

# The European Network for Translational Research in Atrial Fibrillation (EUTRAF): objectives and initial results

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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 remodelling 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 remodelling, 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.

**Keywords** Atrial fibrillation • Pathophysiology • Atrial remodelling • Stroke • Biomarker • Diagnostic tools

## Current challenges in atrial fibrillation 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). Atrial fibrillation is the most common sustained

arrhythmia in the population occurring in 1–2% 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. Atrial fibrillation is associated with an increased risk of stroke, dementia, heart failure, and death.<sup>1,2</sup> In Europe, 1% or even more of the healthcare budget is spent on AF management.<sup>3,4</sup> 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.

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Antiarrhythmic drugs, although reasonably effective for acute termination of AF, are unable to prevent recurrences of AF. Prevention of thrombo-embolic events requires anticoagulation therapy with all the associated risks. Radiofrequency ablation, originally developed for treatment of paroxysmal AF, is not as effective in persistent AF and is associated with potentially serious procedural risks.<sup>5</sup>

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 remodelling process and the occurrence of AF in an individual patient.<sup>6</sup>

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

## Mission statement of EUTRAF

The 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.

## 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 but the research initiated and developed by EUTRAF partners will continue for many more years.

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 (WPs) 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. These partners will define the main biological mechanisms that lead to the slow but progressive process of atrial remodelling increasing the propensity to AF. This atrial disease together with the molecular remodelling occurring as a consequence of AF determine the progression of AF from paroxysmal to persistent forms of the arrhythmia.

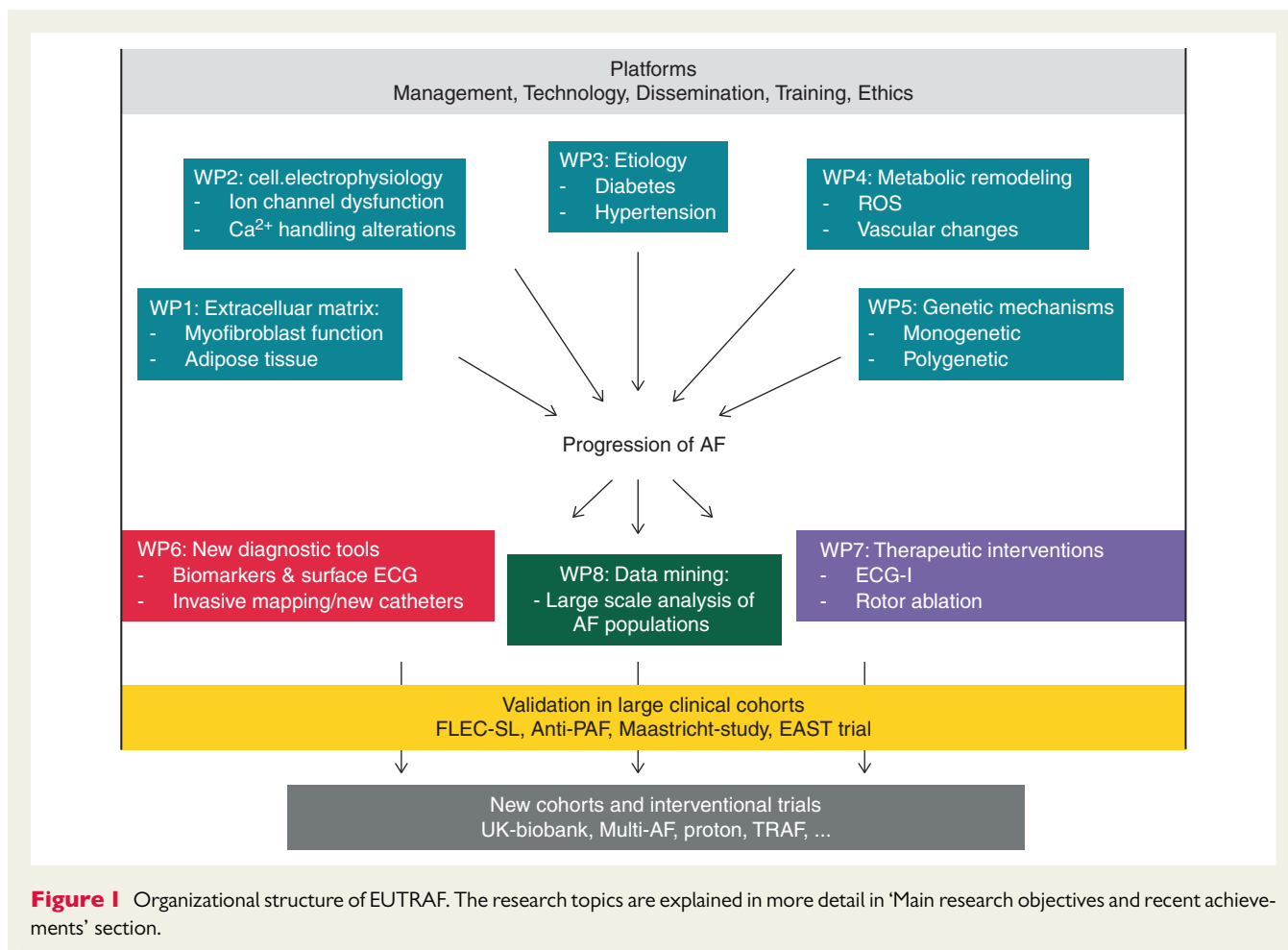
*Figure 1* shows the organizational structure of EUTRAF. The basic research undertaken to define the genetic, molecular, cellular, and structural mechanisms of atrial remodelling are investigated in five basic research WPs. Apart from new insights into the mechanisms of AF, these WPs deliver new biomarkers and new therapeutic targets for AF. These suggestions are explored in two clinical WPs, 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 WP, which at the same time serves as IT platform. The consortium has access to several datasets of large clinical trials that are used for marker validation (e.g. Flec-SL, EAST-Trial, ANTIPAF, and the Maastricht study).<sup>7–10</sup> 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.

