Prediction of successful weight reduction under sibutramine therapy through genotyping of the G-protein β3 subunit gene (GNB3) C825T polymorphism

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Background Sibutramine, a centrally acting noradrenaline and serotonin re-uptake inhibitor, enhances satiety and is frequently used to support weight loss. However, a significant variability exists among individuals concerning the response to sibutramine.

Methods We genotyped 111 participants of a randomized placebo-controlled clinical trial for the GNB3 C825T polymorphism and analysed associations of genotypes with treatment outcome. Patients undergoing a structured weight loss programme were treated with either placebo or 15 mg sibutramine daily for 54 weeks.

Results In the placebo group, the non-pharmacological programme alone resulted in a significantly greater weight loss in individuals with the GNB3 TT/TC genotypes as compared to individuals with the CC genotype (−7.1 ± 1.2 vs. −2.7 ± 1.5 kg, \(P = 0.031\)). Administration of 15 mg sibutramine was more effective in individuals with the CC genotype than in the subjects with the TT/TC genotypes (weight loss: 7.2 ± 2.2 vs. 4.1 ± 2.1 kg, \(P = 0.0013\), sibutramine vs. placebo). In the CC genotype carriers, the odds ratio (OR) for a weight loss greater than 5% (sibutramine vs. placebo) was 6.6 (95% CI 1.8–25.6; \(P = 0.004\)) and for a weight loss greater than 10% was 9.6 (95% CI 1.7–53.8; \(P = 0.010\)).

Conclusion Genotyping for the GNB3 C825T polymorphism is highly predictive for the identification of obese individuals who will benefit from sibutramine treatment. Pharmacogenetics 13:453–459 © 2003 Lippincott Williams & Wilkins

Keywords: obesity, sibutramine, weight loss, GNB3 C825T polymorphism

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Introduction

Obesity is an important risk factor for type 2 diabetes mellitus [1,2], cardiovascular disease [3,4], and overall mortality [5,6]. In view of the spreading epidemic of obesity, efficient strategies to reduce body weight are urgently required. However, current dietary approaches to treat obesity are only of rather limited success. For this reason, weight-lowering drugs were developed to support non-pharmacological measures in obese individuals who experience difficulties in their efforts to lose weight [7].

Sibutramine, a centrally acting noradrenaline and serotonin re-uptake inhibitor, enhances satiety and is used to support weight loss [8–12]. Its efficacy has also been demonstrated in obese patients with type 2 diabetes [13,14]. However, the reported response rates for weight loss greater than 10% during one year are in the range of 20 to 50% only [9–15]. The reasons for the high variability in response to sibutramine are currently unknown. An optimally individualized pharmacother-
subsequent studies also showed an increased risk for obesity in carriers of the 825T variant [18–20]. Potential mechanisms may comprise a reduced lipolytic response to catecholamines in fat cells from 825T allele carriers [21,22].

As sibutramine was originally developed as an antidepressant and since the neurotransmitters serotonin and noradrenaline activate G protein-coupled receptors, we investigated the association between the GNB3 C825T status and treatment outcome in obese patients. For the skin microcirculation was recently shown that 825T allele carriers display an enhanced vasoconstriction on injection of noradrenaline [23]. Moreover, body weight regulation may involve signal transduction pathways in which the β3 subunit of heterotrimeric G proteins appears to be involved [19]. We, therefore, retrospectively genotyped patients who had participated in a multi-centre, randomized, placebo-controlled study in a realistic primary care setting which compared the efficacy of sibutramine 15 mg daily vs. placebo in addition to a comprehensive non-pharmacological treatment programme.

Research design and methods

Patients
In this study, we retrospectively analysed patients who had participated in a multi-centre, randomized, placebo-controlled study in a primary care setting. The study started in autumn 1997 and was terminated in spring 1999. A total of 389 obese subjects aged 18–65 years with a BMI \( \geq 30 \text{ kg/m}^2 \) and \( \leq 40 \text{ kg/m}^2 \) were initially screened, of whom 362 subjects were included in the 54-week study. The intent-to-treat population consisted of 348 individuals, 174 in the sibutramine group (38 males, 136 females) and 174 in the placebo group (51 males, 123 females). Exclusion criteria included cardiovascular disorders such as uncontrolled hypertension or coronary heart disease among others. Primary endpoint of the study was weight loss in kg after 54 weeks versus baseline. A secondary endpoint was, among others, the rate of participants with weight loss greater than 5 and 10%, respectively, at the last visit compared to visit one.

