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CYP2C9, *VKORC1*, *CYP4F2*, *ABCB1* and *F5* variants: Influence on quality of long-term anticoagulation

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ABSTRACT

Aims: The study aims to evaluate the impact of genetic, demographic and clinical data on various measures of outcome of anticoagulation quality in patients.
Patients and methods: The study consisted of 310 patients receiving long-term oral anticoagulation therapy in our hospital. Apart from demographic and clinical variables, 21 SNPs (in 7 genes) were analyzed and compared with the outcomes of anticoagulation therapy. Various outcomes that were measured are; supra therapeutic INRs (INR >3, >6), anticoagulation stabilization, time taken to stabilize and proportion of INRs within (2–3), above (>3) and below (<2) therapeutic range.
Results: Supra therapeutic INRs were influenced by *CYP2C9**2, *3, *CYP4F2* rs2108622, *VKORC1*-1639G>A, 1173C>T, rs55894764 along with concomitant drugs, smoking, body weight and height. Persistently fluctuating INRs/absolute instability correlated with *VKORC1*-1639G>A, gender, height and body mass index. The time taken to stabilize was associated with *CYP4F2* rs2108622, *CYP2C9**14, smoking, clinical indication and concomitant drugs. The overall distribution of INR was influenced by variants in *CYP4F2* rs2108622, *CYP2C9**3, rs9332230, *VKORC1* 1173C>T, −1639G>A, rs55894764, *ABCB1* rs2032582, rs1128503, rs1045642 and *F5* rs6025, age, smoking and concomitant drugs.
Conclusions: Knowledge of factors influencing the quality of long term anticoagulation can help clinicians to customize therapy either by dose variation, therapy with alternate choice of drug, concurrent heparin therapy and/or frequent INR monitoring.

Introduction

The quality of anticoagulation therapy and drug toxicity in patients may be measured by different means. The most commonly studied parameters for drug response are the stabilized dose and occurrence of bleeding events. Apart from these, the anticoagulant efficiency and risk of toxicity may be calculated by the length of time taken to stabilize, time spent (or percent) within and outside the therapeutic international normalized ratio (INR) range, over anticoagulation (elevated INRs > 3.0 or 4.0), severe over anticoagulation (elevated INRs > 5.0 or 6.0), absolute instability or persistently fluctuating INRs. A wide range of therapy-related clinical factors such as the initiation dose, scheme of dose titration, target INR range, quality and frequency of anticoagulation monitoring and concurrent therapy with interacting drugs can contribute to variations in any of the above quality measures. Environmental and demographic causes of variation may be attributed to food intake, body weight and height, age, smoking and alcohol abuse, clinical indication and comorbidities. Most important are the inherent and unvarying variable, i.e.

Abbreviations: OAC, oral anticoagulant; ADR, adverse drug reaction; BMI, body mass index; SD, standard deviation; CI, confidence interval; BSA, body surface area; INR, international normalized ratio; AOD, arterial occlusive disease; CAD, coronary artery disease; AVR, aortic valve regurgitation; FVL, factor V Leiden; ACE, angiotensin converting enzyme.

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genetic polymorphisms can have a large contribution to the efficacy and toxicity of anticoagulants. There is a dearth of data on the plausible genetic variations that contribute to diversity in the quality of anticoagulation therapy in routine clinical setup. This paper aims to divulge the potential genetic and non-genetic factors contributing to measures of quality and toxicity of oral anticoagulation (OAC) therapy.

Patients and methods

Cohort and inclusion criteria

The study was conducted at the Sir Ganga Ram Hospital, a tertiary health care centre in New Delhi, India. The research protocol was approved by the Ethics Board Committee of Sir Ganga Ram Hospital and is in accordance with the ethical standards of Declaration of Helsinki (World Medical Association). All participants gave written informed consent. The inclusion criteria and design of the study have been described elsewhere [26]. In brief, the study cohort consisted of 257 and 53 new users of acenocoumarol warfarin respectively, enrolled in the period between January 1, 2010, and February 31, 2011 for long-term anticoagulation therapy. All patients were followed up for a year or the end of the study period (1st November 2011) whichever occurred first. Demographic and clinical details were collected with the help of standardized questionnaires. Blood samples were collected and DNA was isolated as previously reported [26].

Outcome definition

The present study analyzed seven different anticoagulation outcomes in patients on long-term anticoagulation therapy; (1) over anticoagulation, defined by at least one elevated INR > 3.0 measurement, (2) severe over anticoagulation, defined by at least one supra-elevated INR > 6.0 measurement, (3) absolute instability, defined as failure to stabilize in the study period due to persistently fluctuating INRs; (4) time taken to stabilize (days), where in a patient was classified as being 'stabilized' when the last three consecutive INR values remain within 2.0–3.0 without any change in weekly average dose; (5) Proportion of INRs within therapeutic range of 2.0–3.0, (6) proportion of elevated INRs > 3.0, and (7) proportion of subtherapeutic INRs < 2.0. The proportion of INRs in the last three outcomes were calculated by the percentage of INRs falling in the three categories of INR group for each patient, i.e. 2–3, >3 and <2. Adverse drug reactions (bleeding/haemorrhage) were initially classified as minor (requiring no additional testing, referral, or outpatient visits), or major (requiring medical or surgical intervention, major blood loss requiring blood transfusion of two units or more).

