Project no. **018802**

Project acronym: **CONTICA**

Project title: **Control of intracellular Calcium and Arrhythmias**

www.contica.eu

Instrument: SPECIFIC TARGETED RESEARCH PROJECT

Thematic Priority: Priority 1: LIFE SCIENCES, GENOMICS AND BIOTECHNOLOGY FOR HEALTH

Publishable final activity report

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Start date of project: 01 Feb 2006 Duration: 42 months

Project coordinator name: Prof. Dr. Burkert Pieske

Project coordinator organisation name: Medizinische Universitaet Graz (MUG)

1. Project execution

1.1 Objectives of CONTICA

Sudden cardiac death is a major cause of death worldwide. Only a few years ago, it became clear that mutations in the cardiac sarcoplasmic reticulum (SR) Ca^{2+} release channel, or ryanodine receptor (RyR2), can cause catecholaminergic polymorphic ventricular tachycardia (CPVT), a stress-induced cardiac arrhythmia, which can lead to syncope and sudden cardiac death. At about the same time, it was also found that dysfunction of RyR2 in cardiac disease, such as heart failure, can likewise result in fatal arrhythmias, pointing to RyR2 as a key player in inherited as well as acquired fatal human arrhythmias. The major scientific objectives of CONTICA, therefore, were to determine the mechanisms underlying fatal *inherited* arrhythmias related to RyR2 mutations as well as the mechanisms underlying fatal *acquired* arrhythmias related to RyR2 dysfunction in cardiac disease.

The specific project objectives were:
Objective 1: Identification and characterisation of mutations and polymorphisms in the RyR2 gene and genotype-phenotype correlations in patients.
Objective 2: In vitro expression of mutant and polymorphism-containing RyR2 proteins to study their functional consequences.
Objective 3: Generation and phenotypic characterisation of transgenic mice and rabbits harbouring mutant RyR2.
Objective 4: In vivo and in vitro characterisation of animal models of acquired vs. inherited arrhythmias involving the RyR2 SR Ca^{2+} release channel.
Objective 5: Characterisation of the mechanisms underlying stress-mediated arrhythmias in human myocardium.
Objective 6: Characterisation of a new mathematical model of cardiac excitation-contraction coupling and the development of delayed afterdepolarisations that takes into account defective RyR2 channel gating in inherited and acquired cardiac disease.
Objective 7: Test novel diagnostic techniques for better risk assessment.
Objective 8: Test existing and novel therapeutic compounds directed at normalising ion channel function to prevent triggered arrhythmias.
Objective 9: Improve clinically relevant awareness and risk stratification.