After study entry, patients received individual dietary counselling by a trained dietitian. Energy intake was prescribed on the basis of estimated daily energy requirement minus 500 to 1000 kcal/day. During the first 4 weeks all patients attended four educational sessions to receive information on healthy eating, physical activity, behaviour modification and motivational issues. All subjects were encouraged to participate in 16 group sessions and to increase physical activity.

In 2001, the 31 general practitioners (GPs) and internists who had originally recruited patients were recontacted and asked for their co-operation to re-investigate potential genetic influences on treatment success. Sixteen GPs provided EDTA blood or buccal swabs and completely answered questionnaires of 111 patients, of whom 52 had received sibutramine and 59 placebo treatment. The actual body weight was measured in the GPs’ offices using the same calibrated scales as during the original trial. The questionnaire contained questions on changes in lifestyle including eating habits, medication, severe diseases among others.

The following variables were documented for each patient: gender, age at study entry, current age, body height and weight at study entry, after 54 weeks of treatment or at drop-out, and 2 years after termination of the study. For the follow-up period, none of the patients reported long-term treatment with drugs known to affect body weight or drugs for weight loss.

All participants gave written informed consent, and the study protocol was approved by the ethical committee of the University Hospital Essen.

DNA genotyping
DNA was extracted from whole blood or buccal swabs using commercially available kits (QIAAMP, Qiagen, Hilden, Germany). The GNB3 C825T polymorphism was genotyped using a PSQ 96 system (Pyrosequencing, Uppsala, Sweden). This system allows rapid genotyping of DNA samples on 96-well plates using a direct sequencing reaction.

Statistical methods
Since the phenotype associated with TT and TC genotype is identical and due to the low number of individuals with TT genotype in Caucasian populations, we combined 825T allele carriers (TT+TC) and compared them with homozygous 825C allele carriers (CC genotype). Continuous variables at beginning of study were compared using Student’s t-test. Categorical variables were compared by \( \chi^2 \)-test. Endpoint measures of bodyweight were analysed by univariate analysis of variance with factors for baseline values, centre and sex as covariates (ANCOVA). Effects of treatment or GNB3 alleles were analysed using between subject’s effects and means, standard error of the mean (SEM) and confidence intervals (CI) were adjusted for covariates. Differences between GNB3 alleles and between treatment groups with regard to 5% and 10% weight reduction at end of study were compared by logistic regression analysis with sex, centre, and baseline weight as covariates. Odds ratios (OR) are given with 95% confidence intervals (CI). Results were regarded significantly different at a \( P \)-value < 0.05. All statistical analy-
sis was done using SPSS version 11.0 (SPSS, Chicago, IL, USA).

**Results**

**Genotype-dependent weight loss**

*GNB3* genotype distribution and selected demographic characteristics of the study population are given in Table 1. *GNB3* genotype distributions were in Hardy–Weinberg equilibrium and not significantly different between treatment groups. For the *GNB3* C825T polymorphism, we observed 15 TT (13.5%), 48 TC (43.3%) and 48 CC (43.2%) carriers in the whole group which corresponds with a 825T frequency of 35.1%. This frequency is somewhat higher than that found in non-obese subjects and comparable to that previously found in obese Caucasian subjects [18,24].

Analysis of covariance with baseline weight, sex and centre as covariates revealed that weight loss after 54 weeks (Fig. 1) was significantly greater in the sibutramine group compared to the placebo group (10.3 kg vs. 5.0 kg; *P* = 0.001). The difference was 5.2 kg and 1.5 kg (95% CI: 2.3–8.2) and was consistent with that from other studies [9,10]. In the placebo group, we observed a genotype-dependent difference in weight loss (TT 7.8 kg, TC 6.9 kg, CC 2.7 kg; Table 1). Weight loss in combined 825T allele carriers (TT+TC) on placebo was by 4.3 kg greater than that of homozygous 825C allele carriers (Table 1; Fig. 2).

The data were also analysed to determine which genotype benefits most from adjunct sibutramine administration or may even require drug therapy to achieve substantial weight loss. In individuals with the TT genotype, sibutramine caused no significant additional effect (Table 1; Fig. 3a). With 7 vs. 8 subjects and an assumed difference of 5 kg at a standard deviation of 7 kg, the probability of correctly rejecting the two-sided null hypothesis of no difference at a level of 0.05 is 25%. To achieve 80%, a total of 32 subjects per group would have had to be recruited. However, the concomitant lack of a significant effect of sibutramine in the distinctly larger TC allele group (TT+TC) adds support to the notion that 825T allele carriers do not benefit from adjunct sibutramine therapy (Fig. 3b). In contrast, a strong effect of sibutramine was observed in individuals with CC genotype (*P* = 0.003; Fig 3d). This group lost an additional 7.2 kg compared to 4.1 kg in the subjects with the TT/TC genotype (*P* = 0.0013) when weight reduction was supported by the drug. There was only little overlapping of individual values between the sibutramine and the placebo group in the CC individuals (Fig. 3d).

