CLINICAL INVESTIGATION

The European Network for Translational Research in Atrial Fibrillation

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The initiation and perpetuation of atrial fibrillation (AF) can be regarded as a complication of a progressive transformation of atrial structure and function. This transformation is the result of complex changes at the molecular, cellular and organ levels, leading to the profibrillation arrhythmic mechanisms in AF. Numerous individual and environmental factors are probably involved in this process. Therefore, progress in the diagnosis, prevention and treatment of AF requires highly integrative research from the benchtop to bedside and from specific signaling pathways and electrophysiological mechanisms to population-based studies. The European Network for Translational Research in Atrial Fibrillation was formed to provide this variety of expertise and has identified central research objectives for improvements in AF prevention and therapy.

Keywords: arrhythmogenic substrate • atrial fibrillation • electrophysiology

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Atrial fibrillation (AF) is the most common sustained cardiac rhythm disturbance, occurring in between 1 and 2% of the general population. It is estimated that over 6 million Europeans suffer from this arrhythmia and its prevalence is calculated to increase by at least 2.5-fold in the next 50 years as the population ages. The ischemic strokes seen in association with arrhythmia are often fatal, and those that survive are left more crippled by their stroke and more likely to suffer from recurrent strokes [1].

For the majority of patients, there appears to be an inexorable progression of their AF to a more persistent or even permanent form, associated with further development of the disease that may underlie the arrhythmia [2]. Little advancement has been made in the understanding of the dynamic development of AF from its pre-clinical state as an 'arrhythmia-in-waiting', to its final expression as an irreversible and end-stage cardiac arrhythmia associated with very serious adverse cardiovascular events. This lack of progress has led to inevitable clinical frustration, which has been fuelled by numerous clinical trials that have demonstrated that the strategic aim of maintaining normal sinus rhythm has no demonstrable value when compared with the 'laissez-faire' approach of leaving AF unchecked, apart from lenient restriction of the ventricular rate.

The European Network for Translational Research in Atrial Fibrillation: its aims & objectives

The European Network for Translational Research in Atrial Fibrillation (EUTRAF) is a unique network of successful investigators who have a common interest in the pathophysiology and treatment of AF. An important factor of this condition is the development of a substrate for AF (especially persistent AF), that usually precedes the first episode and the diagnosis of AF. By integrating the complementary strengths and expertise from molecular and cellular

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electrophysiology to the study of large cohorts of patients, this network aims to develop the most modern approaches towards increasing knowledge of this substrate. A better understanding of molecular and cellular mechanisms involved in the substrate biology will lead to the identification of new biomarkers and targets for prevention, diagnosis and management of AF (Figure 1).

To achieve this strategy, the network brings together experts from academic institutions and industry from all over Europe with a wide range of expertise ranging from genetics and molecular biology, cellular electrophysiology and confocal imaging, morphometry and immunohistochemistry, optical and direct contact mapping, animal models and computer modeling, echocardiography and ECG-imaging, gene and ablation therapy and data mining.

The network (Figure 2) is designed to tackle a central question: the nature and the biology of the substrate of AF. Translations from molecular and cellular mechanisms to clinical applications are achieved through well-defined large animal models, computational biological models, tissue from human atria, new electrocardiographic and imaging approaches and large, clinically and biologically well-characterized European patient cohorts.

Figure 1. Model showing the interaction of genetic, clinical and biologic factors leading to the formation of the atrial fibrillation substrate and the perpetuation of AF.
AF: Atrial fibrillation.

Stéphane Hatem and Stephan Rohr from the INSERM (UMRS956) University of Pierre and Marie Curie (Paris, France) and the University of Bern (Bern, Switzerland), respectively, study the mechanisms of conduction disturbances in the atria on a microscopic scale. They aim to identify the cellular and molecular determinants of early conduction disturbances in the atria, a key determinant of the arrhythmogenic substrate of AF.

