Executive Summary:

Fighting Aneurysmal Disease (FAD, HEALTH-2008-200647) project aims to better understand the mechanism of dilation remodelling in the progression of aneurysm toward rupture, from molecules and cells, in order to develop new diagnostic and therapeutic tools in this disease, linked or not to aging. By nature, FAD is a translational project starting from objective interdisciplinary observations of aneurysms, using updated technological approaches, and applying to new tools for diagnosis and treatments. I order to progress in these ways, FAD limited its field of investigations to aneurysms of the aorta in human, including both aneurysms of the abdominal aorta (AAA) of atherothrombotic origin and aneurysms of the thoracic ascending aorta (TAA) of non-atheromatous origin. AAAs summarize some of the different components of atherothrombotic disease progression, including intraluminal thrombus formation and innate and adaptive immunity, whereas TAAs retain several aetiologies, including monogenic diseases, illustrated by Marfan syndrome in young patients, association with bicuspid aortic valves, and degenerative form with aging, leading to monomorphic pathologies: progressive dilation or acute intramural rupture (dissections). Whatever the location, aortic aneurysmal diseases are characterized by proteolytic injuries of the arterial wall, due to both, interactions between individual genetic background and environmental conditions, particularly local hemodynamic specificities, and circulating blood components and specific biological properties of the arterial wall itself. These general functional genomic and pathophysiological concepts lead to the development of new diagnostic tools, including both new biological biomarkers of the diseases, and new functional and molecular imaging tools. FAD was constituted of fifteen partners distributed in twelve EU countries, and structured in five scientific workpackages (WP):

1) clinical database and biological biobanking,
2) genetics and functional genomics (from genes to phenotypes),
3) pathophysiology (from phenotypes to molecular determinants),
4) diagnosis, including biological genomic and circulating biomarkers, and in vivo imaging,
5) therapeutic developments.

Each partner and each WP contributed a lot to the success story of FAD. Partners participated to the constitution of diverse EU clinical subdatabases and rich biological collections involving DNA and plasma collections, but more originally, tissue and cell preparations and collections. These collections are hosted by some partners and shared at a EU level. Clinical database generated new epidemiological informations, including gender aspect (WP1); and Genetic approaches (WP2) lead to the discovery of new genes involved in monogenic forms of TAA and in genetic susceptibility to AAA. Functional genomic approaches lead to the discovery of a new epigenetic phenomenon specific to smooth muscle cells in TAAs. Pathophysiology (from phenotype to cell and molecular determinants, WP3) focused on the role of IntraLuminal Thrombus as an active determinant of the progression of AAAs, and of transmural convection of plasma zymogens and fibronectin modification of expression as active determinants of TAA progression. These determinants are directly related to biomechanics, including biomechanics of the arterial wall and hemodynamic in the aneurysmal sac. Important progresses were also achieved in diagnosis (WP4) including discovery of numerous new circulating biomarkers of ILT proteolytic, oxidant and pro-inflammatory activities in AAA and genomic biomarkers of TAA evolution. In parallel new contrast agents for molecular imaging of thrombi and proteolysis have been developed at a preclinical level, and original clinical investigations was performed using functional approaches of phagocytosis and adventitial inflammation and AAA, and of arterial wall motion in TAA. Lastly medical preventive approaches and new interventional tools have been proposed at a preclinical level as future prospective therapies in human (WP5). The scientific and medical achievements of the FAD period and the follow-up of works initiated in FAD,

will impact both aortic aneurysms and other vascular diseases, including cerebral aneurysms, arterial dissections, other occlusive forms of atherothrombotic diseases, aortic valve pathology, etc

Project Context and Objectives:

Aneurysms of the aorta, including abdominal (AAA) and thoracic (TAA) aortic aneurysms are important health question, in part due to the progressive aging of the EU population.

Epidemiological and clinical context:

AAA occurs in up to 9% of adults older than 65 years of age, causing about 1-2% of male deaths in Western countries1. Early detection and elective AAA repair represent the main aspects to limit the mortality rate from AAA rupture. Although cost effectiveness of AAA screening was shown to be attractive2, the benefit of early detection of AAAs is limited because early repair of small AAA has been demonstrated to be inefficient and there is currently no established treatment for small AAAs3. On the other hand, the indication for elective repair is based upon the maximal diameter of AAA above 5 to 5.5 cm. The diameter of AAA is a surrogate marker of the growth rate that reflects the magnitude of the degenerative process in the vascular wall and infrarenal aortic diameter is an indicator of AAA disease, but is also an independent marker of all-cause mortality, mainly related to cardiovascular disease4. But AAA progression towards rupture is not linear, but usually presents points of acceleration which can appear at any time5, 6. Conversely, aortic dilatations can remain stable and asymptomatic for many years during which aged patients may die of other causes. In women, AAAs are rarer, and smaller, but represent a higher relative mortality than men7. Therefore, if AAA dimensions are canonically used as endpoints for AAA evaluation and treatment, numerous other biomarkers of risk, related to biological activities associated with AAA expansion remain to be discovered in order to ameliorate the prevention and the timing of interventional treatment.

In contrast prevalence of aneurysms and dissections of the thoracic ascending aorta (TAA) is less than AAA but is increasing due, at least partially, to the aging of the population and has recently reached a new case incidence of 10/100 000 person-year8. The clinical epidemiology and nosology of TAA have been recently reviewed9, 10. TAAD rupture has a mortality rate of 97%, and a median survival rate of 3 days11. However, epidemiological data are limited because of the often acute nature of the disease, the high mortality rate, and the absence of a precise diagnosis. TAA prevalence increases with aging of the population8and shares common risk factors with other common arterial diseases, including high blood pressure, smoking, and high physical stress12. The clinical epidemiology of diagnosed TAAD conforms to a trimodal Gaussian distribution of the disease with age, corresponding to three classes of aetiology (monogenic, associated with bicuspid aortic valves, degenerative) leading to a common pathology of dilation and/or dissection of the ascending aorta.

Pathological context:

AAA is a particular, specifically proteolytic and localized form of atherothrombotic disease, initiated by wall lipid retention, sharing the usual risk factors with occlusive atherothrombosis: male gender, aging, possible genetic susceptibility and dyslipidemia. Aging and smoking is the major risk factor in AAA. Among lipid markers, low HDL level is the most sensitive predictor of AAA13. This could be related to the impact of hypercholesterolemia on the initial step of atheroma in aorta, and to the low levels of 1-antitrypsin conveyed by HDL in human AAA14. The presence of AAA in a patient is a marker of atherothrombotic disease elsewhere15, and aortic diameter a predictor of total and cardiovascular mortality16. AAA is characterized by degradation of the media extracellular matrix, the smooth muscle cell disappearance, the presence of a chronic intraluminal thrombus (ILT), and the association with a significant adventitial reaction17. The ILT is a biologically active neo-tissue described as a laminated structure, containing several layers of fibrin clot, underlying a fresh, hematic

and fibrin-rich luminal, and an actively fibrinolyzed abluminal layer. ILT is a dynamic biological balance between clotting at the luminal interface with circulating blood and outward progressive lysis, providing evidence of a spatial topology of temporal events (clotting and lysis). The ILT is traversed from the luminal to the abluminal surface by a continuous network of canaliculi, allowing unrestricted macro-molecular penetration18.

TAAs (ascending aorta) are pathologies involving mainly the medial layer not related to initial atheroma. Despite the molecular diversity of the aetiologies, all forms of TAA present a common histopathological phenotype, including localised extracellular matrix breakdown, smooth muscle cell disappearance, and areas of mucoid degeneration, leading to dilatation, dissections and rupture. The presence of areas of mucoid degeneration is the common pathological feature of TAA, suggesting that aneurysms and dissections are manifestations of a common disease of the ascending aorta leading to two different clinical/phenotypic expressions, depending on whether the destruction of the arterial wall is progressive (aneurysm) or acute (dissection). Areas of mucoid degeneration are characterised by the local accumulation of modified acidic glycosaminoglycans, presence of vacuoles (usually termed cystic degeneration, but they are not cysts), disappearance of smooth muscle cells, and localized breakdown of fibrillar matrix, including elastic and collagen fibres19. The spontaneous appearance of medial areas of mucoid degeneration in the ascending aorta with aging20, and the concomitant progressive physiological enlargement of aorta 21, could be one of the links between aging and the development of degenerative forms of TAAD. Therefore, biological events, upstream or downstream to the formation of areas of mucoid degeneration, are probably the driving forces for the development and evolution of aneurysms and dissections in the ascending aorta.

Whatever localisations and aetiologies, arterial wall degradation leading to aneurysm and rupture is mainly influenced by the physiological mass transport and activation of molecules, occuring mainly by radial hydraulic conductance from the lumen to the adventitia through the wall22.

FAD objectives:

The general translational, cognitive, technological and medical objectives of the FAD project, were to decipher new pathophysiological concepts in aortic aneurysmal remodeling, targeting cells and molecules, linking genetics with aneurysmal phenotypes, leading to the development of new tools for diagnosis, prognosis and therapeutics in aneurysmal diseases. The project consisted of a consortium integrating groups working on both AAA & TAA in a large scale European collaborative research project from Bedside to Bench and back.

FAD took into account the general priorities of the FP 7 of improving Health of European citizens, including competitiveness and boosting innovative capacities, emphasizing the translational approach in the 3 dimensions of descriptive, objective and projective epistemology of new knowledge in the field of aneurismal pathology, including validation of new therapies, and promotion of healthy ageing.

