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Final Report

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Final Report

PROJECT FINAL REPORT

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Final Report

Please note that the contents of the Final Report can be found in the attachment.

4.1 Final publishable summary report

Executive Summary

Biomarkers are considered as tools to enhance cardiovascular risk estimation. However, the value of biomarkers on risk estimation beyond European risk scores, their comparative impact among different European regions and their role in the drive towards personalised medicine remains uncertain. Based on harmonised and standardised European population cohorts we have built significant research collaborations, expertises and an infrastructure in the EU. We applied highly innovative SME-driven technologies and performed large-scale biomarker determination to assess the predictive value of existing and emerging biomarkers.

Selection of emerging biomarkers was based on quantitative proteomic, transcriptomic, metabolomic, and miRNomic datasets established by private and public consortium members. Existing biomarkers were selected based on non-redundancy and their association with cardiovascular risk and phenotypes. SME-guided development of innovative assay systems biomarkers was tested and validated in a stepwise fashion among European populations in primary and secondary prevention. In addition to their impact on risk prediction, their association with lifestyle determinants and cardiovascular phenotypes assessed by ultrasound and MRI technique was and will be evaluated.

We discovered a (preliminary) BiomarCaRE panel of ten established biomarkers:
o Apo A1
o Apo B
o LP(a)
o CRP
o Cystatin C
o NTproBNP
o hsTnI
o Insulin
o Glucose
o Vitamin D
that improve the prediction of cardiovascular diseases in the primary prevention setting when used in addition to the classic risk factors. Additional model development and the addition of more biomarker measurements – that is continued after the end of the project – are expected to further refine the panel.

Summary description of project context and objectives

Cardiovascular risk assessment based on classical risk factors only partially explains the distribution of risk in the general population, and provides variable results across European regions (Kuulasmaa et al., Lancet, 2000; Conroy et al., Eur Heart J, 2003). Novel biomarkers have been associated with cardiovascular risk. However, the improvement of risk estimation needs to be assessed across European regions. Biomarker discovery and validation studies in the field of coronary artery disease have not led to the widespread application of new diagnostic technologies in primary prevention or in a clinical setting so far.

In the BiomarCaRE consortium, private and public partners cooperated to combine (1) innovation in biomarker discovery for cardiovascular disease prediction with (2) a validation of biomarker effectiveness in a large collaboration of well-defined European cohorts in population and diseased cohorts, based on a long-standing history of epidemiological data harmonization. The BiomarCaRE consortium measured success in terms of technology development and subsequent clinical utility. An academic participant coordinated the consortium but the focus of the research plan was constructed around the emerging biomarkers and innovative technology derived from SMEs.

The primary objective of the BiomarCaRE consortium was to establish the value of existing and emerging biomarkers potentially leading to an integrated panel for cardiovascular disease prediction. Innovative assays were developed and tested and could subsequently be commercialised in a clinical setting or as a point-of-care test. To achieve the objectives, the consortium performed a multilevel, integrated, continuous work flow by selection of biomarkers, assay development and assay selection , measurement of biomarkers and statistical analyses.

Description of main S & T results/foregrounds

WP 1 Selection of existing and emerging biomarkers related to cardiovascular disease

Existing biomarkers were selected at the beginning of the project on the basis of the following criteria:
* availability of the assay (dependent on the negotiations with the assay manufacturers),
* relevance (explanatory power – predictive, diagnostic, therapeutic capacities),
* measurement (reproducibility, standardization, measurable in blood derived samples),
* assay (costs, high throughput, sample volume needed),
* IPR (situation clarified).

Priority list of existing markers (Rank, Short Name (Long Name), Assay Manufacturer):
1. NT-proBNP (N-terminal pro-B-type natriuretic peptide), UMC
2. BNP (B-type natriuretic peptide), Abbott
3. hsTnI (high sensitive troponin I), Abbott
4. (hs)CRP (C-reactive protein), Abbott
5. LDL-cholesterol (low-density lipoprotein cholesterol), Abbott
6. HDL-cholesterol (high-density lipoprotein cholesterol), Abbott
7. Trigylceride, Abbott
8. Creatinine, Abbott
9. CysC; cystatinC, Abbott
10. Galectin-3, Abbott
11. GDF-15 (growth-differentiation factor 15), MHH
12. Glucose, Abbott
13. Lp(a) (Lipoprotein a), Abbott
14. apoA1 (apolipoprotein-A1), Abbott
15. apoB100 (apolipoprotein-B100), Abbott
16. 25(OH)D (serum 25 hydroxy vitamin D), Abbott
17. Insulin, Abbott
18. C-peptide (insulin pro-peptide), Abbott
19. Testosterone, Abbott

Emerging markers, further discussed in workpackages 2 to 5, see below:
* 4-plex assay by Cavadis and Future Diagnostics; see WP 2
o SerpG1 (exosome), serine protease inhibitor G1
o CysC (exosome), cystatinC
o CD14 (exosome), cluster of differentiation 14
o SerpF2 (exosome), serpin protease inhibitor F2
* Initial candidates for immunoassay by Fleet, extended during the project, see WP3
o ABCA1, ATP-binding cassette transporter protein A1
o ABCG1 ATP-binding cassette transporter protein G1
o GPCR15, G-protein-coupled receptor 15
o SerpF2, serpin protease inhibitor F2
* p180 Kit AbsoluteIDQ p180 Kit by Biocrates, see WP 4
o 186 metabolites
* micro RNA candidates, by UKE, see WP5 and 6
o miR-1
o miR-133 a
o miR-208 a
o miR-208 b
o miR-21

o miR-212/132
o miR-423-5p
o miR-499

WP 2 Assay development: exosome

The objective of Cavadis (partner 3) was to develop and deliver two performing immunoassays for use in the clinical validation of its proprietary biomarkers in the BiomarCaRE diseased cohort studies and the case-cohort setting. The first assay being developed is the 4-plex which is specifically designed to measure the expression levels of exosomal CD14, Cystatin C, SerpinF2 and SerpinG1 on the Luminex platform. The development work has started at sub-contractor Future Diagnostics' (FDx) R&D site in November 2011 and was completed in May 2013 with the production of 150 ready-to-use 4-plex Luminex assays that were used at the Universitätsklinikum Hamburg-Eppendorf (UKE, partner 1).

Together with partner Fleet Bioprocessing (Fleet; partner 10), Cavadis has developed the second immunoassay in ELISA format to measure expression levels of SerpinF2 in the case-cohort setting. See work packages 3 and 6 for details.

Assay development of the 4-plex assay

A first research phase produced the following conclusions:

* The bulk antibodies and antigens purchased at R&D Systems showed comparable results with the catalogue products (reference) and were used for the development of the IVD 4-plex assay.
* HPE assay buffer from Sanquin was used since mixed results were obtained with the in-house assay buffers produced at FDx.
* The Bio-Plex wash buffer from Bio-Rad was replaced with STAT wash buffer produced at FDx.
* The commercial ExoQuick buffer from SBI was replaced by the in-house buffer developed by Cavadis and produced at FDx.
* The Corning filters used in sample preparation was replaced by Pall fillers that needed less handling.
* A good correlation between the 4-plex assays performed at FDx vs Cavadis was obtained.

A second, optimization phase gave those results

* Sample type: frozen plasma and serum. All samples must be first frozen prior to exosome isolation. This decision was made since the predictive value of the 4-plex obtained in previous verification studies carried out by Cavadis used frozen samples.
* 4-plex assay components:
o Detection antibodies mixture: 0.1 mg/ml of mouse IgG must be added into the detection antibodies mixture to decrease the high blank signals in the assay.
o Standard: Lyophilized standard 1 has to be reconstituted in Roche lysis buffer and 1:1 diluted in the reconstituted standard matrix to generate the standard curves.
o Control: Lyophilized EDTA-plasma will be used as the control in the assay. This control has to be reconstituted in the standard matrix prior to use.
o Assay buffer: HPE stock buffer (5x) can be stored up to 1 year at -18 to -32°C at the customer site.
o Streptavidin-PE: A 1:110 dilution in the HPE buffer (1x) will be used in the 4-plex assay.
* 4-plex assay performance:
o CD14: total precision <15%, LoQ 172 pg/ml, linear range 59-28937 pg/ml
o Cystatin C: total precision ~25%, LoQ 2243 pg/ml, linear range 1317-39153 pg/ml
o SerpinF2: total precision <15%, LoQ 232 pg/ml, linear range 184-154389 pg/ml
o SerpinG1: total precision <15%, LoQ 5951 pg/ml, linear range 1528-247989 pg/ml

* 4-plex assay stability: Lyophilized standards 1, lyophilized controls, 10x beads mixture and lyophilized detection antibodies mixture were stable at 25?C for 6 weeks. In principle, these materials will also be stable at 2-8?C for 24 weeks (6 months).

All components to build the kits such as antibody-coated beads, assay standards and controls were manufactured according to the specifications obtained in the previous optimization phase. Verification procedures involving repeated tests to confirm that the 4-plex assay fulfilled the specified requirements set earlier in Design Input Document (DID) were carried out at FDx.

A “fresh” standard curve was prepared in the same way as the primary standard 1 but without the lyophilization process. For each marker, a good overlay of primary standard 1 (lyophilized) standard curve vs freshly prepared standard curve was obtained. Lyophilized secondary standards that were included in the kit and used by the customers were produced. Stability tests showed no significant loss in performance and acceptable stability for all materials even after 6 weeks storage at 37°C.

The precision for CD14 and SerpinF2 was comparable to the ones obtained in the optimization phase whereas a better performance was observed for Cystatin C. For SerpinG1, higher values for total and inter assay precision were found. This observation was caused by higher variations of measured SerpinG1 in samples between each run. However, the results were within the acceptable limits to discriminate samples with low or high levels of SerpinG1.

Determination of Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantification (LoQ) for all markers were next examined in the 4-plex assay as described in SOP-004; EP17-A at FDx. The LoB and LoD for CD14, Cystatin C, SerpinF2 and SerpinG1 were found to be within the minimum acceptable limits. For LoQ; the CVs were somewhat higher than those previously obtained in phase 2b. However, they were acceptable and within the required specifications.

The LoQ for CD14, Cystatin C and SerpinF2 were actually better whereas the same range was found for SerpinG1 in comparison with phase 2b’s results. This observation suggests that the clinical cut-off value that is measured in the samples will be higher than the LoQ for all markers.

No carry over effect was found for all 4 markers. Correlation studies showed Pearson’s correlation of > 0.9 for all 4 markers.

