The European Network for Translational Research in Atrial Fibrillation

(EUTRAF): Objectives and Initial Results

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Abstract:

Atrial fibrillation (AF) is the most common sustained arrhythmia in the general population. As an age-related arrhythmia AF is becoming a huge socio-economic burden for European healthcare systems. Despite significant progress in our understanding of the pathophysiology of AF therapeutic strategies for AF have not changed substantially and the major challenges in the management of AF are still unmet. This lack of progress may be related to the multifactorial pathogenesis of atrial remodeling and AF that hampers the identification of causative pathophysiological alterations in individual patients. Also, again new mechanisms have been identified and the relative contribution of these mechanisms still has to be established. In November 2010, the European Union launched the large collaborative project EUTRAF (=European Network of Translational Research in Atrial Fibrillation) to address these challenges.

The main aims of EUTRAF are to study the main mechanisms of initiation and perpetuation of AF, to identify the molecular alterations underlying atrial remodeling, to develop markers allowing to monitor this processes and suggest strategies to treat AF based on insights in newly defined disease mechanisms. This article reports on the objectives, the structure and initial results of this network.

1. Current challenges in AF management and resulting research questions

The increase in life expectancy and recent improvements in treatment of acute heart disease have resulted in a major increase in the number of patients suffering from heart failure and atrial fibrillation (AF). AF is most the common sustained arrhythmia in the population occurring in 1 to 2% of the general population. More than 6 million Europeans suffer from this arrhythmia and in an ageing society this number is expected to rise quickly. AF is associated with an increased risk of stroke, dementia, heart failure and death (1,2). In Europe, one percent or even more of the health care budget is spent on AF management (3). Although the socio-economic burden of AF is growing steadily and significant progress has been made in understanding the pathophysiology of this arrhythmia, treatment of AF patients is still far from satisfactory. The success rate of cardioversion is still limited and anti-arrhythmic drugs are unable to prevent recurrences of AF. Prevention of thromboembolic events requires anticoagulation therapy with all the associated risks. Radio-frequency ablation, originally developed for treatment of paroxysmal AF, is not as effective in persistent AF and is associated with potentially serious procedural risks (4).

The authors believe that the relative slow progress in the development of antiarrhythmic, ablation and upstream AF therapies over the past years largely reflects our inability to identify the leading molecular mechanisms of the atrial remodeling process and the occurrence of AF in an individual patient.

The central objective of the large collaborative project EUTRAF (European Network for Translational Research in Atrial Fibrillation) is (1) to identify these main molecular mechanisms of AF progression, (2) to develop serum, imaging and electrophysiological markers allowing to monitor this processes and (3) develop strategies to treat AF based on insights in these disease mechanisms.

2. Mission statement of EUTRAF

The “European Network for Translational Research in Atrial Fibrillation” (EUTRAF) is a framework programme 7 large collaborative project supported by the European Union.

EUTRAF’s general mission is to improve the management of AF in Europe. To achieve this, methods for identification of patients who are at-risk for AF at an early stage need to be developed and a better understanding of the factors leading towards persistent AF need to be obtained. Also, accurate diagnostic tools for identification of disease mechanisms in an individual patient and new therapies for each patient based on these individual disease mechanisms have to be defined.

More specifically EUTRAF will

- provide a multidisciplinary understanding of the diverse pathophysiological mechanisms of AF on the molecular, cellular, tissue and organ level.
- integrate this knowledge into understanding of the electrophysiological mechanisms initiating and perpetuating AF.
- identify and validate markers for the main molecular mechanisms of AF that can be used in patients.
- propose a classification of AF that integrates major disease mechanisms and the electrophysiological characteristics of the fibrillating atria.
- develop new therapeutic strategies targeting individual disease mechanisms.
- disseminate the knowledge about new diagnostic tools and therapeutic techniques in the public domain.

3. Participants and structure of EUTRAF

EUTRAF is a multidisciplinary consortium of expert groups involved in AF research. It was granted an amount of 12 million €. The total project budget is 16 million €. The programme was launched in November 2010. The active funding period will end by the end of October 2015.

The consortium consists of academic research groups and industry partners. Expertise ranges from molecular biology, genetics and experimental electrophysiology, to engineering, computer science and cardiology. EUTRAF partners work in a matrix structure organized around central work packages each of which will utilize the full range of expertise provided by the network.