Genotyping

Twenty one SNPs in seven different genes were analyzed. Nine variants in *CYP2C9* (*2/rs1799853/430C>T/p.Cys144Arg in exon 3; *3/rs1057910/c.1075A>C/p.Ileu359Leu in exon 7; rs9332120, c.331+73T>C in intron 2; rs9332230, c.1291+53A>T in intron 8; rs2298037, c.1291+147C>T in intron 8; *14/rs72558189, c.374G>A/p.Arg125His in exon 2; rs9332172, c.820-73A>G in intron 5; rs1057911, c.1425A>T/p.Gly475Gly in exon 9; and c.610A>C, *57/p.Asn204His in exon 4), four variants in *VKORC1* (−1639G>A/g.3588G>A/rs9923231 in upstream promoter region; rs9934438, c.1173C>T in intron 2; rs7294, c.516G>A/3730G>A in 3′UTR and rs55894764/c.36G>A/p.Arg12Arg in exon 1), *CYP4F2* rs2108622 (c.1297G>A/p.Val433Met in exon 11), three common polymorphisms in the *MDR1*/*ABCB1* gene (rs1128503/c.1236T>C/p.Gly412Gly in exon 12; rs2032582/c.2677T>G/A/p.Ser893Ala/Thr in exon 21 and rs1045642/c.3435C>T/p.Ile1145Ile in exon 26), *APOE* isoforms (e2, e3, e4 distinguished by two non synonymous polymorphisms; rs7212 and rs229358), factor V Leiden variant in *F5* (rs6025/1691G>A/p.Arg506Gln) and prothrombin variant in *F2* (rs1799963/20210G>A in 3′UTR) were genotyped. The rationale of selection of candidate SNPs in *CYP2C9* and *VKORC1* genes and the genotyping methods have been previously described [26].

Statistical analysis

Statistical analysis was performed with SPSS, version 16.0 (SPSS Inc., Chicago, IL). The expected genotype frequencies and the deviation from Hardy–Weinberg equilibrium were analyzed by Chi square test. The presence of any differences between the categorical outcome groups was analyzed by Fisher exact test for categorical factors and by independent samples *t* test for continuous factors. All analyses were conducted at two-tailed α-level of 0.05. For the genetic variables, we coded the wild type as '0', heterozygous as '1' or homozygous as '2' in order to model additive allelic effects. If the comparison with such coded genotypes was not significant or if the prevalence of homozygous variant alleles was low, the heterozygous and homozygous variant genotypes were pooled for comparison of overall SNP effect on respective outcome measures. We also used dummy variables to code demographic factors (sex, smoking status) and clinical variables (indication for anticoagulant therapy, additional comorbidities), and individual concomitant medications. Finally, we used continuous variables for age, body weight, height, body surface area (BSA), body mass index (BMI). Body mass index (BMI), the common used index of weight-for-height to classify underweight, overweight and obesity in adults was calculated as the weight in kilograms divided by the square of the height in metres (kg/m^2).

Results

The baseline demographic and clinical characteristics are provided here (Supplementary Table 1). The study population had a mean age of 42.51 years (standard deviation, SD = 17.36) and an average BMI of 25.82 (SD = 5.8). The mean follow up period was 475.32 days (SD = 172.57) during which an average of 17.04 (SD = 5.31) INR measurements were recorded for each patient. The study population had 32.98% (SD = 18) of INRs within the therapeutic range, 13.28% (SD = 15.61) INRs > 3.0 and 53.73% (SD = 20.89) INRs < 2.0. Among the 294 patients who stabilized on either anticoagulant, the average time taken to stabilize was 82.9 days (SD = 65.31) and the mean stabilized weekly dose was 20.03 mg (SD = 8.21) and 43.01 mg (SD = 16.34) of acenocoumarol and warfarin respectively. The genotype and allele frequency of all SNPs are tabulated and found to fit in Hardy Weinberg equilibrium (Supplementary Table 2).

Comparative analysis for the outcome measures was carried out with all factors listed in the Supplementary Table 1. Only the significant factors associated with different outcome measures are tabulated; over anticoagulation with INR > 3.0 (Table 1), severe over anticoagulation with INR > 6.0 (Table 2), state of stabilization (Table 3), time taken to stabilize (Table 4) and proportion of INR within therapeutic range of 2.0–3.0 (Table 5). Secondary analyses of factors contributing to proportion of sub therapeutic INRs (<2.0) and elevated INRs (>3.0) was also carried out similarly (Supplementary Tables 3 and 4). Adverse drug reactions (bleeding/haemorrhage) were observed more commonly in patients with 30.5–46% (50th centile, median) sub-therapeutic INRs and 29.5–33.33% (50th