Dobromir Dobrev from the University of Mannheim (Mannheim, Germany) leads the research on the alterations of cellular electrophysiology and Ca^{2+} signaling related to AF. In addition, new ion-channel and transporter targets for treatment of AF are being explored.

Andreas Goette from the University of Magdeburg (Magdeburg, Germany) leads the investigation on the etiology-specific aspects of atrial remodeling. Hypertension, aging and diabetes, as main risk factors for AF, are likely to contribute to the occurrence of AF by different mechanisms. Both biomarkers and the etiology-specific action of therapeutic targets are assessed in EUTRAF.

Burkert Pieske from the Medical University of Graz (Graz, Austria) leads the work on a large animal model (porcine) mimicking human risk-factor accumulation for the initiation of atrial remodeling and AF. This work, in concert with comparative *in vitro* cellular and molecular work in human cardiac tissue, aims at a better understanding of the specific impact of remodeling on AF.

Barbara Casadei from the University of Oxford (Oxford, UK) studies the different aspects of intracellular signaling that might trigger the remodeling process. This work targets the changes in metabolism, myocardial nitroso–redox balance and excitation–transcription coupling.

Paulus Kirchhof from the University of Birmingham (Birmingham, UK) leads the study of the role of genetic alterations (polymorphisms and mutations) in transgenic models. In addition, the pathophysiological aspects of the targets identified in the four studies described above will be investigated.

Ulrich Schotten from the University of Maastricht (Maastricht, The Netherlands) leads the development of novel diagnostic tools to develop a classification of AF-based on invasive and noninvasive markers for the degree of electrophysiological alterations in the atria.

Pierre Jais from the University Hospital of Bordeaux (Bordeaux, France) works on new therapeutic strategies. They investigate the findings of the five working groups mentioned above in small proof-of-principle studies and large study populations.

Ali Oto from the company Medical Information

Technology Solutions (Ankara, Turkey) provides an IT infrastructure for standardized and scalable data collection through web-based interfaces. His team will perform data mining on the project data to discover new therapeutic targets, mechanistic relationships between biomarkers and the perpetuation of AF. Ultimately the team aims to adhere to a clinical decision support system.

Uwe Lendeckel, Uwe Völker and Stephan Felix from the Universitätsmedizin Greifswald/ Ernst-Moritz-Arndt University of Greifswald (Greifswald, Germany) provide methodology and equipment for transcriptome analysis, encompassing an Affymetrix as well as an Agilent DNA microarray platform. Subsequent statistical analysis of the generated expression data using the 'Rosetta Resolver' software and further investigation of putative regulated pathways is carried out using the 'ingenuity pathway' software. The application of these technologies for different animal models of AF are aimed at the identification of novel biomarkers that, if appropriate, will be followed up using gel-free and gel-based proteomics approaches, including the identification of proteins of interest by MS.