The consortium is led by Professor John Camm from St. George‘s University of London (UK). Table 1 gives the list of the academic and industrial partners.

The central objective of EUTRAF is to define the main biological mechanisms that lead to the slow but progressive process of atrial remodeling increasing the propensity to AF. This atrial disease together with the molecular remodeling occurring as a consequence of AF determine the progression of AF from paroxysmal to persistent forms of the arrhythmia (figure 1).

Figure 2 shows the organizational structure of EUTRAF. The basic research undertaken to define the genetic, molecular, cellular and structural mechanisms of atrial remodeling are investigated in 5 basic research work packages (WPs). Apart from new insights into the mechanisms of AF these WPs deliver new biomarkers and new therapeutic targets for AF. These suggestions are explored in two clinical work packages, one of which focuses on new diagnostic tools for AF classification while in others new therapeutic approaches are investigated. Data analysis and integration is accommodated in an eighth work package, which at the same time serves as IT platform. The consortium has access to several data sets of large clinical trials that are

used for marker validation (e.g. Flec-SL, EAST-Trial, Anti-PAF, Maastricht-Study) (5-8). The ideas developed by EUTRAF partners have also led to the initiation of new clinical investigations as add-on studies on existing trials, new cohorts or new interventional trials.

4. Main research objectives and recent achievements

This section describes per work package (WP) examples of research projects undertaken by EUTRAF partners in attempt to unravel new mechanisms of AF, to relate them to clinical signs and symptoms, to develop new diagnostic tools for AF und to improve current therapeutics strategies.

4.1. WP1: Extracellular matrix and structure-function relationship

WP1 addresses questions related to the structural determinants of conduction disturbances in atrial muscle. Adverse heterocellular crosstalk between cardiac stromal and parenchymal cells at the structural, mechanical, humoral and electrotonic level are likely to contribute to arrhythmogenesis in fibrotic hearts. Among the different modalities of stroma-parenchyme interactions, it was found that mechanical strain acting on myofibroblasts that are electrotonically coupled to myocytes causes substantial slowing of impulse conduction because of activation of stretch sensitive channels in myofibroblasts resulting in a secondary depolarization of coupled myocytes (9). At physiological levels of strain, as encountered in end-diastole, this mechanism slowed conduction by up to 30%. With respect to humoral interactions, it was found that the main growth factor involved in cardiac fibrotic tissue remodeling, transforming growth factor-β_1 (TGF-β_1), substantially aggravates the arrhythmogenic

effect of myofibroblasts on electrotonically coupled myocytes. While TGF-β_1 had little effect on conduction in myocyte preparations, it caused significant conduction slowing and increased ectopic activity in a fibrosis model (figure 3). In this model TGF-β_1 produced a reduction of the membrane polarization of myofibroblasts that caused secondary depolarization of cardiomyocytes. Together, the findings suggest that arrhythmogenesis in fibrotic hearts in the context of mechanical stress and humoral factors may be dependent on myofibroblasts acting as primary 'sensors' that signal to adjacent myocytes through gap junctional coupling.

Fibroblasts and myofibroblasts can not only crosstalk to atrial myocytes but also to adipocytes located in the epicardial fat tissue (EAT) or infiltrating the myocardium. This cell type is an important source of adipokines that can diffuse freely in the neighboring atrial myocardium. EUTRAF partners found that human EAT secretes activin A, a member of the TGF-β family, that has a marked fibrotic effect on the atrial myocardium by stimulating myofibroblasts. This observation raises the question which clinical conditions are associated with an abnormal biological activity of EAT which could contribute to the formation of the AF substrate. During heart failure, for example, EAT secretes activin A in abundance which could explain the prominent role of fibrosis in the pathogenesis of AF in this clinical setting (10).

4.2. WP2: Ion channel remodeling and Ca^{2+} handling

Primary objective of WP2 is the characterization of molecular indicators ('biomarkers') and regulators of abnormal function of ion channels in the context of AF. For example, EUTRAF partners could show that shear stress increases membrane availability of the Kv1.5-subunit underlying the ultrarapid delayed rectifier current (I_{Kur}) from subcellular pools through an integrin-dependent pathway, thereby