## Main research objectives and recent achievements

This section describes per WP examples of research projects undertaken by EUTRAF partners in attempt to unravel new

**Table 1** Institutions participating in EUTRAF

St. George's University of London (UK)
Maastricht University (The Netherlands)
Université Pierre Marie Curie – Paris (France)
Technische Universität Dresden (Germany)
Centre Hospitalier Universitaire – Bordeaux (France)
Westfälische Wilhelms-Universität Münster (Germany)
University Hospital Magdeburg (Germany)
Universität Bern (Switzerland)
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)



mechanisms of AF, to relate them to clinical signs and symptoms, to develop new diagnostic tools for AF, and to improve current therapeutic strategies.

### WP1: extracellular matrix and structure–function relationship

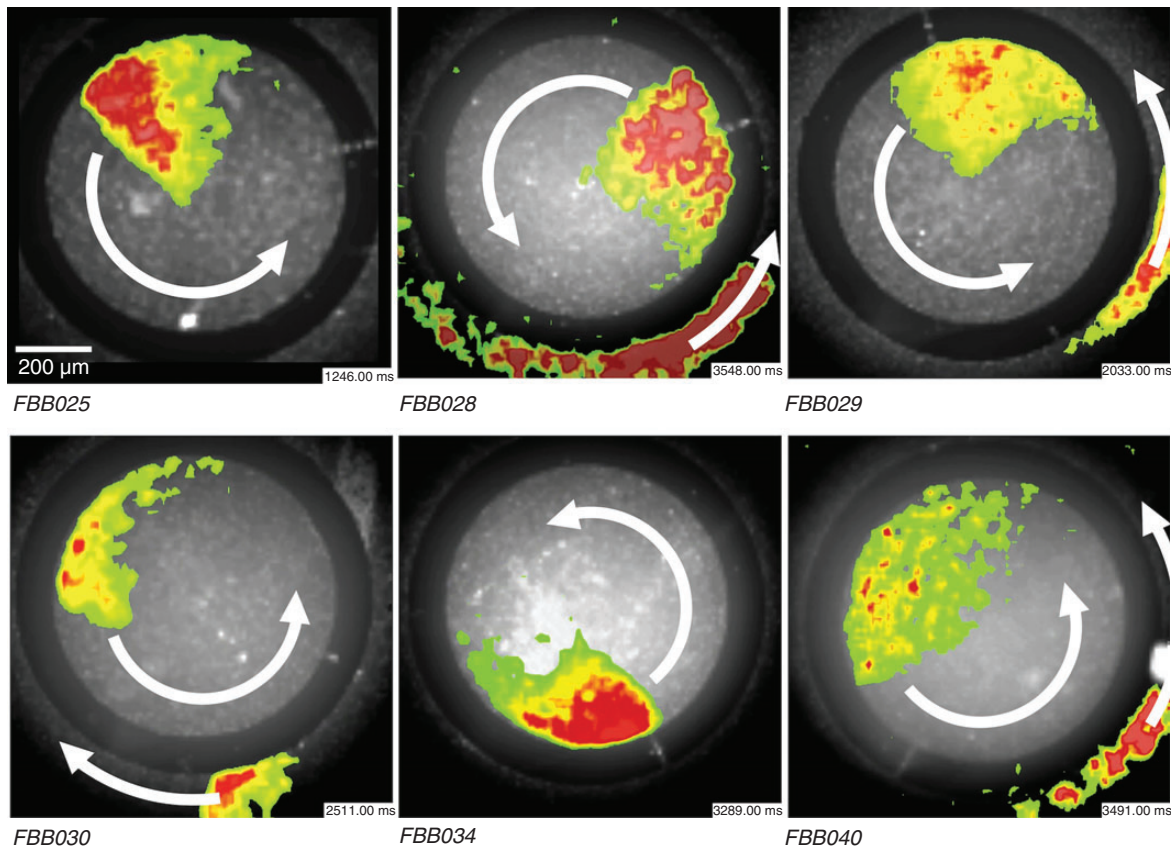
WP1 addresses questions related to the structural determinants of conduction disturbances in atrial muscle. Adverse heterocellular crosstalk between cardiac stromal and parenchymal cells at the structural, mechanical, humoral, and electrotonic level is likely to contribute to arrhythmogenesis in fibrotic 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.<sup>11</sup> With respect to humoral interactions, it was found that the main growth factor involved in cardiac fibrotic tissue remodelling, transforming growth factor- $\beta_1$  (TGF- $\beta_1$ ), substantially aggravates the arrhythmogenic effect of myofibroblasts on electrotonically coupled myocytes. While TGF- $\beta_1$  had little effect on conduction in myocyte preparations, it caused significant conduction slowing and increased ectopic activity in a fibrosis model (Figure 2). 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 adipose tissue or infiltrating the myocardium. This cell type is an important source of adipokines that can diffuse freely in the neighbouring atrial myocardium. EUTRAF partners found that human epicardial adipose tissue secretes activin A (a member of the TGF- $\beta$  family) that has a marked fibrotic effect on the atrial myocardium by stimulating myofibroblasts. EUTRAF partner currently investigate which clinical conditions are associated with an abnormal biological activity of epicardial adipose tissue and how this activity could contribute to the formation of the AF substrate.<sup>12</sup>

### WP2: ion-channel remodelling and Ca<sup>2+</sup> 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.<sup>13</sup> 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.<sup>14</sup> This



**Figure 2** Precipitation of sustained re-entrant activity in models of fibrotic myocardium (myocyte monolayers coated with myofibroblasts). Spontaneously arising functional re-entry (central disc, 800  $\mu\text{m}$  diameter) is, in some preparations, accompanied by anatomical re-entry (peripheral ring of tissue, separated by a gap from the central disc). The frequency of re-entrant activity in the six preparations shown ranged from 3 to 8 Hz (voltage sensitive dye recordings).

