Value of multilocus genetic risk score for atrial fibrillation in end-stage kidney disease patients in a Polish population

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Genetic factors play a key role in the pathogenesis of atrial fibrillation (AF). We would like to establish an association between previously described single-nucleotide polymorphisms (SNPs) and AF in haemodialysed patients with end-stage kidney disease (ESKD-HD) as well as to assess the cumulative effect of all genotyped SNPs on AF risk. Sixteen SNPs were genotyped in 113 patients with AF-ESKD-HD and in 157 controls: without AF (NAF) and with ESKD-HD. The distribution of the risk alleles was compared in both groups and between different sub-phenotypes. The multilocus genetic risk score (GRS) was calculated to estimate the cumulative risk conferred by all SNPs. Several loci showed a trend toward an association with permanent AF (perm-AF): CAV1, Cx40 and PITX2. However, GRS was significantly higher in the AF and perm-AF groups, as compared to NAF. Three of the tested variables were independently associated with AF: male sex, history of myocardial infarction (MI) and GRS. The GRS, which combined 13 previously described SNPs, showed a significant and independent association with AF in a Polish population of patients with ESKD-HD and concomitant AF. Further studies on larger groups of patients are needed to confirm the associations.

Atrial fibrillation (AF) is the most common heart rhythm disorder, and it is associated with increased morbidity and mortality and higher costs for the health care system, mainly due to thromboembolic complications14. AF affects about 6% of the population over the age of 65, and its prevalence increases with age1. According to epidemiological estimations, by the middle of this century, the number of patients with AF will double, and an AF episode will affect approximately 20–25% of people at some point in their lives15. The prevalence of AF is significantly higher in haemodialysed (HD) patients with end-stage kidney disease (ESKD) (ESKD-HD), and according to different data, between 15% and 30% of patients with ESKD-HD are affected6. In addition to classic AF risk factors, such as age, height, male sex, hypertension, diabetes, organic heart disease (myocardial infarction, heart failure and heart valve diseases), obesity and smoking, ESKD-HD patients exhibit new characteristic factors,
including overhydration, anuria, hemodynamic instability during haemodialysis and ionic disorders in respect to
calcaemia, calceemia and magnesemia. In addition, chronic kidney disease (CKD) itself is one of the strongest risk
factors for cardiovascular diseases and cardiac arrhythmias; therefore, these diseases in CKD patients develop
faster, and their symptoms and complications can be more severe.

The disease occurs in the form of the so-called ‘lone AF’ in 10–20% of all patients with AF, that is, despite
the absence of the aforementioned risk factors. Lone AF is a strictly electrophysiologically disorder caused by
disturbances in the flow of ionic currents on the surface of cardiomyocytes and between them. Pathomechanism
of AF becomes more complex, and it is not fully understood when the risk factors occur. It is suggested that
the pathogenesis in this case involves the heterogeneous interactions of many factors, triggering mechanisms
that cause rapid focal discharges and heterogeneous conduction of fibrillation type.

Genetic factors play a key role in the pathogenesis of both ‘lone’ and ‘heterogeneous’ AF. The risk of lone
AF was found to increase rapidly with the number of relatives with AF and with an early disease onset in these relat-
ives. Previous studies showed that the genetic background of lone AF involves mostly rare mutations of genes
encoding components for potassium and sodium channels, but also for nuclear membrane structures, cardiac
transcription factors and cardiomyocyte intercellular structures. For the more frequent forms of ‘heterogeneous
AF, a study of over 5,000 AF patients found that the risk of AF in patients with one parent with AF was more than
time greater than that of patients whose parents did not have cardiac arrhythmias. The risk remained ele-
vated even after the adjustment for classical AF risk factors. Recently, genomic-wide association studies (GWAS)
have provided data indicating the involvement of commonly occurring single-nucleotide polymorphisms (SNPs) in
the genetic predisposition to ‘heterogeneous’ AF.