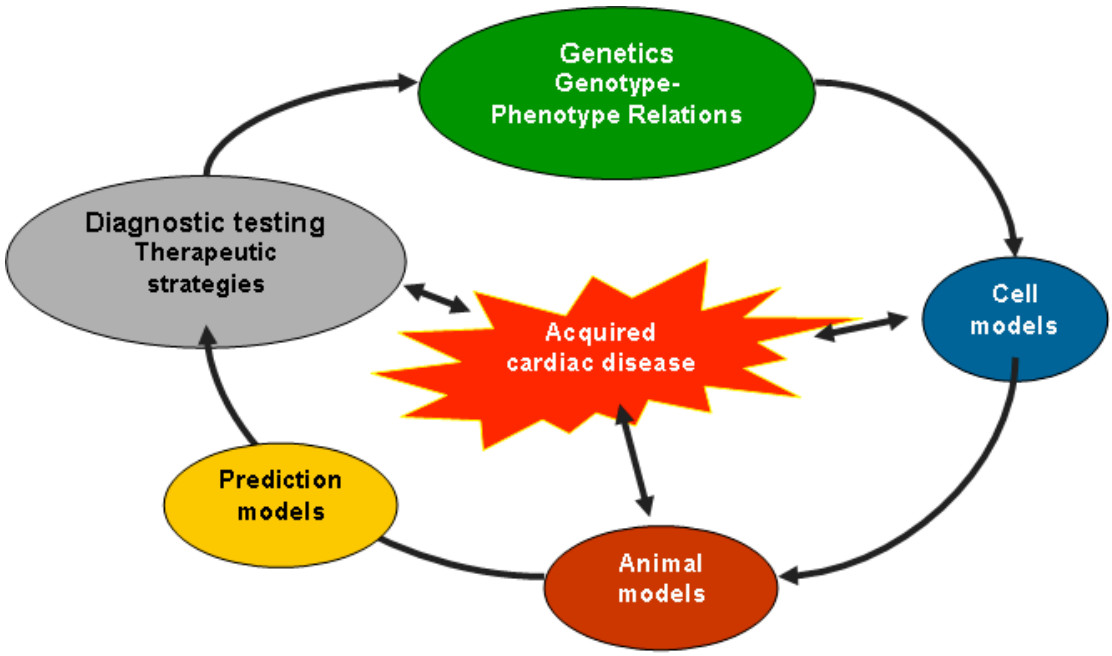

Figure 1. The concept of CONTICA.

1.2 Contractors involved in CONTICA

The project was coordinated by Prof. Dr. Burkert Pieske. During the first period of the project (February 2006 to July 2007), Prof. Pieske was at the Department of Cardiology and Pneumology at the University Medicine Göttingen (UMG-GOE), Germany. He then became head of the Division of Cardiology at the Medical University of Graz (Medizinische Universitaet Graz, MUG), Austria, and coordinated the project in the second period (August 2007 to July 2009) from Graz. His contact details are:

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The contractors involved in CONTICA comprised:
1) Universitaetsmedizin Goettingen - Stiftung Oeffentlichen Rechts (UMG-GOE), Germany
2) University Medical Center Utrecht (UMCU), Netherlands
3) IRCCS Fondazione Salvatore Maugeri - Clinica del Lavoro e della Riabilitazione (FSM), Italy
4) Institut National de la Santé et de la Recherche Médicale (INSERM), France
5) Cardiff University (CU), United Kingdom
6) Ústav molekulárnej fyziológie a genetiky Slovenskej Akadémie Vied (UMFG SAV), Slovakia
7) Agrobiogen GmbH (ABG), Germany
8) IMGM Laboratories GmbH (IMGM), Germany
9) Medizinische Universitaet Graz (MUG), Austria (since 1 August 2007)

1.3 Work performed and end results

Objective 1: Identification and characterisation of mutations and polymorphisms in the RyR2 gene and genotype-phenotype correlations in patients.

The world's largest database with CPVT patients has been extended further. Overall, 405 individuals have been enrolled, genetic screening of the RyR2 has been completed for 350 individuals, and 174 have an established diagnosis of CPVT. The web-based repository of CPVT mutations (www.fsm.it/cardmoc) now includes 75 different allelic variants. The results show that the majority of RyR2 mutations cluster in specific regions of the protein, i.e. the central domain, the N-terminal domain, and the transmembrane segment domain. Genotype-phenotype correlation analysis did not reveal any significant differences in outcomes with respect to the position of the mutations. More refined analyses are still ongoing.

Objective 2: In vitro expression of mutant and polymorphism-containing RyR2 proteins to study their functional consequences.

Four CPVT/ARVD2 mutations and three polymorphism/mutations have been chosen for detailed functional analysis. Selection criteria included that the mutations: a) were present in disparate locations spread across the entire RyR2 sequence, b) occurred in distinct mutational loci, c) had not been previously characterised in the published literature, and d) were of significant interest in the general context of RyR2 structure-function relationships. Expression plasmids have been constructed and expressed in a heterologous expression system (HEK293 cells) for functional analysis. Functional characterization included caffeine activation of Ca^{2+} release, single channel studies in planar lipid bilayers, and FKBP12.6 binding characteristics. In HEK293 cells, mutant/polymorphism proteins were expressed and targeted correctly to the ER. They did not alter cell morphology. Mutant/polymorphism proteins, however, exhibited complex changes in terms of caffeine-induced Ca^{2+} release suggesting that a polymorphism (G1885E) can alter mutant channel function. For single channel studies, expression in HEK293 cells and purification

of hRyR2 channels has been optimized and experiments with reconstituted mutant channels have been conducted. Finally, FKBP12.6 binding experiments revealed that some (R176Q and S2246L) but not all mutations (R4497C) altered RyR2-FKBP12.6 binding under basal conditions. Oxidizing conditions reduced FKBP12.6 binding to all mutants studied to a similar degree. These results suggest that FKBP12.6 regulation of RyR2 function is unlikely to be defective in CPVT.

