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Copy number variation and warfarin dosing: evaluation of *CYP2C9*, *VKORC1*, *CYP4F2*, *GGCX* and *CALU*

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Abstract

Aim—To determine if copy number variants contribute to warfarin dose requirements, we investigated *CYP2C9*, *VKORC1*, *CYP4F2*, *GGCX* and *CALU* for deletions and duplications in a multiethnic patient population treated with therapeutic doses of warfarin.

Patients & methods—DNA samples from 178 patients were subjected to copy number analyses by multiplex ligation-dependent probe amplification or quantitative PCR assays. Additionally, the *CYP2C9* exon 8 insertion/deletion polymorphism (rs71668942) was examined among the patient cohort and 1750 additional multiethnic healthy individuals.

Results—All patients carried two copies of *CYP2C9* by multiplex ligation-dependent probe amplification and no exon 8 deletion carriers were detected. Similarly, quantitative PCR assays for *VKORC1*, *CYP4F2*, *GGCX* and *CALU* identified two copies in all populations.

Conclusion—These data indicate that copy number variants in the principal genes involved in warfarin dose variability (*CYP2C9*, *VKORC1*), including genes with lesser effect (*CYP4F2*, *GGCX*), and those that may be more relevant among certain racial groups (*CALU*), are rare in multiethnic populations, including African-Americans.

Keywords

CALU; CNV; copy number variation; *CYP2C9*; *CYP4F2*; *GGCX*; pharmacogenetics; *VKORC1*; warfarin

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Warfarin is a widely used oral anticoagulant commonly prescribed for prevention of thrombotic disorders among patients with deep vein thrombosis, pulmonary embolism, atrial fibrillation and other indications [1]. The drug has a narrow therapeutic index and wide interindividual variability in therapeutic dose requirements, which is influenced by a number of clinical factors (e.g., age, race, bodyweight and gender amongst others) [2,3]. Several candidate gene studies have identified common sequence variants in *CYP2C9* and *VKORC1* that also significantly influence warfarin dose requirements [4–6]. These genes are involved in warfarin pharmacokinetics and pharmacodynamics, respectively, and since their identification, pharmacogenetic regression algorithms have been developed that include variant *CYP2C9* and *VKORC1* alleles and other clinical variables to predict therapeutic warfarin doses [7–15]. Importantly, a recent study has retrospectively shown that pharmacogenetic-guided dose prediction is more accurate than both empiric dosing (i.e., 5 mg/day) and the US FDA-approved dosing guidelines listed on the warfarin product insert [16].

Given that *CYP2C9*, *VKORC1* and other clinical (e.g., age and weight) and environmental (e.g., concomitant medications) factors together only account for approximately 35–50% of interindividual warfarin dose variation [5,6,13,17], many studies have attempted to identify additional sources of genetic variation contributing to warfarin dose variability. For example, four genome-wide association studies (GWAS) using Caucasian and Japanese patient cohorts identified a single nucleotide variant in a third gene implicated in warfarin dosing, *CYP4F2* [18–21]. Interestingly, no other statistically significant signals were reported among these GWAS; each of which involved approximately 500–2000 patients. However, other candidate gene studies have reported on the role of rare *CYP2C9* variants in warfarin dosing, particularly in non-Caucasian racial and ethnic groups [22–25]. Moreover, nucleotide variants in *GGCX* and *CALU*, additional genes involved in the warfarin anticoagulation pathway, also may be involved in interindividual warfarin dose variability [26–30].

In addition to SNPs, recent attention has focused on the role of heritable copy number variants (CNVs) in disease susceptibility and complex traits [31–41]. CNVs are heritable duplication and deletion alleles, which can have functional consequences on protein abundance and/or enzyme activity. It is estimated that up to approximately 12% of the human genome harbors CNVs [34], which include several known pharmacogenetic genes: *CYP2D6* [42,43], *GSTT1* and *GSTM1* [44,45], *SULT1A1* [46,47] and *UGT2B17* [48]. Although one GWAS on warfarin dosing incorporated CNV detection in a subset of their cases [20], the probe density of their genome-wide platform had low resolution for copy number analyses, limiting the ability to detect smaller CNVs that potentially could influence therapeutic warfarin dose requirements. To date, no study has directly interrogated the copy number of important genes involved in warfarin dose variability. Therefore, we performed a pilot study to determine the role of CNVs in *CYP2C9*, *VKORC1*, *CYP4F2*, *GGCX* and *CALU* in a multiethnic patient population treated with therapeutic doses of warfarin, as well as in healthy individuals from different racial and ethnic groups.