Logistic regression analysis with baseline weight, sex and centre as covariates revealed an overall crude OR...
for 5% weight loss in the whole study group (sibutramine vs. placebo) of 3.5 (95% CI: 1.6–8.1; \(P = 0.003\)) and that for 10% weight loss of 2.9 (95% CI: 1.2–6.8; \(P = 0.014\)). However, this overall effectiveness of sibutramine over placebo was exclusively caused by the strong effect in individuals with CC genotype in whom OR for 5% weight loss (sibutramine vs. placebo) was 6.8 (95% CI: 1.8–25.6; \(P = 0.004\)) and the corresponding OR for 10% weight loss was 9.6 (95% CI: 1.7–53.8; \(P = 0.010\)). OR were not statistically significant for 825T allele carriers.

**Discussion**

In view of the epidemically increasing prevalence of obesity in most parts of the world and together with the fact that obesity is the most important risk factor for type 2 diabetes, effective non-pharmacological and pharmacological strategies to reduce body weight are urgently needed. Due to the limited success of lifestyle intervention programmes weight-lowering drugs are becoming more important but result in a wide variation of response. Therefore, it is highly desirable to identify predictive markers for drug treatment.

In the present study, we have retrospectively genotyped obese individuals who participated in a randomized, placebo-controlled clinical trial to determine the efficacy of sibutramine vs. placebo on weight loss in the setting of a structured weight-loss programme. We show here for the first time that individuals with the CC genotype obviously benefit from adjunct sibutramine therapy for weight loss, whereas no such effect was observed in 825T allele carriers. Furthermore, it turned out that carriers of the 825T allele had a much greater weight loss in response to the non-pharmacological programme alone than the CC carriers.

From the originally participating 348 individuals 111 could be re-recruited for genotype analysis. This raises the questions whether the results reported here were influenced by an unrecognized selection bias occurring at participating study sites or by a non-randomized patient selection for genotyping. However, there is no evidence that this is the case as genotype distributions of the C825T polymorphism in the whole study group as well as in the subgroups treated with placebo or sibutramine were comparable to those found in other obese Caucasian populations [18] and no significant deviations from Hardy–Weinberg equilibrium were observed. Moreover, both patients and referring physicians were unaware of patients’ genotypes at any time of this study. Another argument is that the average
genotype-independent weight loss observed in the sibutramine vs. the placebo group of approximately 5 kg matches exactly the values that were previously reported in comparable studies [9–11,13,25]. This may strongly argue against the notion that our findings are the result of an unintentional preference of certain genotypes in the recruitment process.

The strong dependency of the sibutramine effect on the GNB3 genotype also raises the question on the possible mechanism of action. The phenotype of increased intracellular signal transduction associated with the GNB3 825T allele and the ‘T-haplotype’ are well understood. In general, the 825T allele is associated with enhanced intracellular signal transduction via G protein-coupled receptors which activate G protein heterotrimers presumably involving the G protein β3 subunit. Most pharmacogenetic studies have shown so far that 825T allele carriers – in line with expectations – show increased responses to treatment with drugs which activate G protein-coupled receptors, e.g. α2-adrenoceptors [26,27].

In view of these earlier findings the almost selective responsiveness towards sibutramine in patients with the CC genotype was surprising. We observed here that 825T allele carriers, although being at increased risk for obesity [18,19], show a clear tendency for an increased
weight loss under a non-pharmacological weight reduction programme alone. Nevertheless, this result is in agreement with earlier findings showing that high physical activity almost fully counteracts the susceptibility for obesity in 825T allele carriers [19,28]. Hence, we can only speculate on the reasons underlying the observed effects. Eventually, the presumed set-point for defended body weight is relatively weak and may be easily shifted up- and downwards in for defended body weight is relatively weak and may be easily shifted up- and downwards in

In conclusion, this study demonstrates that genotyping of the GNB3 C825T polymorphism may help to predict the outcome of both non-pharmacological and pharmacological programmes to reduce body weight. The association of weight loss with GNB3 genotypes was surprisingly strong which may indicate a significant role of the gene in the regulation of body weight. Therefore, further studies are required to confirm and examine the strength of this association in other populations. Furthermore, it appears worthwhile to relate treatment outcome with genotypes of other polymorphic genes known to be involved in body weight regulation. Hopefully, this research may ultimately result in an individually tailored drug therapy for obesity.

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