shortening atrial action potential duration (11). Activation of the integrin pathway that enhances Kv1.5 trafficking, may explain the increase in I_{Kur} despite reduced total Kv1.5 during chronic hemodynamic overload. This highlights that not only the shape of the action potential (12) but also the spatial distribution of ion channel pore units play an important role in AF-related ion-current remodeling. There is also accumulating evidence for altered intracellular Na^+-handling and downstream Na^+-dependent ion-channel regulation in AF. In a collaboration between EUTRAF partners, it was identified that both I_{K1} and $I_{K,ACh}$ are regulated by intracellular Na^+ in human atrial myocytes of SR patients, whereas Na^+-dependent regulation of $I_{K,ACh}$ was lost in chronic AF patients (13). Recent EUTRAF studies have identified Ca^{2+}-handling abnormalities including increased sarcoplasmic reticulum (SR) Ca^{2+}-leak and spontaneous SR Ca^{2+} releases leading to delayed afterdepolarizations in AF patients (Ca^{2+} handling instability). However, the molecular underpinnings of Ca^{2+}-handling abnormalities were distinct between paroxysmal AF (increased SR Ca^{2+}-uptake, SR Ca^{2+}-load, RyR2 expression, and RyR2 open probability; unaltered Na^+/Ca^{2+}-exchanger) and chronic AF (unaltered SR Ca^{2+}-load, and RyR2 expression; increased RyR2 phosphorylation, RyR2 open probability and NCX) (14,15). On the other hand, sustained tachycardia and AF as such have also been demonstrated to induce a pattern of changes which reduce the likelihood of Ca^{2+} related proarrhythmic cellular events (Ca^{2+} signaling silencing) (16). It is currently unclear whether Ca^{2+} instability or Ca^{2+} handling silencing prevails in patients with AF. Although these studies have identified novel factors potentially contributing to AF pathophysiology it needs to be established whether and how identification of these mechanisms in an individual patient can be used to tailor AF therapy.

4.3. WP3: Etiological diversity of AF

Many patients with clinical AF suffer from underlying heart disease. Hypertension, heart failure, diabetes and ageing are all strong predictors of AF. Accumulation of clinical factors increases the individual risk of developing AF by adding to AF complexity, presumably via diverse pathophysiological processes related to the underlying conditions. As mentioned above, this etiological diversity may significantly contribute to the limited efficacy of rhythm control therapies. As proposed in a recent consensus paper (17), a classification based on underlying pathophysiology to guide AF therapy may be a superior strategy towards successful outcome.

EUTRAF partners have invested considerable efforts in the development of risk factor-based AF models to systematically identify disease-specific mechanisms of AF. The focus is on intracellular calcium, hypertrophic and fibrotic signaling pathways as primary mechanisms for the initiation and progression of AF. By comparing these mechanisms between animal models of diverse etiology at different stages of the disease process, the ultimate aim is to identify substrate-specific biomarkers related to the pathology and stage of the underlying disease to optimize early diagnosis and therapeutic strategies.

Established animal models include rat models with genetic hypertension or diabetes. These models are particularly suitable for long-term studies addressing the underlying mechanisms and complex interactions between ageing and disease-specific progression of AF. For instance, atria from hypertensive or diabetic rats show distinct age- and disease-dependent alterations in cellular and subcellular calcium homeostasis potentially contributing to both the substrate and the trigger for atrial tachyarrhythmias. In hypertensive rats, atrial hypertrophy and fibrosis develops

independent of age and gender. At the molecular level, there is a significant upregulation of genes that belong to the hypertrophic program. The most striking feature in atria of diabetic rats was apoptosis, which may be one of the mechanisms involved in the loss of contractile mass and function in diabetic cardiomyopathy.

Very few animal studies address the interaction between AF-induced remodeling and disease-specific remodeling during the development of the AF substrate. For this reason, we have established a pig model that combines AF with hypertension. The combined model develops a much more severe phenotype in terms of structural remodeling compared to ´lone` AF (rapid pacing in the absence of hypertension). This is associated with faster onset and progression of sustained AF, and a higher mortality rate. This model is very useful for the identification of specific signaling pathways involved in hypertension-induced structural remodeling stabilizing AF. Once validated, the model may be of high value to test the efficacy of new antiarrhythmic drugs in settings of hypertension.