The FAD project directly addressed the 3 aspects: pathophysiology, diagnostics and therapeutics, expressed in the EU proposal HEALTH-2007-A-1.2.4.2-2: Vascular remodelling in aneurysmal disease, through the 4 workpackages 2-3-4-5, using the WP-1 (human database) as the main translational tool for assessing the objectives. FAD responds to these three aspects by focusing specifically on expansive arterial remodelling, involving both cell and matrix functions and interactions; and exploring both genetic determinants and relevant pathophysiological phenotypes of this specific arterial wall remodelling, and then developing diagnostic and therapeutic applications.

The FAD project integrated the three epistemological dimensions of translational research for medical progress applied to aneurysmal pathology:

- The observation of human disease including the nosological definition of aneurysm, risk factors, clinical epidemiology, and public health concerns.
- The projection of the observed molecular diversity to application to human aneurysms including functional genomic approaches in vitro through molecular and cell biology, and in vivo through experimental models in murine.
- The search for new diagnostic and therapeutic targets through bedside to bench objective research on human pathology, including: genetic exploration of new determinants of susceptibility to aneurysms, human tissue collections, transcriptomic and proteomic methods applied to human tissue and cell biology derived from human tissue.

Translational objectives of FAD included

- The genetic objectives to identify new susceptibility genes for TAA and AAA, and new mutations responsible for familial forms of TAAs (WP2).
- The genomic objectives to rely the observed mutations to the aneurysmal phenotype at a molecular, cellular and tissue levels (WP2).
- The first pathophysiological challenge was to resolve the question raised by the duality between the etiological molecular diversity of TAAs and the phenotypic monomorphism of aneurysm or dissections of the ascending aorta, including VSMC disappearance, areas of mucoid degeneration, and finally extracellular matrix breakdown leading to dilation and/or intraparietal rupture (WP3).
- The second one was to further decipher the spatiotemporal pathophysiology of AAA, including the rheological mechanism of formation of the Intra Luminal Thrombus (ILT) and the role of ILT in the degradation of the aortic media, and the impact of these phenomenons (ILT and media degradation) on the innate and adaptive immunity developed in the adventitia (WP3).
- The search, development and validation of new circulating biomarkers of TAA and AAA progression towards rupture, mainly by using proteomic approaches (WP4).
- and of new functional and molecular imaging tools of predictive value of TAA and AAA progression (WP4).
- The preclinical proof of concept of new medical therapeutic approaches in the prevention of aneurysm development and growth and preclinical search for new devices, biomaterials, cell and gene therapies for interventional treatments of aneurysm (WP5). This WP may open new avenue in original clinical trials in AAA and possibly in TAA (clinical trials are not included in FAD).

The achievements of these objectives was supported by different tools including human clinical database and biological collections shared by numerous partners (WP1), by experimental models in animals, and by further developments of know-how in human genetics & genomics, molecular and

cell biology, protein biochemistry and back to diagnostic for validation and to therapeutics for applications.

These tools were used in respect with ethic's concerns, including personal ethic of researchers and medical doctors, human subject protections in medical research and limitation of animal use and animal welfare (3Rs)

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Project Results:

Fighting Aneurysmal Disease (FAD, HEALTH-2008-200647) was a translational project, aiming to better understand the mechanism of dilation remodelling in the progression of aneurysm toward rupture, from molecules and cells, in order to develop new diagnostic and therapeutic tools in this disease, linked or not to aging.

WP1. Human clinical and biological databases

Objectives

1. To establish, a scientifically and ethically, robust EU web-based database for aortic aneurysms
2. To use the databases to describe for AAA and TAA the incidence and prognosis following diagnoses and operation of AAA and TAA.
3. To give support to clinical investigations in different aspects of aneurismal pathology including biomarkers in the follow-up of aneurysm progression and treatment, correlation to functional imaging, and therapeutic trials.
4. To standardize the European methodologies
5. To exchange data and biomaterials
6. To allow progress of the other WPs: 2, 3, 4, 5, through the use of these sample banks.

Achievements:

Creation of the FAD-database

The creation of the web based database was initially delayed due to summer holiday season in Denmark. In august 2008, an airport meeting in Copenhagen was held between JB Michel, Per Eriksson and Jes Lindholt, in order to prepare the plan and discussion of the database at the Kick off meeting in Liege in September. At that meeting, the variables of the database were decided and standards for some of the key variables as aortic diameter, systemic blood pressure measurement, and ankle brachial systolic blood pressure index (ABI) were agreed together with standards for biobanking. In addition, a none-planned additional subdatabase was agreed upon, in order to supply the consortium with matched controls without aortic aneurysms.

In October 2008, the building started in collaboration between Jes Lindholt, Per Erikson, and an experienced private company serving most public hospitals and research institutions in Denmark due to high quality for low costs. Ultimo November 2008, the database was ready for validation by for some FAD members and clinicians through a demo-database (https://faddemo.opusconsult.dk), which later worked as a training database before doing entries to the real FAD-database (http://www.fad-database.dk)

The database were then revised, and introduced to the Government Board of FAD at the Board meeting in Paris, the 9th of January. This introduction caused further revision, by the 27th of January the database was finished, and released for data entry together with Case Report Forms (See appendices).

The FAD Database Management Board.

A FAD Database Management Board consisting of participants from all partners involved in the database was created at the Liege meeting in order to secure ethical standards and individual partners properties are respected, when transfer of data among partners are requested. The board includes representation from project participants, a statistician and biologist, with appropriate representation of ethnic groups and women.

Each partner can get access to own data, but not other partners' data. Only the Work package 1-leader, Jes Lindholt, can get such access to all data from all participants. Requests of data from other partners are only executed, if accepted by this Board.

The Board members are responsible for their own data including security of the anonymity of cases, validity including that the FAD standards are fulfilled, as well as the administration of users to type in data. Board members and their users are given user names and passwords by the Work package 1-leader, Jes Lindholt, through an established additional consol attached to the database.

Each partner has actually its own database, and defines by himself or herself which ones to be allowed access. All patients are given a database record number automatically at entry, and a Center patient ID number is typed in - this number can be a number which can be referred to the patient for follow up etc, The local administrator can extract these, but they must not be transferred to other partners. This is managed by computing the database not to give Jes Lindholt this number when he

- the only one to be allowed by the database to extract data from other partners
- extract data from other partners, but the database case record number is given. This allows the delivering partner to identify the patient in order to merge other data to the extract because his local extract includes both numbers. Consequently, anonymous data can be transferred from partner to partner from country to country.

Consequently, the FAD database has been organized as intranet private computer connectivity within the consortium to securely share FAD information concerning clinical data available in the different partner centers. The FAD database is implemented, as a Microsoft.NET web application running in a standard web browser, and no installation are required to run the application at client side. The database behind the web application is implemented as a Microsoft SQL2005 database. The web and database server are hosted at a secure location at the University of Aarhus in Denmark. The server room is secured with key cards and armoured windows. The server is protected with firewall, disk mirroring, UPS and redundant power supply. The server is backed up daily and the backup is stored off site in encrypted format.

The FAD database

The FAD database consists of 5 subdatabases, which is one more than planned initially. This fifth subdatabase is for allowing controls to be entered. The other four is

(i) to characterise the phenotype of rapid AAA expansion and need for repair in European of progressive aneurysmal disease as indicated by the late development of endoleaks after endografting;

(ii) to characterise the phenotype of the ultimate termination of AAA - its rupture -

(iii) to characterise the phenotype of genetic causation, from European families with TAA and AAA (with WP2), and to allow age and sex matched controls, known to be with aneurysm.

Finally, familiar disposition for AA is well known but the causality is mainly unknown. Consequently, information concerning familiar disposition is recorded in each subdatabase, so familiar cases can easily be identified (Figure 2).

The webdatabase has it´s own web site (http://www.FAD-database.dk) which can be linked to from the official web site of the FAD project (http://www.fighting-aneurysm.org), but also directly from a personal computer. However, no one can enter without a user name and password. Using this, the user is entering the part of the database belonging to that specific partner (Figure 3). Then an identification number of the patient must be entered. This number locates the patient's previous entries, and thus allows follow up data to be entered. If entered for the first time, some baseline data are requested including choice of one of the three groups: controls, AAA or TAA. After this choice, baseline variables are entered and saved or submitted. If submitted, the data cannot be changed. After saving or submitting baseline data, the possibility of entering follow up data pops up. After saving or submitting follow up data, a second follow up possibility is created etc.

For practice and to experience, how it is working, a demo database has also been created. This demo database can be inspected at http://www.opusconsult.dk/demo/fad and data extraction at http://www.opusconsult.dk/demo/fad/DataExport.aspx.

Everyone can log on the demo-database. One has to simulate that a case is entered. The real database has been established at another location, and access to the demo was maintained after the final version is released the 2th of February.

In order to facilitate as smooth data collection and data entry, 8 case report forms have been created concerning all parts of the database: baseline variable CRFs for all five subdatabases, and 3 follow up CRFs for small AAA, large AAA and AAA treated with EVAR. These CRFs have be constructed as simple as possible with a nice lay out, in order to make easy recording at the clinicians, and easy type in by the users. In this way, data quality is optimised. The CRFs also informs of the possibilities for each variable, so selection can happen easily. In addition, the back side of the CRF is used for more detailed information of some variables, and relevant FAD standards. An example is shown in Appendix 1, but all are available at the FAD intranet.

Consequently, the five FAD databases are now useable to support clinical investigations in different aspects of aneurismal pathology including biomarkers in the follow-up of aneurysm progression and treatment, correlation to functional imaging, and therapeutic trials.