highlights that not only the shape of the action potential<sup>15</sup> but also the spatial distribution of ion-channel pore units play an important role in AF-related ion-current remodelling. There is also accumulating evidence for altered intracellular  $\text{Na}^+$ -handling and downstream  $\text{Na}^+$ -dependent ion-channel regulation in AF.<sup>16</sup> EUTRAF partners demonstrated that both  $I_{K1}$  and  $I_{K,ACH}$  are regulated by intracellular  $\text{Na}^+$  in human atrial myocytes of SR patients, whereas  $\text{Na}^+$ -dependent regulation of  $I_{K,ACH}$  was lost in chronic AF patients.<sup>17</sup> Recent EUTRAF studies have identified  $\text{Ca}^{2+}$ -handling abnormalities leading to delayed afterdepolarizations in AF patients ( $\text{Ca}^{2+}$  handling instability).<sup>18,19</sup> 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  $\text{Ca}^{2+}$ -related proarrhythmic cellular events ( $\text{Ca}^{2+}$  signalling silencing).<sup>20</sup> It is currently unclear whether  $\text{Ca}^{2+}$  instability or  $\text{Ca}^{2+}$  handling silencing prevails in patients with AF.

### WP3: aetiological diversity of atrial fibrillation

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.

EUTRAF partners have invested considerable efforts in the development of risk factor-based AF models to systematically identify disease-specific mechanisms of AF. 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, atrial myocytes from hypertensive rats with early compensated LV hypertrophy showed distinct alterations in subcellular calcium handling consisting of reduced ( $-20\%$ ) L-type  $\text{Ca}^{2+}$  current, reduced ( $-55\%$ )  $\text{Na}^+/\text{Ca}^{2+}$  exchanger current and reduced ( $-33\%$ ) fractional SR  $\text{Ca}^{2+}$  release at increased SR  $\text{Ca}^{2+}$  load.<sup>21</sup>

Very few animal studies address the interaction between AF-induced remodelling and disease-specific remodelling during the development of the AF substrate. For this reason, we have established a pig model that combines rapid pacing with hypertension.

The combined model developed a much more severe phenotype in terms of atrial dilatation compared with 'lone' AF. This was associated with faster onset and progression of sustained AF. Eighty per cent of hypertensive pigs had developed sustained AF (>1 h) after 2 weeks of rapid pacing, compared to (meaning: AF in 80% of pigs with hypertension and in 20% of pigs without hypertension). This model will prove useful for the identification of specific signalling pathways involved in hypertension-induced structural remodelling stabilizing and for testing the efficacy of new antiarrhythmic drugs in the setting of hypertension.

#### WP4: atrial metabolic and redox alterations in atrial fibrillation

In WP4, atrial metabolic and redox alteration are investigated as they may provide so far unexplored targets for upstream therapy of AF. A relevant target for such treatment strategies is the nitric oxide-redox imbalance in the fibrillating atrial myocardium. EUTRAF partners showed 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.<sup>22</sup> 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.<sup>23</sup> This implies that NOX2 inhibition by drugs such as statins may only be effective in preventing new-onset AF or early AF-induced electric remodelling 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 pre-operative atorvastatin treatment for 3 days was sufficient to lower atrial NOX2 activity and superoxide level before changes in LDL cholesterol developed.

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.<sup>24</sup> 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 in the longer term, and may therefore represent another interesting target for upstream therapy.

#### WP5: genetic causes of atrial fibrillation

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, a mutation that increases the late sodium current was studied and the effects of increased expression of CREM in a mouse model of spontaneous AF were characterized. Also, the

functional and gene-expression effects of reducing *pitx2*, the gene that is closest to the genetic variants on chromosome 4q25, were investigated.

Patients with long-QT syndrome develop short-lasting atrial arrhythmias triggered by 'atrial afterdepolarizations'.<sup>25</sup> Using 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 knock-in), it was demonstrated that prolongation of the atrial action potential, early afterdepolarizations and pause-dependent atrial arrhythmias explain this monogenic form of AF.<sup>26</sup>

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.<sup>27,28</sup> EUTRAF partners described an arrhythmogenic substrate preceding AF in CREM-TG mice characterized by distension of atria with disorganized and elongated cardiomyocytes.<sup>29</sup> The atrial remodelling 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<sup>2+</sup>-dependent mechanisms also contribute to the development of AF.

