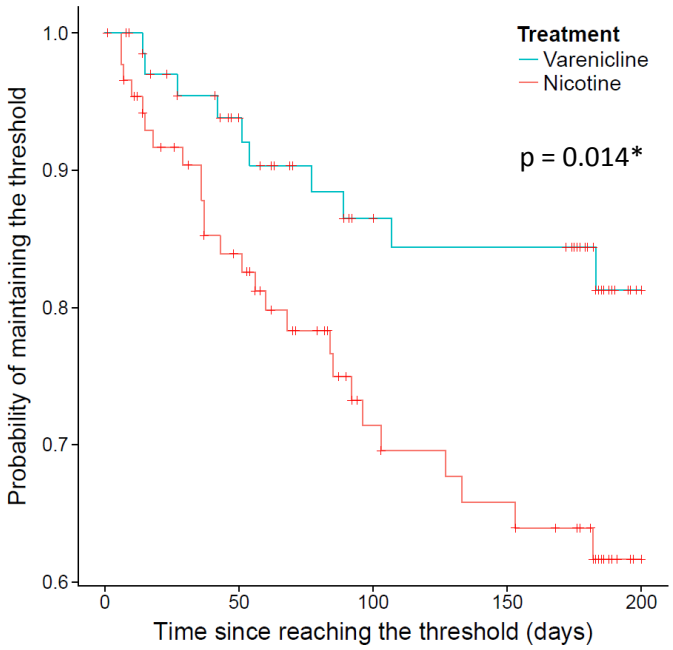
CPD: cigarettes per day
NMR: nicotine-metabolite ratio
NRT: nicotine replacement therapy

Figure 2: Kaplan-Meier estimates of probability of maintaining the threshold of -100% (A), and of -90% (B) in the overall analysis sample. Each cross on the curves represents a lost to follow-up.

A.



B.



Number of participants at risk

Varenicline	71	49	41	41	33
Nicotine	82	50	35	34	18

Number of participants at risk

Varenicline	77	59	46	41	27
Nicotine	87	65	40	36	28

*Log-Rank test

Supplemental Table S1: Description of the population included in the analysis of a 100% and a 90% of CPD decrease by sex

Characteristic	Analysis for a 100% of CPD decrease In males			Analysis for a 90% of CPD decrease In males			Analysis for a 100% of CPD decrease In females			Analysis for a 90% of CPD decrease In females		
	NRT (N=43)	Varenicline (N=33)	p	NRT (N=43)	Varenicline (N=37)	p	NRT (N=39)	Varenicline (N=38)	p	NRT (N=44)	Varenicline (N=40)	p
	N	N		N	N		N	N		N	N	
Nicotine NM based on NMR	23	17	1[2]	23	19	1[2]	29	26	0.7[2]	33	28	0.8[2]
Nicotine NM based on genotyping	40	30	1[3]	40	34	1[3]	32	29	0.7[2]	36	31	0.8[2]
Treatment duration[4]			0.6[2]			0.7[2]			0.4[2]			0.3[2]
Less than or equal to 2 months	17	10		17	12		19	14		23	15	
More than 2 months	25	22		25	24		20	24		21	25	
Subjects with smokers at home	9	9	0.7[2]	9	10	0.7[2]	11	10	1[2]	11	10	1[2]
Ethnicities			0.7[3]			0.7[3]			1[3]			0.8[3]
Caucasian	42	31		42	35		37	37		42	39	
African/African-American	1	1		1	1		1	0		1	0	
Arabic	0	1		0	1		0	0		0	0	
Others	0	0		0	0		1	1		1	2	
	Mean	Mean		Mean	Mean		Mean	Mean		Mean	Mean	

	(sd)	**(sd)**		**(sd)**	**(sd)**		**(sd)**	**(sd)**		**(sd)**	**(sd)**	
Age, years	41 (11)	38 (9)	0.2[1]	42 (11)	39 (10)	0.3[1]	40 (12)	38 (11)	0.4[1]	40 (11)	39 (12)	0.6[1]
Cotinine before the quit date, ng/ml	288 (162)	288 (124)	0.6[1]	288 (162)	288 (120)	0.6[1]	252 (108)	242 (126)	0.7[1]	251 (108)	238 (125)	0.5[1]
3'-hydroxycotinine before the quit date, ng/ml	85 (53)	87 (56)	0.9[1]	85 (53)	87 (56)	0.9[1]	89 (48)	90 (46)	0.8[1]	89 (47)	89 (45)	0.8[1]
Age of onset for smoking, years	18 (6)	17 (4)	0.06[1]	18 (6)	17 (4)	0.05[1]	19 (8)	16 (3)	0.09[1]	19 (8)	17 (4)	0.2[1]
Number of usually smoked CPD	21 (9)	25 (10)	0.03[1]	21 (9)	26 (10)	0.02[1]	19 (7)	21 (11)	0.7[1]	20 (7)	22 (12)	0.5[1]
Number of smoked CPD during the week before the study	20 (9)	22 (10)	0.2[1]	20 (9)	23 (10)	0.2[1]	19 (7)	19 (11)	0.7[1]	19 (8)	20 (12)	1[1]
Number of previous quit attempts	1.7 (0.9)	1.7 (0.7)	0.8[1]	1.7 (0.9)	1.6 (0.7)	0.7[1]	1.6 (0.9)	1.8 (0.9)	0.3[1]	1.5 (0.9)	1.8 (0.9)	0.3[1]
FTND[6]	4.7 (2.2)	5.1 (2.1)[5]	0.3[1]	4.7 (2.2)	5.2 (2.2)	0.2[1]	5.4 (2.0)	5.0 (2.1)	0.4[1]	5.4 (2.1)	5.0 (2.1)	0.5[1]