Standardized European methodologies for contribution to the clinical database, and standardized sample banks of plasma, DNA, cells & tissue associated with the database have been essential to

create in order to make it possible to merge data and biomaterials from several partners. Consequently, methodologies have been standardized concerning methods and intervals for AAA population screening, techniques for provision of DNA and criteria for diagnosis and analysis of endoleaks. Population screening are standardized according to current British and Danish methodologies and this will be applied to other EU and non-EU countries, which are starting population screening (eg Iceland). Standards for biomaterial has been formulated (Appendix 3), and standards for reporting key variables as aneurismal size and endoleak has been proposed by relevant experts, and agreed upon at the kick off meeting in Liege. For instance, the screening procedure for AAA is: The aorta is identified by a longitudinal view, and visualised from as proximally as possible to below the bifurcation. In case of no dilatation, the right-angled anterior-posterior (AP) diameter is measured 2 cm proximally for the bifurcation. In presence of a localized dilatation, the right-angled maximal anteriorposterior diameter will be measured. Digital documentation or thermo-prints are made to demonstrate the visualised morphology and where the AAA was measured for later reproduction, when the patients attend follow up scans. Measurements will be performed in an inner to inner way, from plaque to plaque, if possible, in order to prevent variations in luminal-intramural-extramural measure points and sources of error. The luminal source of error will be the presence of a mural thrombus, and the extramural source will be the anterior longitudinal ligament or the vertebras themselves. All positive findings are being rescanned by a doctor responsible for the surveillance of the screening. In this way the numbers of observers will be kept at a minimum. Annual control scans will be performed of AAAs below 5 cm in maximal AP diameter, and biannually for AAA above 5 cm in maximal AP-diameter. If the AAA exceed 5.5 cm in maximal diameter, or becomes symptomatic, referral for surgical evaluation will be done. Performed operations including indication will be recorded.

In addition, familial cases of AAA, defined as one or more first degree relatives with AAA, will be recorded.

Another example is a standard systemic blood pressure measurement: An appropriate cuff size must be chosen, as well as a calibrated automatic device. After 5 minutes at rest in a quite room, 3 readings at 1 minute intervals are performed on both arms. The average of the 2nd and 3rd measurement at the limb with the highest pressure is noted.

A third example is determination of distal systolic blood pressure: The Doppler ultrasound head is placed over the artery, and when a sufficient signal was obtained, the cuff is inflated until the signal disappeared. Pressure is gradually decreased until the signal re-appeared. If this pressure agreed with the pressure noticed during inflation, this pressure was noted as the systolic pressure of the artery. Otherwise, the procedure is repeated. The pressure is measured bilaterally in the dorsal pedal artery and the posterior tibial artery. All measurements are done twice. The average pressure of positive measurements is recorded as the systolic ankle blood pressure. The brachial systolic blood pressure is simultaneously with the ABP measurements.

Consequently, the essential task given to create this huge database with associated biobanks, so data and biomaterials can, when necessary, be merged and exchanged between groups within the consortium has been completed.

Today (june 2012), a total of 6404 Case Report Forms (CRF) have been entered in the EU database. The repartition between partners and subdatabases are presented in the table (see attachement : DOCUMENT 1)

AAA and TAA the incidence and prognosis following diagnoses and operation of AAA and TAA

One of the aims for WP1 were to use the data from the four subdatabases to describe the incidence and prognosis following diagnoses and operation of AAA and TAA. This has occurred in especially Sweden and Denmark with these publications so far:

P4. Karolinska:

1. Larsson E, Vishnevskaya L, Kalin B, Granath F, Swedenborg J, Hultgren R. High frequency of thoracic aneurysms in patients with abdominal aortic aneurysms. Ann Surg. 2011;253:180-4.

2. Hultgren R, Forsberg J, Alfredsson L, Swedenborg J, Leander K. Regional variation in the incidence of abdominal aortic aneurysm in Sweden. Br J Surg. 2012;99:647-

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P8. Viborg:

4.Lindholt JS, Norman PE. Meta-analysis of postoperative mortality after elective repair of abdominal aortic aneurysms detected by screening. Br J Surg. 2011 May;98(5):619-22.

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The registries of Denmark combined with screening databases in Viborg have supplied data for the most advanced health economical modeling for screening for AAA including rescreening, recently published in BMJ after more than two years of modeling. The extensive data used are presented in a substantial technical report, and parts have been published as individual papers (ref 4, 5, 6 & 7 above). The model is planned so far also be used to evaluate cost effectiveness of screening for AAA in Liege (Partner 6), cost effectiveness of screening for AAA for older woman and as a part of a PhD project of familiar aneurysms in Denmark (see below)

8. Grondal N, Sogaard R, Henneberg EW, Lindholt JS. The Viborg Vascular (VIVA) screening trial of 65-74 year old men in the central region of Denmark: study protocol. Trials. 2010 May 27;11:67.

An ongoing randomized PhD project concerning vascular screening for AAA, PAD and hypertension

Assessing family history and present medication as a risk factor

Another supplemental aim for WP1 was in large case-control studies, utilizing the Swedish and Danish nation wide registries/ Family history.

In Sweden (P4), the nationwide registries of all in-hospital care (IPR=in patient registry) and the cause of death registries will be used to identify all persons diagnosed with, operated for, or having died from AAA in Sweden. For each of these cases, controls are randomly selected from the population; the controls are matched according to birth year, sex and region of residence. This data set will be linked to the Swedish Multigeneration registry in order to identify first-degree relatives of cases and controls. Subsequently, these families will be linked to IPR and the cause of death registry to identify family history of AAA.

Results during Jun 2008 to December 2009: All persons (3183) born after 1932, diagnosed with AAA between 2001 and 2005, and a random selection of 15,943 age-, gender-, and region-matched controls were included. First-degree relatives of cases and controls were identified via the Multigeneration Register. Family history of AAA for cases and controls was assessed by linking the relatives to the Hospital Discharge Register and Cause of Death Register. The data were analyzed by conditional logistic regression. The overall relative risk of AAA associated with family history compared to no family history was 1.9 (95% confidence interval [CI] 1.6-2.2). Comorbidities were more common among the cases than the controls ($P < .0001$) but the relative risks remained unchanged after adjustment for comorbidities. Stratification for absence or presence of comorbidities showed no significant difference between the two groups ($P = .29$). The relative risk of AAA for first-degree relatives was similar for women and men ($P = .22$ for gender differences), ie, the relative risk of AAA was not dependent on the gender of the index person. In conclusion, in this nationwide survey, the relative risk of developing AAA for first-degree relatives to persons diagnosed with AAA was approximately doubled compared to persons with no family history. Neither the gender of the index person nor the first-degree relative influenced the risk of AAA (partner 4, KI, submitted).

In a second study, the role of hereditary and environmental factors to development of AAA in a large population-based sample of twins was studied. The Swedish Twin Registry, containing data on twins born in the country since 1886, was cross-linked with the Inpatient Registry, providing national coverage of discharge diagnoses coded according to the International Classification of Diseases (ICD). All twins with an infrarenal AAA were identified. Concordance rates and tetrachoric correlations were calculated for monozygotic (MZ) and dizygotic (DZ) twins. Tetrachoric correlations were calculated assuming an underlying normal distribution of liability, with multiple factors contributing additively and a threshold value that discriminates between AAA and no AAA. Higher concordance rates and correlations of liability in MZ twins than in DZ twins suggest that genetic factors influence disease development. Structural equation modeling techniques, Mx-analyses, were used to estimate the contributions of genetic effects as well as shared and non-shared environmental factors for development of AAA. There were 172890 twins registered at the time of the study including 266 twins (81% men; mean age 72 years, range 48-94) with AAA. There were 7 MZ and 5 DZ concordant pairs as well as 44 MZ and 197 DZ discordant pairs with AAA. The probandwise concordance rates for MZ and DZ pairs were 24% and 4.8%, respectively. The tetrachoric correlations were 0.71 in MZ pairs and 0.31 in DZ pairs. In the structural equation models, genetic effects accounted for 70% (95% CI: 0.33-0.83), shared environmental effects for 0% (95% CI: 0-0.27), and non-shared environmental effects for 30% (95% CI: 0.17-0.46) of the phenotypic variance among twins. In conclusion, robust epidemiological evidence that heritability contributes to aneurysm formation is provided. Concordances and correlations were higher in MZ compared with DZ twins, indicating genetic effects. A heritability of 70% of the total trait variance was estimated. The

remaining variance was explained by non-shared environmental factors with no support for a role of shared environmental influences

1.Wahlgren CM, Larsson E, Magnusson PK, Hultgren R, Swedenborg J. Genetic and environmental contributions to abdominal aortic aneurysm development in a twin population. J Vasc Surg. 2010 Jan;51(1):3-7; discussion 7.

2: Larsson E, Granath F, Swedenborg J, Hultgren R. A population-based case-control study of the familial risk of abdominal aortic aneurysm. J Vasc Surg. 2009 Jan;49(1):47-50; discussion 51.

In Iceland (P15), generation of a population-based screening of AAA will be initiated by high risk screening. High risk screening will be aimed at; a) 1st and 2nd degree relatives of AAA patients diagnosed individuals since 1958 b) 1st and 2nd degree relatives with PAD patients diagnosed since 1980 c) smokers d) old individuals. However, DeCODEs recruitment of first-degree relatives of AAA patients and high-risk screening has been delayed for practical reasons. The main emphasis of deCODE was placed on new AAA samples already recruited from the Netherlands that became available. This allowed for faster SNP chip genotyping of new samples with the aim of identifying earlier new AAA risk variants as outlined in WP2. A renewed consent form and questionnaire for the recruitment study has been submitted to the Icelandic bioethics committee. Relatives to be called in for screening have been defined using the deCODE genealogy database and recruitment started in January 2010 and ended in 2012. Datanalysis is ongoing.

In Denmark (P8), more than 25,000 men aged 65-74 years old have been invited to population-based screening for AAA, more than 18,000 attended. Attenders fulfilled a questionnaire including a family history of AAA, risk factors, quality of life a.o. In Janury 2012, a PhD project was started in order to study the natural history of familiar AAA, and characterize normals with positive family history. The population based screening study will be complemented with a population based case-control study of more than 2,000 incidently diagnosed AAA.