4.4. WP4: Atrial metabolic and redox alterations in AF

In many patients and in animal models, AF becomes more stable over time and restoration of sinus rhythm correspondingly more difficult. The reason for this lies in progressive, structural and functional remodeling of the atria. In a goat model of AF, we demonstrated that this increase in AF stability corresponds with endomysial fibrosis in the thin epicardial layer of the atria, leading to a more complex, 3-dimensional pattern of fibrillatory conduction (18). Ideally, the progression of AF that results from functional and structural remodeling should be prevented by upstream therapy. A relevant target for such treatment strategies is the nitric oxide-redox imbalance in the fibrillating atrial myocardium. Our findings indicate that the

mechanisms responsible for this imbalance change with the duration of AF. In the first weeks of AF, activity and expression of NOX2 are upregulated in the left atrial myocardium. However, in later stages (months of AF), the oxidase systems underlying the increase in reactive oxygen species in both atria shift from NOX2 to mitochondrial oxidases and uncoupled nitric oxide synthases (19). Correspondingly, ex vivo incubation of atrial tissue with atorvastatin inhibits atrial Rac1 and NOX2 activity in right atrial samples from patients who develop AF after cardiac surgery, but does not affect atrial reactive oxygen species production and nitric oxide synthase activity in a patients with permanent AF (20). This implies that NOX2 inhibition by drugs such as statins may be only effective in preventing new-onset AF or early AF-induced electric remodeling of the atrial myocardium. Indeed, in patients undergoing cardiac surgery, atrial superoxide and peroxynitrite levels were independently associated with an increased risk of post-operative AF and preoperative atorvastatin treatment for 3 days was sufficient to lower atrial NOX2 activity and superoxide level before changes in LDL cholesterol developed.

Remodeling of atrial myocytes during AF is ultimately caused by altered transcriptional regulation in the nucleus. Nuclear Ca^{2+} plays an important role in regulation of transcription and it is well known that *cytoplasmic* Ca^{2+} handling changes dramatically in AF (21). Whether there is also *nuclear* Ca^{2+} remodeling in AF is unknown. Therefore, another aim is to characterize potential nuclear Ca^{2+} remodeling in AF (e.g. with regard to IP3 signaling) (22) and to evaluate its role in atrial myocyte remodeling. Identifying and understanding such changes holds the promise to identify novel upstream therapy targets.

Another related factor is the balance between atrial energy/ oxygen demand and supply. Little is known about how atrial oxygen supply responds to increased demand,

and under which conditions it falls short (supply-demand mismatch). We have recently reported that in normal healthy pigs the left atrium has a lower coronary flow reserve than the left ventricle, but a higher oxygen extraction reserve (23). Although both reserves were recruited during short-term AF, a supply-demand mismatch, as evidenced by increased lactate production, still arose in the left atrium. This imbalance may form a pivotal trigger that induces atrial structural changes on the longer term, and may therefore represent another interesting target for upstream therapy.

4.5. WP5: Genetic causes of AF

Atrial fibrillation shows familial clustering, and common genetic variants on chromosome 4q25 (and others) are associated with AF. To further understand the mechanisms conveying AF in patients with genetic variations, we studied a mutation that increases the late sodium current, characterised the effects of increased expression of CREM in a mouse model of spontaneous AF. We also investigated the functional and gene expression effects of reducing pitx2, the gene that is closest to the genetic variants on chromosome 4q25.

Patients with long QT syndrome develop short-lasting atrial arrhythmias triggered by “atrial afterdepolarizations” (24,25). A unique genetic model expressing a knock-in mutation of the cardiac sodium channel with an exclusive increase in late sodium current (D-KPQ-SCN5A knockin) allowed us to demonstrate that prolongation of the atrial action potential, early afterdepolarizations and pause-dependent atrial arrhythmias explain this monogenic form of AF (26).

The myocyte-directed expression of the splice variant CREM-IbΔC-X of transcription factor CREM in mice (CREM-TG) led to spontaneous-onset AF associated with an

increased CamKII mediated phosphorylation of RyR2 (24-26). EUTRAF partners described an arrhythmogenic substrate preceding AF in CREM-TG mice: Atrial remodeling occurred at week 3 of age characterized as a distension of atria with thin atrial walls of disorganized and elongated cardiomyocytes (27). The atrial remodeling represents a key event in the development of AF in this model that precedes the occurrence of AF and is associated with atrial ectopics and triggering of AF episodes by programmed stimulation in young transgenic mice. Functional changes in CREM-TG atria are related to the electrophysiological properties of atrial myocytes and further alterations suggesting that Ca^{2+}-dependent mechanisms also contribute to the development of AF. Therefore, the expression of CREM-IbΔC-X in mice triggers mutually dependent changes in morphology, electrophysiological properties and regulation of intracellular calcium in atrial myocardium which highlights important aspects of human AF. Interestingly, β-adrenoceptor stimulation provokes a >50-fold up-regulation of novel CREM isoforms (smICER) which leads to the translation of the very same or similar CREM repressors as arise from CREM-IbΔC-X (28). Hence, data from CREM-TG mice leads to the hypothesis that plasma catecholamines contribute to the pathogenesis of AF by triggering the induction of CREM repressors.