CPD: Cigarettes Per Day, NRT: Nicotine Replacement Therapy, NM: Normal Metabolizer, NMR: Nicotine-metabolite Ratio, FTND: Fagerström Test for Nicotine Dependence

[1]: Wilcoxon rank sum test

[2]: Chi-squared test

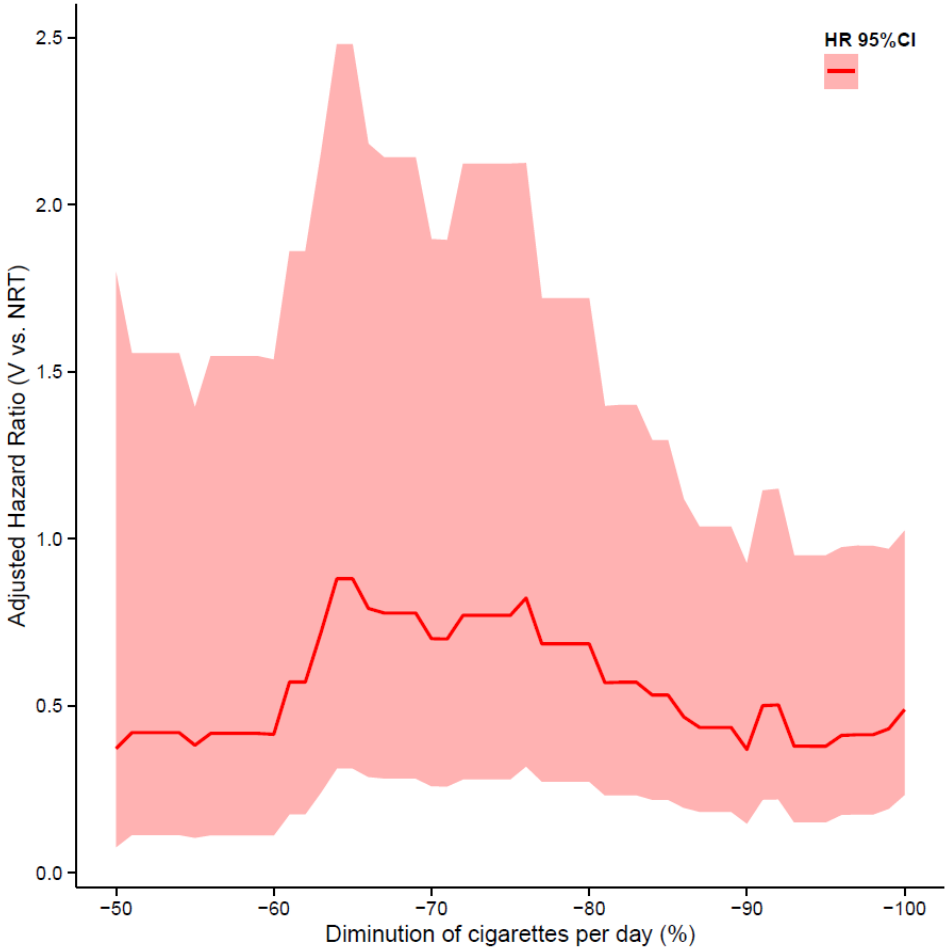
[3]: Fisher exact test

[4]: For both analyses in males, the treatment duration was unknown for one subject in the NRT group and one subject in the varenicline group.

[5]: Missing data for one individual

[6]: Fagerström Test for Nicotine Dependence score ranged from 0 to 10

Supplemental Figure S1: Hazard ratios (HR) depending on the threshold of cigarettes per day decrease used in each analysis. The line represents the hazard ratio; the area represents the 95% confidence interval (95% CI) of the hazard ratio. The sample size (n subjects) and the number of relapse are reported for each analysis with a rounding threshold (-50%, -60%, -70%, -80%, -90%, -100%).



n subjects	170	168	169	166	164	153
n relapses	13	18	24	27	36	53

V: Varenicline, NRT: Nicotine Replacement Therapy