RESCAN collaborators (Sweeting MJ, Thompson SG, Brown LC, Powell JT, Thompson SG, Powell JT, Gotensparre S, Brown LS, Sweeting MJ, Bown M, Buxton MJ, Glover M, Kim L, Greenhalgh R, Naylor R, Hartshorne T, Fowkes FJ, Norman PE, Parvin S, Ashton H, Chalmers R, Earnshaw JJ, Wilmink AB, Scott JA, McCollum CN, Solberg S, Ouriel K, Laupacis A, Vega de Ceniga M, Holdsworth R, Karlsson L, Lindholt JS). Meta-analysis of individual patient data to examine factors affecting growth and rupture of small abdominal aortic aneurysms. Br J Surg. 2012 May;99(5):655-65.

Individual data were collated from 15 475 people under follow-up for a small aneurysm in 18 studies. The influence of co-variables (including demographics, medical and drug history) on aneurysm growth and rupture rates (analysed using longitudinal random-effects modelling and survival analysis with adjustment for aneurysm diameter) were summarized in an individual patient meta-analysis.

The mean aneurysm growth rate of 2.21 mm/year was independent of age and sex. Growth rate was increased in smokers (by 0.35 mm/year) and decreased in patients with diabetes (by 0.51 mm/year). Mean arterial pressure had no effect and antihypertensive or other cardioprotective medications had only small, non-significant effects on aneurysm growth, consistent with the observation that calendar year of enrollment was not associated with growth rate.

Available biosamples associated or not with clinical databases

As described in the grant agreement, for practical and ethical reasons, the standardized biological collections performed by each partner were prepared and conserved by each partner. The table represents all the biological samples available through FAD partnership (see attachment DOCUMENT 2)

These biobanking activities have included:

- Preparation of the samples involving dissection of the arterial tissue, separation between diseased parts and non or less diseased parts, separation of thrombi, intima, media, and adventitia; direct freezing and secondary cryo-pulverization, preparation of conditioned medium, and enzymatic digestion for preparation of primary culture of human endothelial cells, medial smooth muscle cells, and adventitial fibroblasts, and isolation of leukocytes. This step includes quality controls of the initial samples.

- Storage and Conservation of the samples at -80°C, in a specific area (Biological Resource Center) devoted to this activity

- Cession of the samples for experimental researches within the laboratory and between different laboratories through the FAD partnership and outside. These procedures include the signature of a Material Transfert Agreement and a declaration to the national regulatory authorities.

Therefore access to these collections has opened unique opportunities for translational research, due to both, diversity in localization allowing comparison of different forms and sites of aneurysms in human, and diversity of tissue & cell preparation allowing numerous complementary methodological approaches: histology, biochemistry, human mesenchymal and circulating cell biology, mRNA expression, genetics & epigenetics.

Preparation, performance and use of these biobanks integrate numerous translational innovative methods including preparation of DNA, RNA, and chromatin, proteome secretion and extraction, at both tissue and cell levels, permitting downstream genetic and epigenetic analyses.

These collections are hosted in the Biological Resource Center of the Xavier Bichat-Claude Bernard hospital (BRC-BCB), which have been certified (AFNOR certification 2009/34457), in the Fundacion Jimenez Diaz biobank in Madrid (Spain), and in the Viborg Resource center in Denmark. These biobanks and activities are integrated in national Biobank networks, which are partners of EU BBMRI, Biobanking and Biomolecular Resources Research Infrastructure (http://www.bbmri.eu). BBMRI-ERIC (European Research Infrastructure Consortium) has established a node in each EU country supporting the infrastructure.

WP2. Genetics and functional genomics [from genes to phenotype]

Objectives:

To explore the molecular determinants of aortic dilation in humans through genetic and functional genomics in both humans and mice. Specifically WP2 includes:

- identification of new susceptibility genes for AAA and TAA

- identification of new mutations responsible for familial forms of TAA
- exploring the signalopathy associated with aneurysms
- development of animal models to study candidate genes for TAA and AAA.

Genome-wide/candidate gene case-control study to identify new susceptibility genes for AAA.

Genetic susceptibility studies in humans necessitate large cohorts of patients versus controls. So far, two large genome wide association studies (GWAS) have been performed on AAA, both lead by the FAD consortium. A meta analysis using the results of the different GWAS is currently conducted.

One of the main achievement of FAD in identifying genetic susceptibility to AAA was made by DeCODE (partner 15, ref. 17). Using a GWAS approach, a sequence variant was identified within intron 1 of the DAB2IP gene as a powerful genetic marker of AAA risk. DAB2 Interacting Protein is a member of the RAS-GTPase-activating protein family. DAB2IP suppresses cell survival and proliferation and is expressed by vascular cells, particularly by VSMCs. The identified variant could probably influence directly or indirectly the survival of VSMCs in response to proteolytic injury. Partners 4 (Karolinska), 6 (Liege) and 8 (Viborg) all contributed to this discovery.

- In addition, partner 15, identified two new AAA risk variants located within the LPA gene associate with systemic atherosclerosis and coronary atherosclerotic burden but not with venous thromboembolism. These variants were identified through genotyping and testing of a set of CAD associated SNPs on a large sample set of European AAA case-control samples (Ref Helgadottir A et al. 2012)

A second GWAS was recently conducted by partner 2 (London) and the AAA Consortium. Partners 4 (Karolinska), 8 (Viborg) and 15 (DeCODE) contributed to this discovery (ref AJHG 2011). The study identified the low-density-lipoprotein receptor-related protein 1 (LRP1) as a susceptibility gene for AAA. Interestingly, LRP1 has been shown to have a role in the regulation of MMP9 expression and murine models have demonstrated that LRP1 is essential for the maintenance of vascular wall integrity and that this effect is mediated via PDGF receptor beta and Smad signaling

In the years just prior to FAD, DeCODE identified a locus 9p21 as a susceptibility locus for AAA development and for myocardial infarction. The impact of this genetic susceptibility on clinical, molecular and intermediate phenotypes (functional genomics) has since been investigated mainly by the Karolinska partner (partner 4), establishing a relation of this locus with arterial rigidity (ref. 98) and with the expression of several genes within the locus (ref. 14). Several studies are now in progress in this field of research (see the third periodic report for more details)

Identification of new mutations responsible for familial forms of TAA

In contrast to AAAs, a significant fraction of pathologies of the ascending aorta, including aneurysms (TAA) and dissections (TAD), are determined by single mutations in one specific gene. Several mutated genes have been identified as being directly responsible for TAAD, including both syndromic forms, of which Marfan syndrome is the most frequent, and familial forms. This approach of monogenic determinants necessitates the recruitment of informative families by clinicians. Numerous

genes have now been identified: fibrillin (FBN1), SMC myosin (MYH11), actin (ACTA2), TGFBReceptors, SMAD3, a glucose transporter (GLUT10), etc. FAD partners 3 (Gent) and 1 (Inserm) actively participated in the discovery of these genes prior to FAD (background work). New genes have been identified by FAD participants during the last four years. In particular, partner 1 (Inserm) recently identified a mutation in TGF-2 as responsible for a mild form of Marfan syndrome (ref. 150). New informative families have been identified (ref 40), leading to the identification of new genes responsible for FTAA. Also partner 16 has collected several interesting FTAA families suitable for gene identification. (ref 125). At this moment whole exome sequencing is performed using the powerful Next Generation Sequencing approach, on many FTAA families collected during the FAD project. We can assume that this will reveal in the near future many new genes. The important added value of all these findings clearly shows that the extracellular matrix exert not only a structural function but that besides its mechanical role in providing strength and support to the tissues, it also acts as a reservoir for cytokines and growth factors implicated in cellular proliferation, differentiation, migration and survival and therefore has an important regulatory function in the development and homeostasis of body organs and tissues. It is clear that these new insights open perspective to plausible therapeutic approaches for this group of aorta related disorders as it is proposed that treatments which decrease TGF expression, as angiotensin II antagonist, can attenuate of prevent the phenotypes.

In this field of monogenic aortic disease, numerous studies of FAD partners have been published, exploring the genotype/phenotype relationship in order to explain the phenotypic variability of these monogenic forms, particularly focusing on the clinical expression of mutations and their relation to haplo-insufficiency or dominant negative effects. These studies have been published during FAD: ref 51, ref 165, ref 75, ref 9, ref 29: common semiology and different clinical expressions of FNB-1, TGFR and MYH11 mutations, significance of polymorphisms and mutations in currently known genes, impact of facial features, CV risk in the FNB1-mutated population, importance of associated dissections of the descending aorta, neonatal Marfan syndrome. Numerous studies are progressing in this field (for more details see the third periodic report). These studies have been mainly performed by partner 3 (U. Ghent), partner 1(Inserm Paris), and partner 5 (La Charite, Berlin).

TGF signalopathy in TAA (functional genomics)

One major question arising from genetic studies in TAA is how does the molecular diversity in aetiology lead to aortic aneurysms and/or dissections which are all very similar histopathologically. Rapidly it appeared that although diverse mutations in TGF-pathways were important genetic determinants of monogenic forms of TAA, mutations were not limited to this pathway (MYH11, GlUT10, ACTA2), and that changes in expression of TGF-ß molecules, mainly Smad2, were common to all forms of TAA, including forms not genetically detemined.

Alternatively spliced extra domain A (EDA) of fibronectin plays an essential role in tissue repair under the control of the TGF signaling pathway. Partner 4 (KI) has analyzed the expression of fibronectin spliceforms in dilated and non-dilated ascending aorta of tricuspid (TAV) and BAV patients. EDA expression was increased in dilated aorta from TAV patients compared with non-dilated aorta. In contrast, EDA expression was not increased in dilated aorta from BAV patients. The expression of EDA correlated with maximum aortic diameter in TAV but not in BAV patients. TGF treatment influenced the splicing of FN and enhanced the formation of EDA-containing FN in cultured medial VSMCs from TAV patients but not in VSMCs derived from BAV patients. Gene set

enrichment analysis suggested that differences in TGF signaling may explain the impaired EDA splicing in BAV patients (Partner 4).