We also studied pitx2 expression as a marker for “leftness” in the atria. We used a mouse model with reduced expression of pitx2 mRNA to assess the functional and gene expression effects of reducing pitx2 for atrial fibrillation. We could demonstrate that pitx2, a paired homeobox transcription factor that is implicated in left-right differentiation during embryonic development, is expressed at relatively high levels in the adult left, but not right, atrium (29). We also found that reduced pitx2 expression shortens the atrial action potential, especially at short pacing cycle lengths, and predisposes to atrial fibrillation in the absence of gross structural atrial abnormalities

(29). These data already suggest that pitx2 may be relevant to maintain the left-right differentiation in adult atrial tissue. We identified differentially expressed genes in left and right atrium in mice and in human atrial tissue, and identified a list of the "top 10" genes that differ in right and left atrial expression (30). Interestingly, half of these genes are differentially expressed in mouse atria with reduced pitx2 expression (30). We are currently working to identify whether these genes could be relevant as functional mediators conveying AF and/or as biomarkers to identify patients with "polygenic AF" (31).

4.6. WP6: Classification of AF with new diagnostic tools

WP6 of EUTRAF aims at developing non-invasive tools that may provide information on the relative degree of electrophysiological changes in the atria. The slow but steady process of structural remodeling in the atria due to ageing, structural heart disease or AF itself is characterized by hypertrophy, activation of fibroblasts and enhanced collagen deposition. The resulting increase in electrical dissociation between muscle bundles causes conduction disturbances with more and narrower fibrillation waves (32-34). There is ample evidence that this increase in the complexity of the conduction pattern during AF reduces the success rate of any rhythm control strategy. For this reason, in WP6 tools to noninvasively determine AF complexity have been developed. Quantification of the AF substrate by advanced analysis of surface ECGs appears to be a logical step towards non-invasive quantification of the individual degree of electropathological alterations in the atria (35,36).

Within the EUTRAF consortium we developed a large software package allowing for quantification of multiple complexity parameters in the ECG. The analysis of AF

ECGs in more than 500 AF patients using this software package revealed that a combination of different mathematical techniques (e.g. f-wave amplitude and organization index of the power spectrum) and multiple leads has a higher predictive value for outcome than a single method on single leads. Importantly, the ECG predictors had a comparable or higher predictive value than established clinical predictors like age, gender, LV-performance, heart dimensions, or co-morbidities.

Because of the close anatomical relation between the esophagus and the left atrial posterior wall, the EUTRAF consortium developed a technique to determine the degree of electrophysiological changes in the posterior wall of the left atrium by fractionation analysis of a transesophageal ECG (TE-ECG). Figure 4 demonstrates the excellent match between frequency and complexity of the atrial activation in the atrium (LA, gold standard) and the TE-ECG. This technique has recently been patented (EPO Patent 2526861-A1) and a valorization program for this technique has been initiated. In order to stimulate the process of standardization and harmonization of the assessment of AF complexity from surface ECG EUTRAF members hosted the first European Conference for Standardization of Advanced ECG Analysis in Arrhythmia Diagnostics focusing on Quantification of the Atrial Fibrillation Substrate Complexity, in Lugano, Switzerland, in December 2013. At this conference details on technical standards were discussed and common studies for correlating ECG parameters to AF mechanisms and cross-validation of AF complexity measures were discussed and initiated. These activities will be very useful for the development of a clinically useful classification of AF to determine AF mechanisms and to optimize AF treatment.

4.7. WP7: Improving AF therapy by mechanism-based therapies

Treating AF through surgical or catheter ablation is based on the elimination of the triggers initiating the arrhythmia and/or the substrate maintaining it. In paroxysmal AF, ablation successfully targets triggers, which are mainly located in the pulmonary veins (PVs). In persistent AF results are less satisfactory, presumably because of the influence of wider atrial substrate for AF perpetuation (37,38). For determining a therapeutic strategy in persistent AF (localized target vs. global intervention), the key question is whether the activation waves that perpetuate persistent AF individually emanate from few, stable, and localized drivers or whether the waves are transitory, widely distributed and self perpetuating. Localized drivers are difficult to detect in persistent AF with conventional techniques because of limitations in technology for sequential mapping, high degree of electrograms fractionation, intermittent firing and spatial meandering of fibrillation waves (39-41).