Objective 3: Generation and phenotypic characterisation of transgenic mice and rabbits harbouring mutant RyR2.

and Objective 4: In vivo and in vitro characterisation of animal models of acquired vs. inherited arrhythmias involving the RyR2 SR Ca^{2+} release channel.

A transgenic mouse model with a specific human RyR2 mutation (R4497C) has been generated. This mouse model has been characterized in detail both in vivo and in vitro. The heterozygous $RyR2^{R4496C+/-}$ mice reproduced in many aspects the human CPVT phenotype and thus represent the first animal model of CPVT. Characterization of the cellular mechanisms underlying stress-induced arrhythmias in this model confirmed the hypothesis that delayed afterdepolarizations (DADs) and triggered activity may cause the arrhythmias (Liu et al. Circ Res. 2006; 99:292-8). Further in depth studies on the subcellular mechanisms revealed that cardiac myocytes from these mice exhibit an increased frequency of Ca^{2+} sparks and Ca^{2+} waves. Arrhythmogenic SR Ca^{2+} release was further augmented at higher stimulation rates and by β-adrenergic stimulation, while the response to β-adrenergic stimulation per se ($[Ca^{2+}]_i$ transient amplitude) and the expression and phosphorylation of RyR2 protein was unaltered when compared to wild-type myocytes. Experiments on permeabilized myocytes revealed a dramatically increased Ca^{2+} sensitivity of the mutant RyR2. From these studies it was concluded that arrhythmias in the $RyR2^{R4496C+/-}$ mice are caused by the increased Ca^{2+} sensitivity of the mutant RyR2 channels, which results in a lowered threshold for diastolic SR Ca^{2+} release and triggered activity. The results were published recently (Fernandez-Velasco et al. Circ. Res. 2009; 104:201-9).

In addition, a transgenic rabbit model with the same human RyR2 mutation (R4497C) has been generated. This model was phenotyped in vivo and in vitro. The transgenic rabbits with the R4497C mutation did not die prematurely. In vitro analysis confirmed that the transgenic animals had indeed integrated the mutated eGFP-hRyR2 construct into their genome. There was low expression of the mutated RyR2 on the mRNA level. However, there was no evidence for expression of the functional protein, neither in cardiac myocytes nor in non-cardiac cells (liver). This excluded further characterization of the arrhythmogenic mechanisms underlying CPVT in the transgenic rabbits. Therefore, experiments on the transgenic rabbits were stopped and, instead, work on the successful transgenic mouse model was intensified.

Using the heterozygous $RyR2^{R4496C+/-}$ mouse model as a starting point, several other mouse models were generated in order to investigate the effects of gene dosage, physiological and pharmacological modulators, and pathological stimuli on Ca^{2+} handling, RyR2 function, and arrhythmogenesis in our CPVT mouse model.

Gene dosage: Homozygous $RyR2^{R4496C+/+}$ mice have been generated and phenotyped both in vivo and in vitro. Basically, all the effects observed in the heterozygous animals were found to be greater in the homozygous animals, providing clear evidence for a gene-dosage effect. A very high propensity for spontaneous Ca^{2+} sparks was observed in the homozygotes in the absence of β-adrenergic stimulation, suggesting that PKA-dependent phosphorylation of RyR2 is not necessary for the mutant channel to become leaky. This model may be used for pharmacological testing because of its high sensitivity in the absence of β-adrenergic stimulation.