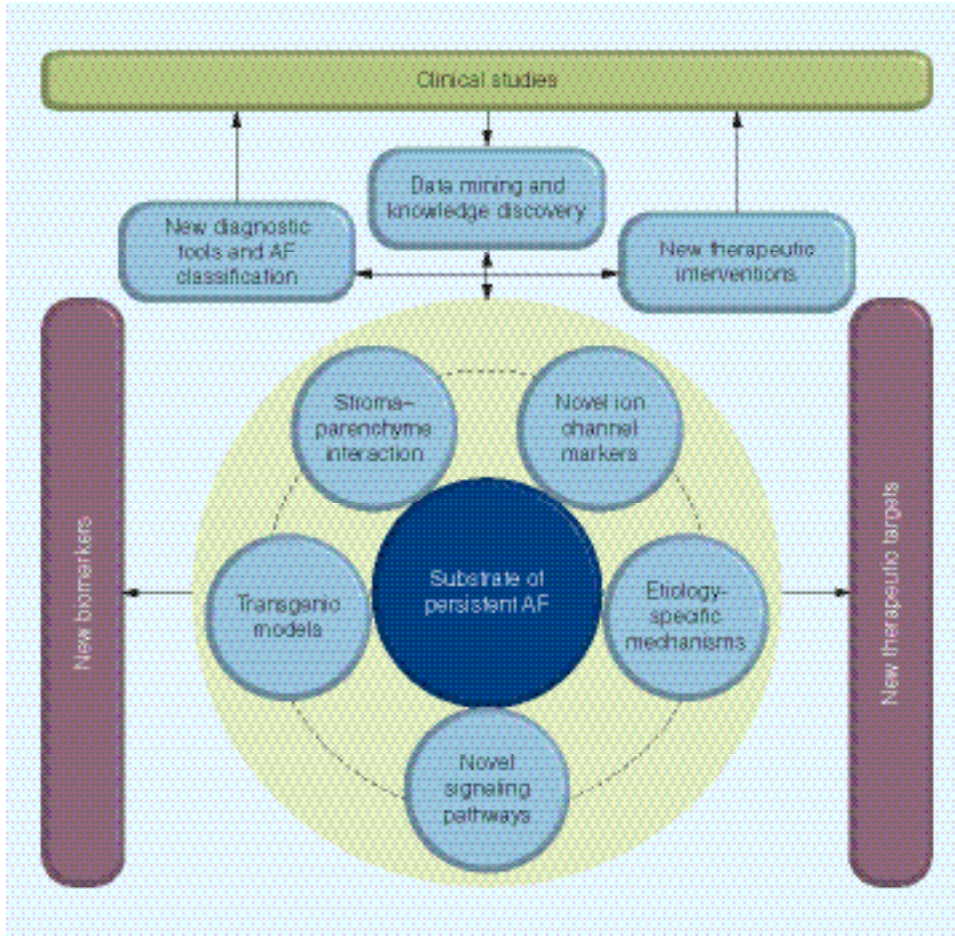


Figure 2. European Network for Translational Research in Atrial Fibrillation strategy.
AF: Atrial fibrillation.

How the network was set up

The EUTRAF network was created when applying to the European FP7 call published in July 2009 and dedicated to AF. Over a period of 5 years, the network will be granted €12 million from the European Commission.

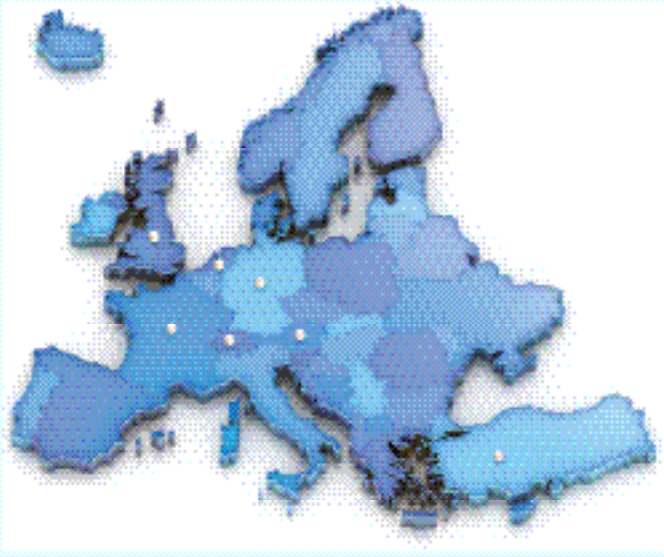
It comprises 19 entities spread over seven countries (UK, Germany, France, The Netherlands, Austria, Switzerland and Turkey). It is composed of ten research centers, four clinical centers or university hospitals, three small and medium enterprises, one global organization and one project management institute (Figure 3).

How the network functions & is structured

The network is led by a steering unit and managed by a management board (Figure 4). The steering unit is composed of the network coordinator, two technical managers, a consortium manager and an exploitation manager. This unit monitors and leads the scientific, contractual and commercial aspects of the project. The coordinator and technical managers ensure that the network work does not deviate from the rationale and objectives initially set and agreed within the group. The consortium manager supports the network with administrative, financial and contractual activities. The exploitation manager monitors the network findings and advises on any commercial opportunities. The coordinator and the consortium manager inform the EC about research progress.