Although many of these loci have been identified, their individual effect on AF risk is modest. For this reason,
a genetic tool allowing for the assessment of the cumulative effect of multiple genetic markers on disease
risk has recently been developed, what is called a genetic risk score (GRS). Previous studies showed that the
combined assessment of multiple markers with low individual impacts provided a better risk estimation, espe-
cially for complex diseases, such as myocardial infarction, stroke, diabetes or rheumatoid arthritis. Tada et al.,
when analysing the Swedish population, reported a GRS comprising 12 SNPs that conferred a two-fold increase
in the risk of AF for patients grouped in the highest quintile of GRS compared to the lowest quintile. Moreover,
the addition of this GRS to classical risk factors resulted in the classification of patients to other AF risk groups.
In another study on a population of American women, Everett et al. showed that GRS based on slightly different
SNPs caused a 2.25-fold increase in AF risk, when compared the highest to the lowest GRS quintile, but adding
this GRS to established risk factors did not alter the classification of patients’ 10-year disease risk assessment.
In a recent study, Lubitz et al. evaluated several different GRSs based on five population studies and found that a GRS
comprising 11 SNPs showed a 1.4-fold increase in AF risk, when compared the highest to the lowest GRS quintile,
while another one consisting of 25 different SNPs showed a maximum increase of 1.6-fold (also comparison of the
highest to the lowest GRS quartile). However, such studies in CKD patients are still lacking.

The aim of the present study was to determine the association of 16 selected SNPs with AF risk in haemodi-
alyzed patients with end-stage kidney disease using a large Polish population in the Mazovian region. We also
wanted to evaluate the cumulative effect of all genotyped SNPs on AF risk and to identify the clinical and genetic
factors that are independently associated with the risk of AF in this group of patients.

Results

The demographic and clinical characteristics of the patients are presented in Table 1. The study and control
groups differed significantly with respect to smoking habits and sex distribution.

A full list of the analysed SNPs are presented in the Supplementary Table 1.

The SNPs’ association with AF are presented in Table 2. None of the individual analysed SNP’s showed the
significant association with AF.

The analysis of the sub-groups showed a trend toward association (nominal association – \( P < 0.05 \), no associa-
tion after Bonferroni correction – \( P > 0.05 \)) of three SNPs – rs3807989, rs2200733, rs10465885 – with permanent
AF (Table 3).

To assess the cumulative risk conferred by multiple loci, the GRS was calculated (Table 4). An example of the
calculation of GRS was placed in the Supplementary Table 2. The GRS was significantly higher in the AF group
and perm-AF group compared to the NAF group.

A logistic regression with nine variables (age, sex, smoking, BMI, presence of diuresis, coronary artery disease,
myocardial infarction, myocardial hypertrophy and GRS) showed that three are significantly associated with
AF: male sex, myocardial infarction and GRS (Table 5). It should be emphasised that OR for GRS in Table 5 is a
0.1 unit odds ratio (i.e. an OR of 1.24 is a ratio for a 0.1-unit increase in GRS). The model including these three
variables (i.e. male sex, myocardial infarction and GRS) showed that it gave the proper classification for 75% of the cases.

Several studies have focused on the genetics of AF in the general population. However, the literature on
genetic risk scores for AF is still scarce and non-existent for ESKD-HD patients. Tada et al. for instance, using a
prospective observational study for a Swedish population (The Malmö Diet and Cancer study), showed that
GRS composed of 12 SNPs (hereafter called Tada-GRS) was associated with at least one AF incident throughout 14 years of follow-up, even after adjusting for non-genetic AF risk factors such as age or hypertension. Patients grouped in the highest quintile of Tada-GRS had a nearly two-fold higher risk of AF than those in the lowest quintile. Furthermore, the effect of Tada-GRS, as an independent AF risk factor, was comparable to one of the strongest classical AF risk factors: hypertension. Patients with high Tada-GRS and without hypertension had a similar risk of AF as those with hypertension and low Tada-GRS. The comparable effect of Tada-GRS and hypertension was observed in both groups below and over 57 years of age. Our work is difficult to compare to Tada et al.’s study for several reasons. The work of Tada et al. is a large observational study conducted on the Malmö local community, though age, sex, BMI, and smoking rates are similar. However, because of the nature of the underlying disease – ESKD – our group of patients is different, with significantly higher rates of hypertensive patients, coronary artery disease (CAD), cardiac hypertrophy or history of myocardial infarction. Moreover, there were only five common SNPs between the Tada-GRS and our GRS. In addition, in the study by Tada et al., there were