*Pitx2* expression was also studied as a marker for 'leftness' in the atria. EUTRAF partners 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.<sup>30</sup> It was demonstrated that reduced *pitx2* expression shortens the atrial action potential, especially at short pacing cycle lengths, and predisposes to AF in the absence of gross structural atrial abnormalities.<sup>30</sup> EUTRAF partners are currently trying to identify whether these genes could be relevant as functional mediators conveying AF and/or as biomarkers to identify patients with 'polygenic AF'.<sup>31</sup>

#### WP6: classification of atrial fibrillation 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 remodelling in the atria<sup>30–32</sup> could possibly monitored by advanced analysis of surface ECGs.<sup>33,34</sup>

Within the EUTRAF consortium, a large software package allowing for quantification of multiple ECG parameters reflecting the complexity of the fibrillatory pattern during AF is developed. 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 such as age, gender, LV-performance, heart dimensions, or co-morbidities.

For example, in 132 patients undergoing step-wise catheter ablation, the predictive value of ECG parameters for acute termination

of AF (f-wave amplitude of aVR and dominant frequency of aVF, area under the receiver operator curve  $AUC = 0.75$ ) was comparable with the performance of best clinical predictors (duration of the current AF episode plus LA diameter,  $AUC = 0.74$ ). The combination of clinical with ECG predictors improved C-statistics to  $AUC = 0.81$ . For long-term outcome, ECG parameters alone predicted long-term success of catheter ablation even better than clinical predictors ( $AUC 0.82$  vs.  $0.68$ , respectively).

Because of the close anatomical relation between the oesophagus and the left atrial posterior wall, a transoesophageal ECG (TE-ECG) has been suggested as a technique to characterize LA electrophysiological properties. So far these studies were limited to a frequency analysis of the oesophageal ECG.<sup>35</sup> The EUTRAF consortium developed a technique to determine the complexity of AF in the posterior wall of the left atrium by fractionation analysis of the TE-ECG. Figure 3 demonstrates the excellent match between frequency and complexity of the atrial activation in the atrium (LA, gold standard) and the TE-ECG. This technique is currently being evaluated prospectively.

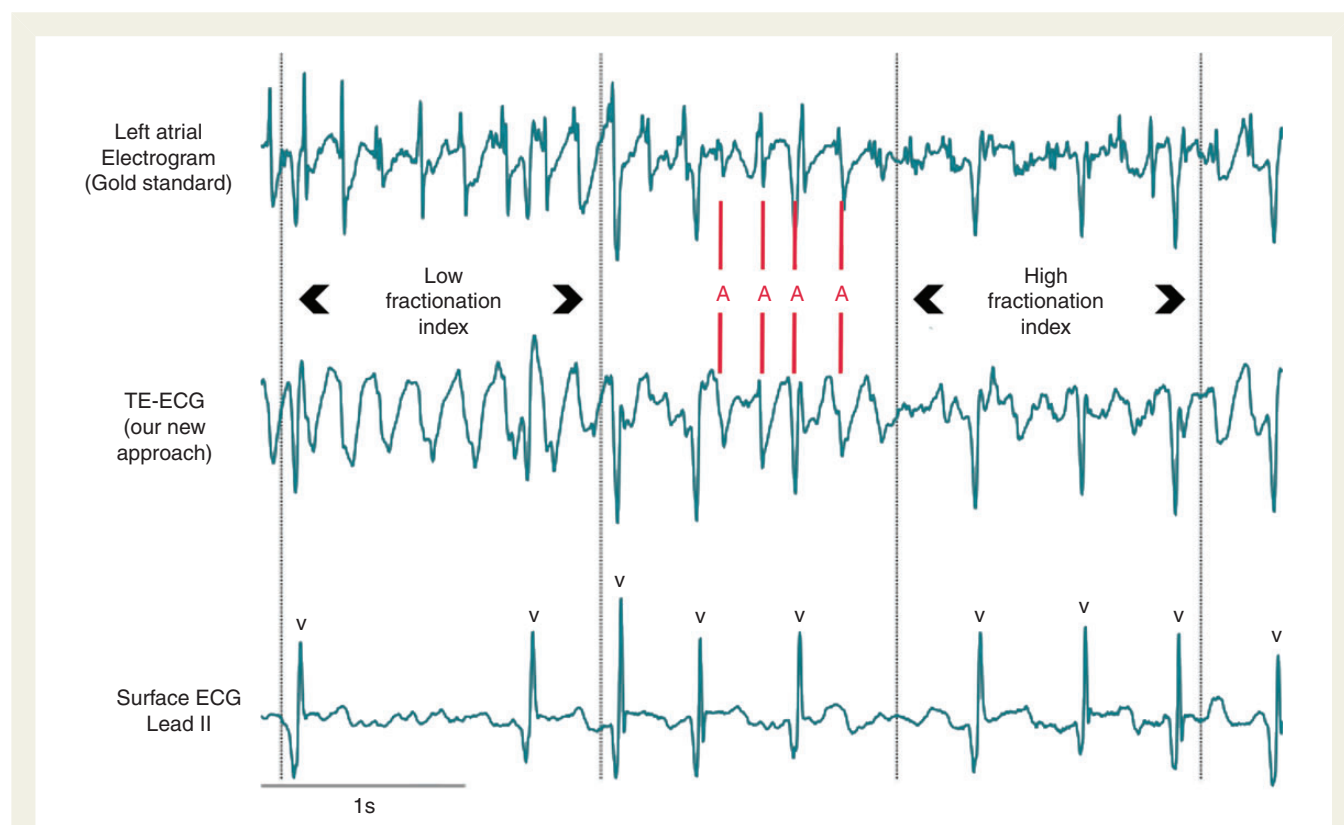
In order to stimulate the process of standardization and harmonization of the assessment of AF complexity from surface ECG EUTRAF members hosted the first European Conference for Standardization of Advanced ECG Analysis in Arrhythmia Diagnostics focusing on Quantification of the Atrial Fibrillation Substrate 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 were initiated.