Partner 1 (Inserm Paris) has investigated the signaling pathway of TGF 1 and its intracellular mediators, the Smad family, in genetic and non-genetic aneurysms of the ascending aorta (ref). A first study demonstrated dissociation between TGF and Smad2 and a second showed an epigenetic regulation of Smad2 constitutive overexpression in VSMCs of aneurysmal wall. The study was performed on human thoracic aneurysms and normal aortas using both medial tissue extracts and primary cultures of SMCs. It was shown that the increase in histone acetylation (H3K9/14ac) is specifically localized on the Smad2 promoter upstream to its first Transcription Start Site. The acetylation process is associated with an increase in Histone Acetyl Transferase (HAT) activity, an opening of the chromatin and the binding of new transcription factors. Partner 1 will further explore the molecular mechanism regulating this overexpression, downstream to the histone acetylation, and the consequences of this up-regulation on protease/antiprotease expression in TAA and dissections. These studies, demonstrating for the first time a specific epigenetic regulation, profoundly modifying the pattern of VSMC gene expression and thereby the constitutive phenotype of the VSMCs, open up new perspectives in the understanding of VSMC physiology in human vascular diseases.

Functional genomics in transgenic mice

Work involving transgenic mice and zebrafish has also been initiated by different FAD partners. The studies of animal models with aortic abonormalities suggested an important role for TGFBeta in the pathogenesis of those disorders. Fbn1 deficient mice as well as the Slc2a10 deficient zebrafish, revealing the arterial tortuosity phenotype, showed each a clear aberrant TGFBeta signalling. To further explore the relationship between the up or down regulation of TGFBeta and the phenotypical characteristics of the disorders partner 3 developed a tgfbr1 knock-in mouse model as well as a transgenic mice for the vascular type of EDS. This latter model spontaneously developed at two months open wounds starting in the shoulder area and penetrating skin and subdermal tissue. These skin lesions evolve fast and also fragility of the arterial vessels is noticed. Partner 3 also knocked down the slc2a10 gene in zebrafish to further investigated the pathogenic mechanisms causing arterial tortuosity. (Ref. Willaert et al. HMG, 2011).Partner 1 developed a different Col3a1 transgenic knock-out mice model (Ehler-Danlos) in order to study the vascular phenotype. At the same time partner 1 developed a myh11 mouse model. These transgenesis approaches were initiated during FAD, and mechanical, functional and clinical studies are currently underway.

WP3. Pathophysiology [from phenotype to molecular determinants]

Objectives:

To explore molecular and biomechanical determinants of aortic dilation and rupture in order to identify new targets for diagnosis and treatment of TAA and AAA. WP 3 includes:

1) Identification of new molecular and cellular targets beyond proteases in TAA and AAA, focusing on the involvement of mucoid degeneration in TAA and on the luminal thrombus as a conveyor of protease activities, pro-inflammatory mediators and neo-antigens in AAA.

2) The inflammatory response to proteolytic injury in AAA, in impeding cellular mechanisms of aneurysmal wall repair, will be explored in this context.

3) The role of cell signaling in the susceptibility for AAA development and ultimate rupture, and the role of elastin derived peptides in inflammation, angiogenesis and calcification, will be elucidated by transgenic approaches in mice.

Outward convection and activation of blood-borne proteases in aneurysmal wall.

Since the contention of the pressurized blood within the arterial conduct is the fact of the insoluble extracellular matrix, synthetized by the VSMC, dilation and finally rupture of the wall is always the fact of proteolytic degradation of the insoluble extracellular matrix. In parallel, molecular transport within the arterial wall is mainly the fact of the outward radial hydraulic conductance, generated by blood pressure through the wall (background). The main achievements of FAD in the pathophysiology of aortic wall dilation and finally rupture focused on the spatio-temporal mechanisms of plasma-borne zymogens, transported through and activated within the aortic wall and consequences for the matrix, the VSMC, and the adventitial inflammatory response. This pathophysiological concept of aneurysmal pathology, whatever the localization and the aetiology (atheromatous versus non-atheroma, aneurysm versus dissection, thoracic aorta versus abdominal aorta, or other localizations) open new avenues for better understanding vascular pathology.(see attachment DOCUMENT 3)

AAA is a specific proteolytic form of atherothrombosis in human. During FAD the partners focused on the spatio-temporal role of intraluminal thrombus (ILT) as a main source of proteolytic and oxidative activities, linked to both release of proteases and agglutination of Red Blood Cells (RBC) (figure above: oxidative activity revealed by Perls reaction and Diaminobenzidine precipitation in the most luminal layer of ILT). In background, FAD partners developed the concept that ILT was a main determinant of AAA progression, due to its ability to generate proteases (MMPs and serine-proteases) (partner 6 & 1), and therefore is associated with more degradation of the media and more inflammation in the adventitia (partner 4).

During the FAD period this concept was further investigated focused on the role of ILT as the site of neutrophil retention, and death and leukocyte proteases release. Partner 10 quantitatively described the leukocyte and the fibrinolytic system in ILT and AAA wall. Partner 1 and 4 demonstrated that ILT is the main source of chemotactic mediators for neutrophils: Leukotriene B4, Leukotriene C4, interleukin 8 and Rantes (human and experimental models). They also identified the luminal layer, interfacing with circulating blood elements as the main source of soluble proteases in ILT, and the release of ADAM-rich microparticles by the abluminal layer as another source of proteases (partner 4). Moreover the progression of AAA toward rupture is staccato (background partner 6). In order to explain this random risk of less or more active progression of AAA, we hypothesized that a preferential trapping of weak pathogens within the ILT could be a transitory event capable to enhance neutrophil chemo-attraction and retention. We demonstrated that Porphyromonas gingivalis (Pg), a weak pathogen of periodontal origin, is frequently present in ILT of human patients, enhancing the retention and activation of neutrophils, and that repeated iv injections of Pg in experimental models of AAA in rats, prevented the spontaneous healing of the model, due to the accumulation of neutrophils in ILT and the impairment of mesenchymal cell recolonization. In agreement, the circulating level of progenitor cells was modified in AAA patients (partners 2 & 9 not yet published).

We also developed the concept that ILT is an important source of oxidative activities due to the release of haemoglobin, including the haeme residue and the globin protein core. We identified globin as a substrate for proteolysis by cathepsin G and hemin as a powerful catalyser of peroxidase activities, compensated by anti oxidant molecules such as peroxiredoxin, thioredoxin, CD163 scavenger receptor of haemoglobin/haptoglobin complexes (partner 7). Both proteolysis and oxidation could generate soluble neo-antigens, which could be outwardly convected, participating to adaptive immune responses taking place in the adventitia.

Similarly in TAA, the proteolytic injury of the arterial wall, come at least in part, from convection and activation of blood borne zymogens through the aortic media. In a first step we identified prothrombin activation in TAA wall (partner 1, background). We identified MMP-3 and MMP-7 as matrix metallo proteinases retained in areas of mucoïd degeneration, and the convection and pericellular activation of plasminogen by VSMC t-PA on the circumference of these areas. This point is particularly important because generated plasmin is able to provoke SMC detachment and death, to activate MMP preforms, and to release TGF-beta from its extracellular matrix storage (LTBP associated with Fibrillin and Fibronectin). In this concept, mutations of genes involved or not in the TGF pathway signalling and hemorheological disturbance enhance the blood-borne proteolytic injuries or decrease the wall resistance to injury by accelerating VSMC death.

Medial and adventitial responses

In TAA, partner 4 established the differential pattern of gene expression in the media associated with bicuspid or tricuspid aortic wall. A set of immune response genes was specifically identified in TAA of tricuspid valves, but not in TAA with bicuspidy, suggesting a more important stimulation of inflammatory response in the former. Similarly the pattern of MMPs expression was established in TAA wall, comparing bicuspidy and tricuspidy, and showing an overexpression of MMP-14 and MMP-19 in dilated aortas. MMP-3 and MMP-7 mRNA were not detected, confirming plasma as their main source. In parallel partner 1 showed an epigenetic modification of expression pattern in VSMC of TAA (see WP2). The consequences of this epigenetic regulation of Smad2 expression on target genes, including proteolytic and antiproteolytic (serpins) effectors, extracellular matrix proteins, are explored in aneurysms and dissection of the ascending aorta.

In AAA, partner 4 established early that the AAA wall covered by a thick ILT shows more signs of matrix degradation compared to the wall free from ILT (background). The FAD partners tried to establish a spatio-temporal relationship between what happened in the ILT and its consequences on the wall, in media and adventitia, sites of the immune response associated with AAA. Partner 4 and 8 focused part of their work on the mast cell response in human tissue as in experimental models in mice. The necessary relation between mast cell and neo-angiogenesis and the release of mast cell proteases (tryptase, chymase, cathepsins) were established. In parallel different products of wall degradation were tested for their ability to provoke inflammation. Partner 5 established that human elastin degradation products are chemotactic for macrophages in vitro. Numerous B cell follicles are detected in the adventitia of AAAs, which are absent from normal aortas. Partner 1 established that immunoglobulins released by adventitial tertiary lymphoid organs (ATLO) of AAA are immunoreactive to proteins/peptides liberated by ILT. Among the recognized peptides, a 2884 Da peptide was identified by mass spectrometry, resulting from the degradation of fibrin by plasmin. Oxidized or proteolyzed Hb was also strongly recognized by AAA adventitial Ig. In collaboration with partner 13 (PhL) and partner 14 (TG), the development of a specific assay of this peptide was

initiated. Unfortunately all the produced antibodies recognized the peptide but also free haemoglobin, limiting the interest of the assay.