### Table 1. Study and control group characteristics. Data are presented as the mean (standard deviation) for continuous variables and number (percentage) for categorical variables. AF: study group (patients with atrial fibrillation). NAF: control group (patients without atrial fibrillation). ESKD: end-stage kidney disease. Uvol: 24-hour urine volume. aData available for 59 AF patients and 120 NAF patients. bTime from the beginning of CKD to end-stage kidney disease. cData available for 96 AF patients and 150 NAF patients. dData available for 111 AF patients. eData available for 106 AF patients and 155 NAF patients. fData available for 105 AF patients and 145 NAF patients. gData available for 104 AF patients and 140 NAF patients. hData available for 92 AF patients and 142 NAF patients.

|                  | AF, n = 113 | NAF, n = 157 | P    |
|------------------|-------------|--------------|------|
| Age, years       | 70.79 (10.34) | 69.41 (8.99) | 0.25 |
| Males            | 74 (65.49%) | 73 (46.50%) | 0.002 |
| BMI, kg/m²       | 26.28 (5.25) | 28.87 (15.46) | 0.1  |
| Ever-smokers*    | 35 (39.32%) | 46 (38.33%) | 0.008 |
| Duration of pre-ESKD period, years | 6.2 (6.21) | 5.47 (5.52) | 0.35 |
| Presence of diuresis* | 76 (79.17%) | 129 (86%) | 0.16 |
| Uvol, mL         | 693.23 (697.84) | 569.87 (504.18) | 0.11 |
| Uvol ≥500 mL     | 60 (62.5%) | 85 (56.67%) | 0.36 |
| Uvol 500–1000 mL | 24 (25%) | 47 (31.33%) | 0.28 |
| Uvol 1000–2000 mL| 30 (31.25%) | 33 (22%) | 0.1 |
| Uvol >2000 mL    | 6 (6.25%) | 5 (3.33%) | 0.28 |
| Type of atrial fibrillation* | 57 (51.35%) | N/A |     |
| -non-permanent   | 54 (48.65%) |     |     |
| -permanent       | 102 (96.23%) | 149 (96.13%) | 0.97 |
| Hypertension*    | 68 (64.76%) | 87 (60%) | 0.44 |
| History of myocardial infarction* | 35 (33.65%) | 40 (28.57%) | 0.39 |
| Myocardial hypertrophy* | 71 (77.17%) | 104 (73.24%) | 0.5  |

Table 2. SNPs’ associations with atrial fibrillation. AF: atrial fibrillation group. CI: confidence interval. NAF: non-atrial fibrillation group. OR: odds ratio. P uncorr: uncorrected P value. RA: risk allele. RAF AF: RA frequency in AF group. RAF NAF: RA frequency in NAF group. SNP: single-nucleotide polymorphism.
10 SNPs nominally associated with AF, possibly due to the large population of patients. Furthermore, in contrast to the study by Tada et al., in our study, hypertension was not significantly associated with AF. Probably, because of the nature of ESKD, it was very common in all groups of patients both with and without the AF. Hence, the fundamental differences between these two studies result from different groups of patients and, in fact, from the presence of ESKD and its consequences; however, Tada et al. and the current study demonstrated the utility of GRS in estimating the risk of AF.