The steering unit is the first point of contact for any matter; it circulates information to the whole network. In case of any issue, the unit investigates the problem and proposes a set of potential solutions to the management board. This board, composed of one representative per organization, decides on the solution to apply by vote.

The steering unit meets once a month via teleconference. Each working group presents its progress to the whole network at annual meetings. Finally, the



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Ernst-Moritz-Arndt Universität	Germany
University of Birmingham	UK

Figure 3. Geographical representation of the European Network for Translational Research in Atrial Fibrillation network.

network submits an activity and financial report to the EC once a year.

Network achievements

The EUTRAF network initiated its collaborative research activities on the 1 November 2010. During the first year, the overall work progressed well and significant results were generated in some working groups.

Stroma–parenchyme interaction

The substrate of AF is composed of profound structural alterations of the atrial myocardium with fibrosis being a common feature. One of our main hypotheses is that this structural remodeling of the atrial myocardium is responsible for abnormal myocyte–myocyte coupling, which at the cellular level is a major arrhythmogenic mechanism. Heterocellular coupling between myocytes and myofibroblasts can be arrhythmogenic by altering the normal electrical properties of the atrial myocardium through various mechanisms, including abnormal electrical coupling and paracrine activity between the two cell types [1]. Several arguments indicate that this alteration in the architecture and cellular organization of the atrial myocardium can also affect the functional polarization of atrial myocytes necessary for the anisotropic propagation of the depolarization wave.

During the first year of the EUTRAF network, several *in vitro* models have been developed to investigate paracrine humoral interactions between stromal and parenchymal cells and to assess the effect of shear stress on atrial myocyte excitability.

A new actor involved in cell–cell contacts and mechanotransduction has been identified. A novel mechanism linking shear stress and changes in the electrical membrane properties has been identified and its role in the regulation of the atrial electrical properties is currently investigated. We also initiated the implementation of a computer model describing heterocellular electrotonic interactions [2].

Identification & validation of novel ion channels & transporters for treatment of persistent AF

At the cellular level, AF is associated with the shortening of both the action-potential duration and the refractory period, which favor the formation of microreentry of the electrical impulse. There is also abnormal calcium homeostasis with calcium leak from the sarcoplasmic reticulum (SR), which results in a high incidence of delay after depolarisations in these cells, and the occurrence of arrhythmogenic triggered activity. An important challenge in this field is to identify the molecular actors underlying these abnormalities. Using human atrial myocytes, experimental systems and transgenic mice models, we study the determinants of altered atrial electrical properties and the formation of the AF substrate. New contributors to AF induction and maintenance have already been identified:

- The multifunctional adapter protein ankyrin-B that interacts with the calcium channel Cav1.3 is

down-regulated in the atrial myocardium during AF, which contributes to the reduction of L-type Ca^{2+} current, a hallmark of electrical remodeling in AF [3];

- Channels are continuously trafficked to the plasma membrane and then recycled. We found that the atrial repolarizing channel, Kv1.5, is recycled via an endocytosis process mediated by the clathrin pathway;
- We have shown that atrial myofibroblasts express several potassium currents, the properties of which differ between patients in sinus rhythm and those in AF;
- Calcium/calmodulin protein kinase-II-dependent calcium leak through ryanodine-receptor channels from the SR is recognized as an important arrhythmogenic mechanism and likely contributes to AF by favoring the occurrence of triggered activity [4]. Inhibition of calcium/calmodulin protein kinase-II phosphorylation of ryanodine-receptor channels prevents AF induction in mice [5], suggesting that SR calcium leak may play a critical role in AF pathophysiology.