WP 3 Assay development: transcriptome

As part of Deliverable 1.1, a panel of novel biomarkers was defined based on the shared expertise of the BiomarCaRE project team, each marker possessing potential interest with respect to cardiovascular risk. This panel formed the subject of Workpackage 3, the aim for each biomarker being:

* To develop a colorimetric Enzyme-Linked Immunosorbent Assay (ELISA) for the biomarker (Task 3.1)
* To manufacture ELISA reagents for shipping to the relevant BiomarCaRE partner to support case cohort testing (Task 3.2)
* To transfer the developed ELISA to the relevant BiomarCaRE partner for case cohort testing, and to provide technical support for the use of the ELISA throughout the case cohort testing program (Task 3.3)

The panel initially agreed (July 2012) for development within Workpackage 3 comprised ELISAs for the following four biomarkers:
* Serpin F2, aka ?2-antiplasmin or plasmin inhibitor
* ATP-binding cassette transporter protein A1 (ABCA1)
* ATP-binding cassette transporter protein G1 (ABCG1)
* G-protein coupled receptor 15 (GPR15, aka GPCR15)
The appropriateness of this panel was reviewed continuously throughout the project, in response both to internal project results and new external learning. As a consequence of this review process, work on ABCA1 and ABCG1 was discontinued in June 2014, and two replacement candidates were

agreed for attention (Lamin A/C, MYADM). Two further candidates (titin and MST-4) were also provisionally considered but not taken forward, as it was not considered feasible to complete relevant activities within the remaining project timescale.

Therefore the remainder of this report provides a brief summary of the activities completed within Workpackage 3 of the BiomarCaRE project for the six biomarkers highlighted in red above.

Serpin F2
The serpin F2 ELISA was adapted from an existing fluorescent multiplex immunoassay developed by CAVADIS in collaboration with Future Diagnostics, the format of which was unsuitable for the BiomarCaRE application. Commercial antibodies from R&D Systems were specified for use by CAVADIS, but these had to be converted to diagnostic-grade equivalents for long-term use. Using these antibodies an effective ELISA format was quickly established and its performance verified, and the final assay format was documented as part of Deliverable 3.1.

Sufficient batches of all the required assay reagents were manufactured by FLEET to support the planned case cohort testing, and a technology transfer programme was executed to transfer the relevant procedure and knowhow to UKE, the partner responsible for the test programme. As is typical for such a programme a number of issues were identified during this exercise, including equipment compatibility, software settings, optimisation of sample dilution factors, and instability of frozen serpin F2 standards. All these issues were successfully addressed, allowing the case cohort test study to be completed satisfactorily. Unfortunately these studies concluded that serpin F2 offered no significant advantage over existing biomarkers in terms of cardiovascular risk prediction.

ABCA1
A range of commercially-available antibodies and partial peptide antigens were evaluated, leading to the establishment of a prototype ELISA using an immobilised partial peptide, and a rabbit polyclonal antibody (the same peptide/antibody combination had been used apparently successfully in Western blot studies reported in the literature). This assay gave a good dose-response to the partial peptide antigen. As there was no commercial source of full-length ABCA antigen, it was not possible to verify a dose response to full-length protein, therefore a number of clinical samples were obtained by Fleet and/or provided by UMCU and tested in the assay. The results obtained were seriously discrepant from the ABCA1 level expected from the relevant clinical status, and on the basis of these results (June 2014), the project team decided to discontinue any further ABCA1 activities.

ABCG1
A range of commercially-available antibodies and recombinant full-length human antigen were evaluated in prototype ELISA formats. None of the combinations tested yielded a convincing dose response to full-length antigen. On the basis of these results (June 2014), the project team decided to discontinue any further ABCG1 activities.

GPR15
A range of commercially-available antibodies and recombinant full-length human antigen were initially evaluated in prototype ELISA formats. None of the combinations tested yielded a convincing dose response to full-length antigen. On the basis of these results (October 2013), the project team decided to discontinue any further GPR15 activities pending the possible availability of a new antigen supply from UKE. In parallel an external supplier (Generon) was contracted to generate a partial peptide GPR15 antigen and to raise 2 x rabbit polyclonal and 2 x mouse monoclonal antibodies.

Once these new reagents became available (January – March 2015) a good dose response for the first batch of UKE GPR15 antigen lysate was obtained by combining Generon and commercial (Abcam) rabbit polyclonal antibodies. However two subsequent batches of UKE GPR15 antigen lysate failed to respond in the assay, and an acceptable response was only recovered using a fourth antigen lysate batch. To allow development to proceed in the meantime, a commercial (Abnova) full-length recombinant GPR15 was introduced for use. This material behaved well, showing a similar dose response to the UKE material, and was therefore incorporated into the final assay design. Despite further delays due to batch-to-batch differences in the UKE antigen, further optimisation of this assay format yielded excellent performance and it was employed to test a range of clinical samples

provided by UKE. The results were in line with clinical expectations, confirming the success of this development. The final assay format was documented as part of Deliverable 3.1.

Lamin A/C
An external supplier (Generon) was contracted to generate a partial recombinant Lamin A/C and to use this to raise 2 x rabbit anti-Lamin A/C polyclonal antibodies. Dot blot analysis of these antibodies vs. the partial recombinant Lamin A/C antigen confirmed that both successfully bound this material. ELISA designs were subsequently evaluated based on displacement of antibody from bound antigen, either by free partial recombinant antigen or by full-length recombinant antigen. Effective displacement of free partial antigen was successfully observed, but not full-length recombinant antigen. It was therefore concluded that these antibodies were not suitable for this application.

MYADM
An external supplier (Generon) was contracted to generate a partial recombinant MYADM antigen and to use this to raise 2 x rabbit polyclonal and 2 x mouse monoclonal antibodies. Dot blot and Western blot analysis of these antibodies vs. the partial recombinant MYADM antigen confirmed that all four successfully bound this material. ELISA designs were subsequently evaluated based on displacement of antibody from bound antigen, either by free partial recombinant antigen or by full-length recombinant antigen. Effective displacement was not observed in either case, and it was therefore concluded that these antibodies were not suitable for this application.

WP 4 Assay development: metabolomics

Biocrates has refined its AbsoluteIDQ® p180 Kit approach to address the specific requirements of the BiomarCaRE project. By the AbsoluteIDQ® p180 Kits use sample measurements can be done in time and according to the Biocrates' quality standards. In more detail, the following adaptations took place:
- Kit adaptation for use by Waters mass spectrometer (MS): the AbsoluteIDQ® p180 Kit has been designed to be used with SCIEX 4000 series triple quadrupole mass spectrometer. Initially it was planned to use a Waters MS at the UKE to perform the BiomarCaRE metabolomics measurements. As a result the AbsoluteIDQ® p180 Kit needed to be adapted for this mass spectrometer. The adaptation includes tuning of the analytes and internal standards (for determination of MS parameters), setting up and checking the full mass spectrometer methods for all injections necessary for a full AbsoluteIDQ® p180 Kit analysis, optimization of several mass spectrometer parameters (e.g. source temperature, gas flow, dwell times) as well as the optimization of LC parameters (e.g. flow, column type, column dimension, injection column, sample dilution, gradient, solvents).
- Reduction of measurement time: Due to the large number of analytes analyzed by the AbsoluteIDQ® p180 Kit, the measurement time was reduced. As the UKE was going to analyze 250 AbsoluteIDQ® p180 Kits in a restricted time period the reduction of measurement time was important. A new UHPLC method, instead of the HPLC method which was used before for the AbsoluteIDQ® p180 Kit, was developed in order to minimize the measurement time per Kit. The development of the UHPLC method started with different UHPLC columns from which the best working one was decided for validation of the method. Furthermore, the FIA method run-time was reduced. As a result, the total analysis time per Kit has been reduced approximately by 50%.
- Kit adaptation for use by the Thermo TSQ Vantage: initially Biocrates has adapted its AbsoluteIDQ® p180 Kit for use by Waters MS. As the UKE finally acquired a Thermo TSQ Vantage, Biocrates adapted the AbsoluteIDQ® p180 Kit for the use with a Thermo TSQ Vantage. Mass transitions for analytes and internal standards were used from the previous adaptation work for the AbsoluteIDQ® p180 Kit use by a Waters MS, but the MS parameters had to be adapted according to the specific demands of the Thermo TSQ Vantage Ion source (HESI II). Adaptation work included setting up and checking the full mass spectrometer methods for all injections necessary for a full AbsoluteIDQ® p180 Kit analysis and the optimization of LC parameters (e.g. flow, column type, column dimension, injection volume, sample dilution, gradient). The validated protocol for the AbsoluteIDQ® p180 Kit on a Thermo TSQ Vantage was used for all measurements performed by the UKE.

Integral part of the AbsoluteIDQ® p180 Kit is Biocrates' proprietary software MetIDQ. The MetIDQ

software was optimized for the specific needs within the BiomarCaRE project. In more detail, the following adaptations took place:
- Biocrates developed an advanced proprietary software solution in the previous years and it is being used to manage the entire analysis workflow, while enhancing the standardization and harmonization of project data processing. Taking into consideration the samples size to be measured within the BiomarCaRE project, Biocrates works on improving already existing features (e.g. MetIDQ Software adaptation for data normalization, QC sample distribution algorithm in worklist generation) and add new features (e.g. data visualization feature in MetSTAT/Display Data). These improvements were released in the beginning of the year 2015 and allow a highly automated data analysis and reporting and therefore a more efficient workflow for the UKE.

Part of the AbsoluteIDQ® p180 Kit is Biocrates' on-site and online based customer support. To get started with the AbsoluteIDQ® p180 Kit the customer support defines the required instrumental and laboratory equipment and gives a detailed on-site training. In order to enable a smooth and constant AbsoluteIDQ® p180 Kit analysis over the metabolomics data analysis within the BiomarCaRE project (20 000 samples), Biocrates' customer support was improved using feedback from this project. In addition various customer documents were developed or improved. In more detail, the following adaptations took place:
- During the initiation period, Biocrates defined all necessary requirements (laboratory equipment, hardware and software), which are needed for smooth AbsoluteIDQ® p180 Kit analytics. Biocrates planned the whole analytical process, including providing advice on plate design and system suitability test on a regular basis, for sample measurements in a standardized and quality controlled way. This is essential for result data normalization within the cohorts. The most robust data normalization procedure was investigated based on different quality control levels. The UKE was advised to determine the most suitable quality control level by statistical variance comparison before and after cohort data normalization. Furthermore, Biocrates offered, in collaboration with Thermo Fisher Scientific, metabolomics training including detailed LC-MS/MS training to implement Kit measurements at the UKE. Previously project partner 1 (UKE) had no hands-on experience neither with LC-MS/MS technique nor with metabolomics. Ever since the UKE moved from the initiation phase to performing routine measurements, Biocrates has been providing support concerning technical and applied analytical issues, statistical data analysis and software issues as they occurred.