In another study on a large European population, Lubitz et al. showed that multi-allelic GRS (hereafter called Lubitz-GRS) combining 12 SNPs that were potentially associated with AF (each SNP was nominally associated with AF risk) and previously confirmed in the genome-wide association studies (GWAS) was associated with a five-fold increase in the risk of AF in the group of patients with the highest number of risk alleles compared to patients with the lowest number of such alleles. Moreover, the study was successfully replicated in a population of Japanese patients with AF; except in this case, the increase of risk associated with AF was four-fold. In both populations, the risk gradually increased as the number of AF risk alleles rose, with the most numerous population (nearly 25%) of patients having an average (9–10) number of AF risk alleles. Furthermore, none of the analysed SNPs were significantly associated either with the survival or the mortality of the patients. It is also hard to compare the study by Lubitz et al. with the current study, mainly because of fundamentally different populations, the underlying disease in our study (i.e., ESKD) and the high heterogeneity of patients’ clinical data in the study by Lubitz et al. The components of both GRSs (Lubitz-GRS vs. Saracyn-GRS) were also different because they overlapped in two of the total of 12 and 13 SNPs used for the analysis in Lubitz et al. and in the current work, respectively. Therefore, Lubitz et al. and the current study have demonstrated the usefulness of GRS in AF risk assessment; however, in Lubitz et al. it was not surprising because almost all their SNPs were nominally and independently associated with AF. Our GRS, with lacking such unequivocal associations for single SNPs, has brought a new value.

| SNP          | RAf   | OR (95% CI) | Puncorr | PAF   | OR (95% CI) | Puncorr | Pcorr |
|--------------|-------|-------------|---------|-------|-------------|---------|-------|
| rs1805127    | 0.66  | 0.96 (0.61–1.59) | 1       | 0.676 | 1.05 (0.64–1.70) | 0.86    |        |
| rs3807989    | 0.62  | 1.29 (0.80–2.07)  | 0.30    | 0.670 | 1.64 (1.01–2.65)  | **0.044** | 0.572 |
| rs2106261    | 0.21  | 1.25 (0.70–2.28)  | 0.45    | 0.190 | 1.08 (0.60–1.94)  | 0.81    |        |
| rs2200733    | 0.15  | 1.01 (0.52–1.97)  | 1       | 0.235 | 1.82 (1.01–3.25)  | **0.043** | 0.559 |
| rs3853445    | 0.74  | 1.12 (0.66–1.90)  | 0.68    | 0.694 | 0.89 (0.54–1.48)  | 0.66    |        |
| rs13376333   | 0.30  | 1.04 (0.63–1.71)  | 0.89    | 0.343 | 1.27 (0.78–2.06)  | 0.34    |        |
| rs1805123    | 0.78  | 0.76 (0.43–1.33)  | 0.34    | 0.833 | 1.07 (0.58–1.99)  | 0.82    |        |
| rs4845625    | 0.48  | 0.94 (0.59–1.48)  | 0.78    | 0.451 | 0.83 (0.53–1.31)  | 0.43    |        |
| rs11047543   | 0.86  | 0.87 (0.45–1.70)  | 0.68    | 0.890 | 1.15 (0.56–2.36)  | 0.71    |        |
| rs10465885   | 0.46  | 1.09 (0.68–1.76)  | 0.72    | 0.602 | 1.96 (1.22–3.15)  | **0.005** | 0.065 |
| rs13038095   | 0.12  | 1.16 (0.55–2.46)  | 0.69    | 0.150 | 1.55 (0.78–3.06)  | 0.21    |        |
| rs800541     | 0.67  | 1.03 (0.63–1.66)  | 0.92    | 0.598 | 0.76 (0.48–1.22)  | 0.26    |        |
| rs251253     | 0.61  | 1.15 (0.73–1.83)  | 0.55    | 0.615 | 1.20 (0.75–1.91)  | 0.44    |        |

Table 3. SNPs’ associations with non-permanent (NP-AF) and permanent (P-AF) atrial fibrillation. A Bonferroni corrected P value is provided for nominal (uncorrected P value < 0.05) associations. CI: confidence interval. NP-AF: non-permanent AF. PAF: permanent AF. OR: odds ratio. Puncorr: corrected P value (Bonferroni correction). Puncorr: uncorrected P value. RAf: risk allele frequency. SNPs: single-nucleotide polymorphisms. P < 0.05 are in bold.

| Variable | OR (95% CI) | P   |
|----------|-------------|-----|
| Male sex | 2.32 (1.13–4.78) | 0.023 |
| Myocardial infarction | 2.66 (1.23–5.75) | 0.013 |
| GRS      | 1.24 (1.07–1.43)  | **0.005** |