Stressor – Cardiac glycosides and elevated intracellular Na^+ concentration: It was also studied how an increase in SR Ca^{2+} load independent of the activation of β-adrenergic receptors and protein kinase A (PKA) might alter the mutant RyR channel. For this purpose, myocytes from $RyR2^{R4496C+/-}$ and wild-type mice were challenged with ouabain, a cardiac glycoside. Cardiac glycosides are inhibitors of the Na^+/K^+-ATPase. They exert a Na^+- and Ca^{2+}-dependent

positive inotropic effect and are used clinically for the treatment of heart failure. Ouabain increased $[Na^+]_i$, as expected. Diastolic and systolic Ca^{2+}, SR Ca^{2+} load, and action potential duration were also increased by ouabain. These ouabain effects on excitation-contraction coupling were identical in wild-type and mutant myocytes. By contrast, ouabain induced significantly more arrhythmogenic events (Ca^{2+} waves, DADs, extra action potentials) in $RyR2^{R4496C+/-}$ myocytes suggesting an increased sensitivity of the mutant channel to cytoplasmic and/or SR luminal Ca^{2+} in the absence of PKA-dependent phosphorylation. Moreover, JTV-519, a stabilizer of RyR2, was able to dramatically reduce the extra action potentials induced by ouabain in the $RyR2^{R4496C+/-}$ myocytes providing evidence that sealing leaky RyR2 channels might be a therapeutic option for future therapy of CPVT.

Stressor – $CaMKII\delta_C$: CaMKII is a Ca^{2+}/calmodulin-dependent protein kinase involved in the regulation of many cellular processes, including excitation-contraction coupling. Recent evidence indicates that CaMKII also plays a key role in the pathophysiology of hypertrophy, heart failure, and associated arrhythmogenesis. Mice with cardiac-specific overexpression of a CaMKII, $CaMKII\delta_C$, develop hypertrophy and severe heart failure associated with serious defects of SR Ca^{2+} handling. It was hypothesized, therefore, that overexpression of CaMKII in $RyR2^{R4496C+/-}$ mice would exacerbate the defects in SR Ca^{2+} handling and further aggravate the phenotype. $CaMKII\delta_C$ overexpression in mutant $RyR2^{R4496C+/-}$ mice (achieved by crossbreeding) resulted in increased mortality of the $RyR2^{R4496C+/-}$ x $CaMKII\delta_C$ animals. Comparison with $CaMKII\delta_C$ overexpressing mice, however, revealed a similar phenotype both in vivo (heart weight to body weight ratio, cardiac dilation, impaired cardiac function) and in vitro (decreases in SR Ca^{2+} load, fractional shortening, and $[Ca^{2+}]_i$ transient parameters as well as force-frequency behaviour). By contrast, $RyR2^{R4496C+/-}$ x $CaMKII\delta_C$ myocytes exhibited an increased SR Ca^{2+} leak and an increased rate of arrhythmogenic events in the absence of β-adrenergic stimulation, which may explain the increased mortality rate. Thus, CaMKII activation has been identified as an important arrhythmogenic factor that may aggravate arrhythmogenesis in CPVT.

Stressor – Pressure overload: Most recent studies have dealt with the role of defective SR Ca^{2+} release (as occurs in CPVT) for the development of hypertrophy and heart failure. For this purpose, $RyR2^{R4496C+/-}$ and $RyR2^{R4496C+/+}$ mice were challenged with transverse aortic constriction (TAC). TAC results in pressure overload of the heart and, thus, mimics aortic stenosis or a chronic increase in blood pressure, as may occur in many patients. If it remains untreated, pressure overload causes remodelling processes of the heart that culminate into hypertrophy and may further progress into heart failure. It was hypothesized that an additional defect in SR Ca^{2+} handling would further aggravate the development of hypertrophy and heart failure during pressure overload. The first in vivo data from this mouse model indeed suggest that impaired SR Ca^{2+} handling per se may be causally involved in the development and progression of heart failure. In WT and $RyR2^{R4496C+/-}$ mice, TAC induced comparable increases in relative heart weight. However, whereas WT TAC mice exhibited concentric left ventricular hypertrophy with preserved cardiac performance 1 week after TAC, $RyR2^{R4496C+/-}$ TAC mice developed eccentric hypertrophy and significant deterioration of phenotypic changes associated with the transition to heart failure. The heart failure phenotype in the $RyR2^{R4496C+/-}$ mice further aggravated 3 weeks after TAC. Moreover, hypertrophy continued to increase 3 weeks after TAC in $RyR2^{R4496C+/-}$ TAC mice, while it saturated in WT TAC mice. In vivo and in vitro characterization of this mouse model is ongoing and will reveal novel insights into the development and progression of heart failure with particular respect to defective SR Ca^{2+} handling.