### WP7: improving atrial fibrillation 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. In persistent AF results are less satisfactory, presumably because of the influence of wider atrial substrate for AF perpetuation.<sup>36</sup> 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.

Recent developments have allowed bi-atrial AF mapping using activation or phase-based analysis of body-surface potentials. For this



**Figure 3** 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.

reason, EUTRAF partners have studied 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.<sup>37</sup> The reconstructed unipolar AF-electrograms were signal-processed to identify the drivers (focal or re-entrant activity) and their cumulative density-map was constructed (Figure 4). The driver domains were catheter ablated using AF termination as procedural endpoint in comparison with step-wise-ablation control group. The maps showed changing beat-to-beat wavefronts and varying spatio-temporal behaviour of driver activities. Re-entries were not sustained and meandered substantially but recurred repetitively. Their locations could be described not as discrete sites but as regions. Ablation of driver regions alone terminated 75 and 15% of persistent and long-lasting AF, respectively. The procedural times were approximately two times shorter when compared with the traditional step-wise approach. 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%). Thus, the non-invasive system can map AF before the procedure and help to shorten invasive procedural time. Further clinical evaluation is currently ongoing.

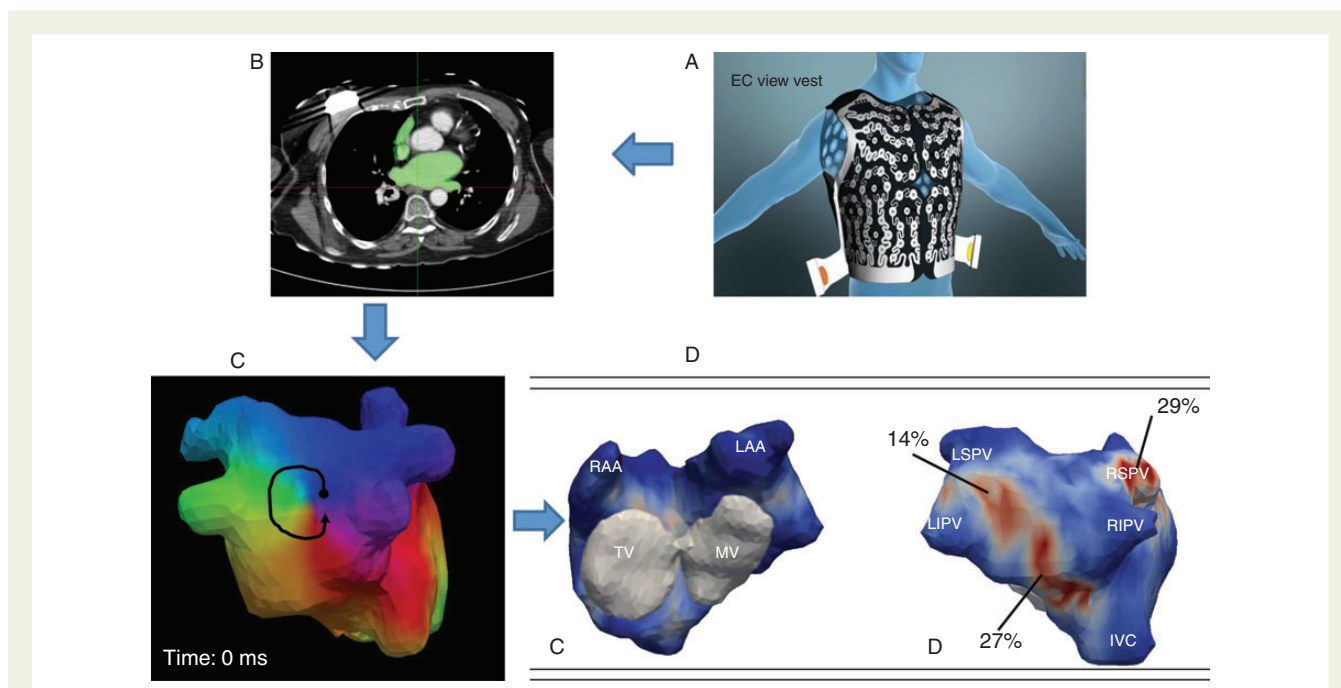
### WP8: insights in atrial fibrillation mechanisms derived from information science and analysis of large-scale datasets

Analysis of large cohorts and databases offers the opportunity not only to validate markers identified in a hypothesis-driven approach

but also to 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 including data warehouse was implemented in the EUTRAF project. An additional clustered data warehouse was built on-site for the TRAF (Turkish Atrial Fibrillation data base) 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.

The objective of these analyses is 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 five different groups in the EUTRAF consortium as well as the German Network of Excellence for Atrial Fibrillation (AF-NET), the Flec-SL, and ANTIPAF clinical datasets as well as datasets collected by the University of Dresden (APDRS) and Maastricht University are analysed. Finally, with the collaboration of MITS, St. George's University London, and the University of Leeds, the largest national cohort of AF patients worldwide, the TRAF database has been built.

For example, the re-analysis of the Flec-SL cohort<sup>8</sup> 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).



**Figure 4** Vest carrying 252 electrodes placed on the patient's chest (A) during CT scan (B) to reconstruct cardiac activation which is then analysed 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.

The TRAF database contains the records of 545 000 patients who had the diagnosis of non-valvular AF within the years 2008–12. 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 database has for example revealed that warfarin significantly increases the rates of hospitalization, and mortality in the low-risk groups ( $\text{CHA}_2\text{DS}_2\text{-VASc} < 2$ ) and decreases hospitalization and mortality in high-risk groups ( $\text{CHA}_2\text{DS}_2\text{-VASc} \geq 2$ ).

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

## Outlook

The authors believe that the scientific achievements of the EUTRAF consortium have been 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 5A).

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 and 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 in Figure 5B.

