Functional and Structural Connectivity of the Aging Brain in Relation to Lifestyle

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Eidesstattliche Versicherung

Ich versichere an Eides Statt, dass die Dissertation von mir selbständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist.

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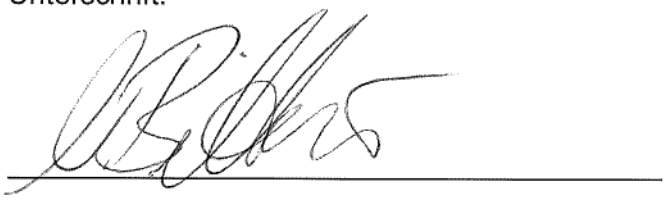

Diese Arbeit widme ich:

Meinen Eltern Michael Bittner und Angela Godek-Bittner,

meiner Schwester Laura Bittner,

sowie

Katharina Hanslik & Kerstin Tenbrink.

Denn ohne Wurzeln wächst kein Baum zum Licht.

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Zusammenfassung

Lebensstil trägt zur interindividuellen Variabilität während der Hirnalterung bei. Vorherige Studien untersuchten dabei hauptsächlich die Effekte einzelner Variablen. Kombinierte und individuelle Beiträge von Alkoholkonsum, Rauchen, körperlicher Aktivität und sozialer Integration zur Variabilität in Hirnstruktur und funktioneller Konnektivität älterer Probanden der populationsbasierten 1000BRAINS Kohorte wurden untersucht. In der ersten Studie wurde ein kombinierter Lebensstil-Risikoscore, der die vier Variablen in einem Wert zusammenführt, entwickelt. Höhere Scores gingen mit geringerer kortikaler Faltung im linken prämotorischen und im rechten Präfrontalkortex einher. Dies wurde von höherem Alkoholkonsum und niedriger körperlicher Aktivität bzw. niedriger sozialer Integration getrieben. Beide Regionen zeigten zusätzlich erhöhte funktionelle Konnektivität zum sensomotorischen und präfrontalen Kortex in Verbindung mit erhöhtem Rauchverhalten. Eine zusätzliche Korrektur für genetisches Risiko, Bildung und depressive Symptomatologie veränderte diese Assoziationen nicht. In der zweiten Studie wurde "BrainAGE" genutzt, ein Imaging-Biomarker, der durch maschinelles Lernen schätzt, ob ein Gehirn auf Grund seiner Anatomie älter (höheres BrainAGE) oder jünger (niedriges BrainAGE) aussieht, als aufgrund des chronologischen Alters zu erwarten ist. Höheres kombiniertes Lebensstil-Risiko war generell mit höherem BrainAGE und somit mit beschleunigter Hirnalterung assoziiert. Dies wurde durch höheres Rauchverhalten und niedrigere körperlicher Aktivität getrieben. Zusammenfassend zeigt die vorliegende Dissertation damit, wie Lebensstil-Verhalten zu alterungsabhängigen Unterschieden in der Hirnstruktur, BrainAGE und der funktionellen Konnektivität beiträgt. Die Ergebnisse helfen zu verstehen, weshalb Menschen so unterschiedlich altern und erleichtern die Entwicklung von lebensstil-basierten Interventionen, um gesundes Altern in der Bevölkerung zu fördern.

Abstract

Lifestyle contributes to interindividual variability in brain aging. Previous studies mainly focused on the effects of single lifestyle variables, though. The combined and individual contributions of four lifestyle variables - alcohol consumption, smoking, physical activity, and social integration - to brain structure and functional connectivity in the older adult population-based cohort of the 1000BRAINS study were studied. In the first study, a combined lifestyle risk score, summarizing all four lifestyle behaviors, was developed. Higher combined lifestyle risk was associated with decreased cortical folding in the left premotor and the right prefrontal cortex. These decreases were driven by higher alcohol consumption and lower physical activity, or lower social integration, respectively. Both regions additionally exhibited higher functional connectivity to sensorimotor and prefrontal cortex in association to smoking. Additional correction for genetic risk, educational level, and depressive symptomatology did not alter the general associations, underlining the relevance of daily habits for brain health. In the second study "BrainAGE" was used, an imaging biomarker that estimates via machine learning, whether a brain appears older (higher BrainAGE) or younger (lower BrainAGE) from its anatomical characteristics than expected at that chronological age. In general, higher combined lifestyle risk was associated to higher BrainAGE, hinting at accelerated structural brain aging. Examining individual lifestyle variables showed that higher smoking, as well as lower physical activity were the driving factors. In summary, the current dissertation provides insights into how lifestyle behavior contributes to age-related differences in brain structure, BrainAGE, and functional connectivity. The current results help to understand why people age so differently and facilitate development of interventions to promote healthy brain aging.