Patients & methods

Study population

Between July 2007 and July 2011, adult patients taking warfarin and managed at institutional anticoagulation clinics at the Mount Sinai Hospital (NY, USA) or Elmhurst Hospital Center in New York City (NY, USA), or referred to the study investigators, were assessed for enrollment. Subjects were included in the analysis if their target international normalized ratios (INR) were between 2.0 and 3.0 or between 2.5 and 3.5, and at least two therapeutic measurements were separated by at least 1 week. Thus, the study sample was

comprised of patients with established warfarin dose requirements. Prior to participation, all subjects signed a written informed consent approved by the institutional review board (IRB) governing research involving human subjects. Subsets of these specimens were used in previously described studies [13,22].

In addition, peripheral blood samples from healthy donors who indicated their racial and ethnic background (African-American, Hispanic, Asian or Caucasian) and gave informed consent for the use of their DNA for research were obtained from the New York Blood Center (NY, USA) with IRB approval [22,49]. Blood samples were also obtained with informed consent from unrelated healthy 100% Ashkenazi Jewish individuals from the greater New York metropolitan area [50,51]. All personal identifiers were removed from these samples and isolated DNA was tested anonymously. Genomic DNA was isolated using the Puregene® DNA Purification kit (Qiagen, CA, USA) according to the manufacturer's instructions.

Multiplex ligation-dependent probe amplification

Multiplex ligation-dependent probe amplification (MLPA) of *CYP2C9* was performed using the P128 CYP450 MLPA kit (Version A1; MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer's instructions. In brief, all DNA samples were diluted to 200 ng with Tris-EDTA buffer and denatured in a thermal cycler for 5 min at 98°C. After cooling to 25°C, probemix (including *CYP2C9* probes for exons 1, 7 and 9; **Figure 1**) and MLPA buffer were added to each sample, mixed and incubated for 1 min at 95°C followed by a 16 h hybridization at 60°C. The ligation reaction was performed at 54°C by adding 32 µl of Ligase-65 mix to each tube followed by heating for 5 min at 98°C. PCR buffer, water and MLPA ligation reactions were mixed in new tubes and maintained in a thermocycler at 60°C while polymerase mix was added to each tube. Exon-specific probes with universal-tagged primers underwent PCR consisting of 35 amplification cycles (95°C for 30 s, 60°C for 30 s and 72°C for 60 s), followed by a 20 min incubation at 72°C. Amplified products were separated by capillary gel electrophoresis and data were analyzed using GeneMarker v1.90 software (SoftGenetics, PA, USA).

CYP2C9 exon 8 (rs71668942) genotyping

The 2226-bp *CYP2C9* exon 8 insertion/deletion polymorphism (rs71668942) was genotyped using a three-primer PCR and standard agarose gel electrophoresis. The use of three primers allowed for a deletion-specific amplicon as illustrated in **Figure 1**. PCR reactions were performed in 25 µl containing approximately 100 ng of DNA, 1X PCR buffer, 2.5 mM MgCl₂, 0.2 mM of each dNTP, 0.4 µM of the forward primer (FWD: 5'-GCACATAGTTTCAGTATCCTGTTACTTTCC-3') and 0.2 µM of each reverse primer (REV-1: 5'-C A G G T C A A G A T C A T C A GGTCAGATG-3'; REV-2: 5'-GATTGAATCATGGGGGATAATTTCC-3'), and 1.0 unit of Platinum® Taq DNA Polymerase (Invitrogen, CA, USA). The PCR amplification conditions consisted of a denaturation step at 94°C for 5 min followed by 35 amplification cycles (94°C for 30 s, 60°C for 30 s and 72°C for 30 s) and a final incubation at 72°C for 5 min. Aliquots of all PCR products were loaded onto 1.5% agarose gels, electrophoresed and visualized under UV light. Representative amplification products were confirmed by bi directional sequencing using individual amplification primers.