In conclusion, the results from these animal studies have provided tremendous and exciting new insights into the mechanisms underlying RyR2-mediated arrhythmias in the presence and absence of additional, physiologically and clinically relevant stimuli and stressors or accompanying cardiovascular diseases. Furthermore, the most recent data also suggest that defective SR Ca^{2+} handling per se, as occurs in CPVT or chronic heart failure, may be causally involved in cardiac remodelling processes that lead to hypertrophy and heart failure.

<u>Objective 5:</u> Characterisation of the mechanisms underlying stress-mediated arrhythmias in human myocardium.

An in vitro arrhythmia model has been established in human heart preparations. It allows the investigation of the cellular and subcellular mechanisms underlying stress-induced arrhythmias in human heart using functional and pharmacological approaches combined with biochemical and molecular biological methods. mRNA expression as well as protein expression and phosphorylation of several proteins involved in Ca^{2+} handling can be measured in individual muscle strips and correlated with the propensity of the very same muscle strips to develop stress-induced arrhythmias. In this model, activation of β-adrenergic receptors by isoproterenol (ISO; used to mimic stress) caused arrhythmogenic events in approx. 50% of the atrial preparations studied. Arrhythmogenic events were entirely dependent on RyR2-mediated SR Ca^{2+} release, reduced by inhibitors of PKA and CaMKII, and associated with increased phosphorylation of RyR2. Increased RyR2 phosphorylation, however, was not sufficient to explain the arrhythmogenic events. Furthermore, the positive inotropic effect of ISO and the expression of major Ca^{2+} handling proteins (including RyR2, SERCA2a, PLB) were not different between muscles with and without ISO-induced arrhythmias. The data suggest that modulation of RyR2-mediated SR Ca^{2+} release is an important contributor to stress-induced arrhythmias in human atrial myocardium, necessary but not sufficient for their induction.

<u>Objective 6:</u> Characterisation of a new mathematical model of cardiac excitation-contraction coupling and the development of delayed afterdepolarisations that takes into account defective RyR2 channel gating in inherited and acquired cardiac disease.

A new mathematical model of cardiac excitation-contraction coupling, delayed afterdepolarisations, and arrhythmias, which incorporates Ca^{2+} diffusion between individual cytosolic (cytosol, subsarcolemmal space, dyadic space) and SR compartments (the junctional and non-junctional SR), has been developed. This in silico approach complemented the in vivo and in vitro approaches of the animal and human arrhythmia models. The mathematical model was directly fed from the results obtained from the CONTICA studies including those from the cellular, animal, and human models and from studies on wild-type and mutant RyR2 in lipid bilayers. The modelling studies were comprised of several parts: 1) model of RyR2 gating; 2) model of Ca^{2+} spark generation; 3) model of Ca^{2+} wave initiation and propagation; 4) model of the cardiac action potential; and 5) model of drug effects. The studies revealed that Mg^{2+} binding/unbinding is an important determinant of RyR2 gating and spark generation. Available RyR2s, i.e. those RyR2s that are kinetically able to unbind Mg^{2+} and open in the course of a Ca^{2+} spark, have 2 Mg^{2+} bound at maximum. Analysis of Ca^{2+} spark data from myocytes isolated from the $RyR2^{R4496C+/-}$ mutant mice revealed that the increase in Ca^{2+} spark frequency could be explained by a 7.5-fold increase in the allosteric coupling between Ca^{2+} binding and channel opening that leads to an increase in the apparent Ca^{2+} sensitivity of the RyR. This could also explain the increased rate of Ca^{2+} waves observed in the mutant myocytes. Furthermore, in a cardiac action potential model, changes of RyR2 gating were simulated. Alterations of the cytoplasmic and luminal Ca^{2+} sensitivity (among other parameters) of RyR2, adrenergic stimulation, and stimulation frequency altered the number of spontaneous action potentials.