Table 5. Variables associated with AF. CI: confidence interval; OR: odds ratio; GRS: genetic risk score; *The 0.1 unit odds ratio, i.e. the ratio for a 0.1-unit change in the predictor (GRS).
In another publication, Everett et al. studied a large prospective cohort study of American women of European descent (part of the Women’s Health Study [WHS]) with a follow-up period of over 12 years. The study showed that GRS (hereafter called Everett-GRS) consisting of 12 SNPs potentially associated with AF increased the risk of AF by 2.25-fold in the group of patients from the highest quintile compared to the lowest quintile. However, the study also demonstrated that the addition of this GRS to pre-established clinical 10-year risk categories of AF did not alter the classification of these patients. Everett et al.’s study and the current one are difficult to compare for several reasons. Most of all, the current study involves a different population, that is haemodialysed patients with ESKD. Moreover, the study by Everett et al. included exclusively women, while the male gender is a strong independent AF risk factor. Additionally, these women were without CVD and heart failure (HF), additional AF risk factors. Finally, our GRS and Everett-GRS had only four common SNPs. In addition, of the 12 SNPs selected by Everett et al., five were nominally associated with AF, which undoubtedly affected the overall GRS estimation. Only seven of the 12 SNPs in the study by Everett et al. were directly genotyped, while the remaining were imputed. In our study, we genotyped all selected SNPs, of which 13 were used in the final analysis.

In the most recently published research, Lubitz et al. once again focused on the issue of GRS and its role in AF risk assessment but used a slightly different material and method. The authors combined the data from five different prospective studies with a 5-year follow-up and found that the GRS combining 25 SNPs (hereafter named as Lubitz-GRS 2) is associated with a 67% increase in the risk of AF when comparing the highest quartile of the GRS to the lowest one. All SNPs used to calculate this GRS were nominally associated with AF. Moreover, the addition of this GRS to the classical AF risk factors in the discriminatory model changed its parameters (C-statistics) only minimally; additionally, it significantly differed between the five analysed cohorts. To compare this work with the current study, we would have to compare our material with the five different cohorts used by Lubitz et al. Our GRS, consisting of 13 SNPs, was associated with a significant increase of AF risk in the group of patients with ESKD-HD, but only with three of 13 SNPs trending toward association with perm-AF.

Of the 13 SNPs that finally qualified for the analysis in our study, three showed a trend toward an association with permanent AF. However, it should be emphasised that none of the SNPs withstood the Bonferroni correction; thus, further studies on larger groups of patients are needed to confirm these associations. Interestingly, the SNPs showing a trend toward an association with perm-AF (rs3807989, rs10465885 and rs2200733) lie within the genes that encode proteins of atrial anatomic structures or proteins involved in their morphogenesis, that is, CAV1, Cx40 and PITX2, respectively.

Caveolin-1 (CAV1) is a basic component of the characteristic cell membrane structure called caveolae. It also actively interacts with the different type of the potassium channels, influences the transforming growth factor-beta 1 (TGF-β1) signalling pathway and regulates the intracellular nitric oxide pathway, disorder of which are one of the pathogenetic mechanisms behind AF. Cell signalling defects as mentioned above can encounter additional mechanisms of arrhythmias in patients with ESKD/HD. During 4 hours of HD, kalemia is able to decrease by approximately 2.5–3.5 mmol/L, on average. Such a difference in potassium concentration in patients without other significant complications usually does not cause a rhythm disorder. However, a coexistence of ultrastructural defects of intercellular junctions, which control calcium flow and HD-dependent changes of calcium concentration during HD sessions, can lead to amplification of strong pathomechanisms of arrhythmias.

Connexin, in turn, is the main component of the gap junctions’ lateralisation. Changes in the number of gap junctions on the cell’s surface and their distribution (lateralisation), as well as structural changes, may result in disturbances of the flow of different signals and the development of arrhythmias. Another critical factor of rhythm disorders in ESKD/HD patients can be a fluctuation of calcemia. Calcium regulates intercellular cross-talk within the gap junction and physiological function of enzymes and cell proteins. During a 4-hour HD session, calcium concentration can decrease by as much as 0.5–1.0 mmol/L (i.e. 50% of the initial blood concentration). Coexistence of ultrastructural defects of intercellular junctions, which control calcium flow and HD-dependent changes of calcium concentration, can lead to serious disorder of calcium currents and development of a heart rhythm disorder.