Beside the above mentioned AbsoluteIDQ® p180 Kit adaptions and support all components, methods, the MetIDQ software, the Kit performance and the use of the AbsoluteIDQ® p180 Kit are described in the Kit documents, e.g. the Kit user manual or the analytical specifications.

Finally, 265 AbsoluteIDQ® p180 Kits were produced and provided by Biocrates to the UKE (Partner 1) for use within work package 6. Biocrates refined and adapted its technology to further optimize its quality and performance.

WP 5 Assay development: miRNA

Biocartis' aim was to develop and transfer to the consortium a customized assay to evaluate the potential of microRNAs as biomarkers for cardiovascular disease risk prediction. After demonstrating feasibility of a proof-of-concept assay the second phase of the project was dedicated to the actual assay development. Several actions were pursued to establish a standard protocol for the sample preparation, increase the assay multiplicity, improve the limit of detection and design the algorithm for data processing and quantification. The activities were based on the subpanel of microRNAs including miRNA controls defined by UKE (miR-423-5p, miR-16, miR-39) and agreed by the consortium after the first annual BiomarCaRE meeting in October 2012. The assay development involved the complete workflow. Improvements in the efficiency of the reverse transcription led to increased sensitivity of the assay. The optimization of the multiplex amplification protocol and the definition of dedicated algorithm for quantitative data processing widened the dynamic range to five orders of magnitude for a 7-plex prototype assay. The default data output was defined as the absolute concentration of each target and control microRNA in serum, upon correction for technical assay-to-assay variability. To facilitate the assay transfer, the amplification protocol for a test assay was transferred to UKE and the protocol description was defined by mutual agreement. Compatibility of the eight PCR instruments of UKE laboratories was tested by applying the test

assay. Within the scope of the assay transfer, guidelines for data normalization were transferred to the consortium and randomization strategy for the testing discussed and defined.

Due to the termination of the grant agreement of partner 11 (Biocartis) by the end of the second report period, the envisioned multiplex assay applied on the Evaluation System could not be finalised and used. Instead, including the knowledge gained above, the advanced miRNA assays of Applied Biosystems were used. This assay includes a one-step RT-reaction with a universal primer and fast PCR reaction. For 21 miRNAs an advanced miRNA assay was designed, including miR-21, miR-150-3p, miR-210, miR-223, miR-423-5p, miR-451a, miR-718, miR-1228-3p, miR-1238-3p, miR-1273f, miR-4298, miR-4459, miR-4787-3p, miR-1825, miR-3135, miR-34a, miR-133a, miR-133b, miR-378, miR-499a, miR-16. The potential of these miRNAs to be used in duplex reactions was tested.

WP 6 Measurements of biomarkers in population based and clinical samples

Work package 6 aimed at the measurement of selected emerging and existing biomarkers. The specific objectives were

1. Transfer of biosample from the Cohort Centres to the BiomarCaRE laboratories to store and coordinated biosamples centrally in one large BiomarCaRE biobank.
2. Implementation of a suitable infrastructure for data handling, quality control purposes and data transfer
3. Acquisition of levels of literature-based and emerging biomarkers in the BiomarCaRE laboratories and performance of accurate documentation of sample and biomarker data during the measurements
4. Performance of an extensive quality control of raw biomarker data to ensure analytical validity and formatting of biomarker data in a pre-specified standard format.
5. Transfer of quality-controlled and validated biomarker values to the BiomarCaRE Data Centres.

Sample Transfer: During the 4.5-years period of the BiomarCaRE projekt samples of 26 different cohorts including the population-based cohorts (Phase I and Phase II) as well as disease cohorts and clinical trials were transferred to the central BiomarCaRE laboratory and were stored in the biobank. Samples of the case cohort set of the respective population-based cohort were selected and used for measurement of the specific biomarkers microRNAs and metabolomics.

Infrastructure: The infrastructure of the BiomarCaRE laboratory was updated and new and/or updated instruments were integrated into the laboratory data management system to automatically transfer biomarker data into a central laboratory database.
For measurement of metabolites, a new mass spectrometry instrument (TSQ Vantage) was installed and trained by ThermoFisher at the laboratory at the UKE. Quality checks were made at several levels including assay specific quality samples as well as own quality controls (mixed gender pooled human serum samples, Seralab) to monitor inter-assay variability.
For data handling, quality control purposes and data transfer a quality control system was programmed in order to format results data for transfer to the central data centres. SOPs for data handling and data quality control as well as a harmonized data transfer format for biomarker results were established at the laboratory.

Measurements: The BiomarCaRE laboratory at the UKE measured biomarkers selected from the literature in Module 1 of the project according to SOPs in these cohorts (n=150.000 samples). These markers included hsTnI (Abbott), Vitamin D (Abbott), c-Peptide (Abbott), Insulin (Abbott), Testosterone (Abbott), Apo A (Abbott), ApoB (Abbott), Creatinin (Abbott), CRP (Abbott), Cystatin C (Abbott), LDL Cholerstol(Abbott), HDL Cholerstol (Abbott), Lp(a) (Abbott), Triglycerides (Abbott), Glucose (Abbott), BNP (Abbott), NT-proBNP (Roche) for the Phase I cohorts as well as Phase II cohorts. In selected cohorts (FinRisk, GHS, Glostrup) markers Galectin 3, ST2 and GDF15 were measured. In the clinical trials mainly hsTnI and BNP/NtproBNP and lipid markers were measured. For each marker, quality performance of the assay kit was determined (linearity, intra-/inter coefficient of variation, internal controls).

Novel, emerging biomarkers measured in the BiomarCaRE project included: CAVADIS exosomal

biomarkers (4-plex: SerpinF2, SerpinG1, Cystatin C, CD14), SerpinF2 (ELISA), Biocrates metabolites (targeted metabolomics, AbsoluteIDQ p180 Kit), microRNAs, transcriptomics-based biomarkers.

Measurement of CAVADIS exosomal biomarkers as 4-plex Luminex assay was performed in the diseased cohorts (n=8,000 samples). For this, a training of UKE laboratory staff for i) exosome isolation and ii) Luminex handling was performed and the Luminex instrument was installed at the laboratory.
Furthermore, a Serpin F2 ELISA had been developed jointly by CAVADIS and Fleet to measure SERPINF2 (as most promising biomarker of the 4-plex) in the case cohort set (n=20.000 samples).

Metabolites were measured in a targeted approach by using the Biocrates AbsoluteIDQ p180 Kit in the case cohort set (n=20.000 samples).

miRNAs were selected i) from the literature (most significant associated and mostly cited miRNAs with respect to the BiomarCaRE endpoints CVD, myocardial infarction, stroke, atrial fibrillation and diabetes and ii) from a screening of circulating serum miRNAs in samples of the Moli-Sani and the Tromso study. This screening was performed at CBC based on microarrays. In total, 21 miRNAs based on literature as well as miRNAs from the miRNA profiling were selected.
For isolation of total RNA from serum samples of the case cohort set a standardized protocol was developed jointly by the UKE and Biocartis. Isolation of total serum RNA has been started in samples of the case cohort set (n=20.000).
For measurements, several technical options were tested including the Microarray Cards of Applied Biosystems, single TaqMan miRNA assays as well as single TaqMan advanced miRNA assays. The best assay performance combined with efficient handling of reagents for RT-reaction and PCR was observed for the advanced miRNA assays of Applied Biosystems. To increase the throughput of the measurements, duplex assays and an efficient 348-well plate layout were designed.

Transcriptomics-based biomarkers were identified by gene expression profiling approaches related to myocardial infarction and blood pressure by the UKE. For a myocardial infarction related marker Fleet generated an ELISA assay that was tested in a pilot phase with samples of early onset myocardial infarction patients, patients with documented coronary artery disease, stable phase, patients with acute myocardial infarction and healthy controls subjects. Levels could only be detected in EDTA plasma samples and highest levels were observed in patients with myocardial infarction (acute myocardial infarction as well as early onset myocardial infarction) and not/at very low levels in patients with coronary artery disease. For confirmation in a larger set of samples more samples were measured.
Levels of the marker related to blood pressure were measured in selected samples of the Moli-Sani and GHS cohort including subjects that suffered incident cardiovascular events. Measurements of protein levels were performed using a commercially available ELISA assay. First data revealed a predictive value of this marker.

Data quality control and Data transfer: All biomarker data were quality controlled according to pre-specified SOPs and were put into a harmonized format for automated reading into the data bases at the central data centres. Quality controlled data of all cohorts were sent for storage in the central BiomarCaRE data base and data analyses to the data centres THL (population-base cohorts) and the IMBS Lübeck (diease cohorts). Quality controlled data of the clinical trials were sent for data analyses to the clinical trial study centres. All data files included biomarker results, date of measurements, units per biomarker, measuring range of assay and specific comments.

WP 7 Data harmonization and statistical analysis

Data harmonization

BiomarCaRE harmonized and collected individual level baseline and follow-up data from large population based cohorts to the data centre at the National Institute for Health and Welfare of Finland. Likewise, BiomarCaRE harmonized data from four patient cohorts to the data centre at the University of Lübeck, Germany. The data harmonization involved the preparation of locally

available data to an agreed format, transfer of the data and related metadata to the data centre, and assessment of quality and comparability of the data. This was usually repeated several times for each cohort until the transferred data met the quality requirements of the Project. The biomarker values measured in BiomarCaRE were appended to the cohort data.

Much of the population cohort data had already been harmonized earlier in the framework of the MORGAM (MONICA Risk, Genetics Archiving and Monograph) project. BiomarCaRE, jointly with the simultaneously ongoing CHANCES (Consortium on Health and Ageing: Network of Cohorts in Europe and the United States) project added several new cohorts, extended the follow-up period for many of the old cohorts, harmonized also data from repeated measurements and added several new disease endpoints (heart failure, atrial fibrillation, peripheral vascular disease, type 2 diabetes and cancer).

