## Project context and main objectives

Cardiovascular diseases are the leading cause of death in the western world. In the second half of the 20th century researchers have recognised the major risk factors that contribute to heart attacks, heart failure, stroke and other cardiovascular diseases. Among these risk factors are hypertension (high blood pressure), diabetes, smoking, hypercholesterolaemia (high cholesterol levels) and others. In clinical practice these factors can be used to estimate a patient's risk for experiencing cardiovascular events. However, precise assessment of current cardiovascular health and precise prediction of cardiovascular risk are not possible.

The aim of EU-MASCARA is to find biomarkers of cardiovascular risk that could help to better assess cardiovascular risk than the currently used risk factors. EU-MASCARA focuses on a range of complex and novel markers that have been shown to provide a comprehensive view of a patient's cardiovascular status. By detecting very early stages of cardiovascular diseases that are not yet causing symptoms these markers could therefore help to assess a patient's risk for developing symptomatic disease. Many of the complex biomarkers in EU-MASCARA derive from recently developed approaches that characterise a large number of molecules in blood and urine samples. Among these are metabolomics (the study of small molecules that derive from the biochemical processes in the body) and proteomics (the study of all proteins in a given sample). EU-MASCARA also looks at other markers such as genetic markers and factors related to cardiac structure and function and inflammation.

The consortium benefits from its partners' expertise with these novel biomarkers. Hence there is no need to develop new biomarkers, and the consortium can quickly move to clinical studies. The first phase of the project was characterised by three main aims.

1. The consortium aimed to build up a data analysis platform to bring together different complex biomarker sets from partner laboratories for integrative analysis. For this purpose a set of approximately 350 blood and urine samples from patients with and without hypertension has been analysed in all partner laboratories. The clinical characteristics are being uniformly assessed and brought together with the biomarker data. This project will define the most promising biomarkers that can be used for further validation in and risk prediction in even larger cohorts.

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2. The consortium aimed to analyse a set of metabolomic, proteomic and cardiac structure and function markers in another cohort with approximately 800 people who were prospectively followed up for changes in cardiovascular parameters and development of symptomatic cardiovascular disease. This experiment provides important large-scale cross-sectional data and a first attempt to translate the novel biomarkers into prospective follow-up and risk prediction.
3. All partners aimed to continue validating specific biomarkers within their specific expertise in population and patient cohorts. These data will inform the prospective follow-up studies that will be performed in the second phase of EU-MASCARA.

## Work performed since the beginning of the project and the main results achieved so far

Italian partners **Istituto Auxologico Italiano** and **Universita' degli Studi di Milano-Bicocca** lead the standardisation of clinical data for analysis across the whole consortium. They have continued collecting patients for biomarker validation studies and read echocardiograms of patients for the consortium-wide biomarker studies. Dutch partner **Universiteit Maastricht** focussed their clinical research activities on patients with heart failure, a common endpoint of a range of cardiovascular diseases.

Belgian partner **KU Leuven** contributed the FLEMENGHO cohort to the consortium. This population-based cohort is characterised by standardised high-fidelity data on cardiovascular health and has been used for the large-scale studies into proteomics, metabolomics and cardiac function/structure.

Partners **FIHCUV (Valencia)** and **FIMA (Pamplona)** in Spain continued recruiting patients with hypertension, heart and kidney diseases but also serve the consortium as core laboratories for metabolomics and markers of cardiac function and structure, respectively. Partner **Medizinische Hochschule Hannover** in Germany complements this work with studies into the prediction of kidney failure. Renal disease is one of the most important risk factors for cardiovascular disease. Austrian partner **Medizinische Universität Graz** continues characterising patients with heart failure and assesses hormone markers involved in the development of cardiovascular diseases.

**Mosaiques Diagnostics GmbH** in Germany performed proteomic analysis in urine from large datasets including the FLEMENGHO cohort (Figure 1). Partner **Charité**, also in Germany, worked on proteomics in serum and plasma and characterised factors that could not only provide a clinical risk marker but also a novel mechanism to explain the development of hypertension. **Randox Testing Services** in the United Kingdom has analysed markers of inflammation using their unique technology that uses very small sample volumes. Dutch partner **ACS Biomarker BV** studied microRNAs; these small

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molecules interfere with the way the genetic information is read and translated into protein.