Aetiology-specific mechanisms in AF

A number of clinical settings (heart failure, hypertension, diabetes or aging) are associated with a high risk of AF and favor the development of the substrate of AF [6]. For instance, hypertension or heart failure is often associated with fibrosis and dilation of the atria. A better understanding of the pathogenic factors responsible for the development of the AF substrate associated with these clinical settings is of major importance in order to define new tools that could be used for an early diagnosis of patients at risk and to prevent the progression of the substrate.

Two models of atrial remodeling and AF associated with hypertension have been developed in rat and pig and their study has recently been initiated. The aim is to study various biological parameters known to be associated with the AF substrate during progression of the disease process. For instance, atria from spontaneous hypertensive rats show alterations in cellular calcium homeostasis that could contribute to the activation of arrhythmogenic mechanisms. We successfully developed a pig model for AF on the basis of hypertension (desoxycorticosterone acetate) and lipid disorders/overweight to induce atrial remodeling; AF is then induced by rapid atrial stimulation via telemetric controlled custom-made pacemakers. During the development of the cardiac remodeling in this model, various approaches will be used to follow the development of the substrate for AF.

Identification & preclinical testing of new 'upstream' therapeutic targets for sustained AF

In EUTRAF, the role of intracellular signaling pathways has been investigated. It was found that alterations of oxidative stress resulting in maladaptive reactive oxygen species (ROS) signaling are very similar in the goat model of AF and in patients with AF. In patients undergoing cardiac surgery, statins could significantly improve ROS signaling and might explain the efficacy of these compounds in the prevention of AF [7].

Another important field of investigation is the effect of AF on the Ca^{2+} homeostasis in the nuclei of atrial myocytes. Ca^{2+} signals in the nuclei of myocytes are known to have an important impact on the gene regulation. We could demonstrate that these Ca^{2+} signals are reduced by AF, probably as a sign of protection of the cell against excessive Ca^{2+} signaling during high activation rates. Also, activation of inositole-3-phosphate receptors, a signaling molecule known to be deeply involved in alterations of signaling during AF, significantly alter nuclear Ca^{2+} signals in AF but not in control myocytes. These observations all point towards a very important contribution of nuclear Ca^{2+} metabolism to gene regulation during AF [8].

Translating genetic contributions to AF into novel therapeutic targets

Although the need for disease-specific targeted therapy is recognized, the notion that different forms of AF exist is not fully understood. There are several

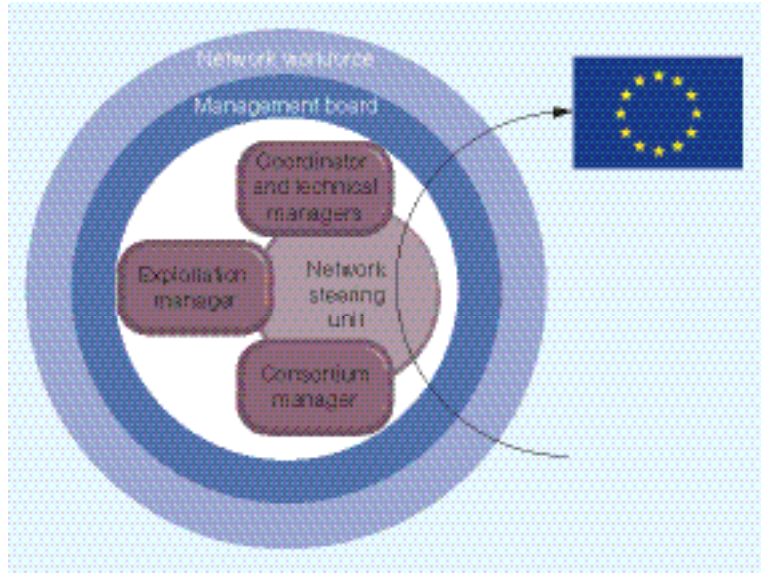

Figure 4. Management structure of the network.