Finally, a paired-like transcription factor (PITX2) is involved in asymmetric atrial morphogenesis. It also influences the proper functioning of ion channels, gap and tight junctions in the cardiomyocyte cell membrane. Recently, it has also been found that PITX2c expression in cardiomyocytes in patients with perm-AF is significantly reduced, confirming the existence of a molecular mechanism linking PITX2 dysfunction with AF development. Patients with ESKD can suffer from chronic overhydration between HDs and from rapid dehydration (even 4.0–5.0 L) during an HD session. These extreme changes of volemia usually provoke rapid blood pressure drops, incidents of tachycardia during HD and, with the coexistence of PITX2-dependent atrium structure and cell membrane defects can also lead to accumulation of these two arrhythmogenic mechanisms and generate a fleet development of atrial fibrillation.

In ESKD-HD patients, these morphogenetic mechanisms appear to be an ‘ideal’ backgrounds for AF development. Based on these genetic predispositions, existing ESKD and renal replacement therapy would include additional risk factors for AF, such as chronic overhydration, rapid dehydration, rapid blood pressure drops, tachycardia episodes and/or ionic disorders, during haemodialysis. As a strength of the current work, it should be emphasised that our study is the first concerning the genetic background of AF in patients with ESKD-HD. Therefore, it is difficult to compare our results with those from previous studies conducted on different populations. The GRS constructed for this study was significantly higher in AF and perm-AF patients compared to the controls. It was also significantly and independently from other risk factors associated with AF. The GRS also differentiated between patients with permanent and non-permanent AF (i.e. help identify patients at risk of disease chronification and patients with a lower chance of heart rhythm normalisation). Although we have found no associations with AF for single SNPs, we have shown a positive association with multiple genetic marker. This new genetic tool, which is composed of 13 previously described SNPs, has been applied for the assessment of AF risk in ESKD/HD patients for the first time. The assessment of cardiac arrhythmias risk during HD can be a critical condition and practical tool for an adequate and safe haemodialysis sessions.
A few limitations of the present work should also be noted. The study group, even though involving haemodialysed ESKD patients from the 5.5 million population in the Mazovian region (approximately one-sixth of the population and area of Poland), was relatively small; therefore, our study had limited power in detecting associations of single SNPs with AF. However, to overcome this problem, we used a combined weighted GRS as the main genetic variable. The constructed 13-SNP GRS was significantly associated with AF independent from other risk factors. In addition, the study and control groups differed in sex proportions and percentage of ever smokers. However, these parameters reflect the clinical data and the clinical distribution of AF risk factors, and we recruited all consecutive patients with AF and ESKD/HD from the Mazovian region.

In summary, although we observed only a trend toward an association of several previously reported SNPs with permanent AF in ESKD-HD patients, the multiple loci genetic risk score, composed of 13 SNPs, was significantly higher in the AF group and permanent AF group compared to the controls. Moreover, the association of GRS with AF was independent of classical risk factors. The disease model comprising three variables (i.e. GRS, age and myocardial infarction) covered nearly 10% of the phenotypic variability, and the post-hoc analysis demonstrated that it allowed for a proper classification of 75% of the cases. This new genetic tool, composed of 13 previously described SNPs, has been applied for the assessment of AF risk in ESKD/HD patients for the first time in literature. The assessment of cardiac arrhythmias risk during HD can be a critical condition and practical tool for an adequate and safe haemodialysis sessions.

**Material and Methods**

The study was approved by the Military Institute of Medicine Ethics Committee. Informed consent was obtained from each patient. All procedures were performed in accordance with the Helsinki Declaration of 1975, revised in 1983.