The resulting database includes data from 234,000 persons with 40,000 deaths, 18,000 incident acute coronary events, 10,000 strokes, and 3,900 new diagnoses of heart failure, 3,400 atrial fibrillation, 3,700 diabetes, 1,200 venous thromboembolism and 4,500 cancers (Table 1).

A case-cohort set of 22,552 persons was selected for the analysis of biomarkers which were not feasible to measure in the entire cohort.

From the four disease patient cohorts participating in BiomarCaRE, 57 variables were available from all cohorts. The data we collected and quality controlled the data. This resulted in a total data set of approx. 9,100 patients with 1,178 secondary cardiovascular events.

Data analysis

After 16 established biomarkers (Apo A1: Apoliprotein A1, Apo B: Apoliprotein B, HDL-cholesterol, LDL-cholesterol, triglycerides, lipoprotein(a), creatinine, C-reactive protein, cystatin C, NTproBNP: N-terminal-pro B-type natriuretic peptide, hsTnI: high sensitivity troponin I, C-peptide, insulin, glucose, testosterone and vitamin D) had been measured for the first nine cohorts in the laboratory at the UKE, their impact on acute coronary heart disease, stroke, cardiovascular disease, heart failure, atrial fibrillation, type 2 diabetes and death was assesed in order to select the biomarkers to be measured in the remaining cohorts. Thirteen of them were significant on top of the others as predictors of at least some of the diseases. After removal of the three less significant predictors and biomarkers which were highly correlated, we got ten biomarkers which together made a meaningful improvement to the prediction of coronary heart disease, stroke and cardiovascular disease when added to the model after the classic risk factors smoking, systolic blood pressure, blood pressure treatment, diabetes and body mass index. Adding also the remaining six biomarkers did not make a meaningful improvement to the prediction. The specific results will be published after validation in the remaining cohorts.
Moreover, in specific cohorts, emerging markers such as Galectin-3, GDF15 and sST2 were measured and analysed regarding their impact an coronary heart disease and cardiovascular disease. The results have been published or are under revision.

Data of 184 metabolites from the AbsoluteIDQ p180 Kit were quality controlled and normalized and prepared for data analysis, which is currently ongoing.

A large number of analysis projects on specific biomarkers and /or disease end-points have been completed or are ongoing. Of the already published results:
* An analysis of the population based Scottish cohort revealed that troponin I, measured in a high-sensitivity assay is an independent predictor of cardiovascular events and might support selection of individuals at risk. The issue is further elaborated in an analysis of ten prospective population cohorts which has been accepted for publication, and in a submitted paper on the association of repeatedly measured high sensitivity assayed troponin I with cardiovascular disease events in a general population. An analysis in the FINRISK cohort revealed that using a super-sensitivity assay, cardiac troponin was detectable in almost all healthy individuals;
* Vitamin D was shown not to explain the seasonal variation of coronary heart disease in the Scottish

cohort. An analysis of the association of Serum Vitamin D Levels and risk of cardiovascular diseases among a large number of European populations is ongoing;
* Midregional pro-adrenomedullin predicted all-cause death, major adverse cardiac events, and especially heart failure even beyond NTproBNP in the population based FINRISK 1997 cohort.
* Using a profiling-replication-validation model, we identified eight miRNAs, which may facilitate the diagnosis of unstable angina pectoris. This case-control study was based on the stenoCardia study and the population based Gutenberg Health Study.
* An analysis of the Brianza cohort revealed that ApoB and ApoA1 could improve coronary risk prediction when used as second level biomarkers in non-diabetic subjects with metabolic syndrome classified at intermediate risk.
* An analysis of the FINRISK 1997 cohort revealed that Galectin-3 levels were predictive for future cardiovascular events but improvements in discrimination and reclassifications were modest.

Additional analyses are ongoing on:
* the value of microvesicle ?-2 antiplasmin (serpinF2) in improving cardiovascular risk prediction in primary prevention;
* prognostic value of the growth differentiation factor 15 (GDF-15), hsCRP, hsTnI, and NT-proBNP in addition to established risk scores based on classic cardiovascular risk factors;
* the value of GDF-15 and other biomarkers (sST2, CRP, and NT-proBNP) to diagnose individuals with heart failure, to discriminate between heart failure patients with reduced (HFrEF) and preserved ejection fraction (HFpEF), and to determine the prognostic value of the biomarkers for all-cause mortality;
* assessment of repeated measurements of GDF-15 as a prognostic marker for heart failure and fatal cardiovascular events;
* seeking better understanding of myocardial infarction among those without, or with only minor, cardiovascular risk factors;
* lipoprotein(a) levels and risk of future cardiovascular disease among European populations;
* the predictive value of testosterone for cardiovascular diseases;
* the predictive value of multiple biomarkers for heart failure and atrial fibrillation;
* sex differences in cardiovascular disease risk factors and associations with atrial fibrillation and heart failure across European cohorts;
* the value of biomarkers for ischaemic and haemorrhagic stroke prediction in European cohorts; and
* the paradigm shift on how to assess the value of biomarkers in cardiovascular risk prediction.

The harmonized population cohort data also revealed that inclusion of education would improve the European SCORE equation for the prediction of risk of cardiovascular death in men. Furthermore we found that educational class inequalities accounted for 343 and 170 additional CHD events per 100?000 person-years in the least educated men and women compared with the most educated. In Central and South European populations the social gradient of CHD was explained largely by inequalities in case fatality of heart attacks whereas in the north they were mostly explained by differences in CHD incidence. The major determinants of the inequalities in CHD incidence were smoking in men and HDL cholesterol in women.

Collaboration between the BiomarCaRE and CHANCES projects revealed that smoking cessation still at older age is beneficial for preventing death, cardiovascular disease and cancer.

For the two largest patient cohorts (AtheroGene and KAROLA) we investigated the predictive potential of the CAVADIS 4-plex biomarker panel which was developed by Partner 3 (CAVADIS) and for which partner 1 (UKE) undertook the measurements. Using Cox regression, Cystatin C showed a significant association with the endpoint total death. For the other three biomarkers no significant association was observed.

In order to facilitate the analysis of survival data with random forests, we developed and implemented a fast and modular framework for random forests (ranger). We validated the new software by comparing results obtained with those from random survival forests (RSF).
To improve the performance of the random forest analysis with survival outcome we developed an approach based on the idea of maximally selected rank statistics to overcome the biased split-point selection problem. To avoid the end cut preference of random forests for survival outcome we developed a split criterion based on Harrell’s C. Both approaches showed promising results for

simulated data, but did not improve the prediction performance in the real data when adding the information from the CAVADIS 4-plex.

WP 8 Retrospective application of the European BiomarCaRE panel in biobanks of clinical trials

Measurements of the preliminary BiomarCaRE biomarker panel (hsTnI (Abbott), ApoA (Abbott), ApoB (Abbott), Vitamin D (Abbott), C-Peptide (Abbott), Insulin (Abbott), Testosterone (Abbott), Cystatin C (Abbott), Lp(a) (Abbott), NT-proBNP (Roche) have been carried out in theIBIS-2 and LIPID clinical trials. For each marker, quality performance of the assay kit was determined (linearity, intra-/inter coefficient of variation, internal controls). For JUPITER hsTnI, and NT-proBNP have been measured. First analysis results have been published (Tonkin 2015, Funke-Kaiser 2014), Further analyses are ongoing.

WP 9 Economic assessment of the BiomarCaRE panel

Can a combined score of CRP, NT-proBNP and Troponin I be effective and cost-effective in selecting patients for preventive statin medication?

A previous EU –project (MORGAM) suggested that a combined biomarker score can improve risk estimation in cardiovascular disease prevention (Blankenberg 2010, Circulation). The BiomarCaRE project aimed at finding new biomarkers that could potentially improve risk estimation further. This cost-effectiveness study extended the evaluation of a combined score beyond the risk prediction to its potential benefit in selecting individuals for preventive statin medication and to the potential long-term effectiveness and cost-effectiveness of using a test panel for the included biomarkers.
The current guidelines of the European Society for Cardiology (Perk et al. 2012, EurHeartJ) recommend lipid-lowering medication for high risk individuals with diagnosed cardiovascular disease or diabetes and in addition for individuals with ESC SCORE >= 10% and LDL-cholesterol >= 1.8mmol/L or ESC SCORE >= 5 % and LDL-cholesterol >= 2.5mmol/L. The ESC SCORE is an equation to predict the 10-year risk of cardiovascular death and is a function of age, gender, smoking status, systolic blood pressure and total cholesterol. For the remaining, larger, part of the population without overt disease further tests may be considered. The current study is looking at the effect of using a test of CRP, NT-proBNP and Troponin I and a combined score of these 3 biomarkers as an additional criterion in the decision for preventive statin medication. We performed evaluations for two subgroups of the population without diagnosed CVD or diabetes: Cohort 1 is below the levels of risk that the guideline uses for assignment of statins and cohort 2 is the group that would receive statins immediately under these guidelines.
To evaluate the combined biomarker score, a decision-analytic model was developed. The model consists of the following parts: 1) A decision tree describing the strategy options that are to be compared. These are: a) Everybody in the target group receives statins, b) Nobody receives statins, c) Statins are given if the combined biomarker score of the individual is higher than a cutoff value (each cutoff value means a separate strategy). 2) A state-transition model for disease incidence and progression. This model describes the cardiovascular events non-fatal myocardial infarction, non-fatal stroke, cardiovascular death and other cause mortality. Individuals transition between states with annual probabilities. Individuals' paths through the model are followed over their lifetime. Risks of events are functions of conventional risk factors. They also vary with biomarker level and statin medication and therefore with the strategy that was chosen. 3) A data base of model parameters to populate the model. 4) Summation of years of life lived, of quality-adjusted life years and of health care costs over lifetime. 5) An examination of different cutoff values for the biomarker score and selection of optimal cutoff levels regarding long-term effectiveness and cost-effectiveness. 6) Construction of an artificial model population based on the FINRISK97 population-based cohort, providing measurements of all needed conventional and novel biomarker risk factors but adapted to resemble the demographics and distribution of conventional risk factors of the German population.

