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STUDY UPDATE

The Rotterdam Study: 2014 objectives and design update

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Abstract The Rotterdam Study is a prospective cohort study ongoing since 1990 in the city of Rotterdam in The Netherlands. The study targets cardiovascular, endocrine, hepatic, neurological, ophthalmic, psychiatric, dermatological, oncological, and respiratory diseases. As of 2008, 14,926 subjects aged 45 years or over comprise the Rotterdam Study cohort. The findings of the Rotterdam Study have been presented in over a 1,000 research articles and reports (see www.erasmus-epidemiology.nl/rotterdamstudy). This article gives the rationale of the study and its design. It also presents a summary of the major findings and an update of the objectives and methods.

Keywords Cardiovascular diseases · Cohort study · Dermatological diseases · Endocrine diseases · Epidemiologic methods · Genetic epidemiology · Liver diseases · Neurological diseases · Ophthalmic diseases · Otolaryngological diseases · Pharmacoepidemiology · Psychiatric diseases · Respiratory diseases

Introduction

The Rotterdam Study was designed in the mid-1980s as a response to the demographic changes that were leading to

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an increase of the proportion of elderly people in most populations [1]. It was clear that this would produce a strong rise in elderly people living with diseases, as most diseases cluster at the end of life, and that to discover the causes of diseases in the elderly one would have to study risk factors of those diseases [2]. A major approach to finding causes is the prospective follow-up study, which has proven quite effective in finding causes of heart disease and cancer.

The design of the Rotterdam Study

The design of the Rotterdam study is that of a prospective cohort study among, initially, 7,983 persons living in the well-defined Ommoord district in the city of Rotterdam in The Netherlands (78 % of 10,215 invitees). They were all 55 years of age or over and the oldest participant at the start was 106 years [3]. The study started with a pilot phase in the second half of 1989. From January 1990 onwards participants were recruited for the Rotterdam Study. Figure 1 gives a diagram of the various cycles in the study. In 2000, 3,011 participants (out of 4,472 invitees) who had become 55 years of age or moved into the study district since the start of the study were added to the cohort. In 2006 a further extension of the cohort was initiated in which 3,932 subjects were included, aged 45–54 years, out of 6,057 invited, living in the Ommoord district. By the end of 2008, the Rotterdam Study therefore comprised 14,926 subjects aged 45 years or over [4–6]. The overall response figure for all three cycles at baseline was 72.0 % (14,926 of 20,744).

The participants were all examined in some detail at baseline. They were interviewed at home (2 h) and then had an extensive set of examinations (a total of 5 h) in a specially built research facility in the centre of their district. These examinations focussed on possible causes of invalidating diseases in the elderly in a clinically state-of-the-art manner, as far as the circumstances allowed. The emphasis was put on imaging (of heart, blood vessels, eyes, skeleton and later brain) and on collecting body fluids that enabled further in-depth molecular and genetic analyses. These examinations were repeated every 3–4 years in characteristics that could change over time. And so there were examination cycles from 1990 to 1993, from 1993 to 1995, from 1997 to 1999, from 2000 to 2001, from 2002 to 2004, from 2004 to 2005, from 2006 to 2008, from 2009 to 2011, from 2011 to 2012, and from 2012 onwards (Fig. 1). In December 2013 the second examination cycle for the third cohort (RS-III-2) will be finished.

The participants in the Rotterdam Study are followed for a variety of diseases that are frequent in the elderly (and many are also in the not so elderly): coronary heart disease, heart failure and stroke, Parkinson disease, Alzheimer disease and other dementias, depression and anxiety disorders, macular degeneration and glaucoma, respiratory diseases, liver diseases, diabetes mellitus, osteoporosis, dermatological diseases and cancer.

The Rotterdam Study has been approved by the institutional review board (Medical Ethics Committee) of the Erasmus Medical Center and by the review board of The Netherlands Ministry of Health, Welfare and Sports. The approval has been renewed every 5 years, as well as with the introduction of major new elements in the study (e.g., MRI investigations).

In the remainder of this article the objectives and major findings will be presented with an update of the research methods for cardiovascular diseases, dermatological diseases, endocrine diseases, liver diseases, neurological diseases, ophthalmic diseases, psychiatric diseases, respiratory diseases, as well as for genetic and biomarker studies and for pharmaco-epidemiologic studies. For relevant recent EJE references see [7–19].

Cardiovascular diseases

Objectives

Research on the epidemiology of cardiovascular disease focuses on the etiology, prognosis, and prediction of cardiovascular disorders (including coronary heart disease, stroke, heart failure) diabetes mellitus and metabolic syndrome. The main emphasis is on prevention and management of a first cardiovascular event but prevention of secondary events is also an area of interest. Putative risk factors include five groups: lifestyle factors, endocrine factors, factors involved in hemostasis, inflammation and endothelial function, metabolomic factors and genetic factors. We have five specific focused themes:

1. Lifestyle: focused on evaluating the role of lifestyle factors (including nutrition, physical activity, sleep and smoking) in maintaining cardiovascular health as well as the interactions that lifestyle factors might have other factors (e.g. genes and medications).
2. Biomarkers and genes: aimed to identify relevant biomarkers for the identification of novel mechanisms of disease. These incorporate both molecular and genetic factors together with their potential interactions. Genomics and metabolomics play a key role.
3. Prediction and women's cardiovascular health: aimed to improve the identification of individuals at increased risk of developing cardiovascular disease in order to point out windows of opportunities that could permit

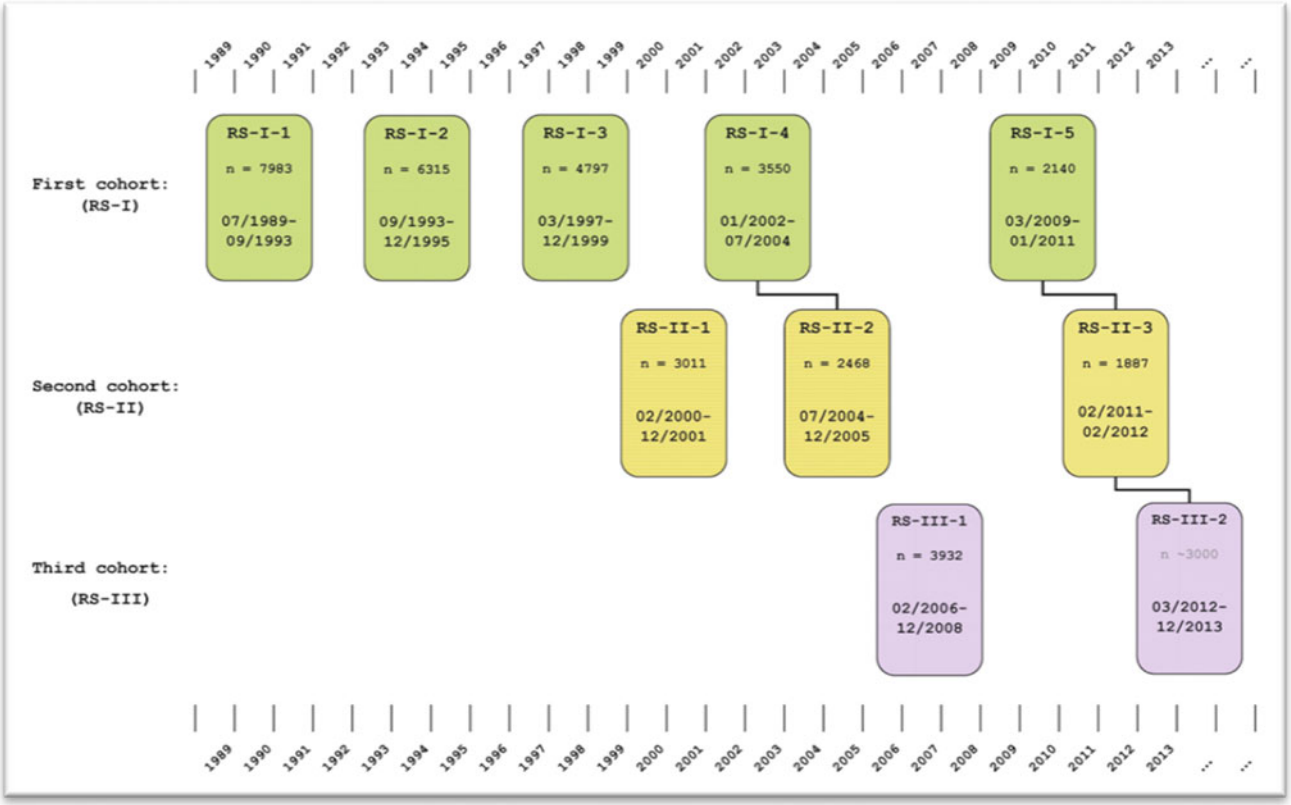

Fig. 1 Diagram of examination cycles of the Rotterdam Study (RS). RS-I-1 refers to the baseline examination of the original cohort (pilot phase 07/1989–12/1989; cohort recruitment 01/1990–09/1993). RS-I-2, RS-I-3, RS-I-4, and RS-I-5 refer to re-examinations of the original cohort members. RS-II-1 refers to the extension of the cohort with persons in the study district that became 55 years since the start of the study or those of 55 years or over that migrated into the study district. RS-II-2 and RS-II-3 refer to re-examinations of the extension cohort. RS-III-1 refers to the baseline examination of all persons aged 45 years and over living in the study district that had not been examined already (i.e., mainly comprising those aged 45–60 years). RS-III-2 refers to the first re-examination of this third cohort which will be completed by the end of 2013. Examination RS-I-4 and RS-II-2 were conducted as one project and feature an identical research program. Similarly, examinations RS-I-5, RS-II-3, and RSIII-2 share the same program items

early preventive interventions and personalised care. A special focus is given to evaluating specific factors and formulating targeted strategies to prevent cardiovascular disease in women.

4. High risk: focused on predictors and prognosis of chronic cardiovascular conditions, like heart failure, pulmonary hypertension, and atrial fibrillation.
5. Imaging: this work theme aims to identify the contribution that new technologies can provide to the maximum benefit of early diagnosis and accurate prognostication. Major focus is on non-invasive assessment of atherosclerosis to improve the understanding of the atherosclerotic process and the prediction of cardiovascular disease, including measurement of coronary calcification with electron-beam and multi-detector CT (MDCT) and carotid plaque characterization by MRI.

Major findings

Recognized and unrecognized myocardial infarction

We found that a high proportion of incident myocardial infarctions remains clinically unrecognized. The incidence rate of recognized myocardial infarction in the Rotterdam Study was 5.0 per 1,000 person years. The incidence was higher in men (8.4) than in women (3.1). The incidence rate of unrecognized infarction was 3.8 per 1,000 person years. Men (4.2) and women (3.6) had approximately similar incidence. Hence, the proportion of unrecognized infarction is lower in men (33 %) than in women (54 %) [20]. We have further identified a two-fold increased risk in developing heart failure or atrial fibrillation among men with unrecognized myocardial infarction [21, 22].

Heart failure and atrial fibrillation

The Rotterdam Study enabled accurate assessment of the incidence and lifetime risk of heart failure and atrial fibrillation in an elderly population [23–25]. It was shown that inflammation and resting heart rate is associated with risk of heart failure [26, 27]. In addition we identified several new risk factors of atrial fibrillation. We found that markers of generalized atherosclerosis in persons without a history of myocardial infarction or angina were associated with a higher risk of atrial fibrillation [28]. Furthermore, high-normal thyroid function [29] and higher levels of dehydroepiandrosterone sulfate, a precursor in the biosynthetic pathway of androgenic and estrogenic sex hormones were associated with incidence of atrial fibrillation [30]. In collaboration with several community-based prospective studies we were able to develop a prediction model for atrial fibrillation, only using variables that are routinely collected in primary care settings [31]. In a large collaborative study as part of the CHARGE consortium, we investigated the genetic variation responsible for 6 traits related to cardiac structure and function. We found two replicated loci for left ventricular dimension and 5 replicated loci for aortic root size [32]. Another topic of interest was the search for genetic determinants of several rhythm and conduction disturbances on the ECG, notably RR-interval, QRS duration, and QT(c)-interval, PR-interval, as well as atrial fibrillation and sudden cardiac death. For example, we identified several new loci for PR interval [33], heart rate [34], and atrial fibrillation [35, 36] in meta-analyses from the CHARGE consortium.

Cardiovascular risk factors and prediction

Endocrine, inflammatory and hemostatic factors and risk of coronary heart disease were addressed in several studies. Subclinical hypothyroidism was an independent risk factor of atherosclerosis and myocardial infarction in older women [37]. In a recent study, we compared the change in the accuracy of risk predictions when newer risk markers, representative of various pathophysiologic pathways, were added to the established clinical risk predictors. Among the biomarkers, improvements in coronary heart disease risk prediction were most significant with the addition of amino-terminal pro-B-type natriuretic peptide (NT-proBNP)[38, 39]. Furthermore, plasma C-Reactive protein (CRP) and lipoprotein-associated phospholipase A2 (Lp-PLA2) activity were independent predictors of coronary heart disease [40, 41]. Earlier findings included the association of tissue plasminogen activator (TPA) with incident coronary heart disease [42]. Recently, we developed and validated a coronary heart disease prediction model tailored for the aging population based on competing risk methodology [43].