Biomechanics of aortic aneurysm

In-vitro biomechanical testing of aneurysm tissue from open surgical repair provided novel biomechanical information of the aneurysm wall as well as the intra-luminal thrombus. This is essential data for reliable organ-level biomechanical simulations with specific importance for the biomechanical AAA rupture risk assessment. The large pool of CT-A data available through the FAD consortium allowed a direct clinical interpretation of biomechanical computed indices, as it is important to integrate biomechanical simulations in the clinical decision making process. Polarized light microscopy of wall samples from open aneurysm repair provided novel data of the collagen organization in the AAA wall. This information is not only important for a qualitative understanding of the AAA wall but also critical input for multi-scale histomechanical constitutive models. Here, the integration of histology and biomechanics provided a basic understanding of load carrying mechanisms of the AAA wall. Hemodynamic simulations of the blood flow through the normal and the aneurysmal aorta showed significant differences, which motivated a novel hypothesis for intra-luminal thrombus development in the AAA. Integrating the coagulation cascade in the analysis further reinforced this hypothesis. The outcome could potentially help to design therapies for a conservative treatment of AAAs. Based on follow-up CT-A data of patients with small AAAs their three-dimensional development was studied. The collected information suggested that AAAs should be monitored all over the aneurysmatic sac in order to identify the site of faster diameter growth. This information could essentially improve monitoring protocols for small aneurysms and towards avoiding expansion-related ruptures. In addition, the collected information is the basis for the development of aneurysm expansion models, which could further improve the surveillance of small AAAs

WP4. Diagnosis

Objectives of this WP4 are to translate WP2 & 3 to diagnostic tools for human aneurysms, using specific new technologies, including proteomics and in vivo imaging technologies involving functional and molecular imaging as intermediary phenotypes in TAA and AAA pathologies. WP4 also involve discovery of new tracers for molecular imaging, establishment of proof of concept at a preclinical level, and initiation of clinical investigations.

Plasma biomarkers in AAA.

Numerous circulating biomarkers of AAA were identified by the partners as background of FAD: MMP-9 (partner 6), fibrinolyic signal (partner 8) platelet activation (partner 1), interleukins (partner 10). In particular, partner 7 identified IGFBP-1 as a circulating biomarker, released by ILT, and associated with AAA size. Studies exploring the value of these biomarkers were performed in larger cohorts. Other pathophysiological biomarkers were discovered in FAD, such as circulating free DNA (partner 1) or NGAL (partner 7) as markers of neutrophil activation, and anti-Pg antibody titration as marker of periodontal infection associated with AAA evolution (partner 1).

Numerous partners of FAD (p1, p2, p4, p7, p8, p11, p14) initiated researches in proteomic in order to characterize proteins release by AAA, and exploring their potential use as circulating biomarkers. Numerous and diverse data were obtained and several methodological progress were done, including exploration of the deep proteome, using Proteominer® (random peptide library), multiplex immune analysis, PAcIFIC, TOF-SIMS (ex vivo molecular imaging), metabolomics and other technologies. Such approaches have also a pathophysiological interest, promoting conceptual implementation of the different parameters involved in the progression of the disease. Among the most relevant data, the identification of plasma biomarkers of oxidation in association with AAA was initiated and developed during FAD. Partner 6 (Ulg) identified an increase in circulating oxidant state in association with AAA. Partner 7 (UAM) identified by proteomics of ILT several anti-oxidants such thioredoxin and peroxiredoxin as biomarkers associated to the response to oxidative stress. This oxidative state is mainly the fact of Red Blood Cell retention and haeme release in the ILT. A more complete analysis of the redox state and iron metabolism is in progress between partners. Partner 7 (UAM) also initiated the first metabolomics studies in AAA. These approaches linked the diagnosis (WP4) to the pathophysiology (WP3)

Circulating cell biomarkers, progenitors & leukocytes

Partners 7 (UAM) and 1 (INSERM) discovered differential expression of a number of redox proteins in circulating neutrophils and in the membrane of red blood cells of AAA patients, and are currently pursuing validation of these in larger cohorts. Among these proteins, catalase and peroxiredoxin were decreased in both cell types, with a concomitant increase in prooxidant factors suggesting the involvement of oxidative stress in AAA pathogenesis. Similarly, partner 1bis performed a profiling of macrophages from AAA (Lamblin N, Ratajczak P, Hot D, Dubois E, Chwastyniak M, Beseme O, Drobecq H, Lemoine Y, Koussa M, Amouyel P, Pinet F. Profile of macrophages in human abdominal aortic aneurysms: a transcriptomic, proteomic, and antibody protein array study. J Proteome Res. 2010 Jul 2;9(7):3720-9). In parallel circulating progenitors were identified and characterized in association with AAA (partner 2, St Georges Hospital, and partner 9, CNRS). Circulating microparticles of platelet and neutrophil origin were also identified in association with AAA.

Functional hemodynamic imaging in TAA.

Beside the usual morphological measurements of aneurysmal dimension (maximal diameter, length, etc.) and the presence of ILT in AAA, FAD developed several new approaches including measurement of aortic rigidity in TAA, using ultrasound and now Magnetic Resonance Imaging (MRI). In particular partner 12 (AMC) developed this imaging technology. MRI also can image non-laminar flow in aneurysm, allowing a more precise analysis of recirculation and vortex phenomenon associated with initiation and progression of aortic dilation. This technology is not yet proposed in TAA due to the complexity of the software and the time of calculation. These approaches link the diagnosis (WP4) to biomechanics (WP3)

Iron traffic in AAA

Since iron gives a powerful negative signal in MRI, this technology allowed us to explore the spontaneous presence of iron in AAA, in ILT and wall, and the ability of phagocytes present in ILT to uptake exogenous, intravenously injected, Small Paramagnetic Iron Oxide (SPIO) particles (partner 6 Ulg and 1 Inserm). This data are important since they demonstrate the ability of MRI to image in vivo in human (WP4) the involvement of iron, and downstream of oxidation in the pathophysiology of AAA (WP3). Nevertheless the data also raised the question of the sensitivity of MRI to detect molecular events.

PETscan and aneurysm progression.

Partner 6, ULg, was the first to show that 18F-DeoxyGlucose uptake, detected by Positron Emission Tomography coupled with Computed Tomography scan (Rx) for morphology (18FDG PET/CT) could be used as prognosis marker in aneurysmal disease (background). During FAD, ULg constituted an important series of 711 18FDG PET/CTscan in in 428 patients presenting aortic diseases, including AAA (n= 301) with or without EVAR, dissections (n=25) of the descending aorta, TAA (n=66) and Aortitis (n=36). The results concerning TAA are mainly negative, except rare exceptions, probably, due to the absence of adventitial inflammatory granuloma in association with TAA. These negative data suggest that TAA do not generate important adventitial immune response. In contrast, data concerning AAA and dissection are particularly rich and interesting. In parallel to PET a biological database (plasma and DNA samples) was constituted and morphology was accessible by the CT data. This triple approach: PET showing localized spots of high 18FDG uptake (high cellular metabolism), associated with CT allowing dimensions measurements and morphology classification, and biology offer an unique opportunity to link the evolution of aneurysmal dilation (CT) to cell function imaging (PET) and biology. Usually the hot spots of 18FDG take place in the external part of the aortic wall. The exploitation of this triple database is now in progress.

In a first study the relationship between 18FDG uptake and localized cellular and molecular alterations was established, suggesting a predictive value of rupture risk in AAA. Taken the opportunity of Open Vascular Aneurysm Repair by surgery, 18FDG PET/CT was performed in AAA patients before surgery and specific biopsies of the hot spots were performed during surgery. The relationship of hot spots of FDG uptake to presence of immune granuloma in the adventitia, and to high expression of MMPs in the wall, was established (article submitted to publication).

In a second study the prognostic value of FDG PET positivity in the aneurysmal evolution of dissection of the descending aorta was established. First the relation of aneurysmal evolution of dissections to partial thrombosed false lumen was confirmed (as compared to patent and totally occluded false lumen). Aneurysmal progression (morphology) is related to FDG PET positivity, and to more active intraluminal thrombus as demonstrated by and elevation of coagulation and fibrinolysis biomarkers. Aortic dimensions measured on CT were correlated with 18FDG Standardized Uptake Values measured on PET, and coagulation and fibrinolysis biomarkers. Moreover in the follow-up of a patient, PET positivity was related to aneurysmal progression and clinical and biological coagulopathy of consumption. All of these markers were reversed by successful EVAR. These data demonstrate a direct link between aneurysmal progession, ILT biological activities and its impact on PET positivity in the adventitia (article in preparation).

Similarly this database is now used for testing the ability of 18FDG PET to follow endoleak's evolution in AAA treated by EVAR (in progress). The total AAA cohort will be also exploited (PET, CT and biology).

This FAD achievement is particularly original, translational and transversal, establishing important link between morphology, cellular imaging, and biological activities associated with aneurismal progression.