'forms' of genetically conferred AF. This has recently been classified by a consensus conference of the Atrial Fibrillation Network and European Heart Rhythm Association. In this work package, we use two approaches, investigating novel AF-causing factors in CREM-IbΔC-X transgenic mice (CREM-TG) and to study the relevance of identified factors/markers and selected genes by targeted therapy. Another approach uses transgenic models that express genetic variants that cause AF in patients, either in inherited cardiomyopathies or in genome-wide association studies.

Overexpression of CREM-IbΔC-X in young TG mice (<8 weeks) led to atrial dilatation combined with distension of myocardium, elongated myocytes, little fibrosis, down-regulation of connexin 40, loss of excitability in depolarized myocytes, atrial ectopics and inducibility of AF [9]. It was concluded that transcription factor CREM is an important regulator of atrial growth implicated in the development of an arrhythmogenic substrate in TG mice. We found that a genetically increased late sodium current causes atrial arrhythmias in scn5a ΔKPQ-knock-in mice. This mutation, which is found in patients with long QT syndrome type 3, prolongs the atrial action potential and provokes atrial after depolarization. Further studies identified that reducing *pitx2* expression, a gene that is located close to common gene variants that associate with AF, predisposes the mouse heart to AF while causing left atrial action potential shortening [10]. This work also identified *pitx2* as a marker for left atrial tissue and as a gene that appears relevant for 'maintaining leftness' of the left atrium. Systematic analysis of gene expression differences between left and right atria in different mouse strains and in human atrial tissue. These findings form the basis for further studies within and outside of EUTRAF.

New diagnostic tools for AF

A very important research objective of the consortium is to develop better means to classify patients with AF. Such a classification exists in many other cardiovascular diseases and is necessary to clarify which patients would respond to a specific therapy [11]. In EUTRAF, a number of tools allowing for such a classification have been developed.

Diagnostic tools for a rapid and fully automated method for the analysis of direct contact electrograms were used to assess the degree of electrophysiological changes in the atria in all animal models used throughout the consortium [12]. For the first time, this technique allows for the direct comparison of electrophysiological changes between patients with AF and an array of animal models with specific pathomechanisms. These may be used as a technical standard.

Another example is a new technique with which an ECG recorded in the esophagus is used to classify AF. In a study with 32 patients, there was a very good correlation between the AF characteristics retrieved from this ECG compared with direct invasive measurements from the heart. In future, the consortium will investigate whether such measurements can be used to guide the therapy of AF.

Another important challenge is to identify the degree of fibrosis in the atrium. In EUTRAF, atrial fibrosis was assessed by MRI of the atria. This technique has recently been developed and now needs to be implemented into clinical practice. For validation, the results in patients were compared with the complexity of the electrophysiological alterations. Surprisingly, fibrosis was associated with less complex activity and not with electrophysiological mechanisms promoting AF. The meaning of this unexpected result requires further investigation.

Future perspective

The EUTRAF collaboration has already begun to plan its long-term objectives. The over-riding concept is to allow rapid translation into the clinical arena of information generated in the basic science laboratory. As such, the partners and work package leadership are forging ties between the entire membership of the collaboration to ensure that the output from EUTRAF is substantially greater than could have been achieved

Executive summary

- Atrial fibrillation is common and is associated with adverse outcomes and, therefore, an important and expensive element of healthcare.
- The European Network for Translational Research in Atrial Fibrillation is hosted in ten research centers, four clinical centres or university hospitals, three small and medium enterprises, one global organization and one project management institute.
- The European Network for Translational Research in Atrial Fibrillation is funded for 5 years through an FP7 grant from the European Commission.
- This Network, which is managed by a Steering Unit and Management Board, concentrates on translational research elements but spreads from the genome to clinical trials and epidemiological studies.
- The first year of research has met all timelines, and provided deliverables on time. The network has now entered its second year of research.

by groups working in isolation. Plans are already in hand for work that extends far beyond the original intention. The ultimate legacy of the group will be deeper knowledge and better patient care for patients with AF.

Financial & competing interests disclosure

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