The perspective of the cost-effectiveness analysis was that of the statutory health insurance in Germany. Costs are for the year 2015. Costs and QALYs (quality-adjusted life years) were discounted at 3%

Results of the cost-effectiveness analysis

Cohort 1 (no immediate statin recommendation in guidelines): Long-term effectiveness is increasing with each strategy that adds individuals to statin medication down to a combined biomarker score cutoff of -0.75 standard deviations (SD) below the mean value. Strategies adding further individuals with a lower score to statin medication have lower effectiveness despite increasing costs and are therefore dominated. Cost-effectiveness depends on the assumed willingness-to-pay threshold. With a threshold of €50,000/QALY, adding individuals above a cutoff of +1.0 SD is cost-effective while a strategy adding all above +0.75 SD has an incremental cost-effectiveness ratio (ICER) just above this threshold. The benefit in long-term effectiveness for adding all individuals with score > -0.75 SD is 0.01 QALY or 3.65 quality adjusted life days per person. The proportion additionally receiving statins under a strategy with cutoff +1SD is 14% of the model population of men and women without diagnosed CVD or diabetes between the ages of 24 and 75 with risk categories currently not recommended to receive statin immediately. If the model population would accurately represent the German population, this would correspond to roughly 6 million men and women. According to our model population, 58% of the 6 million would be women. Including individuals with CVD or diabetes and adding all for whom immediate statins are recommended already, this would mean that 35 % of the population age 24-75 would receive statins.
Cohort 2 (immediate statins recommended in guidelines): Compared to a strategy without statin medication, long-term effectiveness is increasing with each strategy that assigns more individuals to statin medication. All strategies on the cost-effectiveness frontier have ICERs well below €50,000/QALY. In line with the current ESC guideline, the most cost-effective strategy in this population is to provide all individuals with statins. Strategies excluding individuals based on very low biomarker scores have less or equal effectiveness but are more costly due to biomarker testing costs. These strategies are dominated.
In sensitivity analyses, results for cohort 1 were very sensitive to the effect of daily statin intake on the quality of life. This reduction in quality of life is not related to adverse events of statins. It has been studied recently because adherence to statins is low despite a low risk of adverse event.

Further results of this work package

1) Estimation of risk modification through a fixed set of conventional risk factors, consisting of age, gender, smoking status, systolic blood pressure, total cholesterol and LDL-cholesterol

A Cox proportional hazards model for each of the possible transitions between health states in the state transition model was used to describe the population heterogeneity in risk. Hazard ratios were estimated using the R package msm. One novel biomarker was added to the model separately in addition to the conventional risk factors to obtain hazard ratios for each of the three novel biomarkers CRP, NT-pro BNP, and Troponin I.

2) Estimation of the hazard ratio for the combined biomarker score as an additional covariate

The log hazard ratios for each of the three novel biomarkers were linearly combined to create a novel biomarker panel score. The novel biomarker panel score forms a new covariate in addition to the conventional risk factors. The msm models were re-run with the conventional risk factors and the novel biomarker panel score included in the model to obtain output such as hazard ratios and beta parameters.

3) Novel method for estimation of transition probabilities

Annual transition probabilities by age and gender were estimated in the framework of a continuous time state transition model under exponential assumption, using the R routine msm. The msm package was extended by using the output from the models previously run to obtain hazard ratios for the conventional risk factors alone, and with the addition of the novel biomarker panel score. One year transition probabilities between each of the health states were calculated for each year starting at 50 years of age depending on the use of a novel biomarker panel score or not.

4) Reclassification in ten-year risk of cardiovascular death through the additional covariate of a combined biomarker score

Using the one year transition probabilities calculated, the 10 year risk of CVD death was calculated to assess the prediction increment for the new novel biomarker panel score. The 10 year risk of CVD death was calculated using the conventional covariates, and again with the addition of the novel biomarker panel score. The movements for individuals between the different risk groups were observed and net reclassification indices calculated.

Potential impact and main dissemination activities and exploitation results

The major impact of BiomarCaRE is the availability of a harmonized (measured with the same assays) data set of biomarkers across European cohorts. These data sets can be made available for collaborative partners and will enhance the understanding and potential value of biomarkers in cardiovascular diseases.

The data harmonization continued to be a successful process and has produced a valuable data resource and infrastructure not only for BiomarCaRE but also for future collaborative research on biomarkers and cardiovascular diseases. Examples of such ongoing collaborations are the extensive contribution of the MORGAM database to the FP7 projects CHANCES and BBMRI-LPC.

The published findings clearly advance the knowledge about the clinical impact of biomarkers, in particular in the general population, and the data harmonized and generated in the project, after fully analysed, have high potential for significant advances for the medical practice.

BiomarCaRE improved our understanding of the social gradients of cardiovascular diseases in Europe and helps in focusing attempts to decrease them.

The level of impact can be estimated based on the large number of analyses and follow-up sub-projects that were already triggered through the availability of a large data set and harmonized data and is expected to extend in the future and to give significant insight in the importance of biomarkers in cardiovascular diseases.

They include the following topics, some of them already published:
1. Vitamin D as a risk factor in the Scottish cohorts
2. Risk stratification by high sensitivity assayed troponin I among the European population – a path to individualize cardiovascular prevention?
3. The value of microvesicle ?-2 antiplasmin (serpinF2) in improving cardiovascular risk prediction in primary prevention
4. Assessment of the added prognostic value of GDF-15, hsCRP, hsTnT, and NT-proBNP in the context of the European SCORE risk estimation system, the new AHA/ACC ASCVD risk score, and the Framingham risk score
5. Value of GDF-15 and other biomarkers (sST2, CRP, and NT-proBNP) to diagnose individuals with heart failure, to discriminate between heart failure patients with reduced (HFrEF) and preserved ejection fraction (HFpEF), and to determine the prognostic value of the biomarkers for all-cause mortality
6. Assessment of repeated measurements of the growth differentiation factor 15 (GDF-15) as a prognostic marker for heart failure, fatal cardiovascular events in the BiomarCaRE cohorts Glostrup and Caerphilly
7. cryptogenic (unexplained) myocardial infarction: “Explaining the unexplainable”
8. Lipoprotein(a) levels and risk of future cardiovascular disease among European populations
9. Association of repeatedly measured high sensitivity assayed troponin I with cardiovascular disease events in a general population
10. The predictive value of testosterone
11. The predictive value of multiple biomarkers for atrial fibrillation
12. Heart failure risk score in the European general population
13. Gender differences in cardiovascular disease risk factors and associations with atrial fibrillation and heart failure across European cohorts
14. Analysis of the association of Serum Vitamin D Levels and risk of cardiovascular diseases among European populations
15. Analysis of the value of Biomarkers for Ischaemic and Haemorrhagic Stroke prediction in the European General Population

16. Assessment of the paradigm shift on how to assess the value of biomarkers in cardiovascular risk prediction
17. Development of a risk score based on established risk factors for incident coronary heart disease and stroke in Europe
18. Compared predictors on peripheral arterial disease and coronary heart disease
19. Analysis of educational class inequalities in the incidence of coronary heart disease
20. Analysis of educational class inequalities in the incidence of stroke
21. Analysis of the interaction between social classes and risk factors on the risk of coronary heart disease or stroke
22. Socioeconomic patterning of low-grade inflammation and the role of health-related behaviours
23. Impact of smoking and smoking cessation on cardiovascular events and mortality among older adults
24. Smoking and All-cause Mortality in Older Adults
25. Quantification of the smoking-associated cancer risk with rate advancement periods
26. Variations in the effect of alcohol consumption on the incidence of cardiovascular diseases
27. Are detrimental/beneficial effects of alcohol consumption different according to social status?

The availability of a colorimetric ELISA for serpin F2 allowed this biomarker to be thoroughly evaluated within the BiomarCaRE project, albeit with the conclusion that it did not offer any significant advantage over existing commercial biomarkers.

The availability of a colorimetric ELISA for GPR15 is potentially of interest to organisations such as UKE who have wider research interests with respect to this biomarker.

The AbsoluteIDQ® p180 Kit provided by Biocrates to the project partner 1 (UKE) was used for metabolomics measurements of 20 000 samples. Metabolomics data evaluation will be done by project partner 1 (UKE) with the aim to identify new marker for prediction and diagnosis of cardiovascular disease. Very likely results from this unique metabolomics data set analysis will improve the prediction, diagnosis and treatment of cardiovascular diseases.
Detailed feedback given by project partner 1 (UKE) from the use of 265 AbsoluteIDQ® p180 Kits improved the workflow, robustness, sample analyze time, documentation and customer support efficiency for further scientists using the AbsoluteIDQ® p180 Kit. By this samples can be analyzed easier, faster and more efficient by scientists to improve for example the prediction, diagnosis or treatment of diseases on the way to a more personalized medicine. By the validation of the AbsoluteIDQ® p180 Kit for Waters MS and the Thermo TSQ Vantage, the AbsoluteIDQ® p180 Kit is now available for a wider target group of metabolomics researchers.

The cost-effectiveness results of this study contribute to the current discussion on improving the selection of patients for preventive statin treatment. A combined score of multiple novel blood biomarkers has the potential to be effective and cost-effective in a lower risk population, depending on the cutoff level chosen to assign statins to an individual. The result can inform health policy decision making.

Main dissemination and training activities

The consortiums major route for dissemination is the publication of scientific results in peer-reviewed journals and scientific conferences and meetings. Please see the list of publications and other dissemination activities.

The BiomarCaRE laboratory and biobank was presented at several national and internationally meetings in order to provide information about the project itself as well as the specific activities of the laboratory. Moreover, an overview of the markers measured and available data was provided for potential collaborative projects.
The BiomarCaRE project has been represented at the laboratories website (http://www.uke.de/kliniken-institute/kliniken/allgemeine-und-interventionelle-kardiologie/forschung/schwerpunkte/fo

Updates to the MORGAM Manual concerning the harmonization standards; instructions for the new baseline variables and disease end-points; analysis variables derived from these; and descriptions of the participating cohorts were completed and published in the series MORGAM Project e-publications (http://www.thl.fi/publications/). The description and quality assessment of MORGAM data is being completed for publication in the same series.

Two Statistics Workshops were organized, one in Hamburg, Germany on 3-5 June 2013, with 44 participants representing 14 Partners, and the other one in Tromsø, Norway on 17 June 2014, with 27 participants representing 14 partners.

Furthermore, first patents were submitted based on the results of the initial metabolomics data.