Development of new molecular tracers targeting Intra luminal thrombi (partner1)

Preclinical proof of concept that 99mTc-Fucoidan images intraluminal thrombi

P-selectin expression is involved in the pathophysiology of biologically active arterial thrombus, and endothelial activation following transient ischemic event. Fucoidan is a polysaccharidic ligand of P-selectin with a nanomolar affinity (background). In FAD we have proposed a new approach of P-selectin molecular imaging based on radiolabeled fucoidan. Two kinds of experimental models were selected to evaluate the ability of radiolabeled fucoidan to detect P-selectin expression: platelet-rich arterial thrombi (arterial mural thrombus in experimental aneurysm), and myocardial ischemia-reperfusion. These two settings were chosen because they were clinically relevant, and both were associated with an important overexpression of platelet and endothelial P-selectin, respectively. Radiolabeled fucoidan SPECT was able (a) to detect the presence of platelet-rich arterial thrombi in all animals, with a median target to background ratio of 3.6 in mural aneurysmal thrombus (b) to detect a persistent endothelial activation 2 hours after reperfusion; in this latter model the magnitude of the signal was correlated with the extent of myocardium that underwent transient ischemia. The sensitivity of selectivity of the uptake and retention of 99mTc-fucoidan in both settings was excellent. This study supports 99mTc-fucoidan as a relevant imaging agent for in vivo detection of biological activities associated with P-selectin overexpression, such as arterial thrombus and ischemic memory. Given the previously reported wide availability at a low cost, and its low toxicity, fucoidan seems to overcome some of the limitations of previous P-selectin-targeted imaging agents. This approach has been patented by partner 1: WO 2010/116209 A1: Fucoidans as ligands for the diagnosis of degenerative pathologies, published 14 october 2010, extended to USA 02970-24565US02, January 9, 2012; and published (J Nucl Med 2011).

This approach is now extended to Magnetic Resonance Imaging modality using Fucoidan as pharmacophore for intraluminal thrombi and Ultra Small Paramagnetic Iron Oxide (USPIO) as contrastophore for MRI.

We project to develop these imaging programs in human. We will first begin with (99m)Tc annexinV a high affinity pharmacophore for activated platelet, now available for injection in human. This clinical investigation will start in the near future. We will continue with Fucoidan.

Development of new molecular tracers targeting protease activities (partner 13 and partner1)

Serine proteases hold a central role in the pathophysiology of degenerative arterial diseases, but to date there is no suitable agent for molecular imaging. The project was designed to (1) develop a radiolabeled peptide probe for serine proteases imaging, including plasmin and leukocyte elastase,

and (2) evaluate these new agents in vivo in a rodent model of arterial thrombus, including experimental aneurysms.

A plasmin pseudo-substrate of high affinity (D-Val-Phe-Lys; IC50 : 100 pM) was combined with chloromethyl-ketone for covalent binding to the target, and with diethylene triamine pentaacetic acid via a glycine spacer for pertechnetate labeling. The imaging efficiency of this low molecular weight probe for plasmin (VFK, MW: 971 Da) was assessed in a rat model of mural thrombus in abdominal aortic aneurysm. In vivo imaging was performed by SPECT and uptake ratios (UR) were quantified by autoradiography.

VFK demonstrated a labeling efficiency of 90%. After injection, the labeled peptide was rapidly cleared from the blood via kidneys. Two hours after injection, an infrarenal focal uptake was detectable in all animals (n=6) and the UR on autoradiography was 7.6±3.3. In two additional rats, the pre-injection of unlabeled VFK (20 times the radiolabeled mass) resulted in a fivefold decrease of the UR compared to animals without pre-injection (1.3±0.3, p=0.03). The UR obtained in the same settings with a protein inhibitor of serine proteases (radiolabeled Aprotinin, MW: 6.5 kDa) was 5.0±0.9 (p=0.03 vs. VFK).

These results support the ability of radiolabeled VFK to detect plasmin within arterial thrombus. The rapid blood clearance of the tracer is suited for imaging in vascular diseases. A similar approach is now developed for targeting leukocyte elastase. This approach was patented by partner 13 & 1 (AGENTS FOR THE MOLECULAR IMAGING OF SERINE-PROTEASE IN HUMAN PATHOLOGIES, EP11305405, 07april 2011, MICHEL09617MC2, Article submitted for publication)

WP5. Therapeutics

Objectives.

To the validate current treatment and new therapeutics in human aneurismal diseases. WP5 will develop clinical investigations in patients and preclinical testing in experimental models.

This program includes:

1. An evaluation of medical prevention of aneurysm development and progression by existing medications,
2. The development of curative cell and bio-engineered therapeutics at a preclinical level and further extension to human,
3. Further development of new therapeutic strategies.

Medical prevention of aneurysm development and progression (preclinical)

Since there is no specific treatment able to completely prevent or inhibit progression of aneurysm, the FAD WP5 explored new medical therapeutic approaches at a preclinical level. First, since WP3 demonstrated that ILT is one of the main driving forces for AAA progression, the ability of inhibition

of platelet aggregation to limit experimental and clinical progression of AAA was tested. Experimentally platelet inhibition prevented AAA development in an experimental model in rats. This result was retrospectively confirmed in human. Therefore, inhibition of platelet aggregation could be recommended in patients with small aneurysms, in prevention of growth (Partner 1 and 8). In parallel treatments able to impair the retention of neutrophils in ILT or antibiotics, which treated bacterial contamination, were also tested with success in experimental models. Evaluation of AZD 9668, a powerful inhibitor of leukocyte elastase is under progress.

Important preclinical studies have been done showing the ability of therapeutic HDL to prevent development of aneurysm in mice and rats, by different properties, not directly linked to their ability to reversely transport cholesterol (antiprotease, anti-inflammatory activities, endothelial protection) (Partner 1 and 2).

The effect of blockade of the angiotensin system was also experimentally explored in animal (partner 1), and clinical trials with angiotensin II antagonists are in progress in patients with Marfan syndrome in order to prevent TAA development and dissection (partner 1 & 3). In parallel antibodies blocking elastin degradation products and/or indomethacin were tested in experimental models of Marfan in mice (partner 5).

Finally, cyclosporine, a compound able to promote TGF expression and signalling was also tested with success in experimental models (partner 9) and now a clinical trial is initiated (partner 9).

Novel interventional curative therapeutics through cell seeding, gene therapy and biomaterials

Several new models of aneurysms have been developed during FAD for evaluating new interventional therapeutics. These models are transposition of classical model (elastase, decellularized xenograt) to large animals than mice and rats, including rabbit and pigs (partner 1, 9 & 10). A model of EVAR is developed in rats (partner 1). These models were used for testing cell seeding as interventional therapeutic approach in aneurysm, and for developing new hydrogels avec vector of local cell or molecular therapy.

Different types of cells have been test, mesenchymal stem cell of bone-marrow origin, characterized by their ability to be use in autologous systems, and their efficiency to proliferate in vitro. These mesenchymal stem cells were tested in rats, rabbit and pig with success (partner 1 & 9). Other cells were also tested, fibroblasts, which aggravated the evolution of experimental aneurysms, endothelial cells, which are protective (partner 9).

With the participation of chemists, polysaccharide hydrogels have been developed in order to convey cells, nucleic acids, and recombinant proteinS in the aneurysmal sac (partner 1 & 9). In particular sulphated hydrogel was used to heparin binding mediators such VEGF or SDF-1.

New therapeutic strategies

These different approaches allowed us to initiate or to participate to clinical trials, including trial of angiotensin II antagonists in Marfan syndrome, inhibitor of mast cell activation (Cardoz), and cyclosporin in human AAA. Others are in discussion with pharmaceutical industry (leukocyte elastase inhibition with Astra-Zeneca).

Scientific and medical conclusions.

FAD was a highly translational and transversal large-scale program, mobilizing numerous energy in order to better understand what are the biological determinants of aneurysm development in order to propose new diagnosis and therapeutic tools. FAD achievements are diverse, due to the number of partner, each developing its own project. Nevertheless the majority of the works were complementary through the different WPs and the different partners. Two examples are presented in the table beyond (see attachement DOCUMENT 4). The works performed on TGF-beta signalling pathway covered all the WPs, mainly WP1, 2, 3, & 5, and involved height partners among fifteen. Similarly the works performed on the Intraluminal thrombus as the predominant driving force in AAA evolution, covered all the WPs and involved eleven partners among fifteen. Similarly the number of common publications, 81 among 292 (28%), co-signed by 2 partners or more, also provide evidences of the transversality of FAD.

Major achievements have been performed in each WP opening new avenue for future Research and Development (R&D) in the field of aneurismal disease of the aorta:

- Clinical and genetic database and tissue and cell collections were constituted through FAD, These database and collections, shared inside FAD by several partners, but also opened to other teams outside of FAD consortium, allow important translational work in the future.
- Discovery of new gene mutations in aneurismal disease raises the important question of genetic diversity leading to common pathological features of arterial wall and rupture.
- Pathophysiology leads to the general concept of blood-borne injuries of the arterial wall, sensitized by intrinsic defect of the wall components, leading to medial and adventitial response.
- Diagnostic applications are in progress, including circulating biomarkers, but also important progress in functional and molecular imaging of aneurismal pathology. These progress include clinical applications, but also new tracers for which the preclinical proof of concept has been now established. Further developments as first injection in human are programmed.
- Similarly, new therapeutic approaches are in progress through clinical investigations in human patient.

Potential Impact:

The potential impacts, including the socio-economic impact and the wider societal implications.

FAD will have medical and socio-economic impacts in health, medicine, and economic development of new tools.

The development of screening programs will have, with time, consequences on health in aging EU population. Screening for AAA will have important consequences on the care of aging male without symptom. This beneficial effect will extend more than AAA alone. It will be extended to the detection of atherothrombotic disease whatever the localization and the prevention of periodontal diseases in aging population. The relationship between periodontal disease in aging population and the cardiovascular risk is now well established by epidemiological studies (see attachment DOCUMENT 5). Through translational research, FAD provided evidences of the biological relationship between periodontal disease, blood weak pathogen transitory passage, ILT retention of these weak pathogens, leading to AAA progression. Such health programs are now progressing slowly (figure: new tools for mouth-dental hygiene in elderly people), but must be developed with the support of EU for Research & Development. We proposed this transversal project, including cardiologists, neurologists, biologists, odontologists, twice to EU (ERC 2010 and COST), but we didn't succeed to be selected.