Copy number analysis using quantitative PCR

The copy number of selected genes known to influence warfarin dose requirements (*VKORC1*, *CYP4F2*, *GGCX* and *CALU*) was interrogated using commercially available TaqMan® real-time quantitative PCR (qPCR) copy number assays (Applied Biosystems, CA, USA) as per the manufacturer's instructions. In brief, FAM-labeled *VKORC1*, *CYP4F2*,

GGCX and *CALU* TaqMan minor-groove binding probes and unlabeled PCR primers (**Table 1**) were individually run in a duplex qPCR with a VIC-labeled RNase P TaqMan Copy Number Reference Assay (catalog number 4403326, Applied Biosystems). Quadruplicate experiments were each performed in 10 μ l containing 10 ng of DNA, 1X TaqMan Genotyping Master mix, 0.5 μ l each of TaqMan Copy Number and Reference Assays in 384 well plates. Covered plates were run in a 7900HT Fast Real-Time PCR System (Applied Biosystems) and the amplification consisted of a denaturation step at 95°C for 10 min followed by 40 amplification cycles (95°C for 15 s and 60°C for 60 s). Data were captured using absolute quantitation with a manual C_T threshold and autobaseline, followed by analysis using CopyCaller™ v1.0 Software (Applied Biosystems) where the number of copies of target sequence was determined by relative quantitation with the comparative C_T ($\Delta\Delta C_T$) method. This method measures the C_T difference (ΔC_T) between target and reference sequences, and then compares the ΔC_T values of test samples to a calibrator sample with two copies of the target sequence. The copy number of the target was calculated as twice the relative quantity.

Results

Patient characteristics

Patient characteristics are listed in **Table 2**. The mean age was 65 ± 15 years; 106 participants (61%) were male. A total of 88 subjects (49%) were Caucasian, 37 (21%) African-American, 21 (12%) Hispanic and 30 (17%) Asian. The most frequent indications for anticoagulation were atrial fibrillation in 117 patients (66%), and venous thromboembolism in 23 patients (13%). The median therapeutic warfarin dose among all patients was 35 mg/week and the distribution of therapeutic warfarin doses among the INR 2.0–3.0 and 2.5–3.5 cohorts are illustrated in **Figure 2**. Among the warfarin-treated patient population, therapeutic dose requirements ranged from 7–280 mg/week; 21% had therapeutic warfarin dose requirements of less than 21 mg/week, 47% were between 21 and 49 mg/week and 31% were >49 mg/week. Previous analyses of the majority of patients in this cohort with target INR values of 2.0–3.0 identified significant associations between common *CYP2C9* and *VKORC1* variant alleles and warfarin dosing, consistent with other studies [13,22].

CYP2C9 copy number analysis

To interrogate *CYP2C9* copy number, MLPA was performed on all 178 patient DNA samples. The P128 Cytochrome P-450 MLPA kit (Version A1, MRC-Holland, The Netherlands) contained probes for *CYP2C9* exons 1, 7, and 9 (**Figure 1**). All 178 patient samples had two copies of *CYP2C9* based on the interrogated exons compared with control reference probes. A representative MLPA analysis of 50 patient samples is shown in **Figure 3**.

CYP2C9 exon 8 deletion analysis

Given that the *CYP2C9* MLPA probes did not interrogate exon 8 of the gene and a large (~2.2 kb) insertion/deletion polymorphism (rs71668942) encompassing this exon is cataloged in the NCBI SNP database (dbSNP) [101], additional genotyping was performed to interrogate *CYP2C9* exon 8. Using the three-primer PCR strategy detailed in the 'Patients & methods' section, in the presence of a wild-type *CYP2C9* sequence, the three *CYP2C9* primers generate two amplicons of 457 and 2528 bp; however, the PCR conditions did not allow for amplification of the larger 2528-bp product (**Figure 1**). A deletion corresponding to rs71668942 would result in a single 302-bp amplification product. Among all 178 warfarin-treated patient samples tested, no rs71668942 deletion carriers were identified. A representative gel image for 18 patient samples is shown in **Figure 3**. In addition, 350 DNA

samples, each from healthy anonymous African–American, Asian, Hispanic, Caucasian and Ashkenazi Jewish individuals were interrogated and no *CYP2C9* exon 8 deletion polymorphism carriers were identified.

***VKORC1*, *CYP4F2*, *GGCX* & *CALU* copy number analyses**

In addition to *CYP2C9* copy number analyses using MLPA and insertion/deletion genotyping, *VKORC1* copy number was interrogated using TaqMan copy number assays with specific probes and primer pairs for all three *VKORC1* exons. All 178 patients analyzed had two copies of *VKORC1* and the calculated copy number data for 50 representative patient samples are shown in **Figure 4**. In addition, three other genes recently shown to influence warfarin dosing, *CYP4F2*, *GGCX* and *CALU*, were analyzed with TaqMan copy number assays and all patients again harbored two copies of each gene (**Figure 5**).