Introduction[1]

Neuroimaging and the Aging Brain

Western populations nowadays face a shift towards a growing proportion of older adults (WHO, 2017; 2018). Aging is also the most critical risk factor for the development of neurodegenerative diseases, such as Alzheimer's disease (AD; Riedel, Thompson & Brinton, 2016). Cognitive decline, however, is not only present in pathological conditions, but also during the normal aging process. In comparison to younger adults, older adults experience decreases in cognitive performance in the domains of processing speed, working memory and executive functions (Reuter-Lorenz & Park, 2010). These decreases in cognitive performance are accompanied by age-related differences in the brain´s functional and structural architecture (Jockwitz, Caspers, Lux, Eickhoff, et al., 2017; Jockwitz, Caspers, Lux, Jutten, et al., 2017; Liu et al., 2011; Ziegler, Dahnke, Gaser, & Initiative, 2012).

Neuroimaging techniques, such as structural and functional magnetic resonance imaging (MRI), provide an excellent possibility for examining the aging brain. Structural high resolution T1-wheighted anatomical MR images depict gray matter (GM), white matter (WM) and cerebrospinal-fluid (CSF) by different gray values. From these anatomical scans local characteristics of the brain´s structural architecture can be inferred, such as GM volume (Fischl et al., 2002), the thickness of the cortex (Fischl & Dale, 2000) and gyrification (Schaer et al., 2012; Schaer et al., 2008), where gyrification refers to the degree of cortical folding (Figure 1). The human cortex is highly folded, such that a great amount of cortex is not located directly at the interface of CSF and the skull, but buried within the sulci (Zilles, Armstrong, Schleicher, & Kretschmann, 1988; Zilles, Palomero-Gallagher, & Amunts, 2013).

[1]The introduction was partially adopted from manuscripts 1 and 2 in the Appendix.

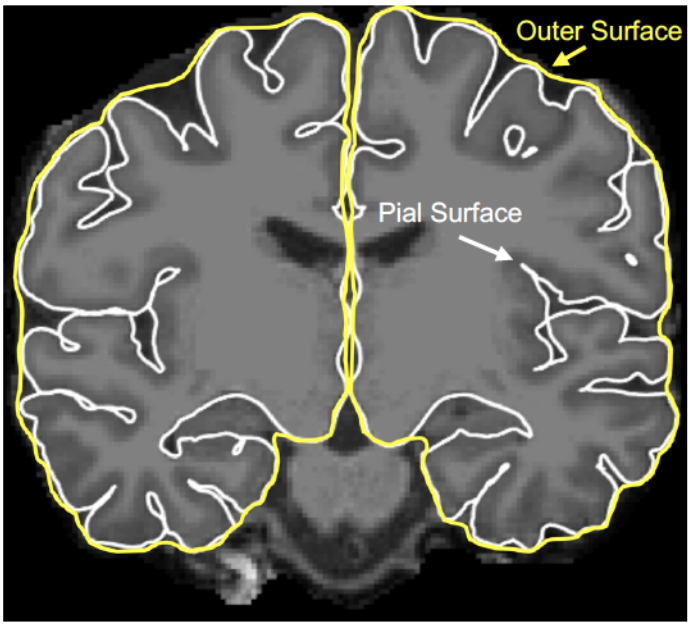

Figure 1. A coronal section depicting the measurement of the local cortical folding index. The pial surface, tightly following the gyri and sulci, is depicted in white. And the created outer surface, wrapping the pial surface without following the sulci, is depicted in yellow. The ratio between the pial and the corresponding outer surface at a given point describes the amount of cortical folding. Illustration derived from the two-dimensional approach of Zilles et al. (1988).

General age-related differences in brain structure between younger and older adults comprise lower cortical and subcortical GM, lower WM volume, and lower cortical thickness, accompanied by increases in CSF in older adults (Fjell & Walhovd, 2010; Fjell et al., 2013; Jockwitz et al., 2019; Kennedy & Raz, 2015). Especially the prefrontal cortex has been reported to be most affected by the factor age (Hogstrom, Westlye, Walhovd, & Fjell, 2013; Raz et al., 2005). Subcortical structures, such as the pallidum or the amygdala, as well as the hippocampal formation seem to be particularly vulnerable to age-related GM decreases as well (Fjell et al., 2013). One sensitive measure for cortical atrophy in older adults is the local gyrification index, which measures the degree of cortical folding (Hogstrom et al., 2013; Jockwitz, Caspers, Lux, Jutten, et al., 2017; Kochunov et al., 2005; T. Liu et al., 2010;

Madan & Kensinger, 2016; Magnotta et al., 1999). Higher gyrification is supposed to promote functional development and brain connectivity more efficiently than increasing cortical GM (Hogstrom et al., 2013). Further, differences in gyrification seem to be more closely related to age than differences in cortical thickness (Hogstrom et al., 2013).