Address of project public website and relevant contact details

Address of website and contact details

http://www.biomarcare.eu

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Table 1. Population cohort data currently in the MORGAM Data Centre

Study	Years of exam.	Re-exam. of	Persons	Follow-up time (years)	Incident events							
					CHD	Stroke	Heart failure	Atrial fibr.	Diabetes	Periph. vasc. disease	Venous thrombo-embolism	Cancer
ATBC	1984-88		29133	14	5929	3509					772	
	1986-93	1984-88	3701									
	1992-93	1984-88	20278									
Finrisk	1997		8444	13	559	363	565	452	602	121	186	664
	2002		9485	9	241	151	226	209	390	52	67	358
Augsburg	1994-95		4692	13	239	171			290			
	1999-01		4221	9	116	82			163			
	2006-08	1999-01	3063									
GHS	2007-10		9949	5								
Tromsø	1986-87		20458	20	1564	767						
	1994-95	1986-87	26914		1027	739						
Moli-sani	2005-10		24325	4	351	90	613	414	352			
SHIP-TREND	2008-12											
HAPIEE Czech	2002-05		8480	8	326	211	248					
HAPIEE Kaunas	2005-08		7161	4	171	93						
HAPIEE Krakow	2002-04		10012	7	277	82						
HAPIEE Novos.	2002-05		9349	6	329	159						
Caerphilly	1989-93		2171	20	529	313						
	2001-05	1989-93	1143									
Belfast	1991-94		2745	16	370	113			263			495
Lille	1991-93		2633	10	156	47						
Strasbourg	1991-93		2612	10	158	27						
Toulouse	1991-93		2610	10	158	23						
Catalonia	1986-88		2571	10	46	8		13				
	1990-92		2934	8	42	4						
N-Sweden	1986		1625	23	264	166	121	169	97			323
	1999	1986	1316									
	1990		1576	20	203	110	91	114	80			218
	1999	1990	1258									
	1994		1893	16	238	147	129	162	84			266
	1999	1994	1562									
	1999		1789	12	134	84	76	107	48			139
	2004		1863	8	73	35	46	67	21			102
	2009		1704	3	15	10	6	11	14			16
Estonia	2001-10		4971	7	815	544						
Glostrup	1982-85		4052	23	641	473	428	388	306		122	
	1987-90	1982-85	3078									
	1993-95	1982-85	2753									
	1986-87		1504	22	181	136	102	105	100		49	
	1991-92		2026	17	236	184	159	167	103		38	
Brianza	1986-87		1659	21	127	52						
	1989-90		1599	18	99	42						
	1993-94		1674	14	67	31						
Friuli	1994		1786	4	12	8						
Rome	1993-96		2519	10	60	70						
	1993-96		1970	10	42	16						
Scotland	1984-87		11573	21	1856	704	860	852	711	413		1519
	1992		1754	15	240	126	167	141	74	72		206
	1995		1656	14	117	49	56	64	46	35		98
	1989		1017	19	122	58	49	61	43	38		112
Total			278118		18130	9997	3942	3496	3787	731	1234	4516

4.2 Use and dissemination of foreground

Section A (public)

Publications

LIST OF SCIENTIFIC PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES										
No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Is open access provided to this publication ?	Type
1	BiomarCaRE: rationale and design of the European BiomarCaRE project including 300,000 participants from 13 European countries 10.1007/s1065 4-014-9952-x	Tanja Zeller , Maria Hughes , Tarja Tuovinen , Arne Schillert , Annette Conrads-Frank , Hester den Ruijter , Renate B. Schnabel , Frank Kee , Veikko Salomaa , Uwe Siebert , Barbara Thorand , Andreas Ziegler , Heico Breek , Gerard Pasterkamp , Kari Kuulasmaa , Wolfgang Koenig , Stefan Blankenberg	European Journal of Epidemiology	Vol. 29/Issue 10	Springer Netherlands	Netherlands	01/10/2014	777-790		Peer reviewed
2	High population prevalence of cardiac troponin I measured by a high-sensitivity	Zeller T, Tunstall-P	European Heart Journal	Eur Heart J.	Oxford University Press		01/04/2014	271-81	Yes	Peer reviewed

	assay and cardiovascular risk estimation: the MORGAM Biomarker Project Scottish Cohort.	edoe H, Sa arela O, Ojeda F, Schnabel RB, Tuovinen T, Woodward M, Struthers A, Hughes M, Kee F, Salom aa V, Kuul asmaa K, B lankenberg S; MORGAM Investigators								
3	Comparison of Three Troponins as Predict ors of Future Cardiovascular Events – Pr ospective Results from the FINRISK and B iomaCaRE Studies	Neumann JT, Havulinna AS, Zeller T, Appelbaum S, Kunnas T, N ikkari S, Jousi lahti P, B lankenberg S, Sydow K, Sa lomaa V	PLoS One	9 (3)	Public Library of Sc ience		01/03/2014	e90063	Yes	Peer revie wed
4	The contribution of educational class in improving accuracy of cardiovascular risk prediction across European regions: The MORGAM Project Cohort Component 10.1136/heart jnl-2013-304664	M. M. Ferr ario , G. Veronesi , L. E. Chambless , H. Tunstall -Pedoe , K. Kuulasmaa , V. Salomaa , A. Borglykke , N. Hart , S. Soderberg , G. Cesana; for the MORG AM Project	Heart	Vol. 100/I ssu e 15	BMJ Publishing Group	United Kingdom	01/08/2014	1179-1187		Peer revie wed
5	Predictive value of midregional pro-adre nomedullin compared to natriuretic pepti des for incident cardiovascular disease and heart failure in the population-based FIN RISK 1997 cohort 10.3109/07853 890.2013.874662	Anne Funke -Kaiser , Aki S. Havulinna , Tanja Zeller , Sebastian A ppelbaum , Pekka Jou silahti , Erkki Vartiainen , S	Annals of Medicine	Vol. 46/Is sue 3	Informa Healthcare	United Kingdom	01/05/2014	155-162		Peer revie wed

		tefan Blankenberg , Karsten Sydow , Veikko Salomaa								
6	Do apolipoproteins improve coronary risk prediction in subjects with metabolic syndrome? Insights from the North Italian Brianza cohort study 10.1016/j.atherosclerosis.2014.06.029	Francesco Gianfagna , Giovanni Veronesi , Luigina Guasti , Lloyd E. Chambless , Paolo Brambilla , Giovanni Corrao , Giuseppe Mancia , Giancarlo Cesana , Marco M. Ferrario	Atherosclerosis	Vol. 236/Issue 1	Elsevier Ireland Ltd	Ireland	01/09/2014	175-181		Peer reviewed
7	Biomarkers of Coronary Artery Disease: The Promise of the Transcriptome 10.1007/s1188 6-014-0513-4	Marten Antoon Siemelink , Tanja Zeller	Current Cardiology Reports	Vol. 16/Issue 8	Current Medicine Group	United States	01/08/2014	513		Peer reviewed
8	Assessment of microRNAs in patients with unstable angina pectoris 10.1093/eurhe artj/ehu151	T. Zeller , T. Keller , F. Ojeda , T. Reichlin , R. Twerenbold , S. Tzikas , P. S. Wild , M. Reiter , E. Czyz , K. J. Lackner , T. Munzel , C. Mueller , S. Blankenberg	European Heart Journal	Vol. 35/Issue 31	Oxford University Press	United Kingdom	14/08/2014	2106-2114		Peer reviewed
9	High-sensitivity cardiac troponin I in the general population – defining reference populations for the determination of the 99th percentile in the Gutenberg Health Study 10.1515/cclm- 2014-0619	Tanja Zeller , Francisco Ojeda , Fabian J. Brunner , Philipp Peitsmeyer , Thomas Münzel , Harald	Clinical Chemistry and Laboratory Medicine	Vol. 0/Issue 0	Walter de Gruyter GmbH & Co. KG	Germany	06/01/2014	epub		Peer reviewed

		Binder , Norbert Pfeiffer , Matthias Michal , Philipp S. Wild , Stefan Blankenberg , Karl J. Lackner								
10	Troponin I and cardiovascular risk prediction in the general population: the BiomarCaRE consortium 10.1093/eurhe artj/ehw172	Stefan Blankenberg , Veikko Salomaa , Nataliya Makarova , Francisco Ojeda , Philipp Wild , Karl J. Lackner , Torben Jørgensen , Barbara Thorand , Annette Peters , Matthias Nauck , Astrid Petersmann , Erkki Vartiainen , Giovanni Veronesi , Paolo Brambilla , Simona Costanzo , Licia Iacoviello , Gerard Linden , John Yarnell , Christopher C. Patterson , Brendan M. Everett , Paul M. Ridker , Jukka Kontto , Renate B. Schnabel , Wolfgang Koenig , Frank Kee ,	European Heart Journal	Epub ahead of print	Oxford University Press	United Kingdom	12/05/2016	ehw172	Yes	Peer reviewed

		Tanja Zeller , Kari Kuul asmaa								
11	Calibrating random forests for probability estimation 10.1002/sim.6 959	Theresa Da nkowski , Andreas Zi egler	Statistics in Medicine	in press	John Wiley and Sons Ltd	United Kingdom	01/01/2016	in press	Yes	Peer revie wed
12	High-Sensitivity Cardiac Troponin I and B-Type Natriuretic Peptide as Predictors of Vascular Events in Primary Prevention: Im pact of Statin Therapy 10.1161/CIRCU LATIONAHA.114.014 522	B. M. Everett , T. Zeller , R. J. Glynn , P. M. Ridker , S. Blankenberg	Circulation	Vol. 131/I ssu e 21	Lippincott Williams and Wilkins	United States	26/05/2015	1851-1860	Yes	Peer revie wed
13	ST2 may not be a useful predictor for in cident cardiovascular events, heart failure a nd mortality 10.1136/heart jnl-2014-305968	M. F. Hughes , S. Appelba um , A. S. Havulinna , A. Jagodzins ki , T. Zeller , F. Kee , S. B lankenberg , V. Salomaa	Heart	Vol. 100/I ssu e 21	BMJ Publishing Group	United Kingdom	01/11/2014	1715-1721	No	Peer revie wed
14	Predictive value of galectin-3 for incident c ardiovascular disease and heart failure in th e population-based FINRISK 1997 cohort 10.1016/j.ijc ard.2015.05.040	Annika Jag odzinski , Aki S. Havulinna , Sebastian Appelbaum , Tanja Zeller , Pekka Jousila hti , Silke Sky tte-Johanssen , Maria F. Hughes , S tefan Blan kenberg , Veikko Sal omaa	International Journal of Cardiology	Vol. 192	Elsevier Ireland Ltd	Ireland	01/08/2015	33-39	No	Peer revie wed
15	Cohort Profile: Estonian Biobank of the Estonian Genome Center, University of Ta rtu 10.1093/ije/d yt268	Liis Leitsalu , Toomas Ha ller , Tõnu Esko , Mari-L iis Tammesoo , Helene Ala vere , Harold	International Journal of Epidemiology	Vol. 44/Is sue 4	Oxford University Pr ess	United Kingdom	01/08/2015	1137-1147	No	Peer revie wed