African–American copy number analysis

Given that variant alleles known to influence warfarin dosing (e.g., *CYP2C9**2, *3 and *VKORC1* c.-1639G>A) are less frequent among African–Americans compared with Caucasians [49] and account for less of the dosing variability in the African–American population [52], an additional 50 DNA samples from healthy African–American individuals were tested for *CYP2C9*, *VKORC1*, *CYP4F2*, *GGCX* and *CALU* copy number [22,49]. Consistent with the multi ethnic warfarin-treated patient cohort and the 37 warfarin-treated African–American patients, two copies of each tested gene were detected in the additional healthy African–American samples tested using MLPA and TaqMan copy number assays.

Discussion

Previous studies investigating the pharmacogenetics of interindividual warfarin dose variability have focused on the role of common SNPs within candidate genes involved in warfarin pharmacokinetics and pharmacodynamics. The paucity of data on copy number in the warfarin pharmacogenetics literature prompted our study to interrogate CNVs in major genes associated with warfarin dose requirements. Using quantitative molecular techniques, we found that heritable variation in the copy number of *CYP2C9*, *VKORC1*, *CYP4F2*, *GGCX* and *CALU* was not common in a multiethnic patient population treated with therapeutic doses of warfarin and additional healthy control individuals. Based on our study sample sizes, CNVs in the tested genes have allele frequencies in the African–American, Asian, Caucasian and Hispanic populations of less than 0.5, 2.5, 0.5 and 2.0%, respectively.

Common *CYP2C9* variant alleles with reduced activity result in delayed warfarin elimination, decreased therapeutic warfarin doses and increased bleeding risks [53,54]. The *VKORC1* enzyme is the direct target of warfarin, and common *VKORC1* haplotypes that result in reduced gene expression have been reproducibly implicated as the major genetic determinant of warfarin dose variability [7,8,11,26,52,55]. Together, *CYP2C9* and *VKORC1* variant alleles account for approximately 35% of the interindividual dose variability [8,11], prompting several GWAS and candidate gene studies dedicated to identifying additional genes and/or variants involved in warfarin dose variability. These studies have confirmed the principal roles of *CYP2C9* and *VKORC1* and also have identified a single variant allele in the *CYP4F2* gene (p.V433M; rs2108622) that accounts for between 2 and 5% of dosing variability [18–21]. Notably, with the exception of one of these GWAS [20], copy number analyses have not previously been incorporated into warfarin pharmacogenetic studies.

Because *CYP2C9* and *VKORC1* are the primary genes known to influence warfarin dosing, our CNV study attempted to cover as much of the coding region of these genes as possible. For *CYP2C9*, exons 1, 7 and 9 (of nine exons) were assessed by MLPA, and for *VKORC1*,

all three exons were interrogated by qPCR. Although MLPA would easily detect any full-gene *CYP2C9* deletion or duplication, it would not detect smaller exon-specific copy number variants not interrogated by the probe mix, including the approximately 2.2-kb exon 8 insertion/deletion (rs71668942) polymorphism. This variant allele was previously identified by computational analyses of DNA resequencing data originally generated for SNP discovery projects [56,57]. Although this particular insertion/deletion variant was not directly validated when first identified, the authors reported a 97% validation rate when confirming other novel insertion/deletion variants [56]. No allele frequencies currently exist for rs71668942 on dbSNP; however, our study indicates that the *CYP2C9* exon 8 deletion allele is rare (<0.1%) in the African-American, Asian, Hispanic, Caucasian and Ashkenazi Jewish populations. Of note, the newer version of the P128 Cytochrome P-450 MLPA kit (Version B1; MRC-Holland, The Netherlands) now contains two additional *CYP2C9* probes that target exon 8 based on these data.

In the coagulation pathway, *GGCX* is directly involved in the post-translational carboxylation and activation of hypofunctional clotting factors with reduced vitamin K serving as an essential cofactor. Variant *GGCX* alleles (e.g., rs11676382) have been associated with warfarin dosing in some studies with modest effect sizes, but have not been consistently replicated [26–28,58]. Although a *GGCX* CNV is cataloged in the Database of Genomic Variants (Variation_35863) [31,59], no previous studies on *GGCX* and warfarin dosing have directly interrogated its copy number. Despite the single entry for *GGCX* in the Database of Genomic Variants, we did not identify any patients or additional population samples that harbored deletion or duplication alleles of this gene, suggesting that *GGCX* CNVs are uncommon in the general population.