Effects of drugs altering RyR2 gating were simulated in the model of Ca^{2+} spark generation and in the model of the cardiac action potential. Important molecular insights into the alterations of RyR2 gating parameters (e.g. elicited by mutations), how these affect the generation of spontaneous action potentials, and how this could be influenced by drugs were derived from these studies.

<u>Objective 7:</u> Test novel diagnostic techniques for better risk assessment.

<u>and Objective 8:</u> Test existing and novel therapeutic compounds directed at normalising ion channel function to prevent triggered arrhythmias.

Beat-to-beat variability of repolarization (BVR), an established prognostic marker in an animal arrhythmia model (i.e. the chronic AV block dog model), was tested for its diagnostic and prognostic value in the mouse model of CPVT with the human RyR2 R4497C mutation. ECG analysis and transmembrane action potentials were recorded from $RyR2^{R4496C+/-}$ mice, and action potentials from isolated cardiac myocytes. No changes of BVR were observed under baseline conditions and following adrenergic stimulation, suggesting that BVR may not be suited as a diagnostic and/or prognostic marker in this mouse model. In patients with congenital long QT syndrome, on the other hand, the beat-to-beat variability of the QT interval was found to be increased when compared to age- and gender-matched controls suggesting that BVR may be a suitable diagnostic tool in humans but not mice.

Existing (flunarizine, verapamil, ranolazine) and novel (JTV-519) drugs targeting RyR2 and other cardiac ion channels were tested for the anti-arrhythmic efficacy in the chronic AV block model. Dofetilide-induced Torsade de pointes arrhythmias (TdPs) and BVR, which preceded the TdPs, were effectively suppressed by flunarizine (a Ca^{2+} current and late Na^{+} current blocker) and verapamil (a Ca^{2+} antagonist). Ranolazine (a late Na^{+} current blocker) also reduced the number of TdPs and partially reversed the increase in BVR. On the other hand, JTV-519 (a stabilizer of RyR2) was unable to suppress the TdPs but rather exerted pro-arrhythmic effects at higher doses. Experiments on isolated cardiac myocytes from the chronic AV block dogs confirmed these findings. Collectively, these data suggest that, in the chronic AV block model, Na^{+} and Ca^{2+} current blocking drugs possess anti-arrhythmic efficacy, whereas JTV-519, which is supposed to stabilize leaky RyR2, does not.

Additional studies on the anti-arrhythmic efficacy of JTV-519 in other arrhythmia models within CONTICA have yielded variable results. While in vivo in $RyR2^{R4496C+/-}$ mice, JTV-519 was unable to prevent ISO/caffeine-induced arrhythmias, it exerted clear anti-arrhythmic effects in isolated myocytes from these mice. In the human in vitro arrhythmia model, JTV-519 exhibited a trend towards less ISO-induced arrhythmogenic events, but this was not statistically significant. Further studies are required to evaluate whether or not, or under which conditions, JTV-519 (or other RyR2 stabilizing drugs) may be anti-arrhythmic. Despite these conflicting results on the anti-arrhythmic actions of JTV-519, however, CONTICA has provided further arguments for the concept of sealing leaky RyR2 channels to prevent life-threatening, Ca^{2+}-mediated arrhythmias.

<u>Objective 9:</u> Improve clinically relevant awareness and risk stratification.

Public awareness of RyR2-mediated arrhythmias has been improved greatly both in the medical as well as the general community. Several newspaper and internet reports on the CONTICA topic (in Austria, Germany, Italy, Slovakia) as well as TV appearances of CONTICA members and CONTICA videos available on the internet (http://www.youris.com/Health/HEALTH-TV/The_Secret_of_Sudden_Death.kl), have greatly increased awareness of sudden cardiac death and its causes. For the medical and scientific community, an entire symposium organized by the coordinator of CONTICA, Prof. Pieske, at the annual meeting of the German Society for Cardiology (DGK) in 2008 dealt with CONTICA. Furthermore, the CONTICA partners have attended numerous national and international conferences, have presented posters and oral presentations, and have, thereby, greatly increased the awareness on the project and its aims in the scientific and medical community.