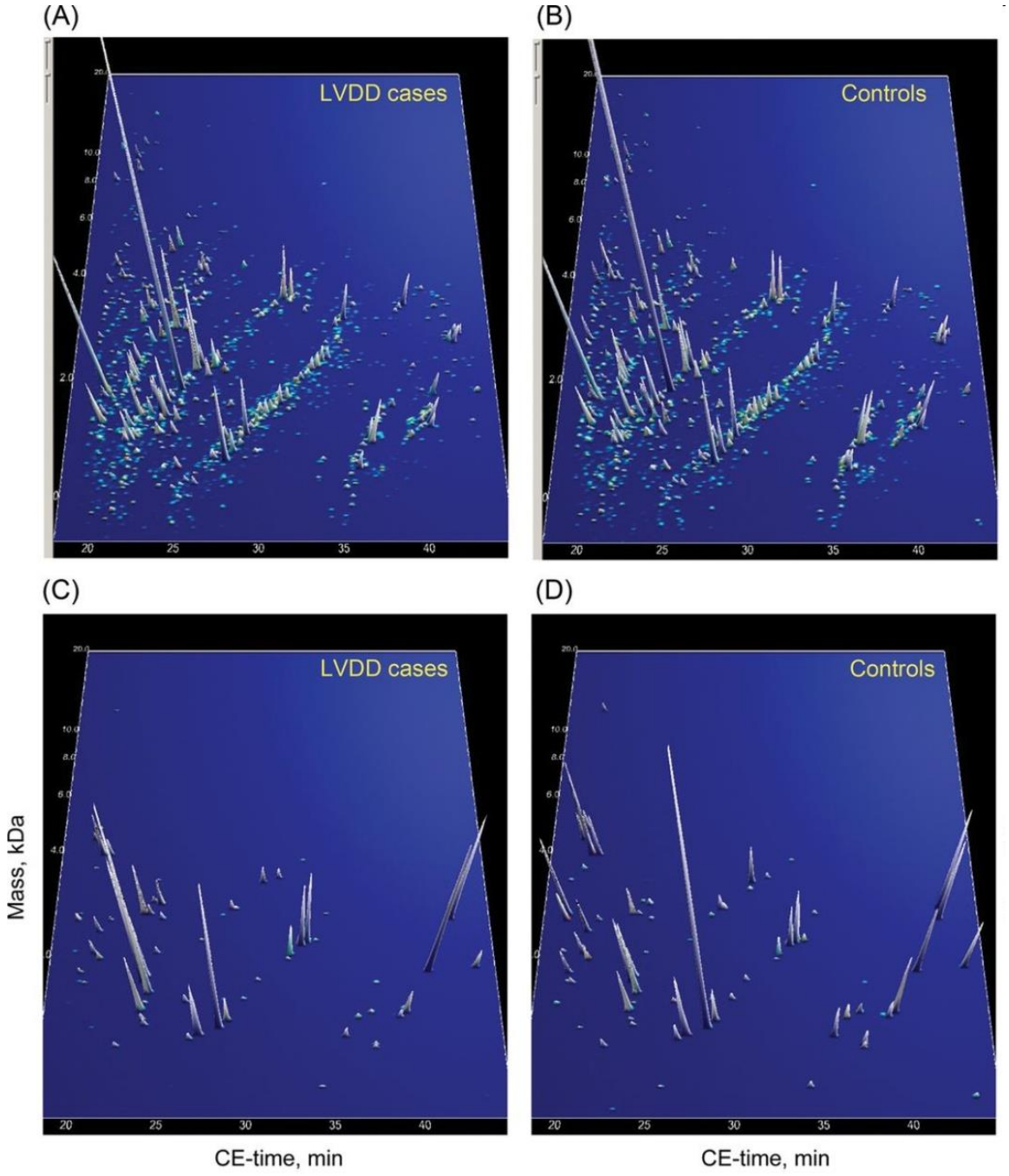


**Figure 1.** Compiled urinary peptide patterns from individuals with normal heart function (Controls, panel B) and patients with impaired heart function (left ventricular diastolic dysfunction, LVDD;

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panel A). The different peptides between patients and controls are shown in panels C and D. From Kuznetsova T et al. Eur Heart J 2012.