Functional magnetic resonance imaging (fMRI) is an excellent technique when it comes to examination of the functional architectonic of the aged brain. FMRI measures changes in blood flow reflecting local changes in neural activity using the blood-oxygen-level dependent (BOLD) signal (Ogawa, Lee, Kay, & Tank, 1990; Ogawa, Lee, Nayak, & Glynn, 1990). It has been shown that the brain of older adults is best characterized by a pattern of increased activation (overactivation) and dedifferentiation (Park & Reuter-Lorenz, 2009; Reuter-Lorenz & Cappell, 2008; Reuter-Lorenz & Lustig, 2005; Reuter-Lorenz & Park, 2010). Further, older adults, who perform as well as younger adults during the execution of cognitive tasks (i.e. episodic memory) recruit additional brain regions and activate those brain regions stronger, than older adults not performing as well as the younger ones (Cabeza, Anderson, Locantore, & McIntosh, 2002; Reuter-Lorenz & Cappell, 2008). This has often been interpreted as compensatory mechanism to maintain cognitive functioning stable (Reuter-Lorenz & Cappell, 2008; Reuter-Lorenz & Park, 2010; Stern, 2012; 2017), especially if the structural integrity of the brain already decreased (Hakun, Zhu, Brown, Johnson, & Gold, 2015; Jockwitz, Caspers, Lux, Jutten, et al., 2017; Reuter-Lorenz & Lustig, 2005).

Further, from changes in regional blood flow, age-related reorganization of functional connectivity (FC) can be inferred (Damoiseaux, 2017; Sala-Llonch, Bartres-Faz, & Junque, 2015). FC (Figure 2) is defined as the co-activation of spatially distinct brain regions, whose BOLD signaling fluctuates simultaneously for a measured amount of time, named timeseries (Beckmann, DeLuca, Devlin, & Smith, 2005; Biswal, F., Haughton, & Hyde, 1995; Smith et al.,

2009). These co-activation patterns can be investigated in humans at rest. At rest, i.e. in the absence of a specific task, this resting-state functional connectivity (RSFC) allows to identify functionally connected brain regions, which constitute neuronal networks corresponding with major functional networks of the working brain (Beckmann et al., 2005; Smith et al., 2009). Crucially, these RSFC networks allow for statements about *general* organization principles of the brain´s functional architecture independent of a specific stimulus.

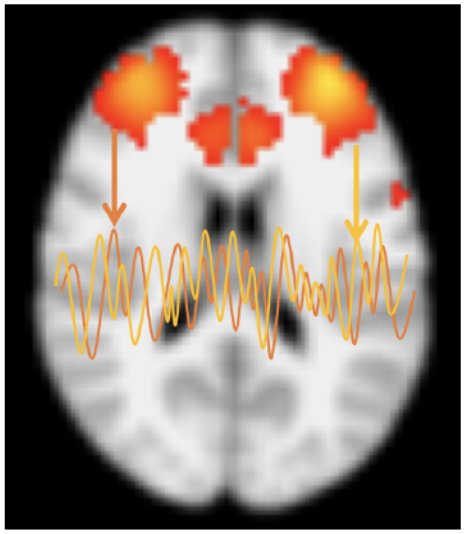

Figure 2. Representation of an executive resting-state network derived from independent component analysis (Beckmann et al., 2005) in a transversal section. The BOLD signals of two regions are represented in yellow and orange, where activation patterns are spatially distinct, but co-activate in temporal correlation.

Another characteristic of the aging brain is the considerable high interindividual variability, which has been observed within age-related neuronal changes and the resulting differences in cognitive decline (Dickie et al., 2013). The most prominent observation illustrating this issue has been made by Katzman et al. (1988). Here, a group of AD patients showed preserved mental ability even though other patients with a comparable amount of AD pathology were suffering from cognitive impairment. Katzman et al. (1988) attributed the preserved mental ability of the first group to higher initial brain weights and greater

number of neurons. Similar observations have been reported in normal aging: Two individuals of the same chronological age may show completely different patterns of brain aging, with one person experiencing strong decline in brain structure, whereas the other exhibits almost none (Bartrés-Faz & Arenaza-Urquijo, 2011; Stern, 2009; 2012; Ziegler et al., 2012). This led to the conclusion that some individuals have the ability to tolerate a great amount of pathology or age-related neuronal loss without experiencing mental deficiencies, which has since been summarized as *brain reserve* (Stern, 2009; 2017). The functional equivalent to brain reserve, however, is *cognitive reserve*, the ability to compensate for age-related differences and structural deficiencies by using neuronal networks and strategies efficiently (Reuter-Lorenz & Cappell, 2008; Reuter-Lorenz & Park, 2010; Stern, 2009; 2012; 2017). Stronger activation of the same or recruitment of additional brain regions in older adults has already been pointed out as one compensational mechanism of the aging brain in order to maintain stable cognitive functioning. If increases in FC occur already during rest, they are thought to reflect higher base levels (Reuter-Lorenz & Cappell, 2008). These higher base levels leave less room for additional increases in brain activity to boost performance during active tasks and seem hence to reflect lower possibility for compensation. Increased RSFC may therefore display dysfunctional disruptions of the brain´s general functional architecture and supposedly reflects lower cognitive reserve (Reuter-Lorenz & Cappell, 2008; Reuter-Lorenz & Lustig, 2005; Stern, 2009; 2012; 2017). Together, brain and cognitive reserve explain, why the same chronological age can manifest very differently in the brain and in cognitive performance. As Leritz et al. (2011) stated:

"It is likely that aging is not necessarily a reflection of number of years per se, but is more probably a manifestation of the cumulative effect of physiological variations []"

How strongly age manifests in the brain is likely not only due to several physiological differences, such as sex (Ritchie et al., 2018; Ruigrok et al., 2014; Scheinost et al., 2015) but psychological and behavioral variations as well, such as higher education. Higher education is generally thought to be protective against age-related decline with higher educated older adults showing lower decreases in GM and therefore less manifestation of age, i.e. higher cognitive reserve (Christensen, Anstey, Leach, & Mackinnon, 2008; Stern, 2009; 2012). Still, these factors explain little of the overall variability in brain reserve (Kennedy & Raz, 2015; Raz et al., 2005; Stern, 2017). Therefore, identification of further factors that are related to variability in brain reserve is still an ongoing task for current neuroscientific research. One factor that has come into the focus of research is lifestyle, where several behaviors have the potential (i) to be modifiable and (ii) to counteract brain decline.

The Role of Lifestyle in Brain Aging

Indeed, some lifestyle behaviors such as smoking and alcohol consumption may pose a serious risk to brain health, whereas others seem to show beneficial effects, e.g. social integration and physical activity (Anaturk, Demnitz, Ebmeier, & Sexton, 2018; Arenaza-Urquijo, Wirth, & Chetelat, 2015; Fratiglioni, Paillard-Borg, & Winblad, 2004).

Physical activity. Physically more active older adults show better cognitive performance (Churchill et al., 2002; Colcombe & Kramer, 2003; Hughes & Ganguli, 2009; Kramer et al., 2003; Kramer & Erickson, 2007; Kramer et al., 1999; Voelcker-Rehage, Godde, & Staudinger, 2010) along with higher task-related activity and more efficient use of brain networks (Colcombe et al., 2004; Voelcker-Rehage, Godde, & Staudinger, 2011). In addition, older adults, who engaged in physical activity training showed increased hippocampal volume (Erickson et al., 2011), as well as preservation of cortical GM volume (Colcombe et al., 2003; Erickson, Hillman, & Kramer, 2015; Erickson, Leckie, & Weinstein, 2014).

Social integration. Stronger social integration of older adults is associated with reduced cognitive decline and reduced risk of dementia (Fratiglioni et al., 2004). Socially integrated AD patients show higher cognitive stability compared to AD patients, who are socially not integrated, despite exhibiting a comparable amount of pathology (Bennett, Schneider, Tang, Arnold, & Wilson, 2006). Further, social network size is correlated with higher amygdala volume (Bickart, Wright, Dautoff, Dickerson, & Barrett, 2011), as well as higher regional and overall GM volumes in humans (James et al., 2012; Mortimer et al., 2012). Additionally, social network size seems to manifest in higher regional GM volumes e.g. in right prefrontal cortex, and higher functional connectivity in monkeys (Sallet et al., 2011). Therefore, both lifestyle habits, physical activity and social integration seem to promote cognitive or brain reserve capacity. In contrast, other lifestyle habits may rather pose a risk onto healthy brain aging.

Smoking. Most studies focus on the direct effect of nicotine and its enhancing effect on attention (Lawrence, Ross, & Stein, 2002), which, in turn, is correlated with increased brain activation (Jacobsen et al., 2004). During rest, though, smokers, compared to non-smokers, showed reduced RSFC (Zhou et al., 2017) as a correlate of generally altered functional brain architecture (Greicius, 2008), between the insula and prefrontal cortex. Additionally, smokers have stronger cortical thinning in prefrontal and temporal regions (Karama et al., 2015) and decreased GM density within cingulum, precuneus, thalamus, and precentral gyrus (Almeida et al., 2008) compared to non-smokers. Hence, the long-term effects of smoking seem to pose a risk to structural, as well as functional brain health. Compared to the high amount of studies investigating the association between physical activity and GM changes in older adults, studies on the effects of smoking on brain health are relatively rare, though.