Recent developments have allowed biatrial AF mapping using activation or phase-based analysis of body surface potentials. The objective of this study was to evaluate the ability of non-invasive mapping to identify driver-domains and characterize them in persistent human AF.

In 103 consecutive patients with persistent AF, accurate bi-atrial geometry relative to an array of 252-body-surface electrodes was obtained from non-contrast CT-scan. The reconstructed unipolar AF-electrograms acquired bedside from multiple windows (duration: 9±1s) were signal-processed to identify the drivers (focal or re-entrant activity) and their cumulative density-map was constructed (Figure 5). The driver domains were catheter ablated using AF termination as procedural endpoint in comparison with stepwise-ablation control group.

The maps showed changing beat-to-beat wavefronts and varying spatio-temporal behaviour of driver activities. Re-entries were not sustained (median 2.6 rotations

lasting 449±89ms), meandered substantially but recurred repetitively. Their locations could be described not as discrete sites but as regions. Totally, 4720 drivers were identified in 103 patients: 3802 (80.5%) re-entries and 918 (19.5%) focal discharges, most of them co-localized. Of these, 69% re-entries and 71% foci were located in the left atrium. Driver ablation alone terminated 75% and 15% of persistent and long-lasting AF, respectively. The number of targeted driver regions increased with the duration of continuous AF: 2 in patients presenting in sinus rhythm, 3 in AF 1-3months, 4 in AF 4-6months and 6 in AF lasting longer. Termination rate sharply declined after 6 months of AF. At 6 months after the ablation, 83% patients with AF-termination were free from AF, similar to control population undergoing step-wise AF ablation (84%,).

The non-invasive system can map AF before the procedure and help to shorten invasive procedural time by identification of AF drivers. The functional role of the regions hosting drivers is demonstrated by prolongation of fibrillatory cycle length during their ablation and AF termination in the majority of patients. This driver based ablation strategy by being more focused allows for a high AF termination rate with significant reduction in radiofrequency-energy-delivery when compared to stepwise ablation. Earlier ablations are to be recommended as they ensure the best clinical outcome with limited tissue destruction.

4.8. WP8: Insights in AF mechanisms derived from information science and analysis of large scale data sets

Analysis of large data sets offers the opportunity to validate markers identified in a hypothesis-driven approach but also find new associations by hypothesis-free strategies, for example by using data mining techniques. The first objective of large-

scale data analysis is standardized and scalable data collection, for which a proper IT infrastructure was implemented in the EUTRAF project, providing an integrated environment for data collection and data warehousing. This system was implemented on an Oracle data warehouse and Sharepoint 2013. An additional clustered data warehouse was built on-site for the TRAF (Turkish Atrial Fibrillation Database) registry in the Social Security Institution of Turkey based on the Microsoft SQL Server Enterprise 2013 database, on which the associated Analysis Services module was run for data analysis. The required security and privacy mechanisms for all the above-mentioned tools have been developed and implemented to provide confidentiality of patient specific information and to address ethical issues based on EU directives.

Following the data collection step, we proceeded with data pre-processing and data cleaning followed by the actual data analysis using various data mining techniques (42,43). The objective was to discover mechanistic relationships between biomarkers and the development and perpetuation of AF, to assess the predictive value of existing biomarkers, and to reveal new valuable biomarkers as well as new therapeutic targets. Through collaborations with 5 different groups in the EUTRAF consortium as well as the German Network of Excellence for Atrial Fibrillation (AF-NET), we analyzed the Flec-SL and ANTIPAF clinical datasets as well as datasets collected by the University of Dresden (APDRS) and Maastricht University. Finally, with the collaboration of MITS, St. George's University London, and the University of Leeds, we have built and analyzed the largest national cohort of AF patients worldwide, the TRAF (Turkish Registry in Atrial Fibrillation) database.