The project is coordinated by the **University of Glasgow** in the United Kingdom who brings a large population cohort of more than 20,000 individuals ("Generation Scotland") into the consortium that will be a crucial part of prospective biomarker studies. In this cohort traditional risk factors have already been measured, and analysis of genetic data is currently being finalised. Health economic analysis will also be coordinated in Glasgow.

The task of data integration and analysis is facilitated by Austrian partner **Emergentec Biodevelopment GmbH** who brings together data from the already available scientific literature, standardises the complex datasets that derive from EU-MASCARA and studies the interaction between different molecules in the form of networks and pathways (Figure 2).

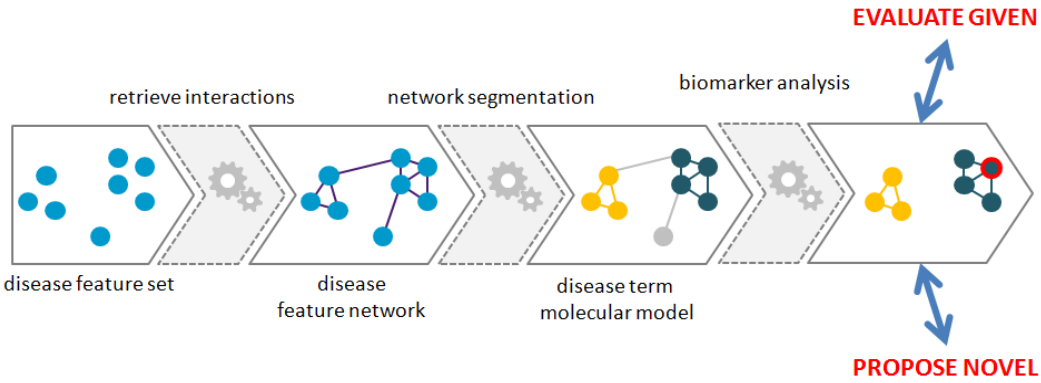


**Figure 2.** Data analysis strategy in EU-MASCARA focussing on interactions between molecules to describe specific features of cardiovascular diseases.

The project management is supported by **Kite Innovation Europe** who coordinate reporting, dissemination and financial aspects of EU-MASCARA and most of the communication with the European Commission.

The consortium met for a kick-off meeting in Glasgow in February 2012 and for a consortium meeting in Vienna in October 2012. There were a large number of bilateral meetings to discuss specific aspects of the project as well as meetings of several partners at scientific congresses and local conferences. Until May 2013 the consortium has already published 49 scientific papers in leading cardiovascular journals.

## Description of the expected final results and their potential impacts and use

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The key objectives of EU-MASCARA are:

**1. To validate the association of emerging biomarkers with cardiovascular phenotypes in cross-sectional disease and population cohorts.** Work towards this objective is on track. The project will lead to a better understanding of the relationship between biomarkers and early stages of cardiovascular diseases and will define biomarkers that provide the best information about current cardiovascular health.

**2. To validate emerging biomarkers as predictors of changes in cardiovascular phenotypes and cardiovascular events in prospective disease and population cohorts.** This objective will be the focus of the next phase of the project. The consortium will generate data on biomarkers that can predict the development of clinically relevant cardiovascular diseases such as heart failure and stroke. EU-MASCARA will deliver data on the predictive value of novel and complex biomarkers but will also see them in the context of the health economic reality.

**3. Integration of emerging biomarkers reflecting different aspects of pathophysiology with established biomarkers into a common predictive model.** This objective is an extension of the second objective of EU-MASCARA and will focus on the development of novel data analysis methods that bring complex data together to form a clinically useful and applicable model to predict development of cardiovascular diseases. The consortium has already developed some of the pipelines for integrative data analysis and is ready to apply them to data that are being generated within EU-MASCARA.

**The key aims for the next phase of EU-MASCARA therefore are:**

- Comprehensive and integrative analysis of data that have been generated in the first phase of the project.
- Based on these data the consortium will decide on biomarkers that will be translated into large prospective studies.
- Assessment of the additional value of novel markers to predict cardiovascular risk compared with the traditional risk factors.
- Health economic analysis to decide how expensive new technology can best be introduced into clinical practice.

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