Alcohol consumption. Chronic alcohol dependence can lead to pathological conditions, e.g. Korsakoff syndrome (de la Monte & Kril, 2014). Further, alcohol dependent patients show pronounced GM loss (Paul et al., 2008), especially in the frontal cortex (Zahr, Kaufman, & Harper, 2011), as well as pronounced WM loss (Topiwala et al., 2017), e.g. in the corpus callosum and cerebellum (Zahr et al., 2011). GM loss, however, has also been reported in older adults with moderate alcohol consumption (Mukamal, Longstreth Jr, Mittleman, Crum, & Siscovick, 2001), not only in alcohol-dependence. To perform similar to controls in a simple motor task alcohol dependent patients need to recruit additional neuronal networks, which seems to be a compensational mechanism to sustain motor performance (Parks et al., 2010) and hints at a underlying disruption of functional brain networks. Hence, alcohol consumption and smoking may both be variables reducing brain reserve and can therefore be classified as risk variables for accelerated brain aging.

The Current Work

Previous studies mainly focused on the isolated effects of single lifestyle variables on brain structure and function. In real life, however, individuals rather engage in a combination of lifestyle habits that could all influence brain reserve, e.g. being a smoker (risk) and socially and physically active person (protective) versus being a smoker and an inactive person. Yet, studies examining combinations of lifestyle, as well as lifestyle in a multidimensional way are rare. For example, Floel et al. (2008) reported that the combination of exercise, dietary habits, BMI, smoking and alcohol intake was a better predictor for memory performance than the individual lifestyle behaviors. Another study found different RSFC patterns, particularly in participants, who both smoked and consumed alcohol, as compared to participants with only one of these risk variables (Vergara, Liu, Claus, Hutchison, & Calhoun, 2017). This underlines the notion that individual lifestyle variables

may have additive or interacting effects on the aged brain. Together with the observation that age-related neuronal differences are likely a manifestation of a cumulative effect of several factors, as has been pointed out in the section "Neuroimaging and the Aging Brain", it is therefore important to examine combinations of lifestyle habits to understand the high inter-individual variability in brain reserve.

Hence, the first aim of the current work was to develop a summary measurement for a combination of lifestyle variables. This approach was inspired by polygenic risk scores (PRS). PRS aggregate the number and effect size of genetic variations, i.e. single nucleotide polymorphisms (SNPs), which are related to one specific outcome, such as Alzheimer´s disease (Desikan et al., 2017) or schizophrenia (Dima & Breen, 2015; French et al., 2015; International Schizophrenia Consortium et al., 2009; Ursini et al., 2018). It has been shown, that accumulating the genetic variations, which an individual carries, provides predictive information beyond each single variation, e.g. to better estimate age of onset of AD (Desikan et al., 2017). Recent studies provide evidence that carriers of higher genetic risk show stronger and faster age-related neuronal decline, i.e. hippocampal volume loss and hippocampal thinning (Fouquet, Besson, Gonneaud, La Joie, & Chetelat, 2014; Harrison et al., 2016). Further specific genetic variations seem to be related to a higher tendency or risk to drink alcohol (Clarke et al., 2017) or to smoke (J. Z. Liu et al., 2010).

The overall goal of the current dissertation was to measure the combined lifestyle risk carried by one individual and to examine its association to differences in brain structure and function in the 1000BRAINS study, a population-based cohort consisting of older adults (Caspers et al., 2014). To measure these differences, a multimodal approach was chosen investigating (i) brain structure using three different parameters, i.e. the degree of cortical folding as a surface-based, sensitive measure for cortical atrophy, the volume of subcortical

structures to complement this surface-based approach, and BrainAGE, a meaningful imaging biomarker, as well as (ii) brain function using functional connectivity to measure the general organization principle of the brain's functional network architecture.

Combining Lifestyle Risks to Disentangle Brain Structure and Functional Connectivity Differences in Older Adults[2]

As pointed out in the introduction, prior studies mainly focused on the individual effects of single lifestyle variables, whose effects may differ from those of possible combinations (Vergara et al., 2017) and which may not reflect the situation in real life. The first study aimed at developing a combined lifestyle risk score reflecting an aggregated measurement of different lifestyle factors, inspired by polygenic risk scores, and examine its association to differences in brain structure, as measured by surface morphology, and subcortical volumes, as well as functional connectivity.