1.4 Impact of CONTICA on its research field

The CONTICA partners are among the world-leading laboratories in CPVT genetics and genotype-phenotype correlations, mouse models of CPVT, construction of RyR2 plasmids and expression in heterologous systems, lipid bilayer studies of single RyR2 channels, mathematical modelling, animal models of arrhythmias, assessment of arrhythmogenic mechanisms in human myocardium, heart failure research, and studies with patients. Thus, they combine the expertise from bench to bedside providing a true and unique translational approach to the topic of arrhythmias and sudden cardiac death.

CONTICA has been exerting a strong and lasting impact on its research field. With the world's largest database with CPVT patients, CONTICA partner FSM is the leading cardiology laboratory in its field. The CONTICA partners have generated the first mouse model of CPVT and have elucidated the molecular and cellular mechanisms underlying CPVT in unprecedented detail. Furthermore, by combining the CPVT mouse model with physiological and pathological stimuli, CONTICA has been gaining novel insights also into the development of hypertrophy and heart failure and arrhythmogenesis in these cardiac diseases. Finally, the CPVT mouse model of CONTICA has been serving as a unique in vivo and in vitro model for testing of anti-arrhythmic compounds targeted against CPVT.

The impact of CONTICA can also be appreciated from its publications. CONTICA has already generated several publications in leading journals of cardiology, physiology, biophysics, and biochemistry. Many more manuscripts by the CONTICA partners have been submitted for publication or are in preparation. It is expected, therefore, that the scientific impact of CONTICA will further increase. CONTICA has also exerted an enormous impact on the general population by increasing the awareness for sudden cardiac death and its causes through press conferences, newspaper, internet, and TV reports reaching several million people in the EU.

2. Dissemination and use

The EC-funded project CONTICA has investigated the calcium-dependent mechanisms underlying inherited and acquired forms of heart rhythm disturbances (i.e. arrhythmias) linked to stress-induced sudden cardiac death, one of the major health burdens in Europe and worldwide. In vivo, in vitro, and in silico studies were performed on patients with inherited and acquired arrhythmias, on isolated human heart tissue, on novel and unique animal models, on cell models, on isolated proteins, and by means of novel computer models using state-of-the-art research techniques. The results have revealed, with unprecedented detail, important and exciting new insights into the molecular, cellular, and in vivo mechanisms underlying calcium-mediated fatal arrhythmias. Novel genetic abnormalities, i.e. mutations and polymorphisms, causing life-threatening arrhythmias were discovered and characterised, novel methods for diagnosis of arrhythmias were developed and verified, and existing and novel anti-arrhythmic drugs were evaluated for their potential to prevent and treat these fatal arrhythmias.

The CONTICA investigators have presented their findings and achievements on numerous national and international scientific meetings and have published the major results of the project in leading basic science and cardiology journals thereby disseminating the scientific knowledge gained from the project to the scientific and medical community worldwide. Furthermore, press conferences, coverage in newspapers and on the internet, and TV reports on the CONTICA project with an estimated overall audience of > 5 million people have fostered the awareness on the topic of sudden cardiac death and its major causes also in the general population within the EU. A useful, informative, and vivid video introduction to the project and its major aims, entitled "The secret of sudden death", can be found on the internet at http://www.youris.com/Health/HEALTH-TV/The_Secret_of_Sudden_Death.kl.

In summary, the project CONTICA has improved the awareness on sudden cardiac death and its underlying causes in the general population and in the scientific and medical community within the EU and worldwide. It has yielded unprecedented novel insights into the molecular mechanisms causing fatal arrhythmias and provided a major contribution to improved diagnosis and therapy of stress-induced cardiac arrhythmias that may cause sudden cardiac death.