GGCX is inhibited by the chaperone protein *CALU*, found in the endoplasmic reticulum. Interestingly, a conserved intronic *CALU* SNP (c.582+133A>G; rs339097) was reported to predict higher warfarin doses in African-Americans [29], which was recently confirmed in a study of Egyptian patients [30]. We did not detect CNVs in the *CALU* gene among the multiethnic patient population or the additional African-American subjects. Since rs339097 is more frequent in African-Americans (~15%) than in Caucasians (<1%), and it is unclear if the allele is a functional or haplotype-tagging SNP, future studies on *CALU* might benefit from extensive sequencing and/or fine mapping in different ethnic and racial patient populations. Microsomal *EPHX1* might also be involved in the warfarin pathway; however, it was not included in our CNV study given the inconsistent reports on its putative association with warfarin dose requirements [60,61].

Conclusion

CNVs in genes involved in drug metabolism and response have received increasing attention since the identification of heritable deletion and duplication alleles [62–65], most notably for *CYP2D6* [42,43]. This is the first study to directly and systematically interrogate the role of CNVs in warfarin pharmacogenetics. However, our data indicate that CNVs in genes involved in warfarin dose variability (*CYP2C9* and *VKORC1*), including genes with lesser effect (*CYP4F2* and *GGCX*), and those that may be more relevant among certain racial groups (*CALU*), are rare in multiethnic patient populations. It is still possible that CNVs in these genes may be present in certain ethnic populations that were not studied here. Additionally, we cannot rule out the possibility of other small exon-specific insertion/deletion polymorphisms that were not interrogated by the primers and probes used in the current study. Moreover, CNVs in as yet unidentified genes in the warfarin pharmacokinetic and pharmacodynamic pathways may still play an important role in interindividual warfarin dose variability.

Future perspective

As the major genetic (e.g., *CYP2C9* and *VKORC1*) and nongenetic (e.g., age, weight, concomitant medication) components of warfarin dose variability only account for approximately 50% of interindividual variation, it is likely that additional genes and variants will soon be discovered that contribute to dose variability. Although CNVs of known genes involved in warfarin pharmacokinetics and pharmacodynamics do not appear to play a major role in interindividual dose variability, it is still possible that CNVs of currently unidentified genes will significantly influence warfarin dosing. Ongoing GWAS will likely identify additional genes and common variants with moderate effects on warfarin dosing in different racial and ethnic groups. Moreover, forthcoming whole-exome and/or whole-genome sequencing studies have the potential to identify novel genes and rare variants with significant effect sizes [66], as well as influential small and large CNVs. As these discoveries are made, pharmacogenetic-guided warfarin dosing algorithms will undoubtedly incorporate the new genetic and nongenetic variables for more accurate, refined and personalized warfarin dose prediction [8,11,12,102].

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Financial & competing interests disclosure

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■■ of considerable interest

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Executive summary

Background

- Common *CYP2C9* and *VKORC1* variant alleles account for approximately 35% of the interindividual variation in warfarin dose requirements; variants in additional genes (e.g., *CYP4F2*, *GGCX* and *CALU*) have smaller effects.
- The absence of copy number data on genes known to influence warfarin dosing prompted our investigation into the role of copy number variants in warfarin dose variability in a multiethnic patient population with known therapeutic warfarin doses, and in additional multiethnic healthy individuals.

CYP2C9, VKORC1, CYP4F2, GGCX & CALU copy number

- Multiplex ligation-dependent probe amplification analysis of *CYP2C9* and targeted genotyping of the *CYP2C9* exon 8 insertion/deletion polymorphism (rs71668942) identified all patients and healthy controls as having two copies of the gene.
- Targeted TaqMan[®] copy number analysis of all three *VKORC1* exons indicated that all tested patients and healthy controls harbored two copies of the gene.
- Targeted TaqMan copy number analysis of *CYP4F2*, *GGCX* and *CALU* indicated that all tested patients and healthy controls had two copies of each gene.

Conclusion

- Our data indicate that copy number variants in genes involved in warfarin dose variability (*CYP2C9* and *VKORC1*), including genes with lesser effect (*CYP4F2* and *GGCX*), and those that may be more relevant among certain racial groups (*CALU*), are rare in multiethnic patient populations.