**Patients and controls.** Initially 115 consecutive AF-HD patients from the group of 1126 ESKD/HD patients were included in the study; however, 2 patients were excluded from the study because of the poor DNA quality. Finally, the study group comprised 113 haemodialysed patients with ESKD and a history of AF. The patients were recruited from the Military Institute of Medicine in Warsaw and Mazovian Centers of Dialysis in Radom, Ciechanów, Grodzisk Mazowiecki, Maków Mazowiecki, Sokół Podlaski, Skiermiewice, Warszawa Międzyłze, Wołomin and Otwock. The inclusion criteria were as follows: (i) ESKD treated by haemodialysis, (ii) presence or history of AF (confirmed by electrocardiography) and (iii) age ≥18 years. The exclusion criteria included the following: (i) congenital heart defect, (ii) history of heart surgery and (iii) history of malignancy. The AF patients were classified as having non-permanent AF (nperm-AF), defined as paroxysmal or persistent (lasting less than 12 months) AF, or permanent AF (perm-AF), defined as AF lasting over 12 months. One hundred and seventy two age-matched controls were enrolled in the study (cases to controls ratio 2:3). However, 5 controls were excluded from the study because of the poor DNA quality, and further 10 controls were excluded because of incorrect enrollment (subjects not fulfilling inclusion/exclusion criteria- mostly concomitant heart arrhythmias other than AF). Finally, the control group consisted of 157 patients with ESKD (non-atrial fibrillation group: NAF group). The inclusion criteria were as follows: (i) ESKD treated by haemodialysis and (ii) age ≥18 years. The exclusion criteria included the following: (i) history or presence of AF, (ii) history of cardiac arrhythmia other than AF and (iii) history of cardiac ablation. A structured questionnaire was used to collect data regarding age, gender, smoking habits, body mass index (BMI), duration of pre-ESKD period, presence and volume of diuresis, coexistence of hypertension or coronary artery disease, history of myocardial infarction and presence of myocardial hypertrophy (confirmed by echocardiography).

**Single-nucleotide polymorphisms selection.** SNPs previously associated with AF were selected for this study. The criteria for SNP selection were the following: (i) association with AF confirmed in GWAS, a meta-analysis or large-scale case-control study and (ii) minor allele frequency (MAF) >0.05 in a Caucasian population (based on data from the HapMap CEU population). Using these criteria, 16 SNPs were selected for genotyping. However, three of the genotyped SNPs were excluded from the analysis because of low genotyping success rate (<80%) or Hardy-Weinberg equilibrium deviation: rs3825214 (TRX5), rs1131820 (KCNN3) and rs11708996 (SCN5A). The complete list of the analysed SNPs is presented in Table S1.

**Genotyping.** DNA was isolated from whole-blood samples using a salting-out method. For SNPs genotyping a custom array was designed (Taquin OpenArray Genotyping Plate, Custom Format 16 QuantStudio 12 K Flex, Life Technologies, Carlsbad, CA, USA) and genotyping was performed according to the manufacturer’s protocols on a QuantStudio 12 K Flex Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

**Statistical analysis.** The PLINK statistical software package was used to test the Hardy-Weinberg equilibrium (HWE) and to assess the differences in allele frequencies of each SNP between the cases and controls. The odds ratio (OR) associated with each individual SNP ORs from previous studies (Table S1) were used to calculate the GRS. Because of missing data, 15 AF patients and 25 NAF patients were excluded from the GRS analysis. The GRS in the study and control groups was compared with a student’s t test. A logistic regression was used to predict the factors associated with AF and calculate the phenotypic variation covered by the model (McFadden R² for logistic regression). The R² was defined as $R^2 = 1 - \ln(L_0)/\ln(L_a)$, where $L_a$ is the value of the likelihood function for a model with no predictors and $L_0$ is the likelihood for the model being estimated.
Data availability. All data generated and analysed during the current study are available from the corresponding author on reasonable request.

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Author Contributions
M.S., R.P., S.N. designed the study. M.S., D.B.-K., W.Z., A.S., W.K., M.S., J.G., M.P., Z.G., R.M., L.N. collected clinical data and blood samples. M.F., M.S. isolated DNA. M.F., L.S. genotyped SNP's. B.K., A.B. performed statistical analysis. M.S., B.K., A.B., W.T., G.K., R.P., S.N. interpreted clinical and genetical data. M.S., B.B., M.K. searched literature. M.S., B.K., R.P., S.N. prepared the manuscript. All authors contributed to final writing, proof-reading of the manuscript. All authors approved the final version of the paper.

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