Analysis 1: Combined Lifestyle Risk and Brain Structure

In the first analysis, 549 participants (301 males, 248 females) aged between 55 and 85 from the population-based cohort of the 1000BRAINS study (Caspers et al., 2014) were included. Then the combined lifestyle risk score as described in manuscript 1one (see Appendix) was developed. In short, self-reported lifestyle behaviors included physical activity measured as the metabolic equivalent per task (Ainsworth et al., 2000; Floel et al., 2010; Ruscheweyh et al., 2011), social integration as social integration index (Alcaraz et al., 2019; Berkman, 2004; Sabbath, Lubben, Goldberg, Zins, & Berkman, 2015), smoking as pack-years (number of packs smoked per day multiplied with numbers of years; Karama et al., 2015) and alcohol consumption in grams per day. Next, in order to obtain a risk score that indicated higher risk with higher values, signs were reversed and the maximum value from each individual measurement for the protective behaviors (social integration and physical activity) was subtracted. In contrast, risk behaviors were analogously transformed into

[2]Parts of this section were derived from manuscript 1 in the Appendix.

positive scores by adding the minimum value to each individual measurement. Hence, all values for risk behaviors were positive (i.e. indicating a higher lifestyle risk). Finally, these linearly transformed values of all individual lifestyle variables were summed up into one combined lifestyle risk score.

To examine brain structure, T1-weighted anatomical 3D images (Caspers et al., 2014) were collected using a 3T Tim-TRIO MR scanner (Siemens Medical System, Erlangen, Germany) at the Research Centre Juelich, Germany. The cortical folding index as a sensitive surface-based measure for studying age-related differences in local brain structure was selected as main parameter of interest and complemented by measuring GM volume of subcortical structures (Fjell et al., 2013). Individual cortical surfaces from all participants were reconstructed to measure cortical folding (Schaer et al., 2012), and GM volume of subcortical structures was extracted (Fischl et al., 2002) using the FreeSurfer Software [version 5.3.0, Athinoula A. Martinos Center for Biomedical Imaging; Fischl (2012)]. Based on the assumption that differences in lifestyle are related to differences in brain structure, the first hypothesis was as follows: Those individuals carrying higher combined lifestyle risk should show decreased regional cortical folding index and GM volume, compared to individuals with a more protective lifestyle.

As expected, higher combined lifestyle risk was associated with a lower cortical folding index. This was found in the left dorsal part of the premotor cortex and the ventrolateral part of the right prefrontal cortex (Figure 3a).

Now, individual contributions of each lifestyle variable to this association were within the focus of interest. Here, each lifestyle variable was stepwise excluded from the combined score, as it is done in epidemiological research, where the contribution of specific variables is tested by stepwise inclusion into a model (Karama et al., 2015; Mukamal et al.,

2001). The hypothesis was that exclusion of those variables that relevantly contributed to the general association between lifestyle risk and brain structure would result in diminishing or vanishing of the respective effect.