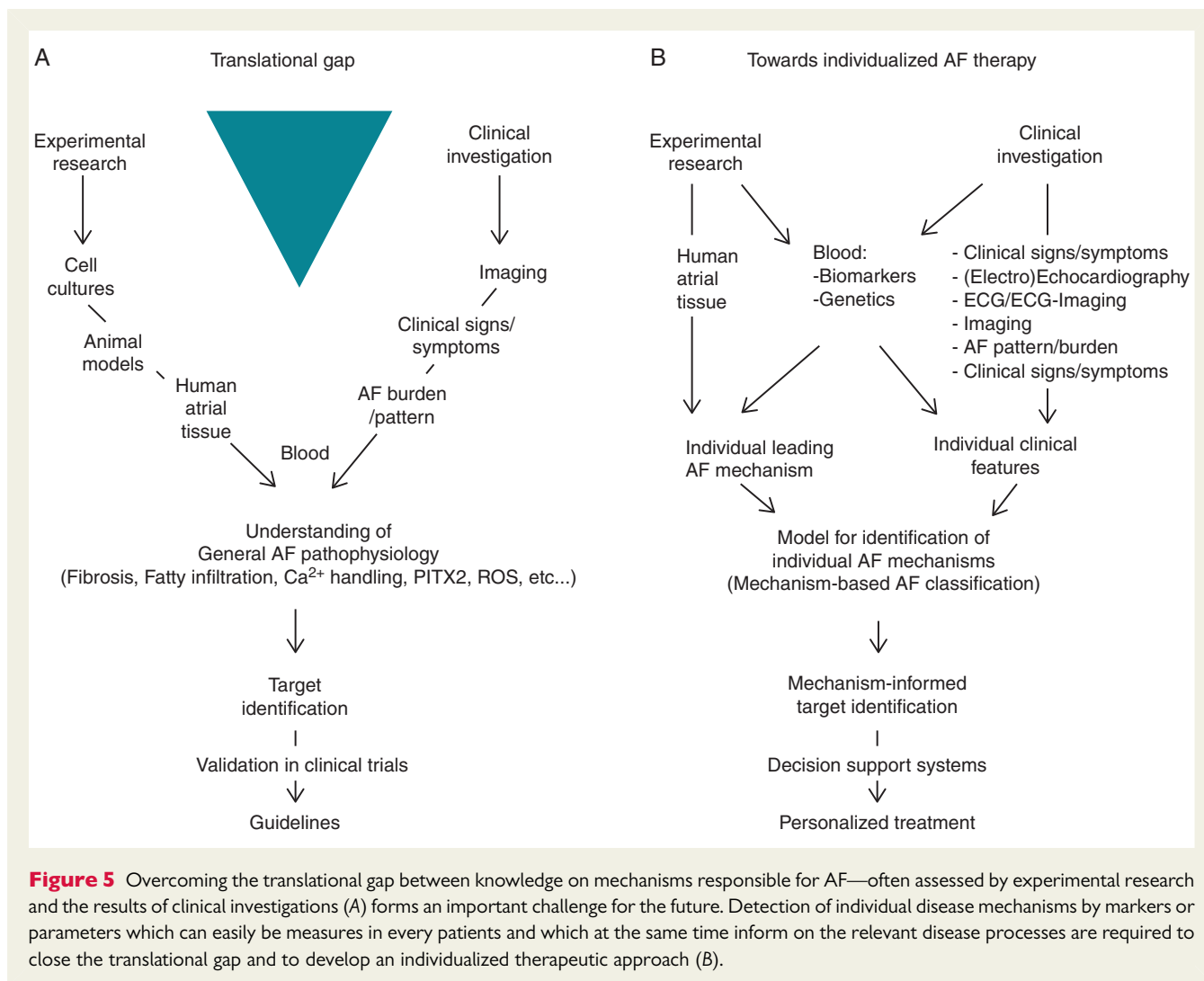
The authors assume that in order to identify the main mechanisms responsible for atrial remodelling and AF and in order to determine the relative contribution of the different disease mechanisms, a combined histological, biochemical, and complex genetics approach is required. In this scenario, it is 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- $\beta_1$ ). Gene-expression profiling could be used to identify differentially expressed genes informing on statistically enriched signalling 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

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

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 recombination 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	Randomized, double-blind, placebo-controlled trial of the effect of perioperative rosuvastatin on post-operative AF and myocardial damage
TRAF	Ankara	545 000	TRAF registry in the Social Security Institution of Turkey. Association between comorbidities, medication, and hospitalization with progression of AF and stroke





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.<sup>38,39</sup> Increasing restrictions in healthcare 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.

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