		Snieder , Markus Perola , Pauline C Ng , Reedik Mägi , Lili Milani , Krista Fischer , Andres Metspalu								
16	An epidemiological perspective of personalized medicine: the Estonian experience 10.1111/joim. 12320	L. Milani , L. Leitsalu , A. Metspalu	Journal of Internal Medicine	Vol. 277/Issue 2	Blackwell Publishing	United Kingdom	01/02/2015	188-200	Yes	Peer reviewed
17	Impact of smoking and smoking cessation on cardiovascular events and mortality among older adults: meta-analysis of individual participant data from prospective cohort studies of the CHANCES consortium 10.1136/bmj.h 1551	U. Mons , A. Muezzinler , C. Gellert , B. Schottker , C. C. Abnet , M. Bobak , L. de Groot , N. D. Freedman , E. Jansen , F. Kee , D. Kromhout , K. Kuulasmaa , T. Laatikainen , M. G. O' Doherty , B. Bueno-de-Mesquita , P. Orfanos , A. Peters , Y. T. van der Schouw , T. Wilsgaard , A. Wolk , A. Trichopoulou , P. Boffetta , H. Brenner	British Medical Journal	Vol. 350/Issue apr20 2	BMJ Publishing Group		20/04/2015	h1551-h15 51	Yes	Peer reviewed
18	Smoking and All-cause Mortality in Older Adults 10.1016/j.amepre.2015.04.004	Aysel Müezzinler et al.	American Journal of Preventive Medicine	Vol. 49/Issue 5	Elsevier Inc.	United States	01/11/2015	e53-e63	No	Peer reviewed
19	Quantification of the smoking-associated cancer risk with rate advancement periods: meta-analysis of individual participant data from cohorts of the CHANCES consortium	José Manuel Ordóñez-Mena et al.	BMC Medicine	Vol. 14/Issue 1	BioMed Central	United Kingdom	01/12/2016	62	Yes	Peer reviewed

	10.1186/s1291 6-016-0607-5									
20	Coding Variation in ANGPTL4, LPL, and SVEP1 and the Risk of Coronary Disease 10.1056/NEJMo a1507652	Stitziel NO et al.	New England Journal of Medicine	Vol. 374/Issu e 12	Massachussett s Medical Society	United States	24/03/2016	1134-1144	No	Peer revie wed
21	Prime mover or fellow traveller: 25-hydr oxy vitamin D's seasonal variation, card iovascular disease and death in the Scottish Heart Health Extended Cohort (SHHEC) 10.1093/ije/d yv092	Hugh Tunst all-Pedoe , Mark Woo dward , Maria Hughes , An nie Anderson , Gwen Kenne dy , Jill Belch , Kari Ku ulasmaa;	International Journal of Epidemiology	Vol. 44/Is sue 5	Oxford University Pr ess	United Kingdom	01/10/2015	1602-1612	Yes	Peer revie wed
22	Educational class inequalities in the in cidence of coronary heart disease in Europe 10.1136/heart jnl-2015-308909	Giovanni V eronesi , Marco M Fe rrario , Kari K uulasmaa , Martin Bobak , Lloyd E C hambless , Veikko Sa lomaa , Stefan Soderberg , Andrzej Pajak , Torben Jørge nsen , Phi lippe Amou yel , Domi nique Arveiler , Wojciech Drygas , Jean Ferrieres , Simona G iampaoli , Frank Kee , Li cia Iacoviello , Sofia Ma lyutina , Annette Peters , Abdonas Tamosiunas , Hugh Tunstal l-Pedoe ,	Heart	Epub ahead of p rint	BMJ Publishing Group	United Kingdom	05/02/2016	heartjnl- 2 015-308 909	No	Peer revie wed

		Giancarlo Cesana								
23	Do little interactions get lost in dark random forests? 10.1186/s1285 9-016-0995-8	Marvin N. Wright , Andreas Ziegler , Inke R. König	BMC Bioinformati cs	Vol. 17/Issue 1	BioMed Central	United Kingdom	01/12/2016	in press	Yes	Peer reviewed
24	ranger: A Fast Implementation of Random Forests for High Dimensional Data in C++ and R. estimation	Wright MN, Ziegler A	Journal of Statistical Software	in press	University of California at Los Angeles		01/12/2016	in press	Yes	Peer reviewed

LIST OF DISSEMINATION ACTIVITIES								
No.	Type of activities	Main Leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
1	Posters	UNIVERSITAETSKLINIKUM HAMBURG-EPPENDORF	Comparison of Contemporary, Highly-sensitive and Supersensitive Troponin Assays as Predictors of Incident Cardiovascular Events — A Prospective Analysis of The Finrisk 1997 Cohort	18/03/2013	New Orleans, USA	Scientific community (higher education, Research)	20000	International
2	Posters	UNIVERSITAETSKLINIKUM HAMBURG-EPPENDORF	Predictive Value of Midregional Pro adrenomedullin Compared to Natriuretic Peptides for Major Adverse Cardiovascular Events in the Population-Based FINRISK 1997 Cohort	18/03/2013	New Orleans, USA	Scientific community (higher education, Research)	20000	International
3	Posters	UNIVERSITAETSKLINIKUM HAMBURG-EPPENDORF	BiomarCaRE – Biomarker for cardiovascular risk assessment in Europe	11/04/2012	Mannheim, Germany	Scientific community (higher education, Research)	8000	Germany
4	Oral presentation to a scientific event	UNIVERSITAETSKLINIKUM HAMBURG-EPPENDORF	Hypertension and Biomarker – new options for diagnostics and therapy	11/04/2012	Mannheim, Germany	Scientific community (higher education, Research)	8000	Germany
5	Oral presentation to a scientific event	UNIVERSITAETSKLINIKUM HAMBURG-EPPENDORF	Heart failure score in FinRisk	12/11/2011	Orlando, FL	Scientific community (higher education, Research)	20000	International
6	Organisation of Workshops	UNIVERSITAETSKLINIKUM HAMBURG-EPPENDORF	Workshop EAST consortium about Biobanking.	01/03/2012	Hamburg, Germany	Scientific community (higher education, Research)	2800	Europe
7	Oral presentation to	TERVEYDEN JA	MORGAM project:	24/09/2013	Leipzig, Germany	Scientific comm	100	Australia, Czech

	a scientific event	HYVINVOINNIN LAITOS	(mentions Biom arCaRE as a major current collabora tion)			unity (higher educat ion, Research)		Republic, Den mark, Finland, France, Germany, Italy, Lithuania, Norway, Poland, Russ
8	Organisation of Workshops	UNIVERSITA ETSKLINIKUM HAMBURG-EPPENDORF	DZHK meets Biom arCaRE	30/10/2014	Hamburg, Germany	Scientific comm unity (higher educat ion, Research)	40	Germany
9	Oral presentation to a scientific event	UNIVERSITA ETSMEDIZIN DER JOHANNES G UTENBERG-U NIVERSITAET MAINZ	GHS Symposium M ainz	01/03/2014	Mainz, Germany	Scientific comm unity (higher educat ion, Research)		Germany
10	Oral presentation to a scientific event	UMIT- PRIVATE U NIVERSITAT FUER GESU NDHEITSWIS SENSCHAFTEN, MEDIZINISCHE INFORMATIK UND TECHNIK GMBH	The BiomarCaRE project, assessment of risk prediction m odels and decision-a nalytic modelling fo r economic asse ssment	04/06/2013	Hall, Austria	Scientific comm unity (higher educat ion, Research)	8	Austria
11	Organisation of Workshops	UMIT- PRIVATE U NIVERSITAT FUER GESU NDHEITSWIS SENSCHAFTEN, MEDIZINISCHE INFORMATIK UND TECHNIK GMBH	Decision-analytic modelling for the economic asses sment of novel biomarker testing st rategies in the Biom arCaRE project	19/10/2013	Baltimore, MD	Scientific comm unity (higher educat ion, Research)		NL, UK, CA
12	Oral presentation to a scientific event	THE QUEEN'S UNI VERSITY OF BELFAST	Can novel bioma rkers be cost-effect ive for preventing C VD? A systematic review	10/06/2014	Antwerp, Belgium	Scientific comm unity (higher educat ion, Research)	100	International
13	Posters	THE QUEEN'S UNI VERSITY OF BELFAST	The Framework of a Decision Analytic Markov Model Assessing the C ost-Effectiveness of	15/05/2014	Belfast, UK	Scientific comm unity (higher educat ion, Research)	100	Irland

			Biomarker Led Strategies in the Prevention of Cardiovascular Disease					
14	Oral presentation to a scientific event	THE QUEEN'S UNIVERSITY OF BELFAST	Improving parameter estimation for a decision-analytic Markov model to evaluate the use of novel biomarker led strategies for primary prevention of CVD	29/09/2014	Hall, Austria	Scientific community (higher education, Research)	15	Austria, Serbia, Croatia
15	Oral presentation to a scientific event	UNIVERSITY OF DUNDEE	Prime mover or fellow traveller: 25-hydroxyvitamin D's seasonal variation, cardiovascular disease and death in the Scottish Heart Health Extended Cohort (SHHEC)	31/08/2014	Barcelona, Spain	Scientific community (higher education, Research)		International
16	Posters	UNIVERSITA DEGLI STUDI DELL'INSUBRIA	Do apolipoproteins improve cardiovascular risk prediction in subjects with cardiometabolic risk? Insights from the North Italian Brianza cohort study	18/04/2014	Rome, Italy	Scientific community (higher education, Research)	100	International
17	Oral presentation to a scientific event	THE QUEEN'S UNIVERSITY OF BELFAST	Can Novel Biomarkers Be Cost-Effective For Preventing CVD? A Systematic Review	29/11/2014	Centre of Excellence for Public Health NI Away Day, Newcastle, Northern Ireland	Scientific community (higher education, Research) - Industry - Policy makers	250	international
18	Posters	THE QUEEN'S UNIVERSITY OF BELFAST	Improving Parameter Estimation for a Decision Analytic Markov Model to Evaluate the use of Novel Biomarker Led Strategies for Prevention of Cardiovascular Disease.	18/05/2015	International Society for Pharmacoeconomics and Outcomes Research (ISPOR) 20th Annual International	Scientific community (higher education, Research)	2000	international