Figure 1. *CYP2C9* multiplex ligation-dependent probe amplification and exon 8 insertion/deletion (rs71668942) genotyping

(A) Schematic representation of the *CYP2C9* gene with asterisks (*) denoting multiplex ligation-dependent probe amplification probe locations. (B) Exon 8 of *CYP2C9* is encompassed by a 2226-bp insertion/deletion polymorphism (rs71668942) highlighted by a gray line. Genomic DNA PCR on a sample without the deletion using two reverse primers (1 and 2) would amplify two products: 457 bp and 2528 bp; however, the larger product is not amplified using the reported PCR conditions. In the presence of the deletion, the second reverse primer would amplify a 302-bp product.

Figure 2. Dose histograms of the patient population

Two target INR populations were included in the study: 2.0–3.0 and 2.5–3.5. Illustrated is the distribution of therapeutic doses among the tested patient population in mg/week and median and mean doses among each target INR population are noted.

INR: International normalized ratio.

Figure 3. *CYP2C9* multiplex ligation-dependent probe amplification analysis and exon 8 insertion/deletion (rs71668942) genotyping

(A) Representative *CYP2C9* multiplex ligation-dependent probe amplification data of 50 patient samples with a pooled reference control sample (46,XY DNA; Promega, WI, USA) and no template (Blank: H₂O) controls. The data are expressed as the ratio of peak heights for the target sequence versus reference sequences known to have two copies in a diploid human genome. Thus, values of ~1.0 represent two copies and values of ~0.5 and 1.5 represent one (deletion) and three (duplication) copies, respectively. All patient samples and healthy controls carried two copies of *CYP2C9* based on probes for exons 1, 7 and 9. Error bars represent the standard deviation of the ratios for the three probes. (B) A representative agarose gel image of 18 patient samples and a PCR-negative control (Blank: H₂O). No deletion carriers were identified as evidenced by only a single PCR product migrating at the expected 457-bp size compared with the 100-bp size markers.

Figure 4. *VKORC1* copy number analysis

Representative TaqMan[®] data for all three *VKORC1* exons of 50 patient samples with two copy (46,XY DNA; Promega, WI, USA) and three copy (47,XY,+16) control samples and no template (Blank: H₂O) controls. The data are expressed as the calculated copy number for the target sequence as determined using CopyCaller[™] software. All tested patient samples and healthy controls carried two copies of *VKORC1*. Error bars represent the standard deviation from quadruplicate experiments.

Figure 5. *CYP4F2*, *GGCX* and *CALU* copy number analyses

Representative TaqMan[®] data for *CYP4F2*, *GGCX* and *CALU* of 50 patient samples with control samples (46,XY, 47,XX,+2 [*GGCX*] and 47,XY,+7 [*CALU*]) and no template (Blank: H₂O) controls. The data are expressed as the calculated copy number for the target sequence as determined using CopyCaller[™] software. All tested patient samples and healthy controls carried two copies of *CYP4F2*, *GGCX* and *CALU*. Error bars represent the standard deviation from quadruplicate experiments.

Table 1

TaqMan® primer/probe sets used for copy number analyses.

Gene	TaqMan copy number assay
<i>VKORC1</i> exon 1	Hs01911614_cn
<i>VKORC1</i> exon 2	Hs02546042_cn
<i>VKORC1</i> exon 3	Hs01545916_cn
<i>CYP4F2</i> exon 10	Hs02635613_cn
<i>GGCX</i> exon 6	Hs02208521_cn
<i>CALU</i> exon 7	Hs01895076_cn

Table 2Subject characteristics[†].

Variable		n (%)
Age (years)		65 ± 15 [‡]
Male		109 (61)
Race	Caucasian	88 (49)
	African-American	37 (21)
	Hispanic	21 (12)
	Asian	30 (17)
	Other	2 (1)
BMI (kg/m ²)		28 ± 7 [‡]
Smoker		11 (6)
Primary indication for anticoagulation	Atrial fibrillation	117 (66)
	DVT or PE	23 (13)
	Other	38 (21)
Number of concurrent medications		5.5 ± 2.9 [‡]
Number of concurrent medications that interact with warfarin	0	55 (31)
	1	71 (40)
	≥2	52 (29)
Goal INR	2–3	158 (89)
	2.5–3.5	20 (11)
Mean therapeutic warfarin dose (mg/week)		42 ± 31 [‡]
Median therapeutic warfarin dose (mg/week)		35 (23 and 53) [§]
Noncompliance score	0	146 (83)
	1	18 (10)
	2	7 (4)
	3	4 (2)
	4	1 (1)

DVT: Deep vein thrombosis; INR: International normalized ratio; PE: Pulmonary embolism.

[†] n = 178.[‡] Data presented as mean ± standard deviation.[§] Data presented as median (interquartile range).