Analysis of the Flec-SL database through machine learning techniques has revealed that successful pharmacological conversion prior to the beginning of the survey

period had the greatest effect in predicting whether a patient would reach the primary endpoint (conversion to persistent AF or mortality). Data mining on the ANTIPAF dataset revealed strong relationships between the glomerular filtration rate, serum creatinine, blood urea nitrogen, and age. The TRAF dataset contains the records of 545,000 patients who had the diagnosis of non-valvular AF according to ICD-10 code I48, within the years 2008-2012. TRAF is extracted from a claims and utilization management system called MEDULA which processes claims for all health insurance funds in Turkey since 2007. Analysis of the TRAF dataset has revealed that warfarin significantly increases the rates of hospitalization, and mortality in the low risk groups ($CHA_2DS_2VASc<2$) and decreases hospitalization and mortality in high risk groups ($CHA_2DS_2VASc>=2$). The same conclusion also holds true for the ischemic stroke/thromboembolism/mortality endpoint. Hemorrhagic stroke and major bleeding rates were found to be increased with warfarin in all risk categories. Further analyses have also included the predictive performance of the $CHADS_2$ and CHA_2DS_2VASc scores, effect of comorbidities on the progression of AF and the analysis of lone AF.

All of these studies will merge and culminate with the development of a data-mining based model and pertaining clinical decision support system (CDSS) to assess the risk of AF and its complications, which is the final innovative and scientific objective of the work package.

5. Outlook

The authors believe that the scientific achievements of the EUTRAF consortium was and will be tremendously helpful for developing better understanding of the general pathophysiology of AF. Hopefully, this understanding will facilitate the development of future therapeutic strategies, such as ion channel blockers, upstream therapy, or

ablation of AF. Some of these concepts are currently tested in clinical trials (table 2). While potentially proving useful in clinical practise, these concepts evolved along the traditional flow of explorative clinical or experimental evaluation, integration in general understanding of pathophysiology, identification of a therapeutic target and validation in clinical trials (figure 7A).

For the future, we foresee a shift in this strategy towards emphasis on individual disease mechanisms. We acknowledge that individual components of pathophysiological processes leading to AF, although assessable by experimental research, as yet have hardly had an impact on the choice of the therapeutic option. Experimental research within EUTRAF but also in many other projects has shown that inflammation, fibrosis, reactive oxygen species activation, or fatty infiltration can all contribute to the development and perpetuation of AF. However, these considerations are usually not taken into account in the choice for an individual therapy. The reasons are manifold and include the presence of multiple mechanisms and temporal variability of pathomechanisms in an individual patient. Also the individual genetic background, with the exception of monogenetic cardiac diseases, is usually not considered in the decision on a therapeutic strategy. To overcome this ‘translational gap’ is one of the most important challenges in the development of a strategy for personalized AF therapy. A potential approach to this is shown on figure 7B.

The authors assume that in order to identify the main mechanisms responsible for atrial remodeling and AF and in order to determine the relative contribution of the different disease mechanisms a combined histological, biochemical and complex genetics approach is required. In this scenario it is essential that blood samples and atrial tissue is available in order to detect the molecular mechanisms leading to AF. At

the same time, detailed clinical characterization of the patients is required so that the molecular mechanisms can be correlated to clinical signs and symptoms.

From blood samples, common gene variants (SNPs) and a list of predefined biomarkers would need to be determined. Histological investigation of the tissue will show degree and distribution of fibrosis, amyloidosis, fatty infiltration, vascular rarefaction, inflammation and other mechanisms. Biochemical studies will reveal ROS production, ion channel alterations, and activity of prohypertrophic, profibrotic, and proinflammatory pathways (e.g. RAS, TGF-β_1). Gene-expression profiling could be used to identify differentially expressed genes informing on statistically enriched signaling pathways. The information from all these investigations will allow for identification of the 'leading molecular mechanisms' in a specific patient. Preferably, the mechanisms should be identified on the tissue or biochemical pathway level. In a second step, a mathematical model to detect the leading molecular mechanisms from parameters measured in blood samples, ECGs, echocardiography, and clinical profile could be constructed. From blood samples, ECGs, echocardiography, and clinical profile information of the same patients (the patients from whom atrial tissue was obtained) a large number of 'features' would be determined in each patient. A feature can be, for example, gender, age, LA size, comorbidity, a common gene variant, elevation of a biomarker, upregulation of a miRNA, an ECG parameter, or a medication. From all this information a model is constructed that assigns a patient to the correct leading molecular mechanisms based on a selection of features. At the end of this step, it is possible to read out the leading molecular mechanisms from a (hopefully) limited list of parameters that can be determined from blood, ECGs, echocardiography, or clinical profile of the patients.