The concept developed in FAD will also impact medicine of other vascular pathologies, including:

- -Genetics of vascular diseases: the effort performed with success in genetics during FAD will be pursued including discovery of new genes through informative family approaches and genetic sensitivity trough GWAS approach in large cohort.

Partner 15, deCODE recently patented one part of these results: Genetic markers for risk management of vascular disease. Case History Report For Matter Number Case History Report For Matter Number P2587PC00. PCT Application date: 22.6.2011, No. PCT/IS/2011/050009. Applicant deCODE Genetics ehf. Inventors: Gudmar Thorleifsson, Solveig Gretarsdottir

- Other localization of aneurysmal diseases: In particular cerebral aneurysms. At the beginning of FAD specific links was established wit ANEURIST (http://www.aneurist.org/). @neurIST -Integrated Biomedical Informatics for the Management of Cerebral Aneurysms - was a European initiative within the Sixth Framework Programme of the Information Society Technologies IST. Alejandro Frangi (Aneurist coordinator) and Juahana Frözen, a Finnish neurosurgeaon, specialist of cerebral aneurysms, were member of the FAD scientific advisory board, and Jean-Baptiste Michel, FAD coordinatore participated to @neurIST meetings in Barcelonna. In particular FAD proposed that the pathogenicity of ILT is common to AAA and cerebral aneurysm.

- New stenting technology: In the context of cerebral aneurysm, a new type of multilayer not covered stent, Flow Diverter (http://www.cardiatis.com/) has been implanted in human patients, particularly in treatment of cerebral aneurysm. The principle was that flow diversion of the aneurysmal sac could provoke sac thrombosis and healing. Unfortunately this technology was not associated with total success. Several secondary aneurysmal ruptures of cerebral aneurysms, but also of aortic and other peripheral aneurysms, impair the results. Since flow diversion is not ILT exclusion, the ILT associated with this type of stents

continued to be biologically active leading to continuous proteolytic degradation of the arterial wall.

The competencies acquired on ILT through FAD allowed Partner 1, Inserm, to be partner in PRESTIGE- PREvention of late Stent Thrombosis by an Interdisciplinary Global European effort (http://www.prestige-fp7.eu/) The project starting in December 2010 is funded under the Seventh Framework Programme (FP7) by the European Commission. Addressed by the topic "HEALTH.2010.2.4.2-1: Reducing in-stent thrombosis", late stent thrombosis represents a major European health care concern.

- Atherothrombotic diseases: Since AAA is an atherothrombotic disease, works performed on AAA also impact directly what we know about occlusive atherothombosis. In particular, works performed in genetics of sensitivity to AAA also impact genetics of coronary and carotid artery diseases in humans.

One other important point is that the studies made on IntraLuminal Thrombus (ILT) during FAD directly impact the pathophysiology of IntraPlaque Hemorrhage (IPH) in occlusive atherothrombosis. IPHs are now well established as the driving force leading to plaque rupture and clinical expression. We wrote important reviews in this field and gave several international conferences on this important subject (EVBO meeting in Krakow, ESC 2012 Munich). It is time now to develop something at the EU level concerning this most important feature in human atherothrombotic diseases. We proposed twice a COST and an ERC project in this field at the EU level (IPH-COST action 2011, ERC Spa-Athero 2012) in order to initiate an EU network in this field. We didn't succeed. We will try again. We wish and hope that this important field of heme/iron metabolism in atherothrombotic diseases will be the object of EU calls at the beginning of FP-8!

The work made on ILT during FAD also allowed us to define new tools for functional and molecular imaging and interventional therapeutic in atherothrombotic diseases. These works allowed us to propose the NanoAthero project applied to atherothrombotic diseases. NANOATHERO-Nanomedicine for target-specific imaging and treatment of atherosclerosis: development and initial clinical feasibility, is now selected to be funded under the Seventh Framework Programme (FP7) by the European Commission. Addressed by the topic «Nano science, Nanotechnology, NMP.2012.1.2-2: Development of novel therapeutic nanotechnology-enabled systems for the diagnosis and treatment of atherosclerosis- Large scale integrated project, Inserm U698 EU coordinator.

- Aortic valve disease: Similarly to the preceding fields, the works performed on TAA particularly on genetics, genomics and biomechanics, allowed to FAD partners to continue collaborations in this field focusing on the relationship between TAA and bicuspid aortic valves. MIBAVA- Mechanistic Interrogation of Bicuspid Aortic Valve associated Aortopathy (http://www.fondationleducq.org/), was selected Under the Transatlantic Networks of Excellence in Cardiovascular Research Program awarded by Fondation Leducq U.S. $6,000,000 over five years for internationally collaborative research. Bart Loeys EU coordinator (partber 3, ex UG), and Karolinska Institue (KI, partner 4) a partner.

- Economic Impact: The most important economic potential impact will be directly linked to the development of applications initiated within FAD. Among the patents generated through FAD the most important and numerous concern diagnostic applications, genetic markers, circulating biomarkers and molecular imaging. The Inserm patent and the corresponding published article on the use of fucoidan as a new pharmacophore for the diagnosis of vascular

disease is probably the more advanced ones. The preclinical proof of concept has been established. The program is now to go to the first injection in human. This must be achieved through several steps including the definitive choice of the fucoidan preparation, the preparation in GMP conditions for first injection in human, minimal toxicologic studies, the writing of the regulatory files, the definition and the writing of the clinical investigation protocole, and finally the financial support to do that. This development is now in progress with a financial support at a national level.

Main dissemination activities and exploitation of results (WP6),

Objectives

Ensure regular training of Junior Scientists and Technical Staff participating in FAD project to acquire new skills and techniques.

In-house training is expected to promote personnel exchanges and amplify excellence and scientific links within the Network.

To disseminate the results generated in FAD in medical science.

To reach all the potential audiences, in particular, pharma companies, industrial partner for diagnostic tools, patient organisations and public health authorities.

During the 4 years of the project, various disseminations activities were performed in order to promote FAD thank to the active involvement of each FAD partners.

A website has been created (ww.fightin-aneurysm.org) to be a showcase of the project, and present the project to the public. The success of this website as a tool to promote FAD can be shown by the statistic of visits of the website, which regularly increase since its launching (see attachment DOCUMENT 6).

This website was a first step to contact the scientific coordinator to obtain more information on the project. It is regularly updated according to the dissemination plan. A leaflet ready to be print is available on the website with synthetic information on the project.

3 training sessions have been organized during the 4 years of the project: Proteomics in aneurismal disease: from sampling to analysis (April 2009), Animal models of AAA: rational to practice (March 2010) and CV molecular imaging (March 2012). The purpose of these training were decided after consultation of the partners on their needs linked to FAD works and centralization of the requests. Each partner has been involved as speakers or as attendees. The participants to these training sessions were French, Danish, English, Sweden, Belgian, Spanish and Czech. Behind the exchange of knowledge between the partners, these courses were a real opportunity for the partners to exchange knowledge and open the door to new collaborations.

300 articles were published (see attachment DOCUMENT 7)by FAD partners, as the fruit of their own work on FAD (articles), of a collaboration with another or others FAD partners (articles) during the 4 years of the project. A article has been published under FAD consortium name (Novel aspects of the pathogenesis of aneurysms of the abdominal aorta in humans. FAD EU consortium. Cardiovasc

Res. 2011 Apr 1;90(1):18-27). This number of article is the demonstration of the fruitful work performed by FAD partners.

These articles have been published in major medical review as Nature Genetics, Circulation, ATVB and were the reflect of the advances and achievements of the work performed on FAD and the demonstration that a real expertise exists in FAD consortium.

The proof of the existence of this expertise resides in the fact that one hundred articles were reviewed by FAD leaders between July 2008 and July 2012. As an example, Jes Lindholt (WP1 team leader, partner 8, Viborg Hospital) is a member of the board at The European Journal of Vascular and Endovascular Surgery and Jean-Baptiste Michel has reviewed 19 articles of the 30 articles on the aneurysms published in ATVB and 10 on 35 in Cardiovascular Research.

Since the beginning of the project, FAD partners have participated to national/European and international congress and have been invited as different meeting to present results obtained with the FAD project. The invitations of international congress of FAD members have been increased and is the reflect of the recognition of the expertise of the FAD members thanks to this international collaboration involved by FAD.FAD project was presented in the most important congress and thanks to the collaboration between the different partner, some researchers involved in FAD have been invited in some events to present their results. For an exemple: Jean-Baptiste Michel (INSERM, partner 1) has been invited byPer Eriksson (Karolinska Institute, partner 4) at the European Society of Cardialogy meeting in Sweden in 2011. FAD logo is always put on the presentation made BY THE Partners to these meetings/congress.

The FAD partners have participate at their level to promote FAD thank to articles in the press (Jean-Baptiste Michel, partner 1, INSERM, Circulation, Cardiovascular research funding; recipients of the European Commission's seventh framework programme-APF Projet de recherché européen pour empêcher le développement de l'anévrisme) or participation to radio and television emissions (Jes Lindholt, partner 8, Viborg hospital, TV2, Regionerne - Jean-Baptiste Michel, partner 1, INSERM, France 5, le magazine de la santé). Behind the participation of congresses, there was a real volontee of each team to talk about FAD as a whole and not only as the individual work of several different teams.

FAD has supported technically and financially the different actions of the partners on the aneurism purpose (International meeting on aneurysms organized by Natzi Sakalihasan, partner 6, Liege University - Workshop on the biochemical analysis of collagen type I, III and IV, collagens responsible for heritable connective tissie disorders organized by Paul Coucke, Partner 3, Ghent University).

This collaboration and exchange of knowledge was one of the success of the project which have create a researcher network which leads to the scientific excellence, which will last beyond the project.

List of Websites:

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