19	Posters	THE QUEEN'S UNIVERSITY OF BELFAST	Estimating Input Parameters for a Decision Analytic Markov Model	11/05/2015	Conference in Applied Statistics in Ireland (CASI), Cork, Ireland	Scientific community (higher education, Research)	100	international
20	Oral presentation to a scientific event	THE QUEEN'S UNIVERSITY OF BELFAST	Can Novel Biomarkers Be Cost-Effective For Preventing CVD? A Systematic Review	29/11/2014	Centre of Excellence for Public Health NI Away Day, Newcastle, Northern Ireland	Scientific community (higher education, Research) - Industry - Policy makers	250	international
21	Oral presentation to a scientific event	THE QUEEN'S UNIVERSITY OF BELFAST	Extending the msm package to derive transition probabilities for a Decision Analytic Markov Model	16/05/2016	Conference in Applied Statistics in Ireland (CASI), Limerick, Ireland	Scientific community (higher education, Research)	100	international
22	Oral presentation to a scientific event	TARTU ULIKOOL	Coronary artery disease related phenotypes at Estonian Biobank	23/10/2015	Estonian Genome Center, Tartu, Estonia	Scientific community (higher education, Research)	12	Estonia
23	Oral presentation to a scientific event	TARTU ULIKOOL	Coronary artery disease related phenotypes at Estonian Biobank	14/04/2016	Estonian Genome Center, Tartu, Estonia	Scientific community (higher education, Research)	30	Estonia, Luxemburg
24	Oral presentation to a scientific event	TARTU ULIKOOL	Sub-topic in a presentation “Hypercholesterolemia in whole genome sequencing dataset of the Estonian Biobank” of CAD-related phenotypes at Estonian Biobank	11/02/2016	FIMM, Helsinki, Finland	Scientific community (higher education, Research)	20	Estonia, Finland
25	Oral presentation to a scientific event	UNIVERSITA DEGLI STUDI DELL'INSUBRIA	Social Inequalities in Stroke Mortality, Incidence and Case-Fatality in Europe	17/02/2016	Los Angeles, CA, US	Scientific community (higher education, Research)		international
26	Oral presentation to a scientific event	UNIVERSITA DEGLI STUDI DELL'INSUBRIA	The contribution of educational class in improving accuracy of cardiovascular risk prediction across European regions	15/05/2015	Lisbon, Portugal	Scientific community (higher education, Research)		international

27	Oral presentation to a scientific event	UNIVERSITA DEGLI STUDI DELL'INSUBRIA	Social inequalities in the incidence of coronary heart disease and ischemic stroke in Europe: a competing risk analysis. The MORGAM Project Cohort Component	15/05/2015	Lisbon, Portugal	Scientific community (higher education, Research)		international
28	Oral presentation to a scientific event	UNIVERSITA DEGLI STUDI DELL'INSUBRIA	Social inequalities in the incidence of coronary heart disease and ischemic stroke in Europe: a competing risk analysis. The MORGAM Project Cohort Component	15/05/2015	Lisbon, Portugal	Scientific community (higher education, Research)		international
29	Posters	UNIVERSITA DEGLI STUDI DELL'INSUBRIA	Social inequalities in Coronary Heart Disease across European populations. The MORGAM Project Cohort Component	03/03/2015	Batimore, MD, US	Scientific community (higher education, Research)		international
30	Posters	UNIVERSITA DEGLI STUDI DELL'INSUBRIA	Exploring social inequalities in ischemic stroke incidence in Europe. The MORGAM Project Cohort Component.	03/03/2015	Batimore, MD, US	Scientific community (higher education, Research)		international
31	Oral presentation to a scientific event	UNIVERSITY OF DUNDEE	Vitamin D and heart disease- Friend or Foe	14/05/2015	EACPR Annual conference in Lisbon	Scientific community (higher education, Research)	200	Europe
32	Press releases	UNIVERSITY OF DUNDEE	Vitamin D	22/06/2015	Dundee, UK	Medias		international
33	Organisation of Conference	UMEA UNIVERSITET	Northern Sweden MONICA forum	21/04/2016	Skellefteå	Scientific community (higher education, Research)	20	Sweden
34	Organisation of Conference	UMEA UNIVERSITET	Northern Sweden MONICA forum	20/04/2015	Skellefteå	Scientific community (higher education, Research)	20	Sweden

Section B (Confidential or public: confidential information marked clearly)

LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS,UTILITY MODELS, ETC.					
Type of IP Rights	Confidential	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant(s) (as on the application)

OVERVIEW TABLE WITH EXPLOITABLE FOREGROUND								
Type of Exploitable Foreground	Description of Exploitable Foreground	Confidential	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use or any other use	Patents or other IPR exploitation (licences)	Owner and Other Beneficiary(s) involved

ADDITIONAL TEMPLATE B2: OVERVIEW TABLE WITH EXPLOITABLE FOREGROUND	
Description of Exploitable Foreground	Explain of the Exploitable Foreground

4.3 Report on societal implications

B. Ethics

1. Did your project undergo an Ethics Review (and/or Screening)?	Yes
If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final reports?	Yes

2. Please indicate whether your project involved any of the following issues :

RESEARCH ON HUMANS

Did the project involve children?	No
Did the project involve patients?	Yes
Did the project involve persons not able to consent?	No
Did the project involve adult healthy volunteers?	Yes
Did the project involve Human genetic material?	Yes
Did the project involve Human biological samples?	Yes
Did the project involve Human data collection?	Yes

RESEARCH ON HUMAN EMBRYO/FOETUS

Did the project involve Human Embryos?	No
Did the project involve Human Foetal Tissue / Cells?	No
Did the project involve Human Embryonic Stem Cells (hESCs)?	No
Did the project on human Embryonic Stem Cells involve cells in culture?	No
Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	No

PRIVACY

Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	Yes
Did the project involve tracking the location or observation of people?	No

RESEARCH ON ANIMALS

Did the project involve research on animals?	No
Were those animals transgenic small laboratory animals?	No
Were those animals transgenic farm animals?	No
Were those animals cloned farm animals?	No
Were those animals non-human primates?	No

RESEARCH INVOLVING DEVELOPING COUNTRIES

Did the project involve the use of local resources (genetic, animal, plant etc)?	No
Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	No

DUAL USE

Research having direct military use	No
Research having potential for terrorist abuse	No

C. Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	1	1
Work package leaders	3	7
Experienced researchers (i.e. PhD holders)	36	37
PhD student	4	4
Other	31	18

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	3
Of which, indicate the number of men:	2

D. Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project ?	Yes

6. Which of the following actions did you carry out and how effective were they?	
Design and implement an equal opportunity policy	Effective
Set targets to achieve a gender balance in the workforce	Effective
Organise conferences and workshops on gender	Effective
Actions to improve work-life balance	Effective
Other:	

7. Was there a gender dimension associated with the research content - i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?	Yes
If yes, please specify:	gender related analyses on biomarker levels according to cardiovascular disease, cardiovascular risk was modeled gender specific

E. Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?	Yes
If yes, please specify:	aggregated harmonized data has been used as an example in undergraduate training

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?	No
If yes, please specify:	

F. Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?	
Main discipline:	3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)
Associated discipline:	3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

Associated discipline:	1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

G. Engaging with Civil society and policy makers

11a. Did your project engage with societal actors beyond the research community? (if 'No', go to Question 14)	Yes
11b. If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?	Yes - in determining what research should be performed
11c. In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	No
12. Did you engage with government / public bodies or policy makers (including international organisations)	Yes - in implementing the research agenda
13a. Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?	Yes - as a secondary objective (please indicate areas below - multiple answer possible)
13b. If Yes, in which fields?	
Agriculture	No
Audiovisual and Media	No
Budget	No
Competition	No
Consumers	No
Culture	No
Customs	No
Development Economic and Monetary Affairs	No
Education, Training, Youth	No
Employment and Social Affairs	No
Energy	No
Enlargement	No
Enterprise	No
Environment	No
External Relations	No
External Trade	No
Fisheries and Maritime Affairs	No

Food Safety	No
Foreign and Security Policy	No
Fraud	No
Humanitarian aid	No
Human rightsd	No
Information Society	No
Institutional affairs	No
Internal Market	No
Justice, freedom and security	No
Public Health	Yes
Regional Policy	No
Research and Innovation	Yes
Space	No
Taxation	No
Transport	No
13c. If Yes, at which level?	International level

H. Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	24
To how many of these is open access provided?	11
How many of these are published in open access journals?	11
How many of these are published in open repositories?	0
To how many of these is open access not provided?	6
Please check all applicable reasons for not providing open access:	
publisher's licensing agreement would not permit publishing in a repository	Yes
no suitable repository available	No
no suitable open access journal available	No
no funds available to publish in an open access journal	Yes
lack of time and resources	No
lack of information on open access	No
If other - please specify	
15. How many new patent applications	0

('priority filings') have been made? ("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).	

16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	
Trademark	0
Registered design	0
Other	0

17. How many spin-off companies were created / are planned as a direct result of the project?	0
Indicate the approximate number of additional jobs in these companies:	0

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:	Difficult to estimate / not possible to quantify, None of the above / not relevant to the project

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (FTE = one person working fulltime for a year) jobs:	0Difficult to estimate / not possible to quantify

I. Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?	No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?	No

22. Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?	
Press Release	Yes
Media briefing	Yes
TV coverage / report	No
Radio coverage / report	Yes
Brochures /posters / flyers	Yes
DVD /Film /Multimedia	No
Coverage in specialist press	Yes
Coverage in general (non-specialist) press	Yes

Coverage in national press	Yes
Coverage in international press	No
Website for the general public / internet	Yes
Event targeting general public (festival, conference, exhibition, science café)	Yes

23. In which languages are the information products for the general public produced?	
Language of the coordinator	No
Other language(s)	No
English	Yes

Attachments	F_WP7_Table1.pdf
Grant Agreement number:	278913
Project acronym:	BiomarCaRE
Project title:	Biomarker for Cardiovascular Risk Assessment in Europe
Funding Scheme:	FP7-CP-FP
Project starting date:	01/10/2011
Project end date:	31/03/2016
Name of the scientific representative of the project's coordinator and organisation:	Prof. Stefan Blankenberg UNIVERSITAETSKLINIKUM HAMBURG-EPPENDORF
Name	
Date	31/05/2016

This declaration was visaed electronically by BiomarCaRE COORDINATOR (ECAS user name ncoordbi) on 31/05/2016