The ultimate goal of this approach is to develop a classification of AF which is based on clinical symptoms and signs and a hopefully short list of biomarkers but which informs on leading pathophysiological mechanism of AF in a specific patient allowing for therapy tailored to these mechanisms. This shift in approach would mean to replace clustering patients in larger groups assumed to have developed comparable disease mechanisms by detecting their individual disease mechanisms. Instead treating them according to guidelines, decision support systems could help to identify the optimal therapy in an individual patient.

Such strategies are more than a daydream. They have been implemented in personalized medicine strategies in various chronic diseases such as Alzheimer's disease or some forms of cancer (44-46). Increasing restrictions in health care budgets call for reinforcement of personalized approaches also in cardiovascular diseases. Taking advantage of their broad and translational expertise EUTRAF partners are well positioned to deliver significant contributions to this process.

Table 1

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University Hospital Graz (Austria)
Medical Information Technology Solutions (Turkey)
University of Oxford (UK)
UK Health & Environment Research Institute (UK)
Sanofi-aventis Deutschland GmbH (Germany)
Osypka AG (Germany)
Xention Ltd (UK)
Universität Duisburg-Essen – Essen (Germany)
Philipps Universität – Marburg (Germany)
Ernst-Moritz-Arndt Universität – Greifswald (Germany)

Table 1: Institutions participating in EUTRAF

Table 2

Study title / acronym	Organizing partner site	Number of patients	Research objective
MULTI-AF	UM	220	Predictive value of new ECG and biomarkers in patients undergoing electrical cardioversion
PROTON	UM	40	Identification of rotors during AF by sinusoidal recomposition and Hilbert transform of endocardial electrograms in patients undergoing catheter ablation for AF
MAPAC	UM	150	Direct contact mapping of patients undergoing open chest surgery, correlation of conduction disturbances with tissue-, serum-, and ECG markers
Late-POAF	UM	100	Identification of tissue-, serum-, and ECG-markers for incident AF in SR patients undergoing open chest surgery.
STICS	Oxford	1922	Randomised, double-blind, placebo-controlled trial of the effect of perioperative rosuvastatin on postoperative AF and myocardial damage
TRAF	Ankara	545000	TRAF (Turkish Atrial Fibrillation Database) registry in the Social Security Institution of Turkey. Association between comorbidities, medication, and hospitalization with progression of AF and stroke.

Table 2: Ongoing clinical studies testing scientific concepts developed by EUTRAF partners.

Figure Legends:

Figure 1: EUTRAF's main objective is to unravel the molecular mechanisms causing progression from paroxysmal to persistent AF in relation to the underlying etiological factors.

Figure 2: Organizational structure of EUTRAF. The research topics are explained in more detail in section 4.

Figure 3: Precipitation of sustained reentrant activity in models of fibrotic myocardium (myocyte monolayers coated with myofibroblasts). Spontaneously arising functional reentry (central disc, 800 µm diameter) is, in some preparations, accompanied by anatomical reentry (peripheral ring of tissue, separated by a gap from the central disc). The frequency of reentrant activity in the 6 preparations shown ranged from 3 to 8 Hz (voltage sensitive dye recordings).

Figure 4: Excellent match between atrial activations (red A) in the left atrial posterior wall (upper tracing) and the TE-ECG (mid tracing). Please note, that the atrial deflections show a low complexity (low fractionation index) in both LA-electrograms as well as in the TE-ECG on the left and a high degree of fractionation (high number of small high frequency deflections) in the right part of the tracing. Such differences are not seen in the surface ECG.

Figure 5: Vest carrying 252 electrodes placed on the patient's chest (A) during CT scan (B) to reconstruct cardiac activation which is then analyzed with a phase

mapping algorithm (C). A rotor is seen on that map (arrow). The rotors and foci are then displayed on cumulative maps (D) to identify the most active region and hierarchize the ablation.

Figure 6: Overcoming the translational gap between knowledge on mechanisms responsible for AF – often assessed by experimental research and the results of clinical investigations (A) forms an important challenge for the future. Detection of individual disease mechanisms by markers or parameters which can easily be measures in every patients and which at the same time inform on the relevant disease processes are required to close the translational gap and to develop an individualized therapeutic approach.

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