Non-invasive measures of atherosclerosis

Multiple studies focused on the predictive value of non-invasive measures of atherosclerosis for risk of coronary heart disease. Strong associations with risk of coronary heart disease were found for carotid intima-media thickness [44], pulse wave velocity [45], and coronary calcification as assessed by electron-beam CT [46]. The relatively crude measures directly assessing plaques in the carotid artery and abdominal aorta predict coronary heart disease equally well as the more precisely measured carotid intima-media thickness [47]. In persons at intermediate risk of cardiovascular disease, coronary artery calcium provided the best increment in coronary heart disease risk prediction and stratification (to reclassify persons into more appropriate coronary risk categories)[39, 48]. The burden of coronary calcification also provides incremental predictive information for heart failure, but not for cerebrovascular disease [49, 50].

Genetic studies

Genetic studies included candidate gene studies [51] and more recently genome-wide association studies of clinical disease and risk factor phenotypes. So far we have contributed to more than 100 Genome-wide association (GWA) studies in the field of cardiovascular disease. These GWA studies are primarily conducted in the framework of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium [52, 53] however in many instances we include further studies. We identified 3 genetic loci associated with uric acid concentration and gout [54].

We also identified a significant association between the UMOD gene which encodes Tamm-Horsfall protein and chronic kidney disease [55]. We found four genes for systolic blood pressure, six for diastolic blood pressure and one for hypertension [56–58]. We found multiple loci that influenced erythrocyte phenotypes in the CHARGE Consortium [59]. In a meta-analysis in more than 80,000 individuals from 25 studies, we identified 18 loci for CRP levels. The study highlighted immune response and metabolic regulatory pathways involved in the regulation of chronic inflammation [60]. Novel associations of multiple genetic loci with plasma levels of factor VII, factor VIII, and von Willebrand factor were also detected [61]. We have also identified genetic loci associated with the measures of subclinical atherosclerosis burden. Our genome-wide association studies on the 3 measures of subclinical

atherosclerosis identified several new genetic loci [62–64]. We have contributed to GWA studies on coronary artery disease [65, 66].

Nutrition and lifestyle

We found that subjects who had fish intake of more than 19 g/day had a significantly lower prevalence of mild/moderate coronary calcification [67]. Also, we found that an increase of 50 g in processed meat was associated with increased CRP levels [68]. In addition to this, the intake of processed meat was associated with a higher risk of type 2 diabetes which was independent of CRP levels [68]. Likewise, we studied whether dietary proteins, amino acids and acid load were associated with the risk of hypertension. It appeared that these factors were not a major determinant of the risk of hypertension in the Rotterdam Study [69–71].Besides main effects of nutrition, we studied gene-nutrient interactions. We found that dietary vitamin E intake may modulate the relation of SIRT1 genetic variants with body mass index [72]. Also, we studied the modification of magnesium, whole grain and a healthy diet score on fasting glucose and insulin by SNPs related to fasting glucose and insulin as part of the CHARGE consortium [73–75].

Methods update

Clinical follow-up

Information on clinical cardiovascular outcomes is collected through an automated follow-up system. The follow-up system involves linkage of the study base to digital medical records from general practitioners in the study area and subsequent collection of letters of medical specialists and discharge reports in case of hospitalisation. With respect to the vital status of participants, information is also obtained regularly from the municipal health authorities in Rotterdam. After notification, cause and circumstances of death are established by questionnaire from the treating physicians. Clinical cardiovascular outcomes are adjudicated according to established definitions based on international guidelines by study physicians and medical specialists in the field affiliated with the Rotterdam Study. Methods of follow-up data collection, adjudication of events, and definitions of cardiovascular end points have been described in detail previously in this journal [14]. Systematic follow-up data collection is done for the occurrence of cardiovascular mortality, coronary heart disease (including coronary death, myocardial infarction, and coronary revascularization procedures), heart failure, atrial fibrillation, and sudden cardiac death [14]. Diabetes mellitus is defined based on guidelines of the American Diabetes Association and the World Health Organization. We defined incident diabetes as fasting plasma glucose level $\geq$7.0 mmol/L, or the use of oral antidiabetic medication or insulin, or treatment by diet and registered by a general practitioner as having diabetes.

Cardiovascular risk factors

Besides traditional cardiovascular risk factors, five major groups of putative risk factors for cardiovascular conditions are examined. The first group are lifestyle factors, including dietary factors, physical activity, smoking, sleep and vitamin D (as described above). The second are endocrine factors, including diabetes, sex hormones, thyroid gland and adrenal gland hormones and natriuretic peptides (e.g. [29, 30, 37–39]. The third group comprises factors involved in hemostasis, inflammation and endothelial function (e.g. [40, 61, 76]). The fourth group covers genetic factors. In addition to the candidate gene approach, studies are more recently conducted through the genome-wide association approach (e.g. [32–36, 54–66, 73–75]). In genome-wide association studies, data from the Rotterdam Study are often combined with those from other studies in the context of the large collaborative CHARGE consortium [52, 53]. Within the fifth group we are applying both proton Nuclear Magnetic Resonance (^{1}H NMR) and Mass Spectrometry (MS) for metabolic profiling in 2,000 participants of the Rotterdam Study including nearly 200 incident cases of coronary heart disease. Furthermore, in this context, special attention has been given to the contribution of different risk factors in relation to cardiovascular disease in women. Data has been collected to evaluate the impact of specific periods of potential vulnerability across a woman's lifespan; menarche, pregnancy, and menopause.

Non-invasive measures of atherosclerosis

At baseline and follow-up examinations, ultrasonographic assessments of carotid intima-media thickness and carotid plaques were conducted in all participants [44]. At these examinations, also measurements of the ankle-brachial index and aortic calcification (on X-rays of the lumbar spine) were obtained [14]. Carotid–femoral pulse wave velocity, a measure of aortic stiffness, was measured in all *participants of RS-I-3, RS-II-1, and RS-III-1 with an automatic device [45]. Measurements of coronary calcification by electron-beam CT and more recently by MDCT were conducted from 1997 onwards in RS-I and RS-II [46, 48]. From 2003 to 2006, MDCT was used to also quantify calcification in the aortic arch and carotid arteries in RS-I and RS-II. Measurement of carotid plaque components using MRI was done from 2007 to 2012 in all participants

from RS-I, RS-II and RS-III with carotid wall thickening on conventional carotid ultrasound. Repeated MRI measures over time were obtained in RS-I and RS-II.

Electrocardiographic, echocardiographic and other ultrasound measurements

At every exam, a 12-lead 10-second resting ECG is made and processed by the Modular ECG Analysis System (MEANS) to obtain a series of ECG measurements [77]. Abdominal aortic diameters were measured by ultrasound at RS-I-1, and from 2002 (RS-I-4) onwards in all three Rotterdam Study cohorts. Also from 2002 onwards (RS-I-4), repeated echocardiographic measurements are conducted of structural and functional left heart parameters [78]. From 2009 (RS-I-5) onwards, measurements of structure and function of the right heart are also collected, including estimates of pulmonary artery pressure. In the same round a 3-minute resting ECG was measured in all participants.

Nutrition and lifestyle

Nutritional data has been collected in RS-I-1, RS-I-5, RS-II-1, RS-II-3 and RS-III-1 by using semi quantitative food frequency questionnaires (FFQ). In RS-I-1 and RS- II-1, participants completed a checklist about foods and drinks they had consumed at least twice a month during the preceding year and a standardized interview using a validated 170-item semi-quantitative FFQ [79]. In RS-I-5, RS-II-3 and RS-III-I, a more comprehensive FFQ was used during the visits as described in detail previously [80–83]. Development and processing of nutrition data is being performed in close collaboration with the Department of Human Nutrition, Wageningen University, the Netherlands. In RS-I-III, RS-I-5, RS-II-I-3 and RS-III-I, physical activity data was assessed by means of an adapted version of the Zutphen Physical Activity Questionnaire and the LASA Physical Activity Questionnaire [84–86]. The questionnaire contained questions on walking, cycling, gardening, diverse sports, hobbies and on housekeeping. According to time spent in light, moderate and vigorous activity, metabolic equivalents of task were calculated.

For additional EJE references concerning cardiovascular disease see [12, 87–136].

Dermatological diseases

Objectives

Dermatoepidemiologic research in the Rotterdam Study focuses on the frequency of the most common skin conditions as well as on genetic and environmental factors associated with these skin diseases. The emphasis is on cutaneous malignancies such as basal and squamous cell carcinomas (BCC and SCC, respectively) and their precursor lesions (actinic keratosis), inflammatory dermatoses such as eczema and psoriasis, and varicose veins. Also, we examine the frequency and determinants including genetics and environmental exposures of skin aging (pigmentation, wrinkling and photodamage).

Methods

In 2010, dermatology studies were introduced in the Rotterdam Study. To the home interview several items have been added questioning ultraviolet light exposure, history of (personal and familial) psoriasis, history of skin cancer, the diagnostic criteria of British association of dermatology for atopic eczema, adjusted diagnostic criteria for psoriatic arthritis.

A full body skin examination by physicians trained in dermatology with a focus on the most common skin diseases is the core contribution of dermatology. The clinical presence and extent of specific skin diseases (i.e., actinic keratosis, malignancies, psoriasis, xerosis, hand and flexural eczema, alopecia, and signs of chronic venous insufficiency based on the 'C' of the CEAP classification) at time of examination is assessed in a standardized fashion. Other dermatological diseases will just be noted.

The extent of skin aging as a global score and broken down in different aspects such as wrinkling, pigmentary spots, and teleangiecatsia are scored using a validated photonumeric scales and computer algoritms. The Norwood-Hamilton classification and the Ludwig classification is used for male and female pattern hair loss, respectively. Fully standardized 3-dimensional photographs (Premier 3dMDface3-plus UHD, Atlanta, USA) of the face are taken to further assess skin characteristics. The colour of the facial skin and at the inner side of the upper arm are measured using a spectrophotometer (Konica Minolta Sensing, spectrophotometer CM-700d, Singapore).

As for other cancers, pathology data of the cutaneous malignancies is obtained from linkage to the national cancer registry and the Dutch pathology database (PALGA). In a further attempt to identify cohort members with psoriasis, medical files and dispenses at pharmacies have been investigated resulting in over 350 psoriasis cases.

Major findings

In the first follow-up study including the skin examinations of more than 2,000 cohort members, showed that actinic keratosis is very common in this elderly population (AK prevalence was 49 % for men and 28 % for women) [137].

After adjusting for other factors, baldness in men was associated with a strongly increased risk of actinic keratosis.

The first prevalence study of single and multiple BCC was done in the Rotterdam Study and showed that a total of 524 patients (4.8 % of included population) had developed this type of keratinocytic cancer and that 31.1 % had developed more than one tumor during observation [138]. A multi-failure survival model suggested that people with red hair and higher levels of education, and those who had their first BCC at younger age were significantly more likely to develop multiple malignancies. A recent update yielded more than 1,350 participants with a history of BCC and approximately 450 with a SCC. In collaboration with researchers from the Nurses Health Study, the association between common genetic variants and these skin cancers is currently being examined.

In a candidate gene study in almost 6,000 people, we confirmed known and identified new variants associated with digital skin colour extraction. Of the two new skin color genes, the genetic variants in UGT1A were significantly associated with hue and variants in BNC2 were significantly associated with saturation [139].

The psoriasis patients within the Rotterdam Study had predominantly mild disease. The distribution of subclinical artherosclerosis measures as well as the cardiovascular events were comparable between the 262 psoriasis patients and the reference population [140]. However, psoriasis patients were significantly more likely to have signs of nonalcoholic fatty liver disease based on ultrasonography than their controls after adjusting for potential confounders (adjusted OR 1.70, 95 % CI 1.13–2.58).

Endocrine diseases

Objectives

The main objective of the programme of endocrine epidemiology research is to study frequency and etiology of major disorders of the endocrine glands (pituitary, reproductive, thyroid, parathyroid, adrenal, and neuro-endocrine pancreas) and the musculoskeletal system. These include diabetes mellitus, osteoporosis, osteoarthritis, reproductive traits (fertility, age-at-menopause), and hypo- and hyperthyroidism. The evaluation of risk factors for the above mentioned conditions includes serum measurements (such as classical hormones and other endocrine molecules) and genetic determinants of endocrine diseases and traits.

Major findings

In the process of obtaining digitized Xrays for all participants at all time-points of follow-up, we have evaluated the 3 major methods to score vertebral fractures: quantitative morphometry, semi-quantitative morphometry, and the qualitative ABQ method [141]. Prevalence of vertebral fractures differed substantially by the different methods, so standardization is crucial for patient care and for large scale epidemiological studies. Using the digitized Xray scores, we have for the first time determined population-based prevalence of Scheuermann's disease in the Dutch population to be 4 % [142].

In the relationship of type 2 diabetes with bone health we observed that diabetic subjects with inadequately controlled glucose control had 1–5 % increased bone mineral density (BMD) and ~50 % increased fracture risk, compared to diabetics with adequately controlled glucose and to non-diabetics [143].

By studying bone health across different types of hip osteoarthritis (OA), we observed that subjects with atrophic OA (i.e., with joint space narrowing but without osteophytes) have ~50 % increased fracture risk as compared to controls without OA [144]. In addition, we found that hip geometry measures had modest ability to predict hip OA, in addition to clinical risk factors [145].

The research line endocrine diseases is also actively involved in collaborating with economists to study the biology of economic behaviour, in particular entrepreneurial behaviour (and related traits such as educational attainment). In one of the first collaborative analyses we found no evidence for a relationship of testosteron levels with entrepreneurial behaviour [146].

Much of the work of this research is made possible by large-scale collaboration in consortia, some of which focus on one particular disease or trait (e.g., GEFOS), while others are more broad spectrum strategic collaborations (e.g., CHARGE, ENGAGE). We are part of several such large consortia studying genetic and epidemiological risk factors for osteoporosis (GEFOS, GENOMOS, CHANCES, CHARGE), osteoarthritis (TREAT-OA, ArcoGen), diabetes (MAGIC), thyroid disease (CHARGE), and reproductive traits (CHARGE, REPROGEN, PCOSGEN).

Major GWAS findings

With >82,000 samples collected within the GEFOS consortium a landmark publication was achieved with the identification of 56 loci influencing bone mineral density in total explaining ~5 % of genetic variation in BMD, and of which 14 loci also were associated with fracture risk [147].

In a meta-analysis of 15,000 hip OA cases and 54,000 controls assembled in the arcOGen consortium, 5 novel loci influencing risk of hip OA were identified [148]. In an analysis of a so-called endo-phenotype of OA, i.e., joint space width narrowing (JSN) a GWAS among 10,000

subjects we identified a novel locus, called DOT1L, to influence cartilage thickness, as well as the risk for hip OA [149]. Interestingly, this gene product is a known drug target for treating leukemia with several drugs under development. Experiments are therefore now ongoing to evaluate the effect of these drugs on features of OA in animal and cell model systems. Finally, in the analysis of another endophenotype of OA, pain, our group led a large scale meta-analysis to chronic wide spread pain in 2700 cases and 14,000 controls and identified for the first time a genetic locus involved in pain, i.e., CCT5 [150].

In the largest meta-analysis of GWAS of age-at-menopause among 62,000 women and which our group led, we identified 13 loci associated with differences in age-at-menopause and explaining ~5 % of the genetic variation [151]. Interestingly, the majority of the loci most likely involves genes in the DNA repair pathway which points to the importance of this system in maintaining an error-free stemcell lineage to which the oocyte belongs.

In a meta-analysis of GWAS data on TSH levels and free T4 levels derived from up to 26,000 subjects, 26 loci were identified explaining 2–5 % of the genetic variation of TSH and fT4 respectively [152]. There was only limited overlap between the loci for TSH and fT4, and evidence was obtained for 5 loci to have sex-specific effects.

An interesting GWAS involved the analysis of Helicobacter pylori serologic status among members of the Rotterdam Study and the SHIP cohort [153]. Two novel loci were identified, TLR1 and FCGR2A, which can help explain inter-individual differences in risk for H pylori infection.

For many endocrine biomarkers GWAS have been performed to identify the genetic loci influencing their serum levels (e.g., homocysteine, testosteron, SHBG, thyroid hormone levels, etc.) and these are also involved in several mendelian randomization analyses in relation to major disease endpoints for which these biomarkers have been suggested to be predictive.

Methods update

For all participants DXA-based bone mineral density (BMD) measurements of the lumbar spine, dual hip and total body BMD, as well as determination of body composition parameters are assessed with a ProdigyTM total body fan-beam densitometer (GE Lunar Corp, Madison, WI, USA). Hip structural analysis (HSA) of DXA scans including hip strength indexes (using software by GE Lunar) is determined for all scans. Since 2009 we perform iDXA measurements (GE Lunar) which measures L1–L4 BMD, bilateral total hip and femoral neck BMD and total body BMD. From the total body scan, we measure lean mass and fat mass body composition, including total body, trunk, arm, legs, and android and gynoid regions of interest. X-ray examinations of vertebral bodies, hips, knees and hand/wrist are since 2009 obtained by a digitalized Fuji FCR system (FUJIFILM Medical Systems) for all participants. Analogue Xray photographs from previous time –points at all follow up measurements (~75,000 Xrays) have now all been digitized. All the Xrays have now been completely assessed for the presence of fractures and/or degenerative changes of the joints (e.g., Scheuermann's Disease). Vertebral fractures and deformities are assessed using the classical quantitative McCloskey-Kanis method, the semi-quantitative Genant method, and a qualitative algorithm-based technique termed the ABQ method. Incident clinical fractures (of all bone sites) are obtained from computerized records of the general practitioners and hospital registries which are regularly checked by research physicians who review and code the fracture information.

Muscle strength is assessed in all participants with a hand grip dynamometer. Muscle mass estimates are derived from whole body iDXA measurements.

The incidence and progression of OA is assessed using Kellgren scores obtained from X-rays of hips, knees, hands, and spine. The complete set of X-rays (all participants, all follow-up time points) has now been evaluated for the Kellgren score at these 4 joints. Novel diagnostic assessments for OA are available using Magnetic Resonance Imaging (MRI) of the knees in a large subset of the population (n = ~ 1,000 RS-III). In addition, pain measurements were added in 2011 in this research line including a quantitative assessment of heat sensitivity on the arm using a standardized device, and indications of (wide-spread) pain in any part of the body using a puppet.

Several specific biomarker assessments in blood/serum/plasma and urine are done for the diagnosis and evaluation of risk factors of endocrine and metabolic diseases (e.g., glucose, TSH, freeT4, steroid hormones, calcium, CTXII, etc.). Fasting blood samples are collected along with challenged samples as part of a glucose tolerance test. Sputum is collected before and after a dexamethasone-suppression test. Finally, validated questionnaires evaluating nutrient intake (e.g., calcium and vitamins) and activities of daily living, allow to evaluate the role of environmental factors in endocrine conditions and locomotor diseases of the elderly.

For additional EJE references concerning endocrine diseases see [154–173].

Liver diseases

Objectives

Fibrogenesis of the liver is most probably not only the result of well known liver diseases, such as viral hepatitis, alcoholic liver disease or non-alcoholic fatty liver disease

(NAFLD), but rather a complex interaction between a genetic predisposition and these liver disorders. Liver research in the Rotterdam Study will concern the association between these known causes of liver disease and the occurrence, magnitude, and progression of fibrosis in combination with genetic and environmental factors. Additional research focus will be on NAFLD. NAFLD is considered the hepatic manifestation of the metabolic syndrome and has become the most common chronic liver disease in Western countries in parallel with epidemics of obesity and type II diabetes mellitus. We aim to study the occurrence and risk factors of NAFLD in a general population and generate insight into the association with cardiovascular morbidity and mortality.

Methods

Abdominal ultrasound

From February 2009 trained technicians perform abdominal ultrasonography in Rotterdam Study participants. Liver, biliary tract, gall bladder, spleen, pancreas, and kidneys in combination with doppler examination of hepatic veins, hepatic artery and portal vein will be evaluated. All images are stored digitally and will be reevaluated by an ultrasound trained physician.

Assessment of steatosis

The diagnosis and grading of liver steatosis will be based on ultrasonographic liver brightness, hepatorenal echo contrast, deep attenuation and vessel blurring [174].

Non alcoholic fatty liver is diagnosed by presence of steatosis on ultrasound and exclusion of excessive alcohol consumption, presence of viral hepatitis, use of fatty liver inducing pharmacological agents, recent bariatric surgery and a history of inflammatory bowel disease.

Assessment of fibrosis

Ultrasonographic evaluation of the liver parenchyma and liver surface will be performed in order to assess severe fibrosis and/or cirrhosis. Additionally, sonographic signs of portal hypertension will be studied (splenomegaly, venous collaterals, portal vein diameter and flow, hepatic venous flow, and the presence of ascites).

To assess and quantify the grade of fibrosis trained technicians will perform elastography in all participants. This test measures non-invasively and quantitatively the liver stiffness using a probe which includes an ultrasonic transducer transmitting a vibration wave through the liver. The velocity of the ultrasonic wave correlates directly with tissue stiffness [175, 176].

Determinants of interest

The association between factors known to influence liver function and the occurrence of steatosis and fibrosis will be studied. Additionally the association of these conditions with age, gender, nutritional intake, concurrent alcohol intake, risk factors for viral hepatitis, BMI, waist-to-hip ratio, serum glucose, insulin, and diabetes mellitus, serum cholesterol and triglycerides will be studied. All clinical information will be obtained by interview (updated with liver specific questions) and clinical examination. For recent EJE references see [91, 177–180].

Neurological diseases

Objectives

Neuroepidemiologic research in the Rotterdam Study focuses on the frequency, etiology and early recognition of the most frequent neurologic diseases in the elderly. We study neurodegenerative diseases (dementia, including Alzheimer disease and Parkinson disease), cerebrovascular disease (both ischemic stroke and intracerebral hemorrhage), and recently migraine and polyneuropathy. In all of these disorders clinical symptoms typically become manifest late in the disease course, the occurrence of clinical disease does not reflect the underlying spectrum of disease-related pathology, and most of the clinical syndromes are etiologically heterogeneous. Therefore, an additional research focus is on the causes and consequences of presymptomatic brain pathology that can be assessed with non-invasive modalities, which include MR-imaging, cognitive testing, gait assessment, and recently electromyography (EMG).

Major findings

Neurodegenerative and cerebrovascular diseases are highly frequent in the elderly. The prevalence increases from age 55–65 years to age 90 years and above from less than 1 % to over 40 % for dementia [181], from less than 0.5 % to more than 4 % for Parkinson disease [182], and from approximately 1 % to nearly 10 % for stroke. The incidence figures follow this pattern of a strong increase with age over the entire age range, with the age-specific incidence of dementia being identical for men and women at least until the age of 85 [183] but with men having a higher age-specific incidence of both stroke and Parkinson disease than women throughout the age range [184, 185].

However, more recent numbers from 2000 onwards suggest that the relative incidence of dementia and stroke may be lower than in the 1990s [186–188]. Still, in absolute numbers these disease will dramatically increase in prevalence over the coming decades.

Vascular pathology and vascular risk factors are associated with worse cognitive performance [189], which also translates in people with vascular pathology or risk factors for vascular disease having an increased risk of dementia, including Alzheimer disease [190]. Moreover, several life style factors are associated with the risk of dementia and Alzheimer disease [191, 192], suggesting that onset of dementia may at least partly be delayed or prevented. However, many of these lifestyle factors have only a short-term effect, suggesting reverse causality to some extent [193]. Commonly used drugs may also have a role in development of dementia [194]. Similar risk factor profiles also underlie cognitive decline prior to the clinical diagnosis of dementia [195, 196].

The classical risk factors for stroke also associate with the risk of stroke in the Rotterdam Study [197, 198]. In contrast, some emerging putative risk factors are not associated with stroke [199], whilst others, such as inflammatory markers, may be etiologically relevant but thus far add little to the identification of people at risk [200]. Possibly underlying this is that a large amount of stroke goes clinically undetected [201]. Nearly 20 % of elderly people have at least one silent brain infarct, and thereby a nearly fourfold increased risk of clinical stroke, a more than doubled risk of dementia including Alzheimer disease, and an increased risk of depression [202].

With the advent of genome-wide association studies, the Rotterdam Study has contributed to large-scale collaborations and contributed to the identification of novel genes underlying the risk of Alzheimer disease and stroke [203–205]. In turn, we have also shown that although currently known genetic variants for dementia associate with cognition in non-demented elderly, these do not improve prediction [206].

Neuroimaging reveals that brain pathology is widespread [207] and can go clinically undetected for a long time. In addition to the silent infarcts, many apparently healthy elderly have ischemic changes in their cerebral white matter, i.e. white matter lesions, that are associated with an increased risk of dementia, stroke and depression. Also brain atrophy, especially of the hippocampus, is already present years before onset of even the earliest sign of cognitive impairment or subjective complaints. This emphasizes the need to shift the attention in etiologic research of neurodegenerative and cerebrovascular disease to the causes of pre-symptomatic and underlying brain changes. Technological advances in image acquisition, optimized imaging sequences and automated post-processing of multispectral MR data are major drivers of the rapid developments in this field. With our current imaging protocol we can now not only investigate established markers of brain pathology, such as infarcts, white matter lesions, and atrophy, but also extend towards novel markers, such as cerebral microbleeds and diffusion tensor imaging [208]. We have shown that risk factors profiles for subclinical disease overlap with those for clinical disease [209–213]. Also, subclinical white matter lesions and silent infarcts improve the clinical prediction of incident stroke [214]. The clinical relevance of subclinical pathology is demonstrated by strong associations of these markers with morbidity and mortality [215–218].

Finally, we have extensively studied the genetic basis of subclinical brain disease, both in genome-wide settings and in candidate-gene studies [219–225] (see further section on population imaging).

Most research on the preclinical phase of neurodegenerative diseases, particularly dementia, focuses on either cognitive performance or brain pathology on neuroimaging. Given that brain pathology is usually diffuse, it is conceivable that brain functions other than cognition will also be affected. In this light, we have shown that gait deteriorates significantly with age. Also, gait and cognition are linked following a specific pattern of certain cognitive domains only associated with specific aspects of gait, but not others [226] .

Methods update

Assessment of dementia and Alzheimer disease

In the baseline and follow-up examinations participants undergo an initial screen for dementia with the Mini Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS), followed by an examination and informant interview with the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX) in screenpositives (MMSE < 26 or GMS > 0), and subsequent neurological, neuropsychological and neuroimaging examinations [181, 183]. Of subjects who cannot be reexamined in person, information is obtained from the GPs and the regional institute for outpatient mental health care. A consensus panel makes the final diagnoses in accordance with standard criteria (DSM-III-R criteria; NINCDS-ADRDA; NINDS-AIREN).

Assessment of Parkinsonism and Parkinson disease

Participants are screened in the baseline and follow-up examinations for cardinal signs of parkinsonism (resting tremor, rigidity, bradykinesia, or impaired postural reflexes). Persons with at least one sign present are

examined with the Unified Parkinson's Disease Rating Scale and a further neurologic exam. PD is diagnosed if two or more cardinal signs are present in a subject not taking antiparkinsonian drugs, or if at least one sign has improved through medication, and when all causes of secondary parkinsonism (dementia, use of neuroleptics, cerebrovascular disease, multiple system atrophy, or progressive supranuclear palsy) can be excluded [182, 185].

Assessment of stroke and stroke subtypes

History of stroke at baseline was assessed through interview and verified in medical records. Putative incident strokes get identified through the linkage of the study database with files from general practitioners, the municipality, and nursing home physicians' files, after which additional information (including brain imaging) is collected from hospital records. A panel discusses all potential strokes and subclassifies strokes into ischemic, hemorrhagic or unspecified [184, 200]. We also systematically collect transient ischemic and neurological attacks [227].

Assessment of cognitive function

Global cognitive function is measured through the Mini Mental State Examination (MMSE) in all surveys. From the third survey (RS-I-3) onwards we added a 30 min test battery that was designed to assess executive function and memory function, and which includes a Stroop test, a Letter Digit Substitution Task, a Word Fluency Test, and a 15 words Word List Learning test. This test battery was expanded from the fourth survey onwards (RS-I-4) to include motor function assessment using the Purdue Pegboard Test. Moreover, from 2009 onwards we expanded further by including the Design Orientation Test (DOT) and a modified version of the International Cooperative Ataxia Rating Scale (ICARS), which assess visuo-spatial orientation and ataxia respectively [228, 229].

Assessment of gait patterns

Halfway through RS-III-1, we successfully implemented the assessment of gait in all participants using the GAITRite walkway (http://www.gaitrite.com/). Gait is assessed using a 5.79 meter long walkway (GAITRite Platinum; CIR systems, Sparta, NJ, USA: 4.88 meter active area; 120 Hertz sampling rate) with pressure sensors. Participants perform a standardized gait protocol consisting of three different walking conditions: normal walk, turning and tandem walk. In the normal walk, participants walk over the walkway at their own pace. This walk is repeated four times in both directions (yielding a total of 8 recordings). In turning, participants walk over the walkway at their own pace, turn halfway and return to the starting position (1 recording). In the tandem walk, participants walk tandem (heel-to-toe) over a line visible on the walkway (1 recording). A total of 30 spatiotemporal gait variables are calculated by the walkway software and downloaded offline for further analysis. Subsequently, principal components analysis on these thirty gait variables is performed to derive summarizing factors, referred to as gait domains. The following gait domains are used: Rhythm, Pace, Phases, Base of Support, Variability, Tandem, and Turn. Gait domains can be compared to cognitive domains, in which each domain reflects a different aspect of the overall concept [226].

Assessment of polyneuropathy

Starting in January 2013, we have successfully implemented a protocol to assess polyneuropathy. This includes a full work-up including questionnaire, neurological exam, and EMG in all participants. In coming years, we will publish on the prevalence, risk factors, and clinical correlates of polyneuropathy in the general population. The continuous measures of conductivity obtained through EMG can also serve as excellent endophenotype for genetic and biomarker studies.

Rotterdam Scan Study: brain imaging within the Rotterdam Study

In 1991, a random sample of 111 participants underwent axial T2-weighted magnetic resonance (MR) imaging to assess presence and severity of white matter lesions [207]. In 1995, a random sample of 563 non-demented participants underwent brain MR imaging in the context of the Rotterdam Scan Study. From August 2005 onwards (RS-II-2 and further), a dedicated 1.5 Tesla scanner is operational in the research center of the Rotterdam Study, and brain imaging is performed in all study participants without contra-indications [208].

Currently, the follow-up of this latter sample extends to up to 7 years. Therefore, in the coming years we will be able to investigate how cerebral microbleeds and DTI-markers relate to incident neurological diseases (see further section on population imaging).

For additional EJE references see [230–241].

Ophthalmic diseases

Objectives

The main objectives of the ophthalmic epidemiology studies are to investigate frequency, genetic and

environmental risk factors, their interaction, (endo)phenotypic characteristics, and potential biomarkers or disease intermediates of frequently occurring complex eye disorders. We particularly focus on age-related macular degeneration, open angle glaucoma, and myopia; other areas of interest are retinal vessel diameters, and diabetic or vascular retinopathy.

Major findings

Age-related macular degeneration (AMD)

Although many major genes for AMD have already been identified these last years, missing heritability was still abundantly present. To identify more genes, we combined our efforts with international study groups which had a total study population consisting of 17,100 advanced AMD cases and > 60,000 control subjects [242]. Within this AMDGene consortium, 7 new genetic regions (*COL8A1/FILIP1L; IER3/DDR1; SLC16A8; TGFBR1; RAD51B; ADAMTS9/MIR548A2; B3GALTL*) for AMD were found. Taken together with the already known genes, a total of 19 AMD genes were identified in this consortium. Highest genetic associations were found for the *ARMS2* and *CFH* genes. The pathogenetic pathways that can be deduced from these genes are the complement pathway, atherosclerosis signaling and lipid pathway, angiogenesis, and extra-cellular matrix remodeling. Further steps to diminish missing heritability are identification of genetic variants by NGSexome sequencing. This is the focus of attention of the newly formed AMDExome Chip consortium.

To perform more extensive analyses involving genetic as well as other risk factors, we also searched for connection with international studies. The 3 Continent AMD Consortium was established, a consortium of four population-based studies: Beaver Dam Eye Study (BDES) [243] and Los Angeles Latino Eye Study (LALES) [244] from the United States; Blue Mountain Eye Study (BMES) from Australia [245], and our own Rotterdam Study (RS) from the Netherlands. The total study population of this consortium is 25,000 subjects aged 45 + years, and many of these subjects have been followed for >15 years. A first effort of the consortium was harmonizing the outcome variable AMD, and a new severity scale was launched. Secondly, a detailed analysis of gene-diet interaction was performed, showing that the major AMD genes interact with the anti-oxidants lutein/zeaxanthin. Thirdly, we developed a prediction model for late AMD based on well-known genetic and environmental risk factors, age, sex, and early AMD phenotype. Incorporation of all these factors provided a predictive value of 0.88 (area under the curve; AUC) to predict late AMD in the discovery cohort the Rotterdam Study, and a predictive value of 0.85 (AUC) in the BDES and BMES at validation. The prediction model enabled distinction between those with virtually no risk of late AMD and those with up to 65 % cumulative risk. We think these findings will be useful for clinicians as well as researchers. They may facilitate recognition of high risk individuals who need life style advice to diminish their risk, as well as identification of a suitable study population for intervention trials.

Research aims for 2013 are evaluation of gene and gene-environment effects on (sub)clinical manifestations of disease as visible on various imaging devices (OCT; fundus autofluorescence; infrared); or as systemic biomarkers; and interaction with medication.

Open angle glaucoma (POAG)

After a first attempt was made to identify genes for optic disc parameters in 2011, we now investigated the genetic factors for intraocular pressure (IOP) in the Rotterdam Study. IOP is a highly heritable risk factor for primary open-angle glaucoma and is the only target for current glaucoma therapy. We performed a GWAS for IOP in 11,972 participants from the 3 Rotterdam Studies and ERF [246]. We then replicated our findings in 7,482 participants from 4 additional cohorts from the UK, Australia, Canada, and the Wellcome Trust Case–Control Consortium 2/Blue Mountains Eye Study. IOP was significantly associated with *GAS7* and *TMCO1*, and risk variants also appeared to associate with glaucoma.

The genes are highly expressed in significant eye structures involved in glaucoma pathogenesis, and we think we have identified two clinically relevant genes involved in IOP regulation. Further action in this realm has been the formation of an international consortium for meta-analyses of GWAS data. Collaboration was found with 16 studies from 7 countries including 45,000 study participants, forming the International Glaucoma Genetics Consortium (IGGC). Genetic meta-analyses are currently being carried out.

Relations with environmental factors were studied with a focus on diet. We found that a low intake of retinol equivalents and vitamin B1 and a high intake of magnesium were associated with an increased risk of OAG [247]. These relations will be further explored.

Imaging of POAG hallmarks on OCT is a relatively new but competitive field. Quantification of the retinal nerve fiber and ganglion cell layer measured on multiple images taken over time would help to establish the early course of disease, and the use of continuous outcomes to estimate progression would facilitate statistical analyses. In a collaborative study with University of Iowa, we investigated test–retest variability of measurements on 3-D OCT data. We found that combined macular RNFL and RGCL

thickness averaged over larger areas had the best test–retest variability, and may be the best outcome parameter to use in future analyses [248].

Future investigations will focus on in-depth elucidation of the genetic background of POAG, identification of metabolic factors, further development of techniques to improve measurements of retinal layers on OCT, risk modeling, and study of gene-environment interactions.

Myopia

After our successful identification of the first gene for common refractive errors and myopia in 2010 [249], we took the initiative to form a consortium with international GWA studies. CREAM (Consortium Refractive Error And Myopia) was formed consisting of 45,000 study participants, and a meta-analysis found 26 significant genomic loci for refractive error [250]. The new loci include candidate genes with functions in neurotransmission (*GRIA4*), ion transport (*KCNQ5*), retinoic acid metabolism (*RDH5*), extracellular matrix remodeling (*LAMA2* and *BMP2*) and eye development (*SIX6* and *PRSS56*). We also confirmed previously reported associations with *GJD2* and *RASGRF1*. Risk score analysis using associated SNPs showed a tenfold increased risk of myopia for individuals carrying the highest genetic load. Our new research is aimed at further clarification of genetic mechanisms, the study of interaction with environmental factors such as education, and search for possible common pathways with glaucoma. Other actions which will be undertaken is development of a classification scheme for myopic retinal changes on various retinal images (photographs; OCT; autofluorescence), and the study of genotype-phenotype correlations using this classification.

Methods update

At baseline and follow-up examinations participants undergo ophthalmic measurements including best-corrected ETDRS visual acuity, refractive error, Goldmann applanation tonometry, keratometry, slit lamp examination of the anterior segment and visual field testing. In pharmacological mydriasis we make 35° color photographs of the macular area, and 20° simultaneous stereoscopic imaging of the optic disc and macular area. Since the fourth follow-up, 35° stereoscopic color photographs of the optical disc and the macular area were made (RS-I-5). Analog fundus photography was replaced by stereoscopic digital imaging of the macular area and optic disc since the third follow-up examination. Optic nerve head analysis with a Heidelberg Retina Tomograph, macular pigment density, and melanin optical density measurements were added during the third follow-up (RS-I-3). At fourth follow-up examination, fourier domain optical coherence tomography of the macular area and optical disc, axial length and width measurements of cornea, anterior chamber, lens, posterior chamber and retina measured with Lenstar; and fundus autofluorescence, infra-red and red-free measurements were added (RS-I-5).

Classification of AMD, POAG, and retinal vessel diameters remained unchanged; refractive error was evaluated as spherical value + half cylindrical value, following clinical standards.

For additional EJE references see [247, 251].

Psychiatric diseases

Objectives

The aim of the psychiatric research in the Rotterdam Study is to investigate the determinants, correlates and consequences of common psychiatric problems in the elderly. The focus has been on depressive disorders but anxiety disorders, sleep disturbances, addiction to smoking, and complicated grief are also being studied.

Study design update

In the first years of the Rotterdam Study, psychiatric data collection was very limited. However, in the second visit most participants were screened for depressive symptoms and from the third examination onwards (RS-I-3), which began in 1997, depressive disorders have been ascertained in all participants. Assessments of anxiety disorders, sleeping disturbances, and complicated grief were added in the subsequent examination (RS-I-4) and have been performed in all follow-up visits of RS-I and II, and in the baseline of RS III. Recent additions to the protocol included a screening for psychotic symptoms and, starting in January 2012, ambulatory polysomnography.

Major determinants

Psychiatric research in the Rotterdam Study focuses on biological risk factors. The vascular depression hypothesis was tested with different measures of atherosclerosis, arterial stiffness and cerebral blood flow [252]. We also examined whether blood levels of vitamins and fatty acids, immune parameters, and markers of folate metabolism increased the likelihood of depression [253]. Diurnal patterns of cortisol secretion were related to psychiatric and other disorders such as subclinical atherosclerosis [254]. Only a few candidate gene studies were performed, currently GWAs data only is analysed in collaborative efforts

focussing on depressive symptoms, sleep, anxiety and cortisol [255, 256]. Several studies of brain morphology are underway [257]. Current data collection includes a dexamethasone suppression test to measure hypothalamic–pituitary–adrenal axis activity in all participants, which is unique in a population-based study. Also, psychiatric problems and psychological traits such as happiness, sleep duration, and depression are increasingly studied as determinants of health and mortality [258].

Major outcomes

Information on depression is obtained from (a) psychiatric examinations, (b) self-reported histories of depression, (c) medical records, and (d) registration of antidepressant use [259]. The psychiatric examination during follow-up visits consists of a screening with the Center for Epidemiologic Studies Depression Scale (CES-D), and in the screen-positive participants a semi-structured interview performed by a trained clinician (Schedules for Clinical Assessment in Neuropsychiatry). To continuously monitor incidence of depression throughout follow-up, trained research-assistants scrutinize the medical records of the general practitioners and copy the information about possible depressive episodes.

The following anxiety disorders are assessed with a slightly adapted Munich version of the Composite International Diagnostic Interview: generalized anxiety disorder, specific and social phobia, agoraphobia without panic disorder, and panic disorder [260].

Sleep quality and disturbance is measured with the Pittsburgh Sleep Quality Index. In addition, sleep duration and fragmentation are assessed with actigraphy, a method that infers wakefulness and sleep from the presence or absence of limb movement [261]. In total, nearly 2000 persons participated in this actigraphy study: they wore an actigraph and kept a sleep diary for, on average, six consecutive nights.

Circadian rhythms: Sleep-wake activity patterns over a week are studied with actigraphy As a marker of circadian rhythms. In more than 1700 persons we calculated interdaily stability, i.e. the stability of the rhythm over days and the intra-daily variability, i.e. the fragmentation of the rhythm.

The Inventory of Complicated Grief is used to identify traumatic grief [262]. This is a condition distinct from normal grief and bereavement-related depression, characterized by symptoms like disbelief about the death and searching for the deceased.

Major findings

Depression: The incidence and recurrence of depression in the elderly was estimated by continuously monitoring depression during a follow-up period of, on average, 8 years [259]. In total, 566 depressive syndromes and 1,073 episodes of clinically relevant depressive symptoms occurred. For depressive syndromes, the incidence rate was 7.0 (95 % CI 6.0–8.3) per 1,000 person-years and the recurrence rate was 27.5 (95 % CI 23.7–32.1) per 1,000 person-years. The recurrence rate of depressive syndromes was equal for women and men.

In a series of initial studies we found some evidence for the vascular depression hypothesis. More severe coronary and extra-coronary atherosclerosis were associated with a higher prevalence of depression, as were cerebral haemodynamic changes [252]. However, our data did not support a specific symptom profile of vascular depression as previously defined [263]. Most importantly, we found no longitudinal relation between peripheral atherosclerosis and incident depression [264]. Recently we prospectively studied cerebral vascular risk factors such as white matter lesions, silent infarcts or blood flow in relation to depression [265]. We found evidence that small vessel disease predicted the onset of depression. This suggests that atherosclerotic processes in the brain are a specific risk factor for depression, but provides little support for a vascular depression phenotype as a distinct entity.

Sleep: We investigated the relationships of sleep duration with both cardiovascular risk factors and psychiatric disorders. We found a marked U-shaped association of actigraphically measured total sleep time with BMI and obesity [266]. We also investigated and aimed to explain sex differences in subjective and actigraphic sleep parameters [267]. If assessed by diary or interview, elderly women consistently reported shorter and poorer sleep than elderly men. In contrast, actigraphic sleep measures showed shorter and poorer sleep in men. These discrepancies were partly explained by sleep medication use and alcohol consumption.

Anxiety: We found that prevalent anxiety disorders fulfilling DSM-IV criteria may be much less co-morbid with depressive disorders than previously thought if the disorders are assessed with different diagnostic instruments. On the other hand, a history of depression is very common in persons with prevalent anxiety disorder [268].

Smoking: Typically, determinants of smoking cessation are studied by comparing former with current smokers [269]. We also used a prospective approach of studying smoking cessation in 1,200 smokers (mean years of smoking: 40 years, minimum: 10 years). Smoking status was repeatedly assessed during follow-up every 3- to 4-years. Thus, an individual could contribute any number of person-years to the analyses. In other words, people were classified as smokers or quitters. This approach enabled us to detect a genetic effect on the incidence of smoking cessation [270].

Complicated grief: In our population-based study of 5741 elderly persons, current grief was reported by 1,089 participants, of these 277 (25 % or 4.8 % of total) were diagnosed with complicated grief, the vast majority of which had no clinical symptoms of anxiety or depression. Persons with complicated grief were older, had a lower level of education, and more often had lost a child [271]. Ongoing work suggests that complicated grief occurs together with structural brain atrophy more often than expected by chance.

Genetics of common psychiatric disorders: In the past year, we have performed a series of genome-wide association studies of the above psychiatric and psychological phenotypes, mostly as part of the CHARGE consortium. Several analyses have yielded no convincing genome wide significant results—clearly the initial studies were underpowered, psychiatric phenotypes do not present very homogenous entities and are highly polygenetic. Disappointingly, the genome wide analyses of intermediate phenotype in the field of psychiatry such as cortisol or executive function have hardly been more successful [256, 272]. To study the genetics of cortisol, we have established a dedicated consortium of population-based studies: CORNET.

Finally, ongoing psychiatric research projects examine whether and how psychological well-being or psychiatric problems contribute to survival. Most importantly, we are interested in whether the effects are specific to certain behaviour or emotions, are independent of confounding by physical disease, or can be explained by lifestyle, immunological or hormonal regulation [273].

For additional EJE references see [94, 120, 132, 274–291].

Respiratory diseases

Objectives

The objectives are to investigate the incidence of respiratory diseases, to study genetic and environmental risk factors for these diseases, and the effect of respiratory diseases on quality of life, hospitalizations and mortality. The respiratory diseases of interest in the RS encompass chronic obstructive pulmonary disease (COPD), asthma, asthma and COPD overlap syndrome, respiratory tract infections, pneumonia and lung cancer. COPD is defined as a chronic airway disease characterized by airflow limitation that is not fully reversible and usually progressive [292]. COPD is a worldwide leading cause of chronic morbidity, disability and mortality; by 2020, COPD will become the third most common cause of death worldwide [293]. In patients with COPD, comorbidities and exacerbations of COPD contribute to the severity and the prognosis of the disease. Asthma is characterized by intermittent attacks of breathlessness, wheezing and cough, often nocturnal or elicited by exposure to specific allergens (such as house dust mites, animal danders and pollens) or to non-specific triggers (such as exercise and cigarette smoke). Although the onset of asthma is most common during childhood, asthma can also occur in adulthood [294]. Asthma in the elderly is underdiagnosed and undertreated, and there is a paucity of knowledge on the clinical course and outcomes of asthma in this population [295].

Major findings

In the first cohort of the Rotterdam Study (RS-I) of 7,983 participants, 648 cases have been identified with incident COPD after a median follow-up time of 11 years, resulting in an overall incidence rate of 9.2/1,000 person-years (PY) (95 % CI 8.5–10.0) [296]. The incidence rate of COPD was higher in smokers than in never-smokers (12.8/1,000 PY; 95 % CI 11.7–13.9 vs. 3.9/1,000 PY; 95 % CI 3.2–4.7) and higher in men than in women (14.4/1,000 PY; 95 % CI 13.0–16.0 vs. 6.2/1,000 PY; 95 % CI 5.5–7.0). In the second and third cohort of the Rotterdam Study (RS-II and RS-III) of 3,011 and 3,932 participants, respectively, a diagnosis of COPD has been validated in 782 subjects based on spirometry, files from the general practitioners and hospital discharge letters.

COPD and comorbidities

Since COPD does not only affect the lungs, but is also associated with extrathoracic manifestations and comorbidities, an important line of research focuses on the association of COPD with other diseases (such as cardiovascular, cerebrovascular, endocrine, oncological and neurological diseases). COPD has been shown to be an independent risk factor for ischemic stroke and the risk increases by severity of airflow limitation. We investigated the prevalence of carotid artery wall thickening by ultrasonography and characterized the carotid artery plaque composition by high-resolution magnetic resonance imaging in subjects with COPD compared with non-smoking and smoking subjects without airflow limitation. COPD subjects had a twofold increased risk of carotid wall thickening compared with control subjects with a normal lung function (odds ratio, 2.0; 95 % CI 1.44–2.85), and the risk increased significantly with severity of airflow limitation [297]. Importantly, vulnerable lipid core plaques were more frequent on magnetic resonance imaging in COPD cases than in control subjects (odds ratio, 2.1; 95 % CI 1.25–3.69). Since vulnerable carotid artery plaques place persons at risk for ischemic stroke through disruption

of the plaque surface and thromboembolism, these observations offer new insights into the link between COPD and ischemic stroke.

COPD and systemic inflammation

We investigate the role of systemic inflammation in the pathogenesis of COPD and its comorbidities. Using a Mendelian randomization approach, we investigated the role of C-reactive protein (CRP) and interleukin-6 (IL-6) in the pathogenesis of COPD. We demonstrated that increased serum levels of high sensitivity (hs) CRP (>3 mg/l), a marker of systemic inflammation, are significantly associated with an increased incidence of COPD (hazard ratio (HR), 1.7; 95 % CI 1.16–2.49) compared with subjects with low CRP levels (<1 mg/l), but that variations (single nucleotide polymorphisms and haplotypes) in the CRP gene are not associated with COPD [298]. Since IL-6 induces the expression of acute phase proteins such as CRP in hepatocytes, we performed a Mendelian randomization study of IL-6 in the Rotterdam Study. Increased plasma levels of IL-6 at baseline were significantly associated with an increased risk to develop COPD [299]. However, genetic variation in the IL-6 gene did not alter the risk for COPD. These findings suggest that IL-6 and hsCRP are *markers* of systemic inflammation, but are not driving the pathogenesis of the pulmonary dysfunction in COPD (*makers* of disease).

Genetic determinants of lung function and COPD

COPD is an obstructive airway disease, characterized by a reduced ratio of forced expiratory volume in one second (FEV_1) to forced vital capacity (FVC) (i.e. FEV_1/FVC). The first genome-wide association study (GWAS) of pulmonary function measures, performed in the Framingham Heart Study, identified single nucleotide polymorphisms (SNPs) near the *HHIP* (hedgehog-interacting protein) gene on chromosome 4q31 which were associated with the FEV_1/FVC ratio. We meta-analyzed GWAS for FEV_1/FVC in 20,890 participants of European ancestry from four CHARGE Consortium studies: Atherosclerosis Risk in Communities, Cardiovascular Health Study, Framingham Heart Study and Rotterdam Study. We confirmed the *HHIP* locus and identified seven novel loci associated with FEV_1/FVC at genome-wide significance [$P < 5 \times 10(-8)$], including *GPR126* (G-protein-coupled receptor 126), *ADAM19* (A disintegrin and metalloproteinase 19), *AGER* (advanced glycation endproducts receptor), *FAM13A*, *PID1, HTR4* (serotonin receptor 4) and *PTCH1* (patched 1, receptor for HHIP) [300]. Thanks to collaboration between the CHARGE and Spirometa Consortium, 16 additional genetic loci have been shown to be associated with lung function, including *MMP15, TGFB2, HDAC4* and *RARB* (retinoic acid receptor B) [301]. Since several identified genes (e.g. *RARB*, *HHIP* and *PTCH1*) play crucial roles in lung development by regulating branching morphogenesis during foetal life, the results of these GWAS suggest that genetic variants associated with lung development and growth might be important genetic determinants of lung function in childhood and adulthood, both in healthy subjects and in patients with airway disease (asthma and COPD) [302].

Since only approximately 20 % of smokers develop COPD, genetic risk factors are suspected to be involved in the pathogenesis of the disease. In the Rotterdam Study, we confirmed the association between the SNPs near the *HHIP* gene and COPD, and demonstrated a significant interaction with the number of pack-years of smoking [303]. In a collaborative effort of fifteen cohorts, we performed meta-analyses of GWAS for airflow obstruction, a key pathophysiologic characteristic of COPD, defined as FEV_1 and FEV_1/FVC both less than their respective lower limits of normal. We confirmed the association of airflow obstruction to the chromosome 15 *CHRNA5/CHRNA3* (nicotinic acetylcholine receptor, subunits alpha 5 and alpha 3) gene cluster, even in never smokers, and implicated the *HTR4* gene in the pathogenesis of COPD [304]. In gene expression studies we confirmed the presence of *CHRNA5/3* in lung, bronchial epithelial cells and airway smooth muscle cells, suggesting that the *CHRNA5/3* region might act as a genetic risk factor for airflow obstruction independent of smoking. Lastly, in genome-wide joint meta-analyses of SNP and SNP-by-smoking associations on FEV_1 and FEV_1/FVC across 19 studies, we identified three novel loci not previously associated with pulmonary function. SNPs in or near *DNER*, *KCNJ2* and *SOX9*, and the HLA region (*HLA-DQB1* and *HLA-DQA2*), were associated with FEV_1/FVC or FEV_1 [305]. These findings demonstrate that joint testing of SNP and SNP-by-environment interaction is able to identify novel loci associated with complex traits that are missed when considering only the genetic main effects.

Methods update

Lung function measurements

Spirometry is performed by trained paramedical personnel using an electronic spirometer with pneumotachograph (Jaeger Master Screen PFT, Cardinal Health, Hoechberg, Germany), according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines. Forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and FEV_1/FVC ratio are measured. The spirogram (volume-time curve) and the maximal

expiratory flow-volume curve are recorded. The spirometries are interpreted by two research physicians according to the ATS/ERS guidelines; a senior respiratory physician decides in case of discordance between both physicians.

Diffusing capacity of the lungs (DLCO) is measured by single-breath determination of carbon monoxide (CO) uptake in the lung using the Jaeger Master Screen PFT Pro Diffusion apparatus according to the ATS/ERS task force on standardization of lung function testing [306]. The test gases used to calculate DLCO include the tracer gas methane, to measure alveolar volume (VA), and carbon monoxide (CO 0.3 %). The DLCO and the diffusing capacity for CO per unit of alveolar volume, also known as transfer coefficient of the lung (i.e. DLCO/VA or KCO), are corrected for blood hemoglobin levels, measured at the same visit of the participants to the Rotterdam Study center.

Clinical assessment of COPD

For the validation of the COPD cases, we have access to spirometry reports, files from the general practitioners, hospital discharge letters and pharmacy dispensing data for subjects participating in the Rotterdam Study. The diagnosis of COPD is based on an obstructive spirometry examination according to the modified Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (FEV_1/FVC < 70 %) and classified into mild, moderate, or severe airflow limitation by FEV_1% predicted of $\geq$80 %, 50–80 % or <50 % respectively. Clinical outcomes are collected during our continuous follow-up and encompass respiratory and non-respiratory death, hospitalizations due to exacerbations of COPD (defined as severe exacerbations) and exacerbations of COPD treated with antibiotics and/or systemic corticosteroids (i.e. moderate exacerbations). In addition, according to the GOLD 2011 update, the influence of respiratory symptoms is evaluated. Dyspnoea score is based on five dyspnoea-related questions and scored from 0 (never dyspnoeic) to 5 (even dyspnoeic at rest). In addition to the spirometric classification of COPD severity according to GOLD 2007 (GOLD grade I, II and III based on mild, moderate and severe airflow limitation), we also assess COPD subjects according to the updated classification of GOLD 2011 [292]. COPD cases are classified as GOLD A, B, C and D according to the combined assessment of airflow limitation, symptoms and exacerbation history. COPD subjects A have few symptoms and low risk (spirometric classification: GOLD grade 1–2 and $\leq$1 exacerbations per year); COPD subjects B have more symptoms but low risk; COPD cases C have few symptoms but high risk (GOLD grade 3–4 as spirometric classification or $\geq$2 exacerbations per year) and lastly COPD cases D report more symptoms and have high risk. Chronic bronchitis is defined as the self-reported presence of cough and sputum for at least 3 months in each of two consecutive years.

For additional EJE references see [307–324].

Genetic and biomarker studies

Objectives

The team in this research line focusses on bio-banking activities of the participants of the Rotterdam Study and investigates biological determinants of disease (i.e., DNA, RNA, proteins, metabolites). Bio-banking involves collecting, storing and managing the biological tissues of participants of the Rotterdam Study at all follow-up measurements. This concerns mainly blood and urine but increasingly also other tissues and bio-materials, such as sputum, hair and faeces. The research of this group concerns assessment of biological determinants of disease (biomarkers) in these bio-materials. The analysis of markers using genomic technologies (using SNP arrays and next generation sequencing) has substantially increased in volume and importance over the past years.

Major findings

Rotterdam Study investigators are playing leading roles in the emerging large global consortia focussed on assessing the contribution of complex disease gene variants by prospective meta-analysis across many epidemiological cohorts, such as in CHARGE and ENGAGE, and in many disease/phenotype focused efforts such as GEFOS, REPROGEN, TREATOA, DIAGRAM, etc. Since 2005 the genome wide association study (GWAs) has changed the field of complex genetics, and identified a still growing list of common variants contributing to disease risk and explaining genetic variance of traits. While this large scale global collaboration has originated from the GWAS era, we now see similar consortia being built around the newer genomics datasets with RNA expression profiles, DNA methylation profiles, and the NGS datasets on DNA, RNA and microbiomes.

The Rotterdam Study has GWAS data for almost the complete dataset summing to over 12,000 DNA samples, and is involved as a major collaborative centre for meta-analysis studies of GWAS data, including national programs (RIDE, NGI-NCHA), EU-funded projects (GEFOS, TREATOA, ENGAGE), and voluntary collaborations (GIANT, MAGIC, CHARGE). Especially, from the CHARGE consortium (the Rotterdam Study together with the Framingham Study, AGES, CHS, and ARIC) many important publications have emerged on a wide variety of phenotypes and diseases from all major research lines in

the Rotterdam Study [325]. You can find them discussed under the subheadings of each individual research line.

Data collection, storage and management

At each examination at the research centre, blood, serum, plasma (citrate, heparine, and EDTA based), and sputum are collected. Fasting blood samples are collected along with challenged samples as part of a glucose tolerance test. Sputum is collected before and after a dexamethasone-suppression test. Sputum is frozen at −196 °C before and after the challenge and stored at −80 °C. To obtain serum and plasma, tubes are centrifuged according to a protocol standardising time and conditions from the drawing of blood to centrifugation. All samples are snap frozen at −196 °C using liquid nitrogen and stored at −80 °C. Overnight urine samples are collected at home, frozen at −196 °C at the research centre and stored at −80 °C.

DNA is isolated from whole blood at one laboratory at Erasmus MC by a manual salting-out protocol and is subsequently stored in Eppendorff tubes at −20 °C. A copy of the complete DNA collection of ∼13,000 samples has been transferred to Matrix 2D-barcode tubes in 96 well format at another location. This copy has been subjected to normalization of DNA concentrations and made suitable for handling in 96- and 384-well micro-titer plates for subsequent downstream genomic analysis.

Starting with the RS-III round of data collection, blood drawing has also been taken place with PAXgene tubes, from which whole RNA is isolated and stored at −80 °C. This is now ongoing for the whole study population following the cycles of visits to the research centre.

Similarly, with the RS-III round, collection of faeces material has been initiated for the intestinal microbiome analysis. A collection pot is distributed at the research centre visit which is to be used at home and then by postal mailed returned to Erasmus MC where DNA is isolated and stored at −80 °C. This is now ongoing for the whole study population following the cycles of visits to the research centre.

For data management, an in-house customized sample-management system has been developed. All genomic data of the Rotterdam Study (e.g., SNP array, RNA expression, Next Generation Sequencing (NGS) DNA and RNA, microbiome NGS) are generated in one laboratory which keeps all raw data, while QC-ed and extracted data are stored and managed in the central data repository of the Rotterdam Study.

Blood serum/plasma assessments

For all participants, serum cholesterol, HDL, LDL, triglycerides, glucose and glucose levels are assessed. In urine, micro albumin and creatinine are determined in all participants. Recently, a new "baseline" serum biomarker dataset has been generated at the Clinical Chemistry Laboratory and the Endocrine Laboratory consisting of RS-I-3, RS-II-2, and RS-III-1 samples. These measurements include a steroid profile by mass-spectrometry (e.g., estrogens, androgens, vitamin D, cortisol), thyroid hormones (TSH, free T4), interleukins, CRP, IGF1, insulin, iron-parameters (iron, ferritin and transferrin saturation), fibrinogen, homocysteine, folic acid, riboflavine, pyridoxine, SAM/SAH ratio, cobalamine, Lp-PLA2, Fas/Fas-L, and abeta42/40.

Human genomics facilities

The Rotterdam Study uses one Erasmus MC laboratory for all its genomic studies on DNA, RNA, methylation, microbiome, etc. The facilities use high-end automated machinery including 2 Caliper pipetting robots (including a TwisterII, and integrated plate reader (OD 260/280), 2 Tecan EVO 150 Freedom pipetting robots, a Deerac Equator NS808 nanoliter liquid dispenser, PCR machines, an ABI7900HT Taqman machine (running 1 ng gDNA in 2 microliter reactions), 2 Illumina iScan micro-array readers, one Roche 450 GS Junior sequencer, and two Illumina HiSeq2000 sequencing machines. DNA sample handling is centred on 384-well plates. Single SNP genotyping studies are done mostly using Taqman and Sequenom genotyping with throughputs at 30,000 genotypes per day. We work with reduced amounts of input genomic DNA of 1 nanogram per genotype. This facility has been generating all GWAS data for the Rotterdam Study as well as its RNA expression profiles, methylation profiles, and all NGS data including whole exome sequences, RNA sequencing, and microbiome sequencing.

Genome-wide association studies (GWAS) datasets

Genome-Wide Association Studies (GWAS) are based on genotyping epidemiological cohorts with high density SNP arrays with 500,000—5 million SNPs. The method has been shown to successfully identify common genetic factors for hundreds of traits and diseases (see www.genome.gov/ GWAstudies). Through a large grant from the Dutch research organisation NWO one of the world's largest GWAs datasets has been facilitated involving over 12,000 DNA samples from the Rotterdam Study cohorts. This GWAS dataset consists of (a) a small dataset of ∼450 women with 500 K Affymetrix arrays (Nsp250 + Sty250), and (b) a large dataset of ∼12,000 samples covering almost all RS-I, RS-II, RS-III DNA

samples consisting of 550 K (RS-I, II; single + duo array format) and 610 K (RS-III; quattro array format) Illumina array genotypes.

The Illumina GWAS dataset of the Rotterdam Study (with approximately 500,000 SNPs having been genotyped) also forms the basis to generate so-called "imputed" datasets derived thereof. In this process the genotypes of SNPs which have been genotyped in reference datasets (such as HapMap with ~8 million SNPs genotyped), are being estimated for all Rotterdam Study samples using the basis Illumina 500 K SNP dataset configurations in each subject. With the advent of large reference datasets becoming available based on whole genome/exome NGS, imputation activities using the Rotterdam GWAS dataset will remain an active area of development. So far, the Rotterdam Study GWAS dataset has been imputed to HapMap version 2 and 3 (with ~7.5 million resulting SNP genotypes in the Rotterdam Study dataset), and the 1000 genome dataset version 4 (with ~18 million resulting SNP genotypes in the Rotterdam Study daaset). Currently, imputations are being generated for the Genome of the Netherlands (GoNL) whole genome NGS dataset [326], and the 10KUK whole genome sequencing dataset.

The Rotterdam Study GWAS dataset is actively being used by all research lines within the Rotterdam Study as can be read under the subheadings of each research line in this review of the Rotterdam Study. In addition, it also serves as a control GWAS dataset for other research centers in- and outside The Netherlands for both SNP frequencies as well as copy number variations (CNVs), in which capacity it has been used in ~100 publications up to date. Most importantly, it has formed the start of a very successful collaboration in the CHARGE consortium (combining GWAS datasets of major epidemiological cohort studies across the world) which has >70 phenotype working groups in which almost all research lines of the Rotterdam Study are active.

Candidate gene SNP studies

In the past, we have genotyped over 300 individual polymorphisms as part of candidate gene studies across the complete Rotterdam Study cohort using Taqman and Sequenom genotyping techniques. These mostly concern individual potentially functional single nucleotide polymorphisms (SNPs) per gene, but sometimes also haplotype tagging SNPs (e.g., ESR1, ESR2, HSD11B1, fibrinogen), and also high density SNP screening (e.g., the vitamin D receptor gene). Currently, for candidate gene/SNP studies we perform look-ups in the GWAS datasets and/or perform individual SNP genotyping (e.g., SNPs not on the array, badly imputed SNPs, functional SNPs).

RNA expression datasets

With the availability of good RNA from Rotterdam Study participants, starting with the RS-III subjects, studies have been initiated analysing the expression pattern of a single gene across samples or of the complete RNA collection in a sample (expression profiling). An expression profiling dataset has now been generated of the first ~1,000 samples of the RS-III dataset, using the Illumina Human HT-12 v4 array containing ~48,000 probes. Subsequently, these same RNA samples are currently being subjected to NGS (see below) so that a very rich expression dataset will become available. While RNA expression is known to differ between tissues, so far we only have RNA isolated from whole blood as a tissue.

Methylation datasets

In ~1,000 samples of the RS-III dataset we have generated DNA methylation profiles of ~480,000 CpG sites across the human genome using the Illumina Infinium HumanMethylation450 array. As this same set of RS-III subjects was also used for the RNA expression profiling, deep genomic studies can now take place in combination with GWAS data and NGS data in these ~1,000 subjects.

New developments: Next Generation Sequencing datasets

A major development in genomics studies has been the introduction in the past few years of high-throughput parallel sequencing methods (also known as Next Generation Sequencing or NGS) which allow DNA (and RNA) sequencing at unprecedented high speed and low costs. This development has brought sequencing of whole genomes within reach of individual laboratories, rather than the large global effort that was needed to sequence the human genome at first pass. This development has led to a revival in Mendelian Genetics by solving many "cold cases" (because causal mutations could directly be found in a few samples, rather than by linkage analysis in many family samples and laborious sequencing analyses of dozens of candidate gene exons in the area). In addition, it has stimulated the cohort studies that had generated GWAS datasets in the past 5 years, to generate NGS data in part or all of their samples to find local/regional variants of interest and variants that are very rare. While whole genome sequencing is often seen as the ultimate goal in these efforts, currently almost all labs turn to Whole Exome Sequencing (WES) because it is much more cost-effective. In WES the parts that code for amino acids as part of the encoded proteins (i.e., the exons) are first captured from the whole genome by hybridization techniques upon which the

selected parts (the exons) are then subjected to NGS. WES costs 5-10 times less euro's than whole genome sequencing (so, more samples can be done with the same amount of money) and generates interpretable findings. (note that for the vast majority of areas in the human genome we have no good idea what they signify as opposed to exons that encode functionally important parts, i.e., the parts that make up the proteins). A large grant from the NGI-sponsored Netherlands Consortium for Healthy Ageing (NCHA), allowed to generate whole exome NGS data for ~3,000 samples by the Erasmus MC genomics facility on the Illumina HiSeq2000 sequencing machines. The samples for this experiment were selected to constitutes a random sample from the RS-I dataset. These NGS data have been generated, and the samples are now in the process of QC and calling of variants. This process is complicated and no golden standard exists, so these steps take place together with colleagues at Baylor College, the Broad Institute, and NGS facilities in Germany and Netherlands. Through a collaborative grant from the NIH Alzheimer initiative we will also obtain an additional ~2,000 samples with whole exome NGS data from RS-I. Also here we collaborate with colleagues in CHARGE since NGS data have been generated in large parts of the ARIC study, the Framingham Study, and the CHS study. Within the several working groups these data are currently being analysed in relation to specific phenotypes.

From the first NGS efforts in reference datasets using whole exome sequencing, many thousands of novel DNA sequence variants were discovered in the exons, mostly rare to very rare and not well-covered on the existing GWAS arrays. Illumina and Affymetrix therefore decided to generate an array devoted to analyse these new exome variants called the Infinium HumanExome array. This array has been used to genotype many DNA samples across the globe and several collaborative analyses are currently using data generated with this array. In the Rotterdam Study we generated exome array genotypes of ~3,000 samples of RS-I (with version 1 of this array), and this dataset is part of several collaborative efforts, including the Dutch BBMRI exome array effort (with ~35,000 collective samples), an effort in GIANT on anthropometric traits, and the CHARGE exome array effort across many different phenotype working groups (with ~60,000 collective samples).

The RNA we have isolated from ~1,000 RS-III whole blood samples is currently being subjected to NGS analysis. This effort is part of the Dutch BBMRI bio-banking initiative together with other Dutch biobanks, such as LifeLines and the Netherlands Twins Register.

We have isolated DNA from faeces of ~1,000 RS-III subjects and this DNA is currently being analysed by 16S RNA sequencing using the Roche 454 Junior NGS machine. We are now setting up the protocols to start running whole DNA sequencing from these samples (called meta-genomics).

For additional EJE references see [9, 121, 277, 285, 327–349].

Pharmaco-epidemiologic studies

Objective

A major objective of the pharmaco-epidemiologic studies is to investigate the role of drugs as determinants of disease in the Rotterdam Study. This includes studying efficacy and effectiveness of drugs, as well as adverse reactions to drugs. As the drugs used in the Rotterdam Study are licensed and often on the market since several years, research focuses on effect modifiers on the association between drugs and disease. A topic of particular interest is pharmacogenetics and genomics as genes may may account for most of the variation in drug response.

Major findings

Important findings have been published on pharmaco-epidemiological topics concerning the main outcomes in the Rotterdam Study. Subsequently, we will summarize pharmacogenetic- and other findings.

Pharmacogenetic findings

Pharmacogenetic activities in the Rotterdam Study can be distinguished into hypothesis-generating 'genome-wide association' (GWA) studies, and in hypothesis-testing candidate gene studies. As the performance of GWA studies on gene-drug interactions require a very large sample size, the Rotterdam Study actively participates in the CHARGE consortium [350]. This is currently 'work under progress' and up till now most comes from GWA studies within the Rotterdam Study on specific topics. A very attractive topic consists of coumarin anticoagulants as the measurement of anticoagulation through the International Normalized Ratio (INR) is not only a very well standardized secondary outcome but also in indirect measure of drug adherence. Interesting were the results of a GWA in users of the anticoagulant phenprocoumon which confirmed the important genetic variant for VKORC1, CYP2C9 and 4F2 in acenocoumarol users [351], and later also in phenprocoumon dosage [352]. Although 4F2 was a relatively new finding, further studies on 4F2 revealed that its contribution was very modest [353]. Another genetic determinant, i.e. GATA-4, did not have a clinically

relevant effect on the maintenance dose of acenocoumarol or phenprocoumon [353]. A recently developed 'European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) acenocoumarol dose algorithm performed as well in the Rotterdam Study as in the original trial [354]. Two analyses in the Rotterdam Stuy investigated the potential interaction between coumarin anticoagulants and other drugs, i.e. antidepressants and proton pump inhibitors. The risk for overanticoagulation during acenocoumarol maintenance treatment was more than doubled in combination with fluvoxamine and venlafaxine. There was no increase in risk for the other SSRIs, but numbers of exposed cases were low for all SSRIs except paroxetine [355]. As for the potential interaction between proton pump inhibitors and coumarins, the risk for overanticoagulation was most pronounced for esomeprazole and lansoprazole [356].

A promising subject for pharmacogenetic research consists of the effect of metformine in diabetics. Previously, we demonstrated that there were important genetic variations in OCT1 and MATE1 transporters with consequences for metformine response in diabetics [357–359]. Recently, we participated in a consortium meta-analyis demonstrating that a gene variant near ATM is significantly associated with metformin treatment response in type 2 diabetic patients from the Netherlands and the UK [360]. Other interesting study results comprised our finding that several statins are substrates for the multidrug resistance-associated protein 2 transporter, encoded by the ABCC2 gene [361], and that the cytochrome P450 CYP1A2 encoding gene variant rs2472299G >A, female sex, and nonsmoking were significantly inversely related to coffee intake [362].

Other findings

Electrolyte disorders such as hyponatremia and hypo- and hyperkalemia are common causes of drug-induced hospital admissions in the aged. Already since decades and in most western countries, antihypertensive diuretics are the most frequent iatrogenic cause of electrolyte disorders [363].

In a cross-sectional analysis in the Rotterdam Study, a total of 776 subjects (15.0 %) had at least 1 electrolyte disorder, with hyponatremia (7.7 %) and hypernatremia (3.4 %) being most common. Diuretics were independently associated with several electrolyte disorders: thiazide diuretics (hyponatremia, hypokalemia, hypomagnesemia), loop diuretics (hypernatremia, hypokalemia), and potassium-sparing diuretics (hyponatremia) [364]. The risk of hyponatremia was increased eightfold in users of thiazides, independent of sex and glomerular filtration rate, but not of age and BMI [365]. Apart from modifying sodium- and potassium levels, thiazides are known to retain calcium and may even be protective against hip fracture [366]. In women, short-term use of loop diuretics was associated with an increased level of FDP, reflecting increased bone resorption by osteoclasts [367].

A productive collaboration between pharmaco-epidemiology and outcome groups in the Rotterdam Study led to a study on the association between corticosteroids and open-angle glaucoma [368]. In the past, the suspicion that corticosteroids may induce glaucoma. Adjusted for age, sex, high myopia and family history of glaucoma, none of the classes of steroids were associated with the incidence of open-angle glaucoma. In this collaboration, two other drug groups were studied for their association with eye disorders. First, an association has been suggested in the past that antithrombotics may also induce open-angle glaucoma. Although there was a significant intra-ocular pressure (IOP)-lowering effect of anticoagulants of -0.31 mm Hg, the IOP-lowering effect of anticoagulants disappeared after additional adjustment for the use of systemic beta-blockers [369]. Second, Long-term use of statins appears to be associated with a reduced risk of OAG, independent of the IOP. These findings are in line with the idea that statins have neuroprotective properties [370]. Apart from its cardiovascular indications, more and more epidemiological data suggest other beneficial effects. Besides biomechanical mechanisms, the pathogenesis of osteoarthritis may involve inflammation, vascular alterations and dysregulation of lipid metabolism. In the Rotterdam Study, statin use was associated with more than a 50 % reduction in overall progression of osteoarthritis of the knee, but not of the hip [371]. Underlining the suggestion that the protective effect of statins on mortality is partly explained by an anti-inflammatory effect, was that long-term statin use (>2 years) was associated with a 39 % decreased risk of death in COPD patients. Stratified according to the level of systemic inflammation, long-term statin use was associated with a 78 % reduced mortality if hsCRP level >3 mg/L, versus a non significantly 21 % reduced mortality if hsCRP level ≤3 mg/L [372].

New developments

Drugs are attractive determinants in clinical epidemiologic research because they are probably the most important therapeutic intervention in health care, and the majority of all marketed drugs have proven biological activity. As a nice consequence of the availability of complete medication histories in Dutch health care, the role of drug exposure can be assessed in a detailed way. In the Rotterdam Study, there is an almost complete coverage of the population as of January 1, 1991, thanks to the fact that all pharmacies which serve the Ommoord district are on one computer network. This facilitates the use of detailed

analyses with the drug as a time-varying determinant [373]. Apart from the performance of collaborative GWA studies on gene-drug interactions, as alluded to above, there are two interesting methodological developments in the pharmaco-epidemiological studies in the Rotterdam Study. First, based on earlier publications on marginal structural modeling [374], we are currently analyzing the association between statins and cardiovascular mortality with such models in which we try to use more precise exposure definitions than use/non-use. Second, in an effort to translate pharmacokinetic data from clinical trials to population-based research, PK/PD-modelling was performed on the association between sotalol and the length of the QTc-interval on ECGs [375].

For additional EJE references see [128, 376–390].

Imaging studies

Objective

Biomedical imaging allows for non- or minimally-invasive assessment of structural and functional changes that may reflect specific pathology. Recent developments in image data acquisition and analysis enable to use these techniques on a large scale. The Population Imaging Unit within the Rotterdam Study aims to assess imaging biomarkers of disease in a pre-symptomatic phase at the population level. Advantages of imaging measures include that they mark early disease, can be assessed reliably and reproducibly, and are quantitative rather than qualitative which makes them more powerful than most conventional outcome measures such as clinical phenotypes.

The main imaging modalities that are currently being applied in the Population Imaging Unit are multidetector computed tomography (MDCT) and magnetic resonance imaging (MRI).

Imaging infrastructure and storage

Mdct

CT imaging is currently performed with hospital-based 16-slice or 64-slice MDCT scanners (SOMATOM Sensation 16 or 64, Siemens, Forcheim, Germany), located at Erasmus University Medical Center. Scanners are operated by clinical technicians. CT images are acquired without contrast-enhancement and according to standardized protocols. Imaging data are transferred from the CT scanner to a securely backed-up research picture archiving system.

Mri

From August 2005 onwards, a dedicated 1.5 Tesla MRI scanner (GE Healthcare, Milwaukee, Wisconsin, USA) is operational in the Rotterdam Study research center. This scanner is operated by trained research technicians and all imaging data are collected according to standardized imaging protocols. Changes or updates in hardware or software configuration are avoided and regular quality checks are performed to secure validity of cross-subject and cross-scan comparisons. Imaging is performed without administration of contrast agents. All imaging data are directly transferred from the scanner facility to the Erasmus University Medical Center. Data are stored on a securely backed-up research picture archiving system, using programmed scripts to check for completeness of the data received.

Data management and processing

Assessment of incidental findings

All imaging data are visually evaluated within days after acquisition by trained physicians for the presence of clinically relevant incidental findings [202]. Expert radiologists are consulted for all abnormal findings and the management of clinically relevant findings is based on protocols defined by expert panels. These protocols are updated on a regular basis incorporating the current best available knowledge regarding treatment and prognosis of the various abnormalities discovered.

Automated processing of MRI data

Though some measurements are still performed manually or scored visually, the majority of imaging data is now processed using semi- and fully-automated computer algorithms. The Population Imaging Unit collaborates with the Biomedical Imaging Group Rotterdam (BIGR) of Erasmus University Medical Center in the application and development of automated processing pipelines for high-throughput of large data quantities. These pipelines comprise on the one end image quality checks and procedures for non-uniformity correction, normalization and image registration and on the other end advanced algorithms to extract image features to use for analyses.

Grid architectures and networked processing pipelines are used to process the large quantities or imaging data that are acquired in the Rotterdam Study.

Major findings

The Rotterdam Study research lines currently applying imaging within the Population Imaging Unit are those on neurological diseases and cardiovascular diseases.

Brain imaging (MRI)

Neurodegenerative and cerebrovascular disease are common disorders in the elderly that exert a large influence on brain functioning. Identifying underlying pathology in a preclinical state may help to recognize persons at risk, assess determinants of disease and develop preventive measures. Main objective for the Population Imaging Unit with respect to brain imaging is therefore to identify and quantify brain imaging biomarkers that mark the development of neurodegenerative and cerebrovascular disease.

From August 2005 onwards (RS-II-2 and onwards), brain imaging in the Population Imaging Unit is performed in all study participants without contra-indications in the context of the Rotterdam Scan Study. The current structural scanning protocol includes 4 high-resolution axial sequences (3D T1-weighted; 2D PD-weighted; 2D FLAIR; and 3D T2* GRE), 2D phase-contrast imaging, and diffusion tensor imaging (DTI). Total scanning time for these sequences amounts to approximately 30 min. Currently, over 5,700 unique brain MRI scans and over 4,800 follow up scans have been acquired with this protocol. Starting 2012, functional imaging is incoporated in the MRI protocol in the form of a resting-state fMRI sequence (8 min), which is added to the existing structural scans. To date, rs-fMRI scans have been acquired in approximately 1,000 individuals and this will continue into 2014.

Fully automated methods are applied to quantify atrophy of brain tissues and structures and the severity of white matter lesions [218, 391, 392]. Automated hippocampal segmentation has been successfully applied on multi-scanner acquired MR images (on scans acquired in the Rotterdam Scan Study in 1995 [211] and follow up examinations in 2006), showing that a decline in hippocampal volume over a 10-year follow up period predicted onset of clinical dementia [393]. Apart from focusing only on supratentorial brain structures, we have more recently also incorporated segmentation of the cerebellar volume into our automated processing pipelines, enabling us to study the cerebellum in aging. In 3,962 persons, we found that smaller cerebellar volumes with increasing age were mainly driven by loss of white matter. Furtermore, diabetes, higher serum glucose and lower cholesterol levels were most important risk factors for cerebellar atrophy in aging.

Phase-contrast imaging allows for assessment of blood flow in the carotids and basilar artery. This yields measures of total brain perfusion [394], which when lower was found to be relate to worse cognition, an association that is mediated by brain atrophy [395].

The 3D T2* GRE sequence that we use was specifically developed to increase the conspicuity of cerebral microbleeds [396]. With this optimized sequence, we found that microbleed prevalence gradually increases with age, from 6.5 % in persons aged 45 to 50 years to 35.7 % in participants of 80 years and older; and that overall, 15.3 % of all subjects over the age of 45 years has at least 1 microbleed; a much higher prevalence than was reported before [370, 371]. We found supportive evidence that deep or infratentorial microbleeds reflect arteriolosclerotic angiopathy, whereas strictly lobar microbleeds are caused by cerebral amyloid angiopathy [397, 398]. We furthermore demonstrated that incidence of microbleeds over a 3-year time interval is high and that risk factors for new microbleeds again differ according to microbleed location, in line with our findings regarding prevalent microbleeds [399]. These findings impact research into the causes of cerebral amyloid angiopathy, as well as fuel the ongoing discussion about the safety of antithrombotic therapy in persons with microbleeds [400, 401]. Our current studies focus on the clinical relevance of cerebral microbleeds, in relation to clinical outcomes such as stroke, dementia and mortality, over a mean follow-up of 5 years.

Diffusion tensor imaging (DTI) allows the assessment of the microstructural integrity of white matter. White matter microstructure looses its integrity with increasing age, but this can largely be explained by presence of white matter atrophy and white matter lesions [402].

Nevertheless, the microstructural integrity in the normal appearing white matter and in white matter lesions relates to cognitive function regardless of concurrent macrostructural changes, emphasizing the importance of the microstructural integrity of white matter [403]. Also, we found that white matter changes in normal appearing white matter are present and can be quantified on diffusion tensor imaging before white matter lesions actually develop. This suggests that white matter lesions develop gradually, and that visually appreciable lesions are only the tip of the iceberg of white matter pathology. We recently developed within the Rotterdam Study a method for fully automated tractography of over 20 major white matter tracts, yielding tract-specific measures of white matter integrity, enabling a more in-depth exploration of structural integrity in relation to functional processes in aging [404].

Imaging of atherosclerotic calcifications (MDCT)

Main objectives with respect to imaging of vascular calcifications are to investigate distribution of and risk factors for atherosclerotic calcifications in the general elderly

population and to study prognosis associated with calcifications in different vessel beds.

From September 2003 until February 2006, all participants from RS-I-4 and RS-II-2 who completed a center visit were invited to a MDCT scan of the coronary arteries and a second scan of the aortic arch and carotid arteries. A total of 2,521 participants (response rate 79 %) were scanned. The cardiac scan reached from the apex of the heart to the tracheal bifurcation. The second scan reached from the aortic arch to the intracranial circulation. Images were analyzed by trained reviewers and calcification in the different vessel beds (coronaries, aortic arch, extracranial and intracranial carotids) were quantified using the Agatston score [405].

As expected, we found that calcification load was higher overall in men compared to women, though aortic arch calcification was more prevalent among women [406]. Age and current smoking were found to be the strongest independent risk factors for arterial calcification [407]. Furthermore, strong and graded associations of prevalent stroke were found with carotid artery, aortic arch and coronary artery calcification, independent of cardiovascular risk factors [408]. When vessel calcification was studied in relation to vascular brain disease (non-invasively imaged with MRI), we found that larger intracranial carotid calcification load related to larger white matter lesion load, and that larger extracranial carotid calcification load related to the presence of cerebral infarcts, independently of ultrasound carotid plaque score. This suggests that calcification of atherosclerotic plaque yields other information in addition to merely the presence of plaques. This importance of vessel calcification is further corroborated by our recent finding that larger calcification volumes are associated with worse cognitive performance in the general population.

Despite extensive research on the identification of lifestyle- and environmental cardiovascular risk factors, a large part of the variability in the total burden of atherosclerosis remains yet unexplained. This inevitably indicates that other, unknown factors also considerably contribute to the development of atherosclerosis. During the last years, it has become clear that genetic factors may play an important role in the development of atherosclerosis. We determined whether previously identified genetic loci for coronary calcification were also associated with calcification in other locations than the coronary arteries Indeed, we found that the genetic basis for aortic arch and carotid artery calcification largely overlaps with that of coronary artery calcification. However, we found that the genetic variants contributed differentially to the amount of atherosclerotic calcification in these vessel beds. This suggests that also on the genetic level, differences in the etiology of atherosclerosis across vessel beds exist. Additionally, we also investigated the genetic basis of atherosclerosis using the genetics of strong risk factors of atherosclerosis, i.e. serum cholesterol levels and blood pressure and demonstrated that both serum lipids and blood pressure share a genetic predisposition with the formation of atherosclerosis in multiple vessel beds. Worldwide, intracranial atherosclerosis is one of the leading causes of stroke, yet it is understudied in population of white descent. We therefore focused specifically on the prevalence and risk factors of intracranial internal carotid artery calcification in our population, and found that the overall prevalence of intracranial atherosclerosis was as high as 82.2 %. Conventional cardiovascular risk factors are associated with intracranial atherosclerosis, but risk factor profiles differed between men and women, with excessive alcohol intake and smoking were strong risk factors in men, whereas diabetes and hypertension were in women. Our current studies focus on long-term risk of disease associated with calcifications in various vessel beds, again with a particular focus on intracranial atherosclerosis.

Carotid plaque imaging (MRI)

Carotid wall thickening and atherosclerosis are highly prevalent at older age and are considered a major cause of cerebrovascular events [197]. Carotid atherosclerotic plaque constituents such as lipid core and hemorrhage, so-called "vulnerable" components, are considered important factors in development of clinical neurological events [409]. With MRI, it is possible to separately identify these plaque components [410]. Main objectives with respect to carotid imaging in the Rotterdam Study are to investigate distribution of and risk factors for carotid plaque components in the general elderly population and to study prognosis associated with specific carotid plaque composition.

From October 2007 onwards, all participants with carotid wall thickening of 2.5 mm or larger on ultrasound (approximately 25 % of the Rotterdam Study population) are invited for carotid MRI. Imaging is performed with a bilateral phased-array surface coil (Machnet, Eelde, The Netherlands), stabilizing subjects in a custom-designed head holder to reduce motion. The imaging protocol consists of a series of high-resolution MRI sequences to image the carotid bifurcations on both sides: a PDw Fast Spin Echo (FSE) Black-blood (BB) sequence; a PDw-FSE-BB with an increased in-plane resolution; a PDw- Echo Planar Imaging (EPI) sequence and a T2w-EPI sequence; and 2 three-dimensional (3D) sequences: a 3D-T1w-Gradient Echo (GRE) sequence; and a 3D phased-contrast MR-Angiography. Total scanning time amounts to approximately 30 min.

Plaques are reviewed by trained raters for the presence of three different plaque components (calcification,

hemorrhage and lipid core). Furthermore, carotid plaque size is quantified by obtaining maximum carotid wall thickness and degree of luminal stenosis using the NASCET criteria [411] on the PDw-FSE images. Postprocessing techniques aimed at automated quantification of plaque volume and identification of different plaque components are currently being developed.

So far, over 1,300 participants underwent a complete carotid MRI scan and data are currently being analyzed. There is a complete overlap between carotid and brain MRI participants, allowing for the investigation of carotid plaque constituents in relation to brain imaging markers. In the first 1,006 scans, we found that intraplaque haemorrhage and lipid core were present in almost 25 % of plaques, respectively, and occurred simultaneously in 9 % of plaques. Different risk factors are associated with these plaque components: hypertension (and in particular high pulse pressure) and current smoking were risk factors for the presence of intraplaque haemorrhage, and hypercholesterolaemia was the only risk factor for lipid core presence.

New developments

As also mentioned above, focus has shifted from purely structural imaging to also including functional imaging data, by incorporating resting-state functional MRI into the brain imaging protocol. Changes in the intrinsic activity of resting-state networks are presumed to represent alterations in functional brain connectivity and may mark neurodegeneration in an early, presymptomatic stage. Our initial studies will focus on the relation between functional brain connectivity and established structural markers in age-related brain diseases such as hippocampal volume, white matter lesions and microbleeds. In a later stage, we will investigate whether functional connectivity can be used an early imaging marker for dementia, by itself or in combination with other imaging markers and risk factors.

Regarding structural imaging markers, an emerging potential marker is Virchow–Robin (VR) spaces, spaces filled with interstitial fluid that surround the blood vessels in the brain and which can be dilated. Despite increasing literature on these dilated VR spaces, a major limitation of current research is the lack of a robust and generalizable rating method on MRI. We recently developed a novel rating method for VR spaces, which we successfully applied in 2 population-based studies, encompassing 3 different scanning protocols. We will use this rating method to explore determinants and consequences of dilated VR spaces in our general aging population.

Besides ever-increasing advances in imaging hardware, software and sequence design, major advances in the short and long run are to be expected from (fully) automated image analysis. Computer processing of images will enable to make fully use of all information contained within the image, introducing new imaging biomarkers. Besides, the vast amount of imaging data that are acquired in population-based studies like the Rotterdam Study renders visual assessment or manual measurements virtually impossible, strengthening the need for (fully) automated methods of data extraction and analysis.

For additional EJE references see [17, 208, 412–415].

Otolaryngological diseases

Objectives

Otolaryngological research in the Rotterdam study focuses on the frequency, etiology and consequences of auditory and vestibular disorders. We are mainly interested in dysfunctions located in the labyrinth of the inner ear, expressed by cochlear hearing loss and a deviant vestibulo-ocular reflex (VOR) for fast head movements. Etiology of both peripheral disorders will be studied, possibly revealing one or more common risk factors as both systems are connected and have similar sensory mechanisms. Additionally, we will investigate possible associations between reduced sensory input and general neuroepidemiological measures.

Methods

From 2011 we started measuring hearing and vestibular function within the Rotterdam Study population. Hearing loss is assessed at both ears by performing pure-tone audiometry in a sound proof room. Hearing thresholds are determined with headphones at frequencies 0.25, 0.5, 1, 2, 4 and 8 kHz. To distinguish between cochlear and middle-ear pathology, also bone-conduction thresholds are measured at frequencies 0,5 and 4 kHz. Additionally, speech perception in noise is tested at the better ear, using a validated triplet digit test [416] with speech-shaped noise at a fixed presentation level of 65 dB SPL. The ability to understand speech in noise is a functional measure that includes both sensory and central aspects of the auditory system.

Peripheral vestibular function is assessed by The Head Impulse Test (HIT), which measures the vestibule-ocular reflex (VOR) for a number of sudden head movements initiated by the tester [417]. Gain and delay are the main parameters that will be used to quantify vestibular function. An advanced infrared video-oculography system is used to track the eye movements simultaneously with the position of the head, enabling VOR recordings, even for fast head

movements. Additionally, central vestibular processing is assessed by measuring the VOR during repetitive slow head movements at frequencies 0.5, 1 and 2 Hz while fixating the eyes at a projected visual target. The oculomotor function is tested by eye tracking of a moving target at frequencies 0.5, 1 and 2 Hz, while the head is fixated.

The general interview contains ten questions related to hearing and balance problems. In case of hearing-aid use, the participant has to answer five additional questions of the International Outcome Inventory of Hearing Aids (IOI-HA) [418]. In case of frequent tinnitus, ten additional questions of the Short Tinnitus Handicap Inventory (THI-S) are added [419].

Determinants of interest

One of our main goals is to find medical and genetic factors that are associated to age-related hearing loss. Age-related hearing loss is a common disorder that deprives older people of key sensory input. It leads to social withdrawal and is even been found to be independently associated with poorer cognitive functioning and incident dementia. Still, little is known about the mechanisms that are responsible for developing hearing loss and the way it affects general cognitive functions within the elderly population. Determinants of interest are genetic factors, cardiovascular disease, use of medication, endocrine diseases and neuroepidemiological factors.

Even less is known about age-related vestibular dysfunction. Problems related to balance and dizziness are frequently reported in elderly people, but not much is known about the prevalence and type of vestibular dysfunction that may cause these problems. This will be the first large cohort study in which vestibular function will be measured. The same determinants as mentioned for hearing will be studied for the vestibular system. Additionally, the relation between hearing and peripheral vestibular function will be extensively investigated.

Management

The Rotterdam Study is directed by a Management Team comprising the scientific principal investigators Sarwa Darwish Murad (PI Hepatic diseases), Cornelia van Duijn (PI Genetic epidemiologic studies), Oscar Franco (PI Cardiovascular diseases and ErasmusAGE), André Goedegebure (PI Otolaryngological diseases), Albert Hofman (chairman, PI Rotterdam Study), Arfan Ikram (PI Neurological diseases), Caroline Klaver (PI Ophthalmic diseases), Tamar Nijsten (PI Dermatological diseases), Robin Peeters (PI Internal Medicine), Bruno Stricker (PI Pharmaco-epidemiology), Henning Tiemeier (PI Psychiatric diseases), André Uitterlinden (PI Genomic studies), and Meike Vernooij (PI Population Imaging); and Jan Heeringa, MD, PhD, study coordinator, Eric Neeleman, head IT, and Frank van Rooij, MSc, head data-management. The study of respiratory diseases is conducted in close collaboration with Prof Guy Brusselle, Department of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium. The study of pulmonary hypertension is conducted in close collaboration with Ardeshir Ghofrani, University of Giessen Lung Center, Giessen, Germany.

Emeritus principal investigators

The following persons are Principal Investigator Emeritus of the Rotterdam Study:

Frank van den Ouweland (PI Internal Medicine 1990–1992), Diederick Grobbee (PI Cardiovascular diseases 1990–1996), Albert Hofman (PI Neurological diseases 1990–1996), Paulus de Jong (PI Ophthalmic diseases 1990–2005), Huibert Pols (PI Internal Medicine 1993–2006), Monique Breteler (PI Neurological diseases 1997–2010), Gabriel Krestin (PI Population Imaging 1998–2010), Johannes Vingerling (PI Ophthalmic diseases 2005–2010), Jacqueline Witteman (PI Cardiovascular diseases 1997–2011), Ernst Kuipers (PI Internal Medicine 2007–2013), Harry Janssen (PI Hepatic diseases 2007–2013).

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