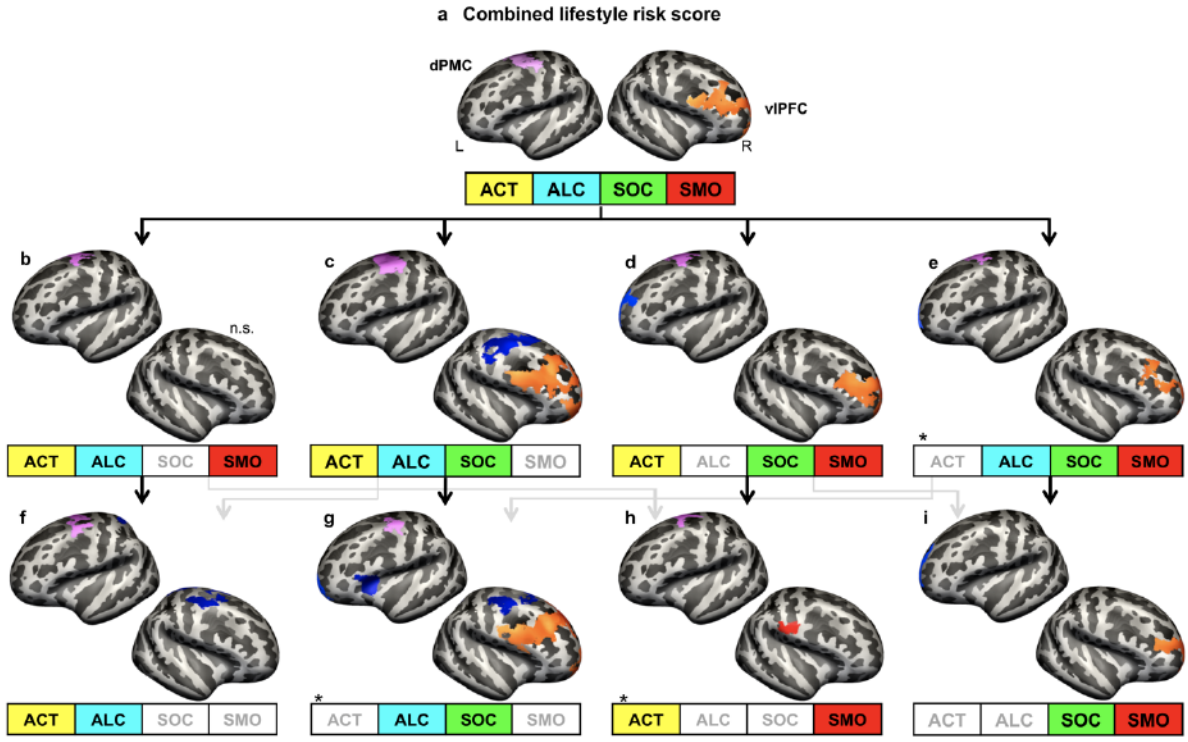

Figure 3. Differences in local brain surface structure as measured via the cortical folding index associated with lifestyle risk. Small boxes represent different combinations of the lifestyle variables. Negative associations are represented in blue, positive associations in red. The recurrent negative associations between higher lifestyle risk and reduced cortical folding index in left dorsal premotor cortex (dPMC) and right ventro-lateral prefrontal cortex (vlPFC) are colored in pink and orange, respectively. Abbreviations: ACT = physical activity, ALC = alcohol consumption, SOC = social integration, SMO = pack years of smoking, L = left hemisphere, R = right hemisphere. The Figure was not modified and is available under a creative commons public license 4.0 (https://creativecommons.org/licenses/by/4.0/), and was originally published in manuscript 1, Bittner et al., 2019, https://doi.org/10.1038/s41467-019-08500-x, *Copyright 2019 by Nature Communications.*

As hypothesized, excluding individual lifestyle variables from the combined score resulted in such alterations of the observed results. On the one hand, the observed decrease in cortical folding index in left premotor cortex was no longer found when excluding both, alcohol consumption and physical activity (Figure 3i). On the other hand, the association between lifestyle risk and cortical folding index in the right ventrolateral prefrontal cortex was no longer found, when excluding social integration (Figure 3b, f, h). Excluding smoking from the combined score, however, did not result in systematic alterations of the results.

Further, higher combined lifestyle risk was associated to lower GM volume of subcortical structures, as hypothesized. The strongest association, however, was observed between enhanced social integration as a single lifestyle variable and greater GM volume of the left hippocampus.

Analysis 2: Combined Lifestyle Risk and Functional Connectivity

Certain lifestyle behaviors have already been related to differences in RSFC (Vergara et al., 2017; Voss et al., 2016; Zhou et al., 2017), such as smoking-dependent RSFC increases in middle-aged adults (Janes, Nickerson, Frederick Bde, & Kaufman, 2012). In addition to that, RSFC increases have also been described to be present in the case of structural atrophy (Hakun et al., 2015; Jockwitz, Caspers, Lux, Eickhoff, et al., 2017; Reuter-Lorenz & Cappell, 2008). Hence, structural differences associated to higher lifestyle risk may be accompanied by alterations in RSFC. Here, those regions that showed structural differences in the cortical folding index, namely the left premotor cortex and the right ventrolateral prefrontal cortex, were expected to exhibit differences in RSFC.

To assess this hypothesis, the left dorsal premotor and the right ventrolateral prefrontal cortex were used as seed regions. RSFC was calculated as the Spearman correlation between the timeseries/BOLD-signal of the seed regions and the

timeseries/BOLD-signal of all other GM voxels. This correlation was then related to lifestyle risk. Since not for all participants RSFC measurements were available, the sample was slightly smaller than in analysis one (n = 501). As expected, higher combined lifestyle risk scores were associated with variations in RSFC, in the present study increases, between left dorsal premotor cortex (dPMC) and bilateral primary somato-motor networks. The seed in ventrolateral prefrontal cortex (vlPFC) showed increased RSFC to right anterior superior frontal gyrus in relation to higher combined lifestyle risk.

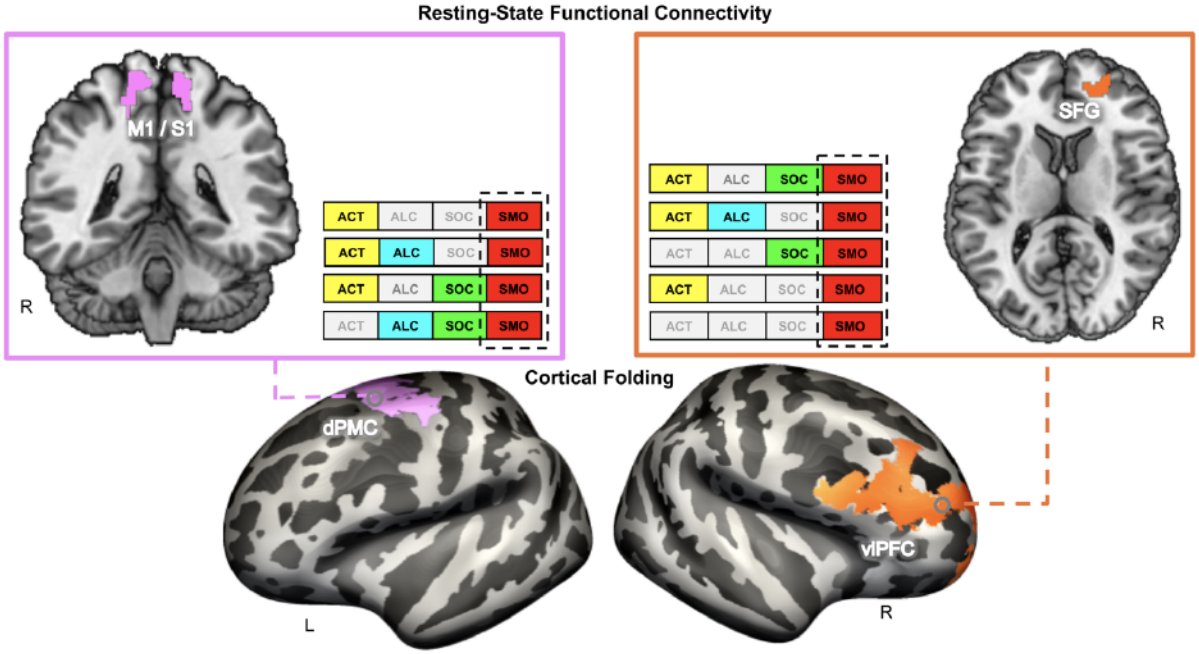

Figure 4. Lifestyle-risk-associated increases in RSFC to the seed in the left dorsal premotor cortex (dPMC) and right ventrolateral prefrontal cortex (vlPFC). Abbreviations in the small boxes refer to the same variables as in Figure 3. S1 = primary somatosensory cortex, M1 = primary motor cortex, SFG = superior frontal gyrus. Figure was modified from Bittner et al., 2019. The original figure is available under a creative commons public license 4.0 (https://creativecommons.org/licenses/by/4.0/), and was published in manuscript 1, Bittner et al., 2019, https://doi.org/10.1038/s41467-019-08500-x, *Copyright 2019 by Nature Communications.*

Again, the hypothesis was that stepwise exclusion of one or more variables would reveal their contribution to the association between lifestyle and RSFC. Figure 4 shows that smoking was the driving factor for the associations between higher lifestyle risk and higher RSFC in both regions. All combinations of lifestyle variables that included smoking as risk factor were significantly related to increases in RSFC to the primary somato-motor network (seed region: dPMC) or right anterior superior frontal gyrus (seed region: vlPFC). The same results were obtained for smoking as a single lifestyle variable. In contrast, if smoking was excluded from combinations of lifestyle variables, systematic variations in RSFC to these regions were no longer found.

Analysis 3: Additional Adjustment for Non-lifestyle Variables

Based on the literature described above, genetic risk (Clarke et al., 2017; J. Z. Liu et al., 2010; Thorgeirsson et al., 2010; Tobacco & Genetics, 2010), educational level (Christensen et al., 2008) and depressive symptomatology (Fratiglioni et al., 2004) were hypothesized to represent possible confounding factors within the associations of combined lifestyle risk and the aged brain. To test this hypothesis, education was measured as defined in the International Standard Classification of Education (UNESCO, 2003) and depressive symptomatology using Beck´s Depression Inventory II (Hautzinger, Keller, & Kühner, 2006). Genetic risk was assessed via a polygenic risk score (PRS) for alcohol consumption and smoking.

For PRS calculation, studies reporting genome-wide significant associations between specific genetic loci and lifestyle variables were reviewed. Those loci meeting predefined criteria (see manuscript one in Appendix) were selected and aggregated into the PRS. As there were no reported loci for social integration and physical activity meeting our criteria

for inclusion, these traits were not covered by the PRS. Since genetic information was not available for all participants, this analysis was carried out with a sample of n = 488.

Entering the PRS, general level of education or depressive symptomatology as covariates into the analysis investigating the relation between lifestyle risk and brain structure, did not change the general pattern of lifestyle risk-related decreases in the cortical folding index. Nevertheless, for the association between combined lifestyle risk and cortical folding index, an additional correction for PRS revealed an additional effect in right precuneus. Additionally, the results of the RSFC analysis remained largely unaffected after adding PRS as covariate.

Discussion[3]

The aim of the first study was to develop a measurement for combined lifestyle risk and to explore its contribution to variability in brain structure and functional connectivity of older adults. Four core findings emerged:

First, the newly developed approach revealed that older individuals carrying higher combined lifestyle risk load seem to be at higher risk for structural brain atrophy. Importantly, systematic differences in regional cortical folding index were found for the combined lifestyle risk score, but not for the single lifestyle variables, likely due to over-additive effects, which are only revealed, when investigating the variables in combination.

Second, with regard to associations between lower cortical folding index in left dorsal premotor cortex and higher lifestyle risk, a combination of lower physical activity and higher alcohol consumption seems to be particularly important. This is in line