Topics in Population Imaging

The Rhineland Study

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List of abbreviations

2D	2 dimensional
3D	3 dimensional
ANT	Advanced normalisation tools
ASEG	automated segmentation
ATC	Anatomical therapeutic chemical
BMI	Body mass index
CAIPIRINHA	Controlled aliasing in parallel imaging results in higher acceleration
CI	Confidence interval
cm	Centimetres
CVD	Cardiovascular disease
d	Dice score coefficient
DDR	DNA damage response
DICOM	Digital imaging and communications in medicine
dMRI	Diffusion magnetic resonance imaging
DNA	Deoxyribonucleic acid
DVA	Developmental venous anomaly
EPI	Echo-planar imaging
FDA	U.S. Food and Drug Administration
FLAIR	Fluid-attenuated inversion recovery
fMRI	Functional magnetic resonance imaging
FOV	Field of view
$HbA1_c$	Haemoglobin $A1_c$

HT	Hormone therapy
Hz	Hertz
ISMRM	The International Society for Magnetic Resonance in Medicine
ISCED	International Standard Classification of Education
IQR	Interquartile range
lacunes	Lacunes of presumed vascular origin
m	Metres
ME-MPRAGE	Multi-echo magnetisation prepared rapid gradient-echo sequence
min	Minutes
mm	Millimetres
mmHg	Millimetres of mercury
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
ms	Milliseconds
mT	Millitesla
n	Number
OR	Odds ratio
PHASES	Population – hypertension – age – size of aneurysm – earlier subarachnoid haemorrhage – site of aneurysm
Px	Pixel
QSM	Quantitative susceptibility weighted
SD	Standard deviation

SE-EPI	Spin-echo echo-planar imaging
SVD	Cerebral small vessel disease
T	Tesla
TA	Time of acquisition
TE	Echo time
TI	Inversion time
TR	Time of repetition
TSE	Turbo-spin-echo
UK	United Kingdom
US	United States
USA	United States of America
WM	White matter
WMH	White matter hyperintensities of presumed vascular origin

1. Abstract

Population imaging is the large-scale data acquisition and analysis of medical images in population-based cohorts. When combined with other data acquired in the cohort, it provides the unique potential to characterise disease burden and identify persons at risk. In setting up new population imaging studies, one has to address several challenges, such as the minimisation of selection bias and how to deal with incidental findings. Population imaging enables the investigation of a wide range of topics of interest, including age-related diseases as cerebral small vessel disease (SVD), of which white matter hyperintensities (WMH) are the archetypical example.

In the first part of my thesis, I focus on how to conduct population imaging in an ethically and valid manner. I performed my research in the context of the Rhineland Study. I successfully broadened standard magnetic resonance imaging (MRI) eligibility criteria, allowing eligible participants with medical implants without MRI safety certificate, tattoos and permanent make-up to undergo 3 Tesla MRI. None of the participants reported any adverse events, suggesting that most medical implants, tattoos, and permanent make-up are MRI suitable. Including participants with such indications is crucial to reduce selection bias, thereby improving generalisability of research findings. The handling of incidental findings, i.e., if and which abnormalities should be reported back to participants, requires insights in the frequency and clinical relevance of the finding. I investigated the prevalence and clinical relevance of incidental findings on neuroimaging in the Rhineland Study. While we observed incidental findings in almost 25 % of the participants, only 5 % of the detected abnormalities required diagnostic work-up.

In the second part of my thesis, I examine the effect of biological sex and, in women, the effect of menopause on WMH. In the Rhineland Study, I found that sex differences in WMH burden exist, which were modified by menopause. After menopause, women presented with more WMH and a steeper increase in WMH burden with advancing age, compared to premenopausal women and men. I conclude that sex differences need to be considered both in research and clinical practice.

2. General introduction and aim

The percentage of the population in Germany aged 65 and above is projected to increase from the current 21 % to 34 % within the next 50 years.[1] As a direct consequence the prevalence of age-related diseases, such as neurodegenerative and cerebrovascular diseases, will rise as well. Many age-related diseases have a long preclinical phase in which the patients are asymptomatic and do not seek medical attention.[2] During this phase, pathological changes and symptoms can manifest and become irreversible. Importantly, the preclinical stage provides crucial opportunities to intervene with treatments and disease-modifying therapies.

Cerebral small vessel disease (SVD) is commonly seen on neuroimaging of the elderly, and it is aetiologically involved in neurodegenerative diseases for which it might be an important early biomarker.[3,4] The vascular burden to the brain accumulates over the life span, and in order to understand the underlying mechanisms, large-scale prospective population-based studies covering the adult life span are needed. The Rhineland Study is such a study, providing an ideal platform to investigate SVD in an early stage using population imaging.

POPULATION IMAGING

Population imaging refers to the large-scale data acquisition and analysis of medical images in population-based cohorts.[5] In combination with other data acquired, population imaging has the unique potential to uncover new markers for early disease diagnosis, identify persons at risk of developing a disease, and unravel underlying risk factors.[5] Magnetic resonance imaging (MRI) has proven to be a powerful non-invasive and safe imaging technique, due to non-ionising radiation, which enables the detection and characterisation of structural and functional brain changes. It thereby allows a unique opportunity to execute population imaging studies with repeated measurements of each participant.

Population neuroimaging is different from clinical imaging.[5] Whereas the main goal of clinical imaging lies in diagnosing a patient and therefore acquires only specific, hypothesis-driven data (i.e., single imaging examination), population imaging acquires multidimensional MRI

data utilising the same protocol across participants, and offers the possibility to combine imaging data with other data acquired from the participants, such as cognition, -omics, lifestyle, etc.. However, designing new population neuroimaging studies presents several challenges, specifically the reduction of selection bias and handling of incidental findings.

Selection bias is an important topic, especially for population-based studies. For most MRI research studies, it is recommended to exclude people with medical implants who cannot provide an MRI safety certificate,[6] or who have tattoos or permanent make-up[7] as heating of the ferromagnetic material may occur.[8,9] Excluding these people a priori, however, introduces selection bias and can jeopardise the validity of a study.

Incidental findings are previously unidentified abnormalities of potential clinical relevance, which are unexpectedly discovered and unrelated to the specific research purposes of a study itself.[10] Guidelines for the assessment and handling of incidental findings on high spatial resolution neuroimaging research need to be established for each new study.

WHITE MATTER HYPERINTENSITIES

One topic of special interest in this thesis is SVD, which encompasses degenerative processes that affect the small vessels of the brain, including small arteries and veins, arterioles, and capillaries.[11,12] At present, these small vessels cannot be visualised on conventional neuroimaging, therefore researchers study parenchymal lesions that are considered to be a consequence of pathological changes of the small vessels.[11] White matter hyperintensities of presumed vascular origin (WMH) are the most prominent marker of SVD on neuroimaging (**Figure 1**). They are defined as hyperintense signal abnormalities in the white matter tracts on fluid-attenuated inversion recovery (FLAIR) and/or T_2-weighted images, and isointense or hypointense on T_1-weighted images.[13] They can range from small focal lesions to confluent areas, and are typically located in the periventricular or deep white matter. WMH have been found to be present to some extent in almost every individual above the age of 60 years.[14] However, since most studies were conducted in middle-aged and older adults, little is known about the prevalence of WMH in younger people on a population level.

Epidemiologic research over the past decades has majorly contributed to current insights into consequences and causes of SVD. Clinical consequences of SVD can range from none, to distinct neurological symptoms including stroke,[15] motor[16,17] and mood disturbances,[18-20] to cognitive dysfunction.[15,21-23] WMH have been associated with hypertension, diabetes, dyslipidaemia, and smoking.[24-27] Exposure to those risk factors in mid-life has been suggested to be of particular impact on incidence of dementia[28] and white matter integrity.[29,30] Unfortunately, these insights are not sufficient to provide effective prevention strategies or treatment options. For example, while it is known that sex differences exist in vascular risk factors[31,32] and that they play a role in the development of dementia,[33] sex-specific trajectories of WMH remain understudied. However, investigating and understanding these trajectories across the adult life span is an essential cornerstone to improve our knowledge of the observed lesion burden.
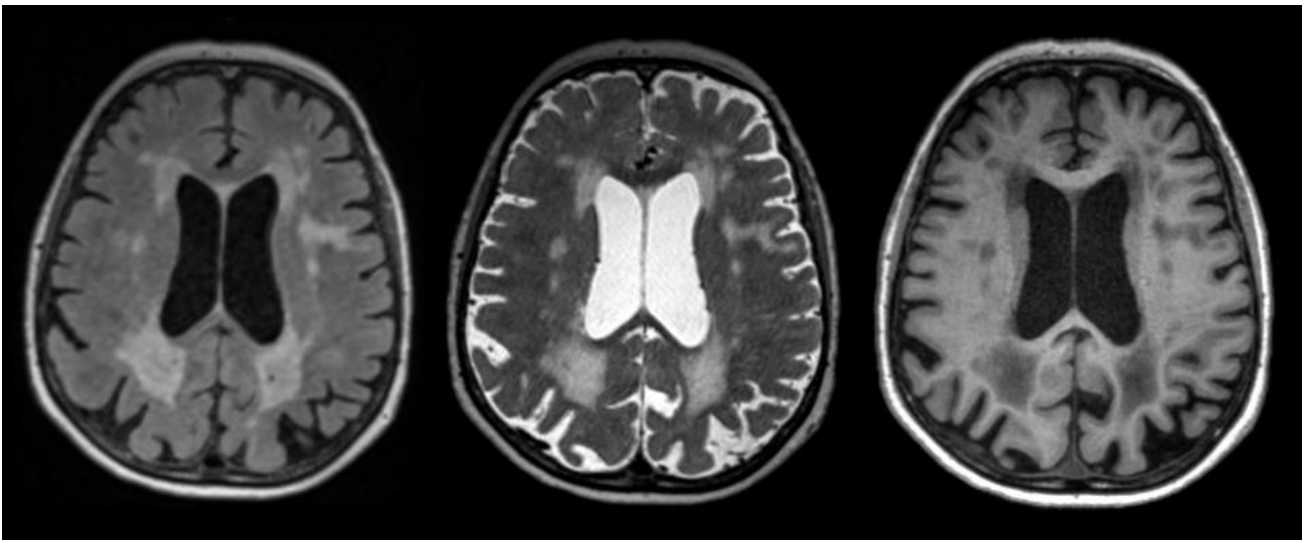

Figure 1 | White matter hyperintensities of presumed vascular origin on high spatial resolution magnetic resonance imaging. Confluent white matter hyperintensities (WMH) in a 70-year-old person. WMH appear hyperintense on fluid-attenuated inversion recovery (FLAIR) (left) and T_2-weighted images (middle), and hypointense on T_1-weighted images (right).

AIM OF THIS THESIS

The first aim of this thesis was to assess how to conduct brain imaging in the Rhineland Study in a valid and ethical way, thereby solving two current challenges in population imaging (**Chapter 3**). One challenge was how to deal with medical implants and tattoos. In **Chapter 3.1**, I assessed the frequencies of these in the Rhineland Study cohort, developed guidelines on how to clarify MRI eligibility, and reported on the medical implants, tattoos and permanent make-up that were safely scanned. Another challenge was how to handle incidental findings on high-resolution neuroimaging. **Chapter 3.2** delineates the assessment, prevalence and clinical management of incidental findings in this cohort.

One topic of special interest in population imaging in the Rhineland Study was WMH as a marker of SVD. Therefore, the second aim of this work was to characterise sex-specific differences in the WMH disease burden in study participants from the age of 30 years and above. Importantly, I examined how menopause alters age-specific trends **(Chapter 4.1)**.

This thesis concludes with **Chapter 5,** in which I discuss the utilised methodology of my work and implications for further research.

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3. Challenges in Population Imaging

3.1. Safety of tattoos, permanent make-up, and medical implants in population-based 3 T magnetic resonance brain imaging: the Rhineland Study

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ABSTRACT

Excluding persons from magnetic resonance imaging (MRI) research studies based on their medical history or because they have tattoos, can create bias and compromise the validity and generalisability of study results. In the population-based Rhineland Study, we limited exclusion criteria for MRI and allowed participants with passive medical implants, tattoos or permanent make-up to undergo MRI. Thereby, we could include 16.6 % more people than would have been possible based on common recommendations. We observed no adverse events or artefacts. This supports that most passive medical implants, tattoos and permanent make-up are MRI suitable and can be scanned in research settings.

INTRODUCTION

Magnetic resonance imaging (MRI) is widely used in both clinical practice and research over the past decades. Millions of MRI scans are acquired every year in the U.S. and adverse reactions of medical implants for MRI are rare. The U.S. Food and Drug Administration (FDA) receives only 300 reports on adverse events yearly.[1] Most of them describe heating or burns, and projectile accidents by moving objects due to the magnetic field. Based on the potential for heating,[2-5] the FDA does not recommend MRI for research purposes for persons with passive devices, including stents, coils, and filters, who cannot provide MRI safety certificates.[1] Additional to these guidelines, non-clinical research studies often incorporate other resources to determine the MRI eligibility of passive implants. A powerful online resource to look up MRI eligibility of implants is the website www.mrisafety.com, which provides a comprehensive list of implants and devices with conditional MRI safety information.[6] However, in order to look up an implant, the exact type of the implant must be identified first, and not everyone might be aware of what medical device they have been implanted. In clinical practice the presence of medical implants hardly ever poses a problem, since the expected benefit from the imaging procedure outweighs the potential risk for the patient. In non-clinical research settings and especially in studies using high-field MRI, however, such participants are still often excluded as a precaution.

Tattoos and permanent make-up are also a frequent MRI safety concern. Case reports have contributed to the awareness of tattoos being a potential risk in patients undergoing MRI.[7-11] However, these case reports might bias the awareness of the potential risks as they do not provide information on the number of persons with tattoos who underwent MRI without adverse events. Although a recent study (n = 330) showed that there is only a low risk for adverse reactions in persons with tattoos, this study still excluded persons with larger or neck or head tattoos.[12] Another retrospective survey in 135 patients using 1.5 T MRI systems did include tattoos independent of location. They reported tattoo-related adverse events in 1.5 % of the patients, which included a slight tingling before the MRI examination started or burning sensation before entering the magnetic field.[13]

Whilst safety of a participant in the MRI is of utter importance, stringent eligibility criteria introduce selection bias, which may jeopardise the validity of a study. Together with experts from the field of MR physics, neuroradiology and epidemiology, we investigated whether we could safely broaden eligibility criteria for 3 T MRI examination in a large population-based study, allowing eligible participant with passive medical implants (even without MRI safety certificates), tattoos and permanent make-up to undergo 3 T MRI.

MATERIALS AND METHODS

Study Population

The study is based on the first 5,000 participants of the Rhineland Study, a prospective, single-centre, community-based cohort study. We invite all inhabitants aged above 30 years from two geographically defined areas in Bonn, Germany, to participate in the study. The sole exclusion criteria was inability to provide informed consent.

Approval to undertake the study was obtained from the ethics committee of the University of Bonn, Medical Faculty. The study is carried out in accordance with the recommendations of the International Council for Harmonisation Good Clinical Practice standards. We obtain written informed consent from all participants in accordance with the Declaration of Helsinki.

Clarification of MRI Suitability

We established an MRI expert committee that developed the procedure for clarification of MRI suitability. This committee included scientists from Population Health Sciences (VL, MB) and MR Physics (TS) from the DZNE, Bonn, and the Clinic for Neuroradiology (EH, SE), University Hospital Bonn. Depending on the nature of the implants, other experts were consulted (e.g., ophthalmologists, urologists).

Our procedure was as follows (**Figure 1**): Active implants (e.g., pacemakers), pregnancy, intrauterine devices, non-medical metal and metal splinters were considered absolute MRI contraindications. Tattoos and permanent make-up were not considered contraindications.
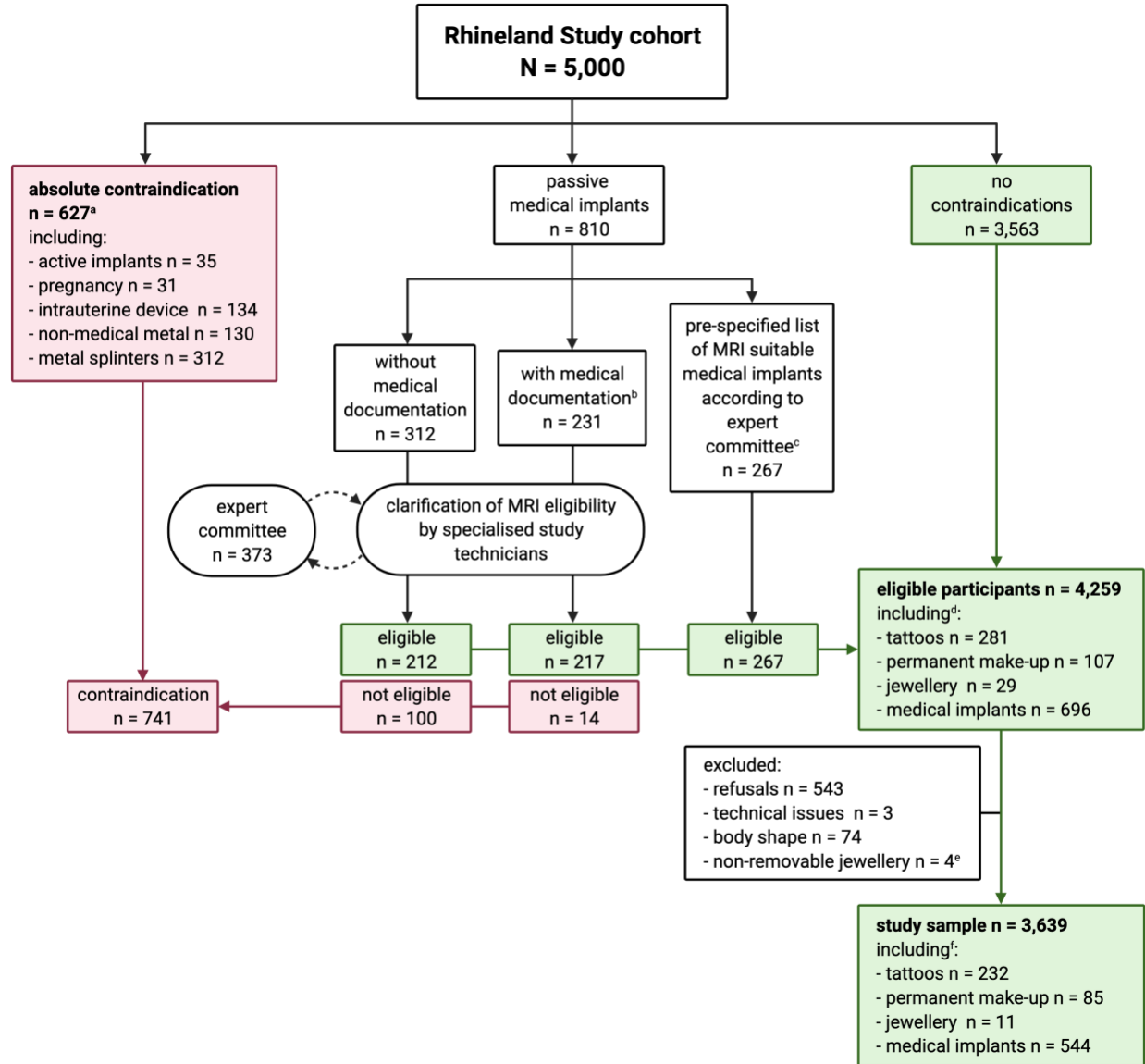
Figure 1 | Flowchart of the process of clarification of MRI suitability in the Rhineland Study. [a] Participant could have more than one absolute contraindication. [b] Only three participants had MRI safety certificates for their medical implants. [c] After evaluating our procedure after one year, the expert committee considered the following medical implants, if implanted after 2005, as MRI suitable without checking further documentation: hip and knee replacements, stents, bypass, breast implants filled with silicone, and screws, plates and stiffening of the spinal cord < 13cm. [d] 376 participants had tattoos and / or permanent make-up, of whom 45 also had medical implants. [e] Participants who were excluded according to stricter exclusion criteria at study start and could not be contacted for reinvitation. [f] 305 participants had tattoos and / or permanent make-up, of whom 35 also had medical implants.

We did, however, ask for age, size, location, colour, and material of the tattoos and permanent make-up. If participants indicated having passive devices, we asked them to bring relevant medical documentation for these (surgery or release reports, implant pass, etc., including age, size and material of the implant). If needed, and with the explicit consent of the participant, we called the hospital which implanted the passive device to ask for further information. Specialized study technicians decided on MRI suitability based on available information, and referred to the MRI expert committee where needed. The expert committee decided on MRI suitability based on current knowledge in both scientific and clinical practice, with the guiding principle to do no harm to participants. In cases of doubt or whenever a possible MRI contraindication could not be ruled out, participants were excluded from MRI.

One year after the introduction of this procedure, the MRI expert committee evaluated it. During this period, 169 participants with medical implants had been discussed by the expert committee and subsequently been scanned without any problems. Based on these experiences, the MRI expert committee made a list of medical implants that from then on could be considered as MRI suitable by the study technicians without further consulting the MRI expert committee. This list included the following medical devices, if implanted after 2005, with or without relevant medical documentation: hip and knee replacements, stents, bypass, clips, breast implants filled with silicone, and screws, plates and stiffening of the spinal cord < 13 cm. The 2005 cut-off was chosen because in recent years such implants are typically made of titanium. A medical implant had to be implanted at least 6 weeks before the MRI examination.

MRI Data Acquisition

All eligible participants underwent a one-hour MRI examination of brain structure and function on 3 T MRI scanners (Siemens Prisma Magnetom, Erlangen, Germany). The scanners were equipped with an 80 mT/m gradient system and a 64-channel phased-array head-neck coil. All MRI sequences and protocols were either developed in-house for the purpose of the Rhineland Study or based on Siemens product sequences. The MRI protocol included the

following sequences: a 3D T1-weighted Multi-Echo Magnetisation Prepared RApid Gradient-Echo sequence (ME-MPRAGE; acquisition time (TA) = 6.5 min, time of repetition (TR) = 2,560 ms, inversion time (TI) = 1,100 ms, flip angle 7°, field of view (FOV) = 256 x 256 mm, 0.8 mm isotropic);[14,15] a 3D T2-weighted Turbo-Spin-Echo (TSE) sequence (TA = 4.6 min, TR = 2,800 ms, echo time (TE) = 405 ms, FOV = 256 x 256 mm, 0.8 mm isotropic);[16,17] a 3D T2 FLuid-Attenuated Inversion Recovery (FLAIR) sequence (TA = 4.5 min, TR = 5,000 ms, TE = 393 ms, TI = 1,800 ms, FOV = 256 x 256 mm, 1.0 mm isotropic); a motion robust quantitative susceptibility weighted (QSM) sequence based on a 2D-segmented 3D gradient echo-planar imaging (EPI) sequence using multiple echo times (6 echo times, TA = 5.7 min, TR = 32 ms, flip angle 14°, FOV = 212 x 212 mm, 0.8 mm isotropic);[18] for simultaneous-multi-slice diffusion weighted MRI (dMRI), a spin-echo echo-planar imaging (SE-EPI) sequence applying threefold slice-acceleration and a compressed-sensing diffusion spectrum imaging protocol (TA = 11.4 min, TR = 5,500 ms, TE = 105 ms, band width 1,624 Hz/Px, FOV = 210 x 210 mm, 1.5 mm isotropic);[19-22] a 3D EPI sequence using 2D Controlled Aliasing In Parallel Imaging Results IN Higher Acceleration (CAIPIRINHA) sampling with variable echo train lengths, rapid water excitation and fat-selective inversion recovery was applied to collect resting-state fMRI data (TA = 10.5 min, TR = 570 ms, TE = 30 ms, TI = 240 ms, flip angle 16°, FOV = 216 x 216 mm, 2.4 mm isotropic);[23] and abdominal MRI was performed for 72 axial slices centred in the middle of the third lumbar vertebra using a breath-hold two-point Dixon sequence while the participants were in supine position with arms placed at side (2 echo times, TA = 0.2 min, TR = 4.12 ms, flip angle 6°, FOV = 500 x 437 mm, resolution 2.0 x 2.0 x 5.0 mm). The T1- and T2-weighted sequences employed twofold parallel imaging acceleration using CAIPIRINHA and elliptical sampling.[24,25]

Before the MRI examination, we verbally informed all participants with medical implants, non-removable jewellery, tattoos and/or permanent make-up about the possibility of adverse events, including tingling sensations, (slight) heating and burning. They were instructed to squeeze the alarm ball during the MRI examination as soon as they would feel any tingling sensation. In case of an adverse reaction, we would ask about their symptoms and document these as well, and provide first aid if needed.

For participants with head implants or permanent make-up, we checked all scouts for possible artifacts which would require immediate stopping of MRI data acquisition. For permanent make-up, this would include any artifacts on the scouts; for head implants any artifact that would make the scan of the brain unreadable. Additionally, all T1-weighted, T2-weighted, and FLAIR scans have been visually inspected for quality during the initial quality assessment of the Rhineland Study, where two raters independently checked for artifacts that might affect the quality of automated brain segmentations.

Sample Size and Minimum Detectable Effect

We have calculated the proportion of adverse events that we could have detected with 90 % and 80 % confidence given our sample size of people with tattoos or medical implants (n = 305 and n = 544, respectively).[26]

RESULTS

Figure 1 gives an overview of MRI suitability in the Rhineland Study. Of the 5,000 participants, 3,563 (71.3 %) had no contraindications, 627 (12.5 %) had an absolute contraindication, and 810 (16.2 %) had a passive medical implant. We ultimately deemed 696 (85.9 %) of the passive medical implants MRI suitable. The expert committee discussed 373 cases and considered 352 of those as MRI suitable. We excluded participants who could not provide enough information to assess suitability.

In total, 4,259 (85.2 %) participants were considered eligible for MRI, of whom 3,639 (85.4 %) were actually scanned (mean age 54.5 (SD = 13.7) years, 57.8 % women (**Table 1**)). Of those we scanned, 544 (14.9 %) had passive medical implants; 305 (8.4 %) had either tattoos (6.4 %), permanent make-up (2.3 %), or both (0.3 %); 35 (1.0 %) had medical implants and tattoos; and 11 had non-removable jewellery (wedding rings, piercings).
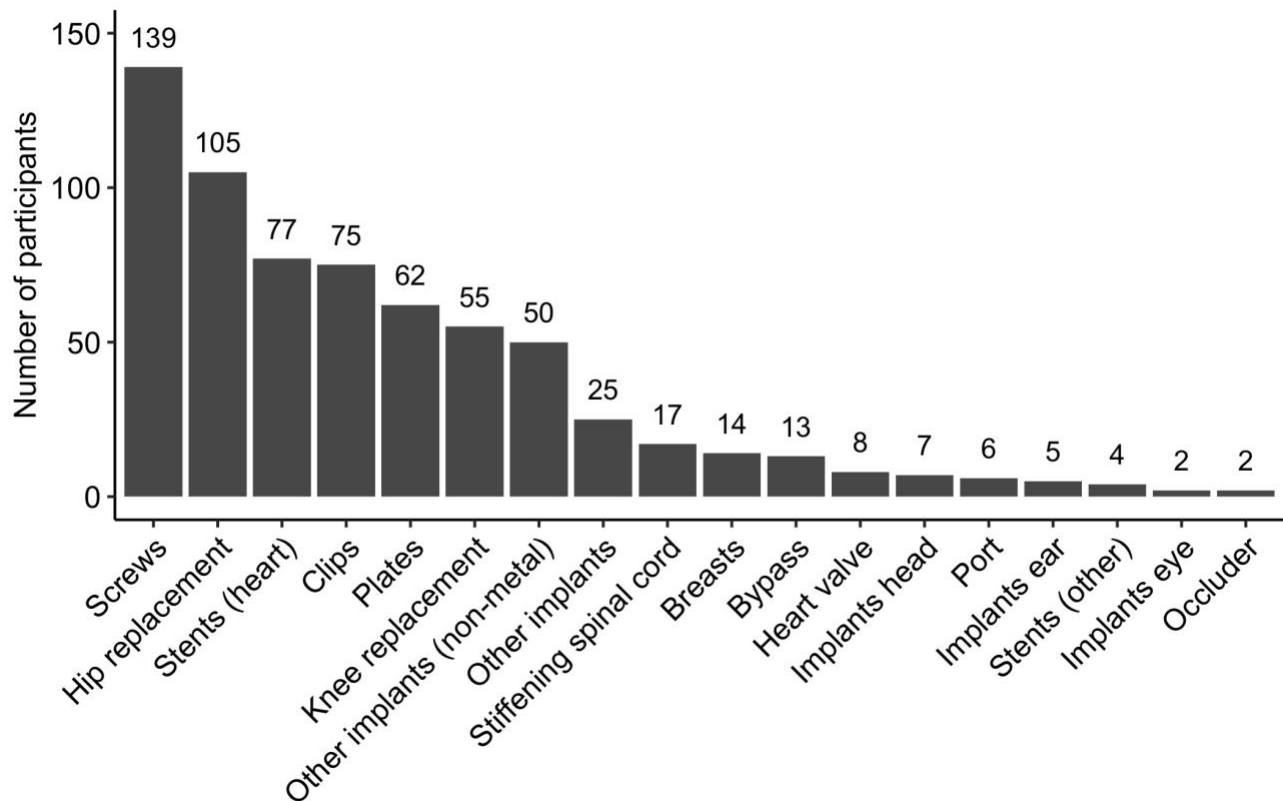
Figure 2 | Frequency of eligible medical implants that were scanned at 3T in the Rhineland Study. Participants could have multiple plates or screws, these were each counted as one implant. *Other implants* included: wire cerclage, threads made from titanium, patches made from Teflon, urinary tract implants, broken dental files. *Other implants (non-metal)* included: hernia mesh, neobladder, artificial bone mass, gastric band.

Participants had up to six medical implants, mostly plates, screws, stents, clips, or hip- or knee-replacement (**Figure 2**), which were up to 48 years old with a median age of 7 years (interquartile range (IQR): 3 – 13 years).

Among participants with tattoos, the number of tattoos (including permanent make-up) per person ranged from one to eight, at in total 532 individual body locations. Most frequent locations were on the torso (33.8 %), arms (20.5 %), or legs (lower leg: 9.2 %; upper leg: 5.3 %) (**Table 1**). The majority of the tattoos (78.8 %) was located above the waist and hence within the main or fringe field of the radiofrequency transmitting body coil and the gradient coils of the MRI scanner, 21.2 % were located in the head coil. Tattoos and permanent make-up were between 1 and 41 years old, with a median age of 10 years (IQR: 4 – 20 years).

Table 1 | Characteristics of the participants of the Rhineland Study who underwent MRI.

	Rhineland Study cohort (n = 3,639)
Age in years (mean (SD))	54.5 (13.7)
Women (n (%))	2,103 (57.8)
Passive medical implants (n (%))	544 (14.9)
Non-removable jewellery (n (%))	11 (0.3)
Tattoo and / or permanent make-up (n (%))[a]	305 (8.4)
Tattoo (n (%))	232 (6.4)
Permanent make-up (n (%))	85 (2.3)
Total tattoo size in cm2 (median [IQR])	100.0 [30.0 – 450.0]
Individual tattoos / permanent make-up (n (%))	
1	181 (59.3)
2	64 (21.0)
3	35 (11.5)
4	9 (3.0)
5	9 (3.0)
6	1 (0.3)
7	1 (0.3)
8	3 (1.0)
Unknown[b]	1 (0.3)
Disappeared[c]	1 (0.3)
Body location (n (%))	
arm	109 (20.5)
eyebrows	51 (9.6)
eyelid	56 (10.5)
foot	19 (3.6)
hand	7 (1.3)
lips	11 (2.1)
lower leg	49 (9.2)
neck	12 (2.3)
private parts	2 (0.4)
torso	180 (33.8)
unknown	2 (0.4)
upper leg	28 (5.3)
wrist	6 (1.1)

Note. *SD* = standard deviation; *IQR* = interquartile range. [a] This includes 12 participants who had both tattoos and permanent make-up. [b] No information provided by participant. [c] Tattoo was not visible anymore after two years.

Median size was 100 cm^2 (IQR: 30 – 450 cm^2), ranging up to 7,960 cm^2, with 72 (17.7 %) tattoos being larger than 20 cm in dimension (**Table 1**). We scanned 24 participants with tattoos covering more than 5 % of the mean sex-specific body surface area.[27] Most tattoos were mono-coloured (64.3 %), most used colours were black (52.1 %), brown (5.5 %), black-red (4.9 %), black-blue (4.5 %), black-red-green (2.4 %). Most participants were not aware of the material of the tattoo (73.2 %), only 2.2 % reported that it was tattoo ink that did not contain any metal, 1.2 % reported that their tattoo was self-made, and 1.0 % did not know the material of their tattoo, but spontaneously reported that they got it outside of Europe or the USA.

None of the participants reported adverse events nor was the quality of any of the MR scout images reduced by any implants or permanent make-up. There were no artifacts seen during the initial quality assessment due to permanent make-up or medical implants in the head which made the brain images unreadable.

Comparison to Previous Recommendations

With regard to tattoos, if we had followed the procedure from a recent study on MRI safety of tattoos, we would have had to exclude 182 of 376 participants who we considered eligible, because of tattoo location (head: n = 108, neck: n = 15, genital area: n = 2), tattoos covering more than 5 % of the total body area (n = 28), tattoos bigger than 20 cm in diameter (n = 60), or tattoos < 20 cm apart from each other (n = 21) (multiple reasons possible).[12]

If we had followed most recent recommendations by the FDA that require an MRI safety certificate,[1] we would have had to exclude all but 3 participants for their medical implant (807 of 810 participants). Following our procedure, we only excluded 114 of 810 participants, yielding an additional 693 eligible participants.

Thus, compared to these established practices and FDA guidelines we classified an additional 830 participants with tattoos or medical implants (45 had both) as MRI eligible (16.6 % of our source population). Of these, 703 participants underwent MRI.

Of note, the FDA guidelines can be interpreted more loosely, allowing for an implant to be identified as MRI suitable based on other medical documentation. Had we used those criteria, we still would have had to exclude 589 of our 810 participants with passive medical implants.

Sample Size

With our given sample size for tattoos and medical implants, we would be able to detect with 90 % confidence adverse reactions in 0.8 % and 0.4 %, respectively, and with 80 % confidence in 0.5 % and 0.3 %, respectively.

DISCUSSION

In this large population-based study, we allowed participants with passive medical implants without MRI safety certificates, tattoos, or permanent make-up to undergo 3 Tesla MRI. We did not observe any adverse events or artifacts that notably reduced quality of the brain scans. Through our relaxed MRI eligibility criteria, we could include 16.6 % more people than would have been possible based on FDA guidelines[1] and recommendations from a previous study.[12]

Older case reports described adverse reactions in people with tattoos undergoing MRI,[7-11] yet a more recent study in 330 persons reported that the probability of having a tattoo-related adverse reaction was only 0.17 %.[12] However, that study excluded participants with tattoos on head, neck or genital area, bigger than 20 cm in diameter, not 20 cm apart from each other, and covering more than 5 % of the total body area, because of fear of adverse reactions. In a retrospective survey among in 135 persons with tattoos including head and neck tattoos who underwent clinical MRI, 1.5 % reported adverse reactions before the actual MRI scanning, which, however, were not long-lasting.[13] In our study, we included all persons

with tattoos and permanent make-up regardless of size or location. None of the participants reported any adverse events.

The FDA recommends to exclude people from MRI for research purposes if their medical implant cannot be identified as MRI eligible.[1] Of course, most studies do not solely base their guidelines for MRI eligibility on the FDA recommendation, but rather on a combination of resources, including expert knowledge or websites such as www.mrisafety.com. Nevertheless, it is essential to be able to identify medical implants in order to confirm eligibility. We found that < 0.5 % of those with a passive medical implant had an MRI safety certificate. Most of our participants had no relevant documentation to identify the medical implant, and would therefore have been excluded had we strictly followed the FDA recommendations. We were able to classify two thirds of these participants as MRI eligible, based on information the participant provided verbally. In the excluded cases, participants could not tell us what exact procedures they underwent nor when. Therefore, we could not out rule any potential risks for the participant to undergo MRI.

Our approach emphasizes the importance of MRI expert panels involved in the clarification of MRI eligibility. Due to the combined knowledge on clinical and physical MRI, we were able to increase the number of participants undergoing MRI. We propose that new (population-based) research studies establish MRI expert panels to determine MRI safety of passive devices, incorporating recent advances in the scientific communities (e.g., ISMRM,[28] www.mrisafety.com[6]) as well as clinical practices, thereby reducing selection bias in research studies.

Here, we defined adverse reactions as pressing the alarm ball during the MRI examination. Previous studies have asked participants afterwards about their experience in the MRI. We refrained from doing so since we instructed our participants extensively before entering the scanner to press the alarm ball whenever something would feel off.

A limitation of our study is that only 24 of our scanned participants had tattoos covering more than 5 % of the total body area. Although we asked participants about the material of their tattoos, most of them did not know. Unfortunately, we did not specifically ask for the country

where the tattoos had been made. Additional studies are therefore required to investigate the MRI suitability of full-body tattoos, and preferably including information on country where and material with which the tattoos were done. While we visually checked the brain scout for artifacts in participants with head implants and permanent make-up at the beginning of the MRI examination, we did not use automated metrics or quantitative assessments for this. However, there were no artifacts that made the images unreadable.

CONCLUSION

We conclude that most passive medical implants (even without MRI safety certificates), tattoos, and permanent make-up are eligible for 3 Tesla MRI research studies. Our procedure could guide new research studies in the clarification of MRI suitability. This is crucial to reduce selection bias in, and thereby increase generalizability and validity of, MRI research studies.

DECLARATIONS

Data availability statement

The datasets presented in this article are not readily available because of data protection regulations. Access to data can be provided to scientists in accordance with the Rhineland Study's Data Use and Access Policy. Requests for additional information and/or access to the datasets can be send to RS-DUAC@dzne.de.

Ethics Statement

The studies involving human participants were reviewed and approved by University of Bonn, Medical Faculty. The participants provided their written informed consent to participate in this study.

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3.2. Incidental findings on 3 T neuroimaging: cross-sectional observations from the population-based Rhineland Study

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ABSTRACT

Purpose: Development of best practices for dealing with incidental findings on neuroimaging requires insight in their frequency and clinical relevance.

Methods: Here, we delineate prevalence estimates with 95 % confidence intervals and clinical management of incidental findings, based on the first 3,589 participants of the population-based Rhineland Study (age range 30–95 years) who underwent 3 Tesla structural neuroimaging (3D, 0.8 mm^3 isotropic resolution). Two trained raters independently assessed all scans for abnormalities, with confirmation and adjudication where needed by neuroradiologists. Participants were referred for diagnostic work-up depending on the potential benefit.

Results: Of 3,589 participants (mean age 55 ± 14 years, 2072 women), 867 had at least one possible incidental finding (24.2 %). Most common were pituitary abnormalities (12.3 %), arachnoid cysts (4.1 %), developmental venous anomalies (2.5 %), non-acute infarcts (1.8 %), cavernoma (1.0 %), and meningiomas (0.7 %). Forty-six participants were informed about their findings, which was hitherto unknown in 40 of them (1.1 %). Of these, in 19 participants (48 %) a wait-and-see policy was applied and nine (23 %) received treatment, while lesions in the remainder were benign, could not be confirmed, or the participant refused to inform us about their clinical diagnosis.

Conclusion: Nearly one quarter of participants had an incidental finding, but only 5 % of those required referral, that mostly remained without direct clinical consequences.

INTRODUCTION

Magnetic resonance imaging (MRI) has been widely used in both research and clinical practice over the past decades. As a consequence, people had to develop best practices for dealing with incidental findings. An incidental finding is a previously unknown abnormality of potential clinical relevance that is unexpectedly discovered and unrelated to the specific research purposes of a study itself.[1]

The prevalence of incidental findings on neuroimaging varies across studies depending on the age distribution of participants and the imaging modalities used.[2] So far, population-based studies have reported incidental findings mostly in older people and using 1.5 Tesla neuroimaging[3-7] with only a few studies using at least one 3D imaging sequence.[5-7] To the best of our knowledge, the Study of Health in Pomerania study is the only population-based study that reported on incidental findings on MRI covering a broad age range by including participants aged between 21 and 88 years; however, their imaging protocol was limited to 2D MR images.[8]

Based on the large, single-centre population-based Rhineland Study, we here report on the prevalence of incidental findings detected on brain neuroimaging using 0.8 mm^3 isotropic 3D imaging sequences across the adult life span, and provide information about clinical management of incidental findings that were reported back to the participant.

METHODS

Study population

This study is based on all participants who underwent structural brain MRI out of the first 5,000 consecutive participants of the Rhineland Study (n = 3,589, shown in **Figure 1**). The Rhineland Study is an ongoing, prospective, single-centre, community-based cohort study. All inhabitants aged 30–100 years of two geographically defined areas in Bonn, Germany, are invited to participate in the study. The sole exclusion criterion is insufficient command of the German language to provide informed consent.
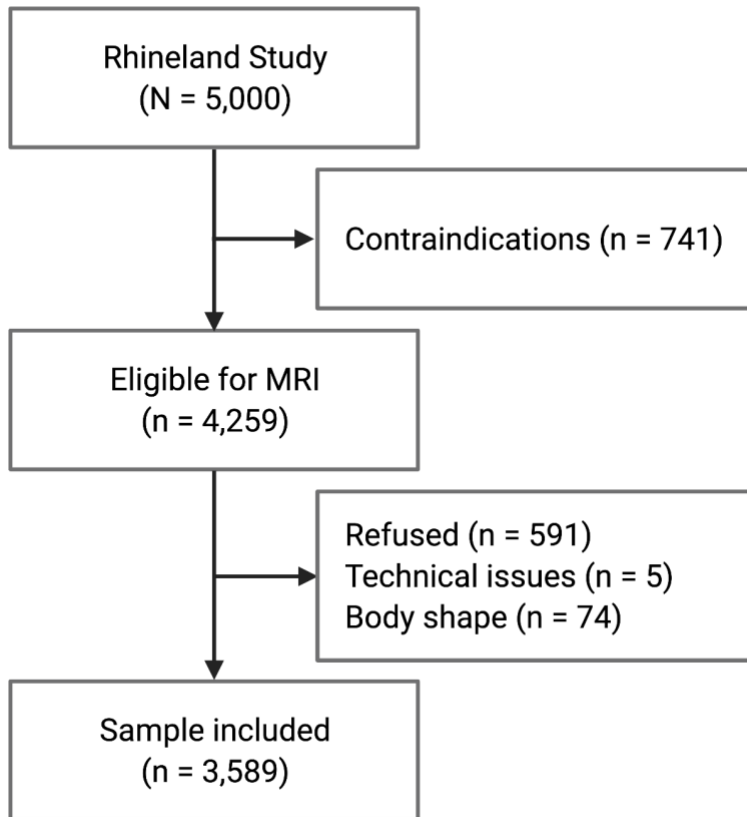
Figure 1 | Flowchart showing inclusion and exclusion criteria of the study. *Body shape* indicates participants who did not fit into the MRI scanner.

Magnetic resonance imaging data acquisition

MRI data was acquired on 3 Tesla MRI scanners (Siemens Prisma Magnetom, Erlangen, Germany) equipped with an 80 mT/m gradient system and a 64-channel phased-array head-neck coil, including the following in-house developed sequences: a 3D T1-weighted multi-echo magnetization prepared rapid gradient-echo (ME-MPRAGE) sequence (time of acquisition (TA) = 6.5 min, repetition time (TR) = 2,560 ms, inversion time (TI) = 1,100 ms, flip angle 7°, field of view (FOV) = 256x256 mm, 0.8 mm isotropic);[9,10] a 3D T2-weighted Turbo-Spin-Echo (TSE) (TA = 4.6 min, TR = 2,800 ms, echo time (TE) = 405 ms, FOV = 256x256 mm, 0.8 mm isotropic);[11,12] and a 3D T2 fluid-attenuated inversion recovery (FLAIR) pulse sequence (TA = 4.5 min, TR = 5,000 ms, TE = 393 ms, TI = 1,800 ms,
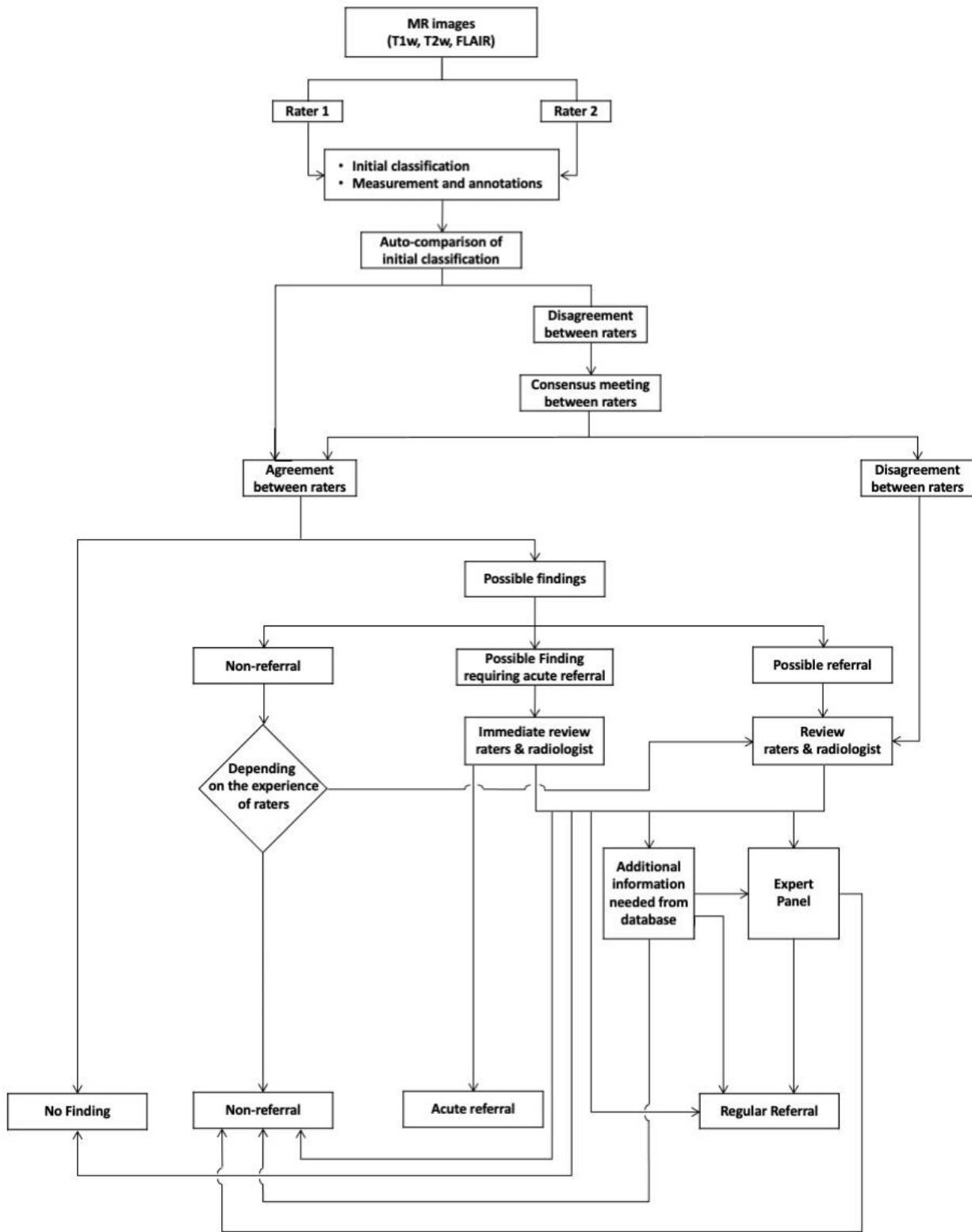
Figure 2 | Workflow assessment of incidental findings in the Rhineland Study.

FOV = 256x256 mm, 1.0 mm isotropic). All sequences employ parallel imaging acceleration with CAIPIRINHA sampling[13] and elliptical sampling.[14]

For the initial screening of incidental findings, all images were reconstructed to a resolution of 2.5 mm isotropic to reduce the workload of the reader. When an abnormality was seen, the reader had direct access to the original images for detailed assessment.

Assessment and clinical management of incidental findings

The workflow of the assessment of incidental findings in the Rhineland Study is depicted in **Figure 2**. Criteria for what constitutes an incidental finding and which findings should be reported back to the participant were developed by an expert committee based on clinical guidelines, state-of-the-art scientific evidence, and ethical considerations (see **Table 1**). Possible incidental findings that were explicitly, but not exclusively, checked for included infarcts, haemorrhage, malignant tumours, parenchymal brain lesions, intraventricular lesions, pituitary lesions, brainstem lesions, lesions involving a cranial nerve, meningiomas, arachnoid cysts, aneurysms, arteriovenous malformations, cavernous malformations, developmental venous malformations, developmental abnormalities, and white matter hyperintensities that were presumably not due to cerebral small vessel disease (including multiple sclerosis). The latter was based on the dark appearance of white matter hyperintensities on T1-weighted images as well as the clinical experience of the neuroradiologists. Initial readings with this prespecified protocol were performed with OsiriX MD, an image processing application for DICOM images, by two of three independent raters (VL, cognitive neuroscientist with 6 years of experience (until end of study); RL, radiologist with 7 years of experience (until August 2019); specifically trained medical student with 1 year of experience (from August 2019 onwards)). The initial raters had previous experience in MR image reading in clinical routine or for research purposes. Additionally, before the start of the study, they joined the Clinic for Neuroradiology in Bonn for two weeks to get more specific training in the detection of brain abnormalities, and had specific training sessions with neuroradiologists (e.g., to distinguish between normal variations and cystic lesions of the

Table 1 | Protocol for the referral of incidental findings for further diagnostic work-up in the Rhineland Study.

Incidental findings that need to be referred for diagnostic work-up

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Acute findings

       Acute infarct

       Intracranial haemorrhage

Mass

       Malignant tumours, inclusive glioma

       Any brain parenchymal lesion (including cystic) with oedema/ hydrocephalus/ midline shift/ nerve or vessel impairment

       Any intraventricular lesion that might cause a hydrocephalus

       Solid/semi-solid pituitary lesion > 1 cm or any cystic lesion with mass effect > 1 cm

       Solid/semi-solid lesion or any cystic lesion with mass effect in brainstem

       Lesions with involvement of a cranial nerve

       Meningiomas

           Convexity meningiomas > 2 cm

           All non-convexity meningiomas regardless of size

Vascular disease

       Aneurysm with PHASES score ≥ 5

       Aneurysm in posterior circulation including posterior communicating artery with PHASES score < 5 should be discussed in the Panel to make the final decision

       Sub-acute intracranial haemorrhage bleeding (including subdural haematoma, epidural haematoma, intracerebral haemorrhage, subarachnoid haemorrhage, intraventricular haemorrhage)

Incidental findings that do not need diagnostic work-up and are not communicated to the participant

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       Mass and vascular diseases not mentioned in the list above

       Arachnoid cysts

       Non-acute cerebral infarcts

       Arteriovenous malformations

       Cavernous malformations

       White matter hyperintensities, including multiple sclerosis

       Developmental abnormalities

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pituitary gland). To train new raters they developed an initial training set including 110 MRI scans from the Rhineland Study, which included both scans with and without abnormalities. The third rater got trained using this initial training set as well as 150 additional random MRI scans from the Rhineland Study. The training set is still increasing in size as raters continue to include interesting cases.

The initial ratings were done blinded to the medical history of participants, usually within 1 working day by at least one of the raters. Next, both ratings were compared. In case of persistent disagreement, an incidental finding that possibly would require referral, or whenever further clarification was needed, an experienced (neuro-)radiologist also read the images and made a final decision on the classification of the finding (SJE, radiologist with 7 years of experience; EH, neuroradiologist with 23 years of experience). All judgements were solely made on the basis of the MRI scans.

The decision whether or not to refer a participant with an incidental finding to a medical specialist for clinical work-up depended on the potential benefit for the participant, which was defined a priori by the expert committee mentioned above (**Table 1**). In case of ethically challenging findings, further experts could be consulted. When referral was needed, a study physician informed the participant and, with the consent of the participant, their general practitioner. Note that we only received feedback on the detected brain abnormality from the persons who we approached for referral. Therefore, we cannot exclude that some of the non-referred lesions were already known to the participant, and therefore in sensu stricto not an incidental finding, even though they had not been reported during the interview.

To obtain information on clinical management of referred abnormalities, we asked the participants to send relevant medical letters or to give consent for us to contact their practitioner to review medical records directly. We only considered clinical diagnoses made by medical specialists after clinical neuroimaging.

Assessment of demographic variables

Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or antihypertensive medication use; diabetes as fasting plasma glucose level ≥ 7 mmol/l, $HbA1_c$ ≥ 6.5 % or use of antidiabetic medication. History of multiple sclerosis and stroke, smoking status (current/non-smoker), and education (low: ISCED 0-3; middle: ISCED 4-6; high: ISCED 7-8)[15] was self-reported.

Data Availability

The data for this manuscript are not publicly available due to data protection regulations. Access to data can be provided to scientists in accordance with the Rhineland Study's Data Use and Access Policy. Requests for additional information and/or access to the datasets can be send to RS-DUAC@dzne.de.

Statistical Analysis

We calculated the prevalence with 95 % confidence intervals (CI) for each incidental finding in our study population. For the most frequent incidental findings we further evaluated whether prevalence differed between sexes and across age using logistic regression. Multiple similar incidental findings within one participant were counted as a single finding (e.g. multiple arachnoid cysts). P-values < 0.05 were considered as statistically significant. All statistical analyses were performed using R version 4.0.2.[16]

RESULTS

Mean age of the study population was 54 ± 14 years, 58 % were women (**Table 2**). Men compared to women were on average more often higher educated (65 % vs. 48 %, p = 0.001), were more likely to have diabetes (6 % vs. 3 %, p < 0.001) and hypertension (40 % vs. 34 %, p < 0.01), and a higher body mass index (26.1 vs. 25.3, p < 0.001). Participants who underwent MRI were on average younger (55 vs. 56 years, p < 0.001), more

Table 2 | Characteristics of the study population.

Characteristics	Whole cohort	Sample with MRI				Sample without MRI	
		Overall	Women	Men	p-value[†]		p-value[‡]
	(n = 5,000)	(n = 3,589)	(n = 2,072)	(n = 1,517)		(n = 1,517)	
Age in years (mean (SD))	55 ± 14	54 ± 14	55 ± 14	54 ± 14	0.23	56 ± 15	<0.001
Women (n (%))	2,824 (56)	2,072 (58)				752 (53)	<0.01
Age group (n (%))					0.11		<0.001
30-39 years	833 (17)	627 (17)	335 (16)	292 (19)		206 (15)	
40-49 years	926 (19)	676 (19)	390 (19)	286 (19)		250 (18)	
50-59 years	1,358 (27)	988 (28)	593 (29)	395 (26)		370 (26)	
60-69 years	1,009 (20)	736 (21)	440 (21)	296 (20)		273 (19)	
70-79 years	666 (13)	450 (13)	252 (12)	198 (13)		216 (15)	
80+ years	208 (4)	112 (3)	62 (3)	50 (3)		96 (7)	
Education (n (%))					<0.01		<0.001
low	101 (2)	61 (2)	48 (2)	13 (1)		40 (3)	
middle	2,232 (45)	1,532 (43)	1,015 (49)	517 (34)		700 (50)	
high	2,621 (53)	1,969 (55)	988 (48)	981 (65)		652 (47)	
Diabetes (n (%))	261 (5)	161 (5)	67 (3)	94 (6)	<0.001	100 (7)	<0.001
Hypertension (n (%))	1,867 (38)	1,283 (37)	684 (34)	599 (40)	<0.001	584 (42)	<0.001
Smoking (n (%))	621 (12)	459 (13)	252 (12)	207 (14)	0.22	162 (12)	0.09
BMI in kg/m^2 (mean (SD))	25.9 ± 4.5	25.6 ± 4.2	25.3 ± 4.7	26.1 ± 3.5	<0.001	26.7 ± 5.2	<0.001
Self-reported MS (n (%))	25 (1)	21 (1)	15 (1)	6 (0)	0.19	4 (0)	0.13
Self-reported Stroke (n (%))	78 (2)	47 (1)	24 (1)	23 (2)	0.33	31 (2)	0.1

Note. *SD* = standard deviation; *BMI* = body mass index; *MS* = Multiple Sclerosis. [†] P-values are adjusted for age where applicable and show differences between women and men. [‡] P-values are adjusted for age and sex where applicable and show differences between participants with and without MRI.

Table 3 | Overview of incidental findings in the Rhineland Study.

Incidental finding (n (%))	Overall (n = 3,589)	Women (n = 2,072)	Men (n = 1,517)	p-value[†]
Any, n (%)	867 (24.2)	505 (24.2)	362 (23.9)	0.08
Pituitary Abnormality, n (%)	443 (12.3)	267 (12.9)	176 (11.6)	0.25
Arachnoid Cyst[¥], n (%)	148 (4.1)	69 (3.3)	79 (5.2)	0.01
Developmental Venous Abnormality[¶], n (%)	89 (2.5)	50 (2.4)	39 (2.6)	0.77
Non-acute infarcts[#], n (%)	64 (1.8)	29 (1.4)	35 (2.3)	0.04
Other[þ], n (%)	43 (1.2)	23 (1.1)	20 (1.3)	0.55
Cavernoma[‡], n (%)	35 (1.0)	22 (1.1)	13 (0.9)	0.56
Other Mass, n (%)	30 (0.8)	20 (1.0)	10 (0.7)	0.32
Meningioma[§], n (%)	26 (0.7)	20 (1.0)	6 (0.4)	0.06
Haemorrhage[*], n (%)	14 (0.4)	7 (0.3)	7 (0.5)	0.55
Developmental Abnormality, n (%)	14 (0.4)	3 (0.1)	11 (0.7)	0.02
MS-like Lesions, n (%)	16 (0.4)	9 (0.4)	7 (0.5)	0.97
Unknown White Matter Disease, n (%)	11 (0.3)	8 (0.4)	3 (0.2)	0.33
Aneurysm[¬], n (%)	8 (0.2)	4 (0.2)	4 (0.3)	0.66
Other Vascular Disease, n (%)	7 (0.2)	4 (0.2)	3 (0.2)	0.97
Inflammatory White Matter Disease, n (%)	6 (0.2)	4 (0.2)	2 (0.1)	0.67
Malignant Lesion, n (%)	3 (0.1)	1 (0.0)	2 (0.1)	0.41
Arteriovenous Malformation, n (%)	2 (0.1)	2 (0.1)	0 (0.0)	0.99
Intraventricular Lesion, n (%)	2 (0.1)	1 (0.0)	1 (0.1)	0.82
Brainstem Lesion, n (%)	4 (0.1)	2 (0.1)	2 (0.1)	0.77
Cranial Nerve Lesion, n (%)	3 (0.1)	1 (0.0)	2 (0.1)	0.44

Note. Other also includes post-operative changes (n = 19) and post-traumatic defects (n = 6). [¥]There were 161 arachnoid cysts in 148 participants, 137 participants had one arachnoid cyst, 9 had two, and two had three. [¶]There were 92 developmental venous abnormalities (DVA) in 89 participants, 86 had one DVA, three had two DVAs. [#]There were 84 non-acute infarcts in 64 participants, 49 participants had one post-ischemic lesion, twelve had two, two had three and one had five non-acute infarcts. [þ]There were 44 other abnormalities in 43 participants, 43 had one abnormality, one had two. [‡]There were 40 cavernomas in 35 participants, 33 had one cavernoma, one had two cavernomas, and one had five. [§]There were 27 meningioma in 26 participants, 25 had one meningioma, one had two. [*]There were 28 haemorrhages in 14 participants, eight had one haemorrhage, one had two, two had three, and three had four haemorrhages. [¬]There were nine aneurysms in eight participants. Seven had one aneurysm, one had two aneurysms. [†]P-values are adjusted for age and show differences between women and men.

often higher educated (55 % vs. 47 %, p < 0.001), were less likely to have diabetes (5 % vs. 7 %, p < 0.001), or hypertension (37 % vs. 42 %, p < 0.001), and had a lower body mass index (25.6 vs. 26.7, p < 0.001), compared to those who did not. Also, more men than women (47 % vs 42 %, p = 0.005) were excluded from or refused MRI.

In total, 867 of 3,589 participants had at least one possible incidental finding (24.2 % [95 % CI: 22.8–25.6 %]) (**Table 3**). This did not differ between women (505 of 2,072 with incidental finding (24.4 % [95 % CI: 22.5–26.3 %]) and men (362 of 1,517 (23.9 % [95 % CI: 21.7–26.1 %]) (p = 0.764). The maximum number of incidental findings for a single person was four; one participant had an arachnoid cyst, a developmental venous anomaly, a cavernoma and a possibly malignant lesion; another participant had an arachnoid cyst, cystic lesion of the pituitary gland, inflammatory WM lesions and cystic lesions around the brainstem. Most frequent incidental findings were pituitary abnormalities (12.3 % [95 % CI: 11.3–13.5 %]), arachnoid cysts (4.1 % [95 % CI: 3.5–4.8 %]), developmental venous anomalies (2.5 % [95 % CI: 2.0–3.0 %]), non-acute infarcts (1.8 % [95 % CI: 1.4–2.3 %]), cavernoma (1.0 % [95 % CI: 0.7–1.4 %]), and meningiomas (0.7 % [95 % CI: 0.5–1.1 %], mean size of the largest dimension, 14.9 ± 6.8 mm). Men had more non-acute infarcts, more arachnoid cysts, and more developmental abnormalities than women (2.3 % vs. 1.4 %, p = 0.040; 5.2 % vs. 3.3 %, p = 0.006; 0.7 % vs. 0.1 %, p = 0.015, respectively). Women had slightly more meningiomas, but because of small numbers the difference was only borderline significant (1.0 % vs. 0.4 %, p = 0.056). The presence of non-acute infarcts increased with age (prevalence odds ratio (OR): 1.06 [95 % CI: 1.04–1.08] per year, p<0.001), as did the frequency of cavernomas (OR: 1.03 [95 % CI: 1.00–1.05] per year, p = 0.044), and of meningiomas (OR: 1.05 [95 % CI: 1.02–1.08] per year, p = 0.002). For other incidental findings we saw no effect of age on prevalence.

Most of the 433 pituitary anomalies that we found were pituitary cysts (mostly pars intermedia cysts; 95.9 % [95 % CI: 93.7–97.6 %]), the remainder were (semi-)solid lesion with or without a mass effect, most likely to be microadenomas. The prevalence of pituitary cysts did not significantly differ between men (10.9 % [95 % CI: 9.4–12.6 %]) and women (12.5 % [95 % CI: 11.1–14.0 %]) (p = 0.174) and was stable across the adult life span (OR: 1.00 [95 % CI:

0.99–1.01] per year, p = 0.805). The prevalence of other pituitary anomalies did not differ between sexes (women: (0.4 % [95 % CI: 0.2–0.8 %]); men: (0.7 % [95 % CI: 0.3–1.2 %]); p = 0.187) but increased with age (OR: 1.04 [95 % CI: 1.01–1.08] per year, p = 0.024).

The raters had initial disagreement in the reading of the MR images in approximately 12 % of the cases, where one of the raters had missed an abnormality. Persistent disagreement occurred in less than 1 %, where clarification by the neuroradiologist was needed.

Referrals and clinical management

Table 4 shows the subsequent clinical management of the 40 participants who we referred for further diagnostic work-up. They underwent clinical MRI which led to a wait-and-see policy for 19, and treatment for nine participants. In four participants the findings were confirmed but classified as benign lesions that did not require further therapy or follow-up. Three participants refused to give information on their clinical diagnosis.

The initial finding on basis of the research examination was not confirmed in five of the 40 participants (13 % [95 % CI: 4–27 %]). In these five participants, we found signal changes of unclear pathogenesis. In two participants, we found cystic lesions of which one could possibly affect the brainstem and the other might possibly cause a hydrocephalus. In two participants, we observed signal changes around the amygdala and in another one changes in the anterior communicating artery which were surrounded by an artefact. In all those cases, we could not rule out malignant pathology and therefore referred these participants for clinical work-up.

Additionally, we found abnormalities that would have required referral according to our protocol in six participants, but were already known and under treatment, and hence by definition no incidental finding.

We did not find any acute lesions that required immediate medical attention, nor any ethically challenging findings for which we would have needed to consult further experts.

Table 4 | Clinical management of 42 different incidental findings that were reported back to 40 participants.

Incidental findings and type of management	Clinical diagnosis	Number of findings
Meningioma†		**9**
Wait and see	Meningioma	7
Surgery	Meningioma	2
Brainstem lesion		**6**
Wait and see	Atypical cystic lesion (n=1), unclear lesion (n=1), calcified cavernous malformation or microbleeding (n=1)	3
No therapy needed	Vascular encephalopathy (n=1), cavernous malformation (n=1)	2
Not confirmed	/	1
Aneurysm‡		**7**
Operative clipping	Aneurysm	4
Endovascular coiling	Aneurysm	1
Not confirmed	/	1
Refused to give information	unknown	1
Other mass		**5**
Wait and see	cystic porencephalic lesion (n=1), unclear lesion (n=1)	2
Surgery	Pilocytic astrocytoma	1
No therapy needed	Benign cyst aqueduct	1
Refused clinical follow-up	unknown	1
Pituitary abnormalities		**3**
Wait and see	Macroadenoma	3
Cranial nerve Lesion		**2**
Wait and see	Vestibular schwannoma (n = 1), cystic lesion (n = 1)	2
Intraventricular mass		**2**
Not confirmed	/	1
Refused to give information	unknown	1

Table 4 | continued

Incidental findings and type of management	Clinical diagnosis	Number of findings
Possible malignant lesion		**3**
Wait and see	Unclear lesion	2
No therapy needed	Gliosis	1
Unclear lesions		
Not confirmed	/	2
Wait and see	Unclear lesion	1
Venous malformation		**1**
Wait and see	Haemangioma	1
Dural fistula		**1**
Surgery	Dural fistula	1

Note. Multiple similar incidental findings within one participant were counted as single finding (e.g. aneurysms). [†]Eight participants had non-convexity meningiomas, one had a convexity meningioma bigger than 2cm in the longest dimension. [‡]Based on the PHASES score (mean PHASES score 6.6, SD=1.4). In one of these participants, image quality was insufficient and there was an artefact around the suspicious aneurysm.

DISCUSSION

In this population-based neuroimaging study among 3,589 participants of the Rhineland Study, we found incidental brain abnormalities on MRI in approximately one quarter of all participants, with pituitary cysts being most common. Based on a prespecified protocol, we had to refer 1.1 % of all participants for further diagnostic work-up, mostly because of meningiomas, lesions affecting the brainstem, aneurysms, and mass. Subsequent clinical management in the majority of these participants was confined to a wait-and-see policy. One-fifth of those who were referred, or 0.3 % of the total sample that had brain imaging, underwent treatment which was successful and without complications.

Consistent with previous reports,[5,17-19] we found that men had more arachnoid cysts and non-acute infarcts than women, whereas women had slightly more meningiomas, and that the prevalence of non-acute infarcts and meningiomas increased with age. Contrary to a previous

population-based study in older adults, we observed an effect of age on the prevalence of cavernomas.[20] However, the other study only assessed axial T2*-weighted (slice thickness 3.3 mm) or standard T2-weighted images, and their reported prevalence of 0.4 % may have been too low to detect age-dependencies.

The prevalence of incidental findings is highly dependent on imaging modalities, with more abnormalities being detected when using at least one high spatial resolution 3D sequence.[2,21] We found pituitary cysts in 11.8 % and arachnoid cysts in 4.1 % of our population, which is indeed much higher compared to previous studies reporting frequencies in the range of 0.8–1.8 % and 1.4–3.6 %, respectively.[4-8] This is likely due to the high spatial resolution of our 3D T2-weighted sequence. The prevalence of aneurysms (0.2 %) in our cohort is low compared to previous large cohort studies,[4-6] which, however, used different imaging modalities, including 2D T2-weighted images or time-of-flight angiography. Our imaging protocol was indeed not optimized to detect aneurysms. Particularly, our highly accelerated 3D T2-weighted sequence is prone to pulsation artefacts interfering with regular intraluminal flow void, making it less suitable for detecting aneurysms.

Discrepancies in prevalence estimates of incidental findings might also be due to classification of what constitutes an incidental finding. For example, we did not include lacunar stroke in non-acute infarcts nor did we track any normal variants (e.g. megacisterna magna).

While the raters initially disagreed in approximately 12 % of the cases, this persisted in only less than 1 % after an initial consensus meeting. This highlights that it is common for non-radiologist raters to miss small abnormalities on brain MRI scans, and the importance of the four-eye-principle in the reading of MR images in large cohort studies. As the clinical neuroradiologists were not involved in the initial ratings, we could not compare their performance with that of the study raters.

Following our protocol to only refer participants for clinical work-up if this would be of clear potential benefit for the person involved, we only referred 1.1 % of the participants suggesting that most abnormalities have no direct clinical consequence. This is in line with reports on

potentially clinically relevant incidental findings in adults from a recent meta-analysis and another German cohort.[8,21] Five of the findings we referred were not confirmed on clinical MRI. Here, we could not rule out possible malignant pathogenesis based on our MRI sequences which were developed for the specific research purposes of the Rhineland Study and not used in clinical settings before. Our prospective follow-up may show whether the lesions we found were indeed false-positive ratings, or that our sequences are more sensitive to subtle changes that are not detectable yet on clinical MRI scans.

Participants included in this study were relatively healthy, as we had to exclude older and sicker people due to MRI contraindications. Additionally, roughly 14 % of eligible participants refused MRI. This may have resulted in a further selection bias and the prevalence estimates should be considered a conservative estimate of the true population prevalence of incidental abnormalities.

Major strengths of this study were that it involves a large number of participants drawn from a population-based cohort with a wide age range. We performed state-of-the-art brain MRI including 3D T2-weighted, 3D T1-weighted, and 3D FLAIR sequences, contacted those affected with abnormalities, and followed-up concerning their clinical course. All images were reviewed within one working day by at least one experienced reader.

When interpreting the results of this study, some issues should be considered. Our rating of incidental findings was limited to T1-weighted, T2-weighted, and FLAIR images and we did not apply any contrast agents. This may have restricted the number of incidental findings detected and may explain some differences in our prevalence estimates compared to previous studies. Furthermore, we do not have longitudinal data on the natural course of incidental findings yet. The prospective nature of the Rhineland Study, however, will allow us to obtain these in the future.

CONCLUSIONS

In conclusion, incidental findings on neuroimaging across the adult life span are common, yet direct clinical consequences are rare. With the number of research studies using high spatial

resolution 3D MR neuroimaging sequences rapidly increasing, it is important to have prespecified guidelines on assessing and managing incidental findings. Our procedure and findings can help guiding in the further development of protocols for new research studies.

DECLARATIONS

Author Contributions

Author contributions included conception and study design (VL, RL, EH, TS, and MMB), data collection or acquisition (VL, RL, SJE, and EH), statistical analysis (VL), and interpretation of results, drafting the manuscript work or revising it critically for important intellectual content, approval of final version to be published and agreement to be accountable for the integrity and accuracy of all aspects of the work (all authors).

Availability of data and material

The data for this manuscript are not publicly available due to data protection regulations. Access to data can be provided to scientists in accordance with the Rhineland Study's Data

Use and Access Policy. Requests for additional information and/or access to the datasets can be send to RS-DUAC@dzne.de.

Compliance with Ethical Standards

Approval to undertake the study was obtained from the ethics committee of the University of Bonn, Medical Faculty. The study is carried out in accordance with the recommendations of the International Council for Harmonization Good Clinical Practice standards. We obtain written informed consent from all participants in accordance with the Declaration of Helsinki.

Consent to participate

All participants signed informed consent for their data to be used for research purposes.

Consent for publication

All participants signed informed consent for their data to be used for research purposes.

Conflict of Interests

The authors declare no competing interests.

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4. White matter hyperintensities as marker of cerebral small vessel disease

4.1. The relation between sex, menopause, and white matter hyperintensities: the Rhineland Study

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ABSTRACT

Background and objective: Mounting evidence implies that there are sex differences in white matter hyperintensity (WMH) burden in the elderly. Questions remain regarding possible differences in WMH burden between men and women of younger age, sex-specific age trajectories and effects of (un)controlled hypertension, and the effect of menopause on WMH. Therefore, our aim is to investigate these sex differences and age-dependencies in WMH load across the adult life span, and to examine the effect of menopause.

Methods: This cross-sectional analysis was based on participants of the population-based Rhineland Study (30 – 95 years) who underwent brain MRI. We automatically quantified WMH using T1-weighted, T2-weighted and FLAIR images. Menopausal status was self-reported. We examined associations of sex and menopause with WMH load (logit-transformed and z-standardised) using linear regression models, while adjusting for age, age-squared, and vascular risk factors. We checked for an age*sex and (un)controlled hypertension*sex interaction and stratified for menopausal status comparing men with premenopausal women (persons aged ≤ 59 years), men with postmenopausal women (persons aged ≥ 45 years), and pre- with postmenopausal women (age range 45 – 59 years).

Results: Of 3,410 participants with a mean age of 54.3 years (SD = 13.7), 1,973 (57.9%) were women, of which 1,167 (59.1%) were postmenopausal. We found that the increase in WMH load accelerates with age and in a sex-dependent way. Premenopausal women and men of similar age did not differ in WMH burden. WMH burden was higher and accelerated faster in postmenopausal women compared to men of similar age. Additionally, we observed changes related to menopause, in that postmenopausal women had more WMH than premenopausal women of similar age. Women with uncontrolled hypertension had a higher WMH burden compared to men, which was unrelated to menopausal status.

Discussion: After menopause, women displayed a higher burden of WMH than contemporary premenopausal women and men, and an accelerated increase in WMH. Sex-specific effects of uncontrolled hypertension on WMH were not related to menopause. Further studies are

warranted to investigate menopause-related physiological changes that may inform on causal mechanisms involved in cerebral small vessel disease progression.

INTRODUCTION

White matter hyperintensities (WMH) have been associated with distinct neurological symptoms including stroke,[1] motor[2,3] and mood disturbances,[4,5] and cognitive dysfunction.[1,6,7] Previous studies investigating sex differences in WMH burden have reported inconsistent results. While studies in the elderly mainly found that women exhibit higher levels and faster progression of WMH burden,[8-10] a study in middle-aged and older individuals found that men have more WMH than women.[11] Although studies typically adjust for sex differences in WMH, the underlying sex-specific mechanisms remain poorly understood.

Menopause is a key event in women's life and age at natural menopause has been associated with cardiovascular disease,[12] dementia,[13] stroke,[14] and all-cause mortality.[15,16] Additionally, it has been suggested that postmenopausal status is associated with higher WMH burden in women.[11,17] What remains unclear, however, is whether sex is associated with WMH burden at younger ages, if there are sex-specific age trajectories, and whether menopausal status underlies later-life sex differences.

Hypertension, especially when uncontrolled, is a main risk factor for WMH.[18-20] Whereas sex differences in hypertension are recognised,[21] it is unknown whether the effect of hypertension on WMH burden differs by sex and menopausal status.

In this study, we aimed to examine the extent to which sex differences exist in WMH load across the adult life span in a population-based cohort. Specifically, we determined if these sex differences are modified by menopause. Additionally, we investigated sex-specific age trajectories and effects of hypertension on WMH.

METHODS

Study population

The current study is based on the first 5,000 consecutive participants enrolled in the Rhineland Study. The Rhineland Study is an ongoing, prospective, single-centre, community-based cohort study. All residents aged 30 – 95 years from two geographically defined areas

in Bonn, Germany, are invited to participate. The sole exclusion criterion for enrolment is insufficient German language proficiency or lack of mental capacity to provide signed informed consent. Participants who had active implants (e.g., pacemakers), passive medical devices of which we could not confirm MRI eligibility, intrauterine devices, non-medical metal and metal splinters, or were pregnant, were excluded from the MRI examination due to MRI contraindications.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the ethics committee of the University of Bonn, Faculty of Medicine, and is conducted in accordance with the recommendations of the International Council for Harmonisation Good Clinical Practice. At time of enrolment, we obtained written informed consent from all participants in accordance with the Declaration of Helsinki.

Magnetic resonance imaging

MRI data was acquired on 3 Tesla MRI scanners (Siemens Prisma Magnetom, Erlangen, Germany) equipped with an 80 mT/m gradient system and a 64-channel phased-array head-neck coil. The protocol included the following sequences: a 3D T1-weighted Multi-Echo Magnetisation Prepared RApid Gradient-Echo sequence (ME-MPRAGE; TA = 6.5 min, TR = 2,560 ms, TI = 1,100 ms, flip angle 7°, FOV = 256 x 256 mm, 0.8 mm isotropic),[22,23] a 3D T2-weighted Turbo-Spin-Echo (TSE) sequence (TA = 4.6 min, TR = 2,800 ms, TE = 405 ms, FOV = 256 x 256 mm, 0.8 mm isotropic),[24,25] and a 3D T2 FLuid-Attenuated Inversion Recovery (FLAIR) sequence (TA = 4.5 min, TR = 5,000 ms, TE = 393 ms, TI = 1,800 ms, FOV = 256 x 256 mm, 1.0 mm isotropic). All sequences employ twofold parallel imaging acceleration using CAIPIRINHA and elliptical sampling.[26] [27]

Assessment of WMH

We defined WMH as hyperintense signals in the white matter tracts on T2-weighted images (see supplement for description of method in full detail).[28] In brief, we automatically outlined WMH using an in-house developed pipeline using DeepMedic,[29] based on image information from the T1-weighted, T2-weighted, and FLAIR sequences. The algorithm was trained on 30 and tested on 10 images, which were manually segmented by one rater, and visually quality controlled by an experienced neuroscientist. To ensure the quality of the automated WMH segmentation, we manually assessed a subset of 908 participants, and additionally excluded 110 participants with other pathology present (e.g., stroke, multiple sclerosis), 23 due to insufficient image quality, and 20 because of pipeline failures. White matter volume was extracted using FreeSurfer's automated segmentation (Aseg).[30]

Sex, menopause and covariates

Sex refers to biological sex, with women being biological female and men biological male at birth. Menopause was assessed as status (yes / no) at baseline examination and was self-reported. Women who indicated they underwent bilateral oophorectomy or had no menstruation for more than a year not due to pregnancy, breastfeeding, or contraception, and women above the age of 60 years were classified as postmenopausal. We excluded women who underwent hysterectomy without bilateral oophorectomy as their menopausal status could not be determined. We defined hypertension as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or antihypertensive medication use; we thereby distinguished between 'controlled hypertensive' participants who had systolic blood pressure < 140 mmHg and diastolic blood pressure < 90 mmHg while using regularly antihypertensive medication, and 'uncontrolled hypertensive' participants who had systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg. Diabetes was defined as fasting plasma glucose level ≥ 7 mmol/l, $HbA1_c$ ≥ 6.5% or use of antidiabetic medication. History of coronary heart disease (angina pectoris, myocardial infarction, coronary bypass operation, coronary artery stenting), valve disease, intermittent claudication, heart failure, and arrhythmia was

self-reported and summarised as prevalent cardiovascular disease. Smoking status was obtained from a self-reported questionnaire, classified as current or non-smoker. Missing smoking status (n = 197) was imputed based on the levels of the nicotine metabolite cotinine in blood, which were measured by the Metabolon HD4 platform.[31] We set the 97.5th percentile of cotinine levels in non-smokers as a cut-off value. Based on this, we then assigned the participants as either current smokers or non-smokers. Other covariates were body mass index (BMI) and use of lipid lowering medication. Education was defined as the highest, self-reported educational attainment and categorised based on the International Standard Classification of Education (ISCED 2011) as low (lower or secondary education), middle (completed secondary education up to completed Bachelor's degree or equivalent), or high (completed Master's degree, equivalent or higher).[32] Use of hormone therapy (HT) in postmenopausal women was assessed based on the Anatomical Therapeutic Chemical (ATC) code of the self-reported medication.

Statistical analysis

WMH lesion load was calculated as WMH volume divided by white matter volume to account for brain atrophy. We logit-transformed WMH due to its skewness, and z-standardised it before further analysis. We assessed differences in WMH load between men and women using linear multivariable regression. All models were adjusted for age (mean-centred), sex, and vascular risk factors (hypertension, diabetes, prevalent cardiovascular disease, smoking, body mass index and use of lipid-lowering medication). Additionally, we checked for non-linear relationships with age by including age-squared as an independent variable, and for an interaction between age and sex by including an age*sex to the models.

First, we tested for overall sex differences in WMH between men and women. Next, we stratified for menopausal status. In our study population, premenopausal women were between 30 – 59 years old, and postmenopausal women between 41 – 95 years old. In this stratification, we excluded postmenopausal women younger than 45 years, because they experienced early menopause (n = 7), therefore postmenopausal women in the stratification

were between 45 – 95 years old. We thus compared lesion load between (1) men (reference group) and premenopausal women (persons aged ≤ 59 years), (2) premenopausal (reference group) and postmenopausal women (age range 45 – 59 years), and (3) men (reference group) with postmenopausal women (persons aged ≥ 45 years). Here, we adjusted the models for age, vascular risk factors, sex (model (1) and (3)), and menopause (model 2). Finally, we examined whether WMH load differed between postmenopausal women who did or did not receive hormone therapy.

All analyses were performed in R version 4.0.2,[33] and p-values less than 0.05 were considered statistically significant. Missing covariates were imputed based on nonparametric missing value imputation applying random forest using the R package 'missForest' (version 1.4).[34]

Data availability

The data for this manuscript are not publicly available due to data protection regulations. Access to data can be provided to scientists in accordance with the Rhineland Study's Data Use and Access Policy. Requests for additional information and/or access to the datasets can be send to RS-DUAC@dzne.de.

RESULTS

Characteristics of the study population

Selection and characteristics of our study population are shown in **Figure 1** and **Table 1**, respectively. Mean age of the 3,410 participants included in this study was 54.3 ± 13.7 years, 1,973 (57.9%) were women. Of the women, 1,167 (59.1%) were postmenopausal of whom 216 (18.5%) were on HT. Hypertension was present in 1,208 (35.4%) participants, of whom 660 (51.7 %) had uncontrolled hypertension. Participants for whom no MRI was available, or where the MRI did not pass quality assurance were on average significantly older, less educated, more often male, and had a higher BMI and more often hypertension or prevalent

cardiovascular disease. In the 45 – 59 year age range, postmenopausal women were older than premenopausal women, and had a higher BMI and lower education.

Median WMH volume in the whole cohort was 0.5 ml [Interquartile range (IQR): 0.2 ml – 1.2 ml], and median WMH load was 0.1% [IQR: 0.1 – 0.3%]. **Table 2** shows the WMH burden characteristics of the study population and the subgroups stratified by sex and menopausal status.
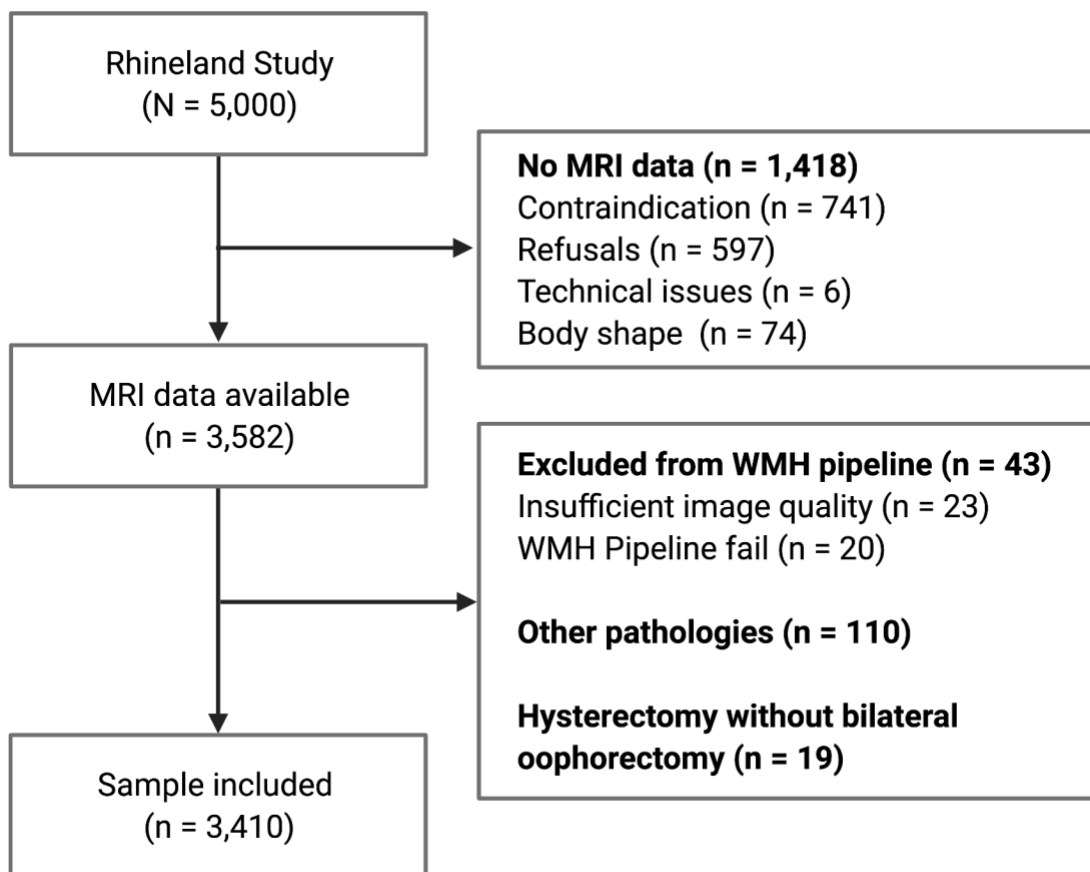

Figure 1 | Cohort selection. Note. *Body shape:* Participants who could not be placed inside the MRI due to size of head, shoulder, or waist circumference being too big for the scanner. *WMH:* White matter hyperintensities.

Table 1 | Characteristics of the study population.

Characteristics	Whole cohort	Participants included in the analyses				Excluded participants	
		Overall	Women	Men	p-value†		p-value‡
	(n=5,000)	(n=3,410)	(n=1,973)	(n=1,437)		(n=1,590)	
Age in years (mean (SD))	55.1 (14.0)	54.3 (13.7)	54.6 (13.6)	53.9 (13.9)	0.412	56.9 (14.4)	<0.001
Women (n (%))	2,824 (56.5)	1,973 (57.9)			<0.001	851 (53.5)	0.003
postmenopausal*	1,640 (58.1)		1,167 (59.1)			473 (55.6)	0.004
postmenopausal HT*	279 (17.0)		216 (18.5)			63 (13.3)	
Education (n (%))					<0.001		<0.001
high	2,621 (52.9)	1,889 (55.8)	951 (48.7)	938 (65.5)		732 (46.6)	
middle	2,232 (45.1)	1,436 (42.4)	955 (48.9)	481 (33.6)		42 (2.7)	
low	101 (2.0)	59 (1.7)	47 (2.4)	12 (0.8)		796 (50.7)	
BMI in kg/m^2* (mean (SD))	25.9 (4.5)	25.6 (4.2)	25.2 (4.6)	26.1 (3.4)	<0.001	26.7 (5.2)	<0.001
CVD (n (%))	960 (19.3)	576 (16.9)	339 (17.2)	237 (16.5)	0.011	384 (24.3)	<0.001
Smoking* (n (%))	621 (12.5)	437 (12.8)	239 (12.1)	198 (13.8)	0.091	184 (11.6)	0.384
Diabetes* (n (%))	261 (5.4)	149 (4.4)	63 (3.2)	86 (6.0)	<0.001	112 (7.0)	0.015
Hypertension* (n (%))	1,867 (38.2)	1,208 (35.4)	648 (32.8)	560 (39.0)	<0.001	659 (42.7)	0.197
Uncontrolled	959 (51.4)	660 (54.6)	349 (50.4)	311 (55.5)	<0.001	299 (42.7)	<0.001
Dyslipidaemia* (n (%))	598 (12.1)	365 (10.7)	171 (8.7)	194 (13.5)	<0.001	233 (14.7)	0.262

Note: Data are number of participants (percentages) or mean (standard deviation). HT: Hormone therapy, BMI: Body mass index, CVD: Cardiovascular disease. Participants were excluded if no MRI was available, or if their MRI scan did not pass quality assurance.* Participants with missing data: Menopause: n = 11 (0.4%); HT in postmenopausal women: n = 13 (4.7%); Education: n = 46 (0.9%); Body mass index: n = 24 (0.5%); Smoking: n = 16 (0.3%); Diabetes: n = 156 (3.1%); Hypertension: n = 110 (2.2%); Use of lipid lowering medication: n = 75 (1.5%). † P-values comparing women and men, adjusted for age where applicable. ‡ P-values comparing participants with and without MRI, adjusted for age and sex where applicable.

Table 2 | White matter hyperintensity burden characteristics of the study population, and the subgroups used in our analysis stratified by sex and/or menopausal status.

	Number	WMH volume (10-1 ml), median [IQR]	WMH load (10-1% of WM), median [IQR]
Whole study population	3,410	5.1 [2.3, 12.2]	1.1 [0.5, 2.7]
Women	1,973	5.1 [2.2, 12.6]	1.2 [0.5, 3.1]
Men	1,437	5.0 [2.5, 11.7]	1.0 [0.5, 2.5]
Subgroup ≤59 years			
Premenopausal women	800	2.4 [1.3, 4.5]	0.6 [0.3, 1.0]
Men	932	3.4 [1.8, 6.0]	0.7 [0.4, 1.2]
Subgroup ≥45 years			
Postmenopausal women	1,166	9.4 [4.5, 24.7]	2.3 [1.1, 6.1]
Men	1,045	7.2 [3.5, 15.0]	1.5 [0.7, 3.3]
Subgroup 45-59 years			
Premenopausal women	310	3.3 [1.7, 6.3]	0.7 [0.4, 1.4]
Postmenopausal women	448	5.1 [2.6, 9.7]	1.2 [0.7, 2.2]

Note. Data represent median [Interquartile range (IQR)]. In the subgroup ≤59 years, we included only premenopausal women to men of the same age range, whereas in the subgroup ≥45 years, we included only postmenopausal women with men of the same age range. WMH: White matter hyperintensities; WM: White matter.

Overall sex differences in WMH

Figure 2.A shows the WMH burden stratified by sex within our population. We saw that with increasing age WMH load increased exponentially, and that sex effects changed over the age span (age*sex interaction: $p < 0.001$), with sex differences becoming more pronounced after menopause.

Age effects were stronger in women than in men (age, b per year increase = 0.05 [95% CI: 0.04 – 0.05]; non-linear age dependency age-squared, b per 10^{-2} $year^2$ = 0.06 [95% CI: 0.04 – 0.07]; and age, b per year increase = 0.04 [95% CI: 0.04 – 0.04]; non-linear age dependency age-squared, b per 10^{-2} $year^2$ = 0.05 [95% CI: 0.03 – 0.07], for women and men respectively).
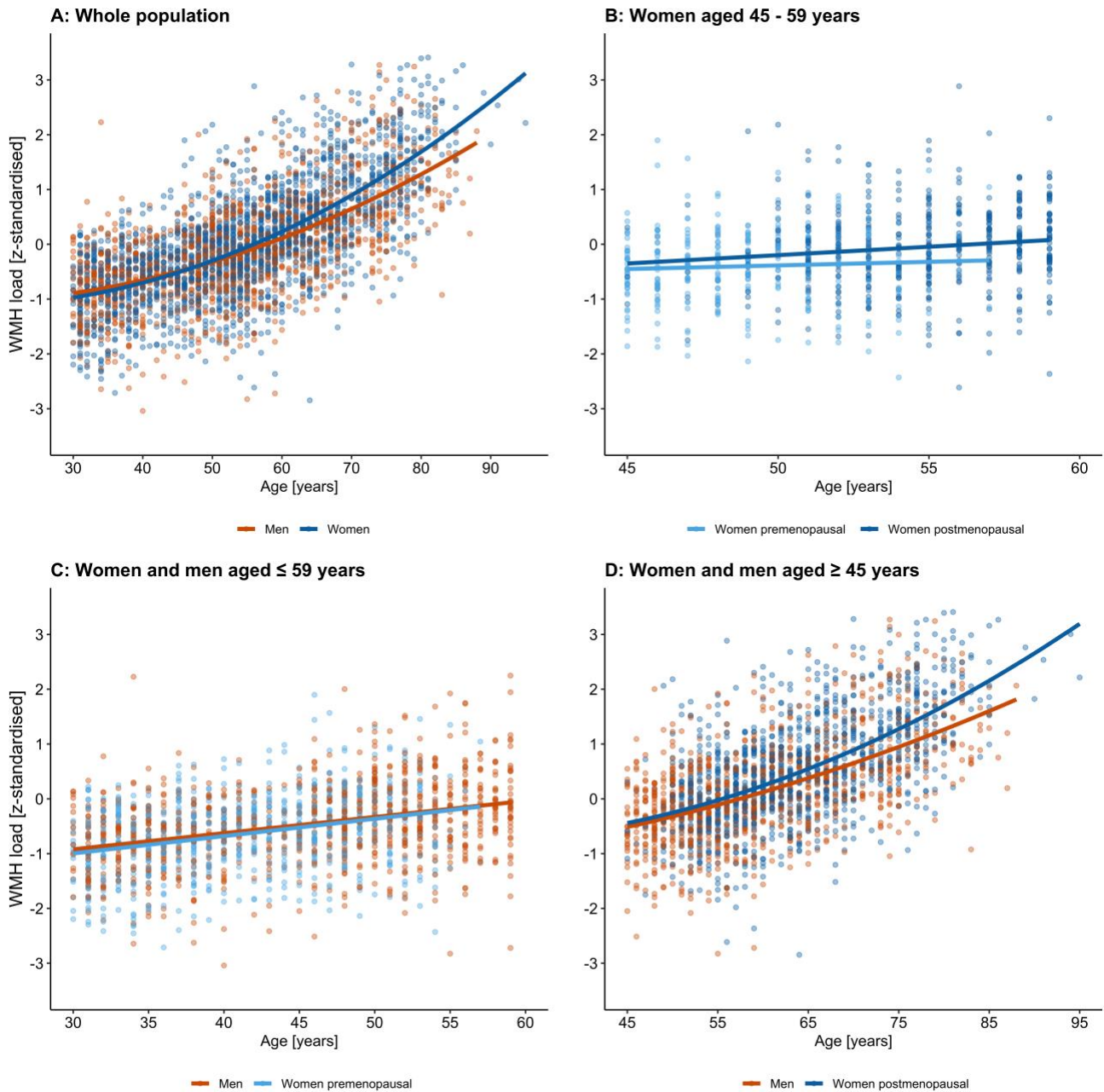
Figure 2 | WMH burden in the Rhineland Study. *A* shows the WMH load for men and women across age in the whole study population. *B* shows differences in WMH load for premenopausal and postmenopausal women aged 45 – 59 years. *C* shows differences between premenopausal women and men younger than 59 years. *D* shows differences between postmenopausal women and men older than 45 years. Note. WMH loads are logit-transformed and z-standardised.

Stratification by menopausal status

Figure 2.B-D shows WMH burden across the subgroups and effect estimates of sex, menopause, and age, respectively.

Subgroup analysis showed that premenopausal women and men until age 59 did not differ in WMH load, and that WMH load increased linearly with age (b per year increase = 0.03 [95% CI: 0.02 – 0.03]).

Postmenopausal women, however, had a higher WMH load compared to men of similar age. Increase in WMH burden accelerated with age, which was different for men and women (age*sex interaction: p = 0.03). Non-linear age effects were stronger in women (age, b per year increase = 0.05 [95% CI: 0.05 – 0.06]; non-linear age dependency age-squared, b per 10^{-2} $year^2$ = 0.07 [95% CI: 0.02 – 0.12]) compared to men (age, b per year increase = 0.05 [95% CI: 0.04 – 0.05]; non-linear age dependency age-squared, b per 10^{-2} $year^2$ = 0.05 [95% CI: 0.00 – 0.09]).

Postmenopausal women had also a higher WMH burden compared to premenopausal women of the same age range (b = 0.21 [95% CI: 0.07 – 0.35]). WMH burden increased linearly with age for both premenopausal and postmenopausal women aged 45 – 59 years (b per year increase = 0.02 [95% CI: 0.01 – 0.04]).

Effects of uncontrolled and controlled hypertension on WMH burden in overall population

Participants with controlled and uncontrolled hypertension had more WMH than normotensive participants, which was dependent on sex (sex*uncontrolled hypertension interaction: p < 0.005). This association was stronger in women (controlled hypertension, b = 0.15 [95% CI: 0.05 – 0.25]; uncontrolled hypertension, b = 0.30 [95% CI: 0.21 – 0.40]) than men (controlled hypertension, b = 0.12 [95% CI: 0.01 – 0.25]; uncontrolled hypertension, b = 0.01 [95% CI: 0.00 – 0.21]).

Effects of uncontrolled and controlled hypertension on WMH burden stratified by menopausal status

We found an interaction between sex*uncontrolled hypertension in premenopausal women and men until age 59 (p = 0.02). Uncontrolled hypertension compared to normotension was associated with more WMH in premenopausal women aged 30 – 59 years (b = 0.31 [95% CI: 0.11 – 0.51]), but not in men (b = 0.05 [95% CI: -0.08 – 0.18]).

In postmenopausal women and men aged 45 years and above, participants with controlled and uncontrolled hypertension had more WMH than normotensive participants, which was depending on sex (sex*uncontrolled hypertension interaction, p = 0.02). This association was stronger in postmenopausal women (controlled hypertension, b = 0.21 [95% CI: 0.08 – 0.33]; uncontrolled hypertension, b = 0.31 [95% CI: 0.20 – 0.43]) than in men (controlled hypertension, b = 0.13 [95% CI: -0.00 – 0.26]; uncontrolled hypertension, b = 0.12 [95% CI: 0.01 – 0.24]).

In premenopausal and postmenopausal women within the same age range, uncontrolled hypertension was associated with increased WMH (b = 0.31 [95% CI: 0.11 – 0.51]), regardless of menopausal status.

HT and WMH in postmenopausal women

There was no difference in WMH load between postmenopausal women using HT and postmenopausal women who did not (b = 0.03 [95% CI: -0.08 – 0.15]).

DISCUSSION

In this population-based cohort, we found that 1) the effect of sex on WMH load changes over the adult life span, 2) postmenopausal women have a higher WMH load compared to men as well as premenopausal women of the same age range, and 3) the increase in WMH burden accelerated with advanced age for both men and women, where the acceleration is faster in women.

Our results imply that WMH evolves differently for men and women, where menopause is a defining factor. In this population-based cohort, we showed that there was no difference between premenopausal women and men of similar age with respect to WMH burden. Postmenopausal women had a higher WMH burden than men of similar age. This agrees with previous reports from the literature, where it has been shown that in the elderly, i.e. predominantly postmenopausal women, women had more WMH than men.[8-10,35-38] However, a study using UK biobank data found that men have more WMH than women.[11] This study, however, also found that woman had larger total brain volume which is not only contradictory to other cohorts[39] but also to other studies using UK biobank data.[40,41] Additionally, we showed that postmenopausal women also had more WMH compared to premenopausal women of the same age range, which agrees with previous smaller studies.[11,17]

Moreover, with increasing age, the WMH burden in the brain exponentially increases for both men and women, suggesting non-linear age-dependencies need to be taken into account in future studies.

We found that participants with uncontrolled hypertension had a higher WMH burden than participants without or with controlled hypertension. This is in line with previous studies.[18,20] Additionally, we showed sex-specific differences in the effect of uncontrolled and controlled hypertension on WMH burden, suggesting that sex differences, which are also underlying cardiovascular diseases, such as hypertension, are also contributing to the vascular burden in the brain. We found that especially women are susceptible to increased blood pressure and WMH burden, even in midlife. These sex-specific differences, however, were not related to menopause.

The effect of menopause on WMH burden suggests that women, after menopausal onset, become more susceptible to vascular changes and disease in the brain. While the mechanisms that underlie these sex differences are still unclear, our findings agree with prior studies that proposed a protective nature of oestrogen.[42,43]

However, we observed no differences in WMH load in postmenopausal women using HT compared to those who did not, suggesting that HT after menopause does not continue this

protective effect on the brain. This is supported by recent work that reported no preventive effect of HT with respect to the development of vascular dementia.[44]

Menopause has been associated with physiological changes beyond hormone levels. Reportedly, menopausal age is associated with methylation levels,[45] and an earlier onset of menopause has been associated with an increase in epigenetic age, a biological marker of accelerated ageing.[46] Accelerated ageing could thus be another mechanism explaining the increase in disease burden in women after menopause.

An alternative explanation for the relation with menopause may lie in the causes, rather than the consequences, of menopause. A recent study has identified loci that are associated with early or delayed onset of natural menopause, by engaging in the so-called DNA damage response (DDR).[47] The DDR is the primary biological pathway regulating age of menopause. Moreover, this study identified DDR pathways which were leading to cell death,[47] which might be the underlying mechanism explaining the increased WMH burden in postmenopausal women.

There are some limitations in our study. Our baseline questionnaire did not capture sex and gender identity of our participants in sufficient detail to account for the full and diverse spectrum.[48] We investigated biological sex differences in WMH burden and we did not take into account gender differences. E.g., biological females, who were assigned male or intersex at birth and used gender affirming hormones, may display different trajectories than the observations reported here. Therefore, our results cannot be generalized to a gender diverse population. Data on menopausal status was self-reported and we did not have information on the age of menopausal onset, or whether participants were perimenopausal. For the stratification in our analysis, we excluded postmenopausal women who were younger than 45 years to exclude women with early menopause. Because we did not ask for age at menopause, we cannot rule out that some older postmenopausal women had also experienced early menopause. Whereas we consider it unlikely that this has biased our findings, future research is required to further disentangle the effects of perimenopause and time of menopausal onset on WMH burden. Additionally, we had no data on how long postmenopausal women had been using HT, nor the type or dose, and the comparison

between women with or without HT is limited by the small sample size. Furthermore, participants within the Rhineland Study cohort demonstrated a low burden of WMH and were in general quite healthy and well educated. Compared to the overall German population, the age and sex distribution of the Rhineland Study cohort shows the same distribution. However, participants of the Rhineland Study were more educated than the German population (high education: 52.9% compared to 18.5%), were less likely to have diabetes (5.4% compared to 9.2%), or to smoke (12.5% compared to 22.4%). The prevalence of hypertension was higher in our cohort (38.2% compared to 31.6%), whereas the proportion of controlled hypertension was similar.[49] Additionally, approximately one third of the Rhineland Study cohort did not undergo MRI (**Figure 1**). These participants were less healthy than the ones who did undergo MRI. To the extent that this may have biased our estimates, we consider it most likely that it led to an underestimation of the true effects of sex and menopause on WMH burden rather than an overestimation.

Strengths of this work include the use of a large sample size drawn from a population-based cohort, which covered a broad age range (30 – 95 years). This allowed us to examine overall sex differences in and specifically the effect of menopause on WMH comparing premenopausal women, postmenopausal women and men of similar age, which prior to this study remained an open question. Importantly, the study protocol includes comprehensive, standardised high spatial resolution neuroimaging data. The Rhineland Study is an ongoing, prospective study. While this works presents cross-sectional baseline associations, the Rhineland Study has the potential to investigate the association between sex, menopause and WMH burden longitudinally in the future. This is essential as both WMH and menopause are a manifestation of an ageing process, with the latter being additionally associated with a deterioration in white matter health. A longitudinal future study comparing WMH progression between women with different ages of menopausal onset might shed light into the causal pathways underlying vascular brain health in women.

Within this large population-based cohort covering the adult life span, we identified sex differences in WMH which were dependent on menopausal status, and showed that increase in WMH burden accelerates with age, especially for women. This highlights the need of sex-

specific analyses to enhance our understanding of the disease burden. WMH are being investigated as biomarkers for disease and disease outcome, e.g. in stroke.[1] Our results demonstrate the necessity to account for different trajectories for men and women, and menopausal status. This further underscores the importance of sex-specific medicine, and the requirement for a more attentive therapy for older/postmenopausal women, especially with advanced vascular risk factors.

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5.   General discussion and outlook

Magnetic resonance imaging in population-based studies, also called population imaging, is key in characterising disease burden and identifying persons at risk.[1] Several aspects need to be considered when designing new population imaging studies, such as the Rhineland Study, including how to conduct the study in an ethical manner and how to study particular diseases at the population level. One disease of special interest in this thesis is cerebral small vessel disease (SVD), particularly because of its high frequency, clinical relevance, and relation with age.

Part of my PhD research focussed on the methodological set up in the Rhineland Study. Together with experts from MR physics, neuroradiology, and epidemiology, I developed standardised procedures on how to conduct population imaging in an ethically and valid manner **(Chapter 3)**. With the high amount of neuroimaging data collected, there was a need for an automated segmentation of white matter hyperintensities of presumed vascular origin (WMH), the most prominent marker of SVD. I worked on this segmentation pipeline together with experts in image analysis (**Chapter 4**).

In **Chapter 3** of this thesis, I showed that most passive medical implants, tattoos and permanent make-up are eligible for magnetic resonance imaging (MRI). Including persons with medical implants, tattoos and permanent make-up is essential to reduce selection bias. I presented prevalence estimates and clinical management of incidental findings on neuroimaging across the adult life span. In **Chapter 4**, I explored WMH across the life span. I found that WMH were prevalent in almost all participants of the Rhineland Study. Moreover, I found that sex differences in WMH load were modified by menopause, and that the increase in WMH burden accelerated with advanced age, especially for women.

REDUCTION OF SELECTION BIAS

Selection bias is a systematic error that results from the selection of the participants for a study and from factors that influence their participation in the study.[2] MR brain imaging can be very susceptible to selection bias due to stringent MRI eligibility criteria, or participants

refusing the examination because of claustrophobia or the inability to remain still in a supine position for the duration of the examination. In general, older people are more likely to have medical implants that might be considered an MRI contraindication. Whilst safety of participants in MRI studies is of highest priority, the presence of these medical implants can lead to a strong selection bias and hence jeopardise the validity of any epidemiologic study. Together with experts from the field of neuroradiology, MR physics and epidemiology, I broadened standard inclusion criteria for MR imaging in research studies, allowing participants with eligible medical implants (also without MRI safety certificates), tattoos, and permanent make-up to undergo 3 T MRI. I showed that most passive medical implants are MRI compatible, and that tattoos and permanent make-up can safely be scanned. By additionally including these participants in the MRI examination, I was able to increase the total sample size by almost 17 % relative to recommendations from the U.S. Food and Drug Administrations (FDA) and other studies.[3,4] Participants with passive medical implants were mostly older participants with a deterioration in health status, whereas the ability to include participants with tattoos and permanent make-up significantly increased the number of MRI data in especially younger adults. In the past twenty years, tattoos have become more prevalent in Germany. While twenty years ago 8.5 % of Germans had a tattoo, this has increased to approximately 20 % today.[5,6] In the Rhineland Study cohort, 9.4 % of the participants had at least one tattoo and were on average younger than participants without tattoos. It is of importance to include participants with tattoos and medical implants in MRI examinations to ensure generalisability.

The guidelines for the clarification of MRI contraindications in 3 T neuroimaging studies can easily be applied in future studies. Although our guidelines with respect to MRI eligibility are not entirely new, especially not to clinicians, the only published guidelines are – to the best of my knowledge – provided by the FDA.[3] The main problem we experienced was that people oftentimes do not know which specific medical device was implanted, which makes identification through resources such as www.mrisafety.com impossible. Because of the discrepancy between the FDA guidelines and clinical and research practice, we decided to adapt the guidelines in the Rhineland Study (**Chapter 3.1**). I propose that future (population-

based) studies establish MRI expert panels to clarify MRI eligibility of participants, and that these panels should incorporate recent insights in clinical practice as well as the scientific community, including repositories such as www.mrisafety.com or the ISMRM safety weeks.[7,8]

HANDLING OF INCIDENTAL FINDINGS: WHEN TO REFER FOR CLINICAL WORK-UP

The rapidly increasing use of high-resolution 3D MR neuroimaging in research studies potentially leads to a dramatic increase in the number of previously undetected abnormalities. The decision whether or not to report such incidental findings back to the participant needs to be based on the overarching principle to not do any harm and on current scientific and clinical advances. Therefore, an educated assessment of the potential benefit for the participant is required, for which insights into the frequency and clinical relevance of incidental findings are needed. Our protocol was developed a priori by an international expert committee. In the Rhineland Study, we only reported back incidental findings, when we determined a potential benefit for the participants with respect to treatment options. In **Chapter 3.2,** I have shown that incidental findings in the Rhineland Study were common (24.2 %), whereas direct clinical consequences, i.e., surgery, were rare (0.3 %).

Some of the abnormalities that were reported back to the participant did not require further treatment, based on a subsequent clinical assessment. For example, none of the pituitary macroadenomas required direct treatment. Therefore, one can argue that knowing about these incidental findings does not have the potential benefit for the participant that we had expected. More importantly, being informed about such a finding might be extremely stressful for the participant and thus has the opposite effect of what we intended.

A few of the abnormalities that we referred for clinical work-up could not be confirmed using clinical sequences. Longitudinal neuroimaging data in the Rhineland Study will show, whether these findings were indeed false-positives or if our MRI sequences were more sensitive to detect subtle brain changes compared to those used in the clinic.

After publishing our guidelines, we received criticism on not reporting back non-acute infarcts and WMH.[9] While we agree that these cerebrovascular diseases are clinically important, we

regarded the potential benefit of the participant in knowing about this finding as low, as there are currently no treatments available.

These ethical considerations regarding the information of participants about incidental findings are far from new.[1,10-15] However, to be able to evaluate the potential benefit for the participant, robust data on clinical follow-up of incidental findings is needed, which I presented here. I expect that the Rhineland Study will acquire more information about the natural course as well as clinical management of these brain abnormalities in the upcoming follow-up examinations. This will, together with future advantages in treatment opportunities, contribute to further evolve and improve guidelines for the handling of incidental findings.

SEX, MENOPAUSE, AND WHITE MATTER HYPERINTENSITIES

In the second part of my thesis, I investigated WMH, a prominent marker of SVD (**Chapter 4.1)**. I demonstrated that there were sex differences in WMH disease burden, and that these were related to menopause. I showed that the WMH burden accelerated with increasing age, which was overall faster in women, but especially after menopause. These results suggest that there might be different mechanisms involved in WMH for men and women, which need to be acknowledged and further explored in future studies (e.g., exploration of sex-specific risk factors).

Menopause has been suggested to lead to accelerated ageing.[16,17] Biomarkers of ageing, e.g., DNA methylation ageing or the epigenetic clock, can distinguish between the chronological and biological age of a person.[18-22] Biological age takes the physiological health status of a person into account and therefore might be a better predictor of ageing, compared to chronological age alone. The exploration of biological age as a driving factor in the susceptibility of postmenopausal women to vascular disease burden in the brain is therefore of great interest in future studies, and will be investigated within the Rhineland Study.

While biological age represents a promising research direction, an alternative explanation might lie in the causes of menopause. A recent study has identified genetic loci that are associated with onset of menopause. These loci have been associated with the so-called

DNA damage response, which results in cell death,[23] and might therefore be the underlying mechanism in the increase in WMH burden. Future studies to explore this are needed.

Sex and Gender

Currently, clinical guidelines for treating SVD lie mainly in the management of vascular risk factors and are not sex-specific.[24] Efforts must be made to incorporate sex-specific patient risk profiles into the daily clinical routine, as the lack of appreciation for biological sex differences might harm both men and women. Additionally, there is a general underrepresentation of women in clinical trials, for example in stroke patients.[25] Future clinical trials in patients with SVD need to warrant the possibility to study e.g. drugs or treatment for WMH in women or men separately. An underrepresentation of women included in such trials might otherwise lead to an undertreatment in women in clinical practice.

While the study presented in this thesis was about biological sex, future research should also unravel the effects of gender on WMH. The term sex describes the biological characteristics of an individuum, based on genetic, biologic and physiological expressions and is usually categorised as male and female, whereas gender describes a social construct based on gender identity, expressions, roles and stereotypes, and is not a binary construct.[26] Sex therefore reflects mainly genetic components of a disease, whereas gender also reflects social and psychological components.

Although sex and gender are associated with health and well-being, sex and gender differences are still regularly overlooked in (the conceptualisation of) research studies.[27] Previous studies investigated sex and gender differences in major chronic diseases such as heart disease, cancer and dementia,[26] however, studies focussing on cerebrovascular diseases are lacking. Unfortunately, the baseline questionnaire in the Rhineland Study did not capture gender identity of the participants in sufficient detail to account for the full and diverse spectrum. This should be implemented in the follow-ups to capture this important topic in future research.

Approximately 0.6 % of American and 0.3 % of Dutch people identify as transgender, meaning their assigned biological sex at birth differs from their own perceived gender identity.[28,29] For Germany, no estimates on the prevalence of gender dysphoria have been published yet.[28] Some transgender people opt to use gender affirming hormonal treatment or gender reassigning surgery. A recent review suggested that transgender women have an increased risk of ischemic stroke after gender affirming hormonal therapy compared to transgender men.[29] Future studies should investigate whether gender affirming treatment also affects the age-specific prevalence and severity of SVD.

Methodological considerations

Within the framework of this thesis, I focused on investigating WMH as a marker of SVD. Other classical markers of SVD include lacunes of presumed vascular origin, cerebral microbleeds, and enlarged perivascular spaces.[30] Additionally, an alternative marker of cerebrovascular disease lies in the microstructural white matter changes, which can be captured with diffusion imaging. Microstructural changes, described through diffusion metrics, often appear before classical neuroimaging markers of SVD and hold the promise of representing reliable early biomarkers when predicting, e.g., cognitive impairment in SVD.[31-33] With regard to the results presented in this work, future studies may therefore investigate sex-specific and menopause-related patterns in early microstructural changes in the white matter.

With high throughput MRI data acquisition, there is also need for automated image analysis pipelines.[1] We used an in-house developed pipeline based on deepMedic for the automated segmentation of WMH. To minimise misclassification, we implemented an extended quality control procedure for this pipeline in which we manually confirmed the quality of segmentations in approximately one third of all scans. While the Dice coefficient, which measures similarity between automated and manual reference segmentation, for the WMH segmentation pipeline was relatively low, as it is to be expected in small lesion

segmentations, our extensive manual quality assurance confirmed that the pipeline segmented the lesions accurately.

The data used for this work were cross-sectional baseline data of a healthy study sample, and therefore allow only limited conclusions to be drawn about causality. The Rhineland Study, however, will increase in sample size and will conduct multiple follow-up assessments over the next decades. This will enable us to study the vascular burden in the brain longitudinally, while additionally focussing on new imaging markers (e.g., diffusion spectrum metrics), and new -omics biomarker, such as the aforementioned epigenetic clock.

CONCLUSIONS

In this dissertation, I have demonstrated that we can perform high-resolution neuroimaging in population-based studies without any adverse events due to medical implants, tattoos and permanent make-up. It is crucial to include participants with implants and tattoos to assure generalisability. Furthermore, I have shown that although incidental findings on high-resolution neuroimaging were common, clinical consequences, such as surgery, were rare. This information is relevant for developing future guidelines for feedback to participants of a research study. Lastly, I investigated the archetypical marker for cerebrovascular diseases and found sex differences in WMH burden, which were modified by menopause. This observation underscores the need for future research to elucidate the role of sex and gender across the full spectrum of SVD and beyond.

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6.   Supplementary material

Supplementary material to 'The relation between sex, menopause, and white matter hyperintensities: the Rhineland Study'

White matter hyperintensity segmentation

White matter hyperintensities of presumed vascular origin (WMH) were defined as hyperintensities in the white matter on T2-weighted images.[1] We automatically outlined WMH using an in-house developed pipeline based on DeepMedic,[2] relying on image information from the T1, T2, and FLAIR sequences. For each participant, sequences were bias-field corrected (Advanced Normalisation Tools (ANTs))[3] and co-registered to the FLAIR sequence using FSL FLIRT.[4] The segmentation algorithm employs an ensemble method, where initial segmentations were created using a combination of one (FLAIR), two (FLAIR and T1 or T2), and all three (FLAIR, T1, and T2) sequences. The algorithm was trained on 30 and tested on 10 images. These were manually segmented by one rater, and visually quality controlled by an experienced neuroscientist. Automated segmentations were evaluated using the Dice score coefficient (d). The Dice score coefficient measures similarity between the automated and manual reference segmentation. It ranges from 0 to 1, with a higher score representing a better agreement.

Accuracy

Accuracy of the automated WMH segmentation with respect to the manual reference standard was assessed on the 10 test images with a Dice of d=0.69.

Quality assurance

After the automated WMH segmentation was complete, we visually inspected the quality of the segmentation in a subset of 908 participants. This subset included participants with a

WMH volume further than two standard deviations away from the model estimate at a given age, estimated using a linear model of log-transformed WMH with age;[5] participants who were previously flagged because of concerns with respect to image quality in at least one of the sequences; and a random set of 10 % of the remaining participants. We excluded 110 participants with other pathology present on neuroimaging (e.g. stroke, multiple sclerosis), and 23 participants because of insufficient image quality. Furthermore, we identified 20 participants with pipeline failures (over-/undersegmentation of WMH: n = 9, imaging artefact segmented: n = 1, hyperintense cortex segmented: n = 2, brain mask extraction fail: n = 8).

Discussion

Lesion load within our participants is generally low. This explains the relatively small Dice coefficients in comparison to other segmentation tasks: Especially for small structures, small deviations between the manual reference and automated prediction are more severely penalised, leading to relatively small Dice coefficients.

However, the performance of the segmentation method used for this study performs at least on par with other WMH segmentation algorithms reported in the literature.[6] Importantly, to ensure high WMH segmentation quality, we implemented an extensive quality control procedure. During visual inspection, only a small number of failure cases were identified, suggesting accurate WMH volume estimates within the entire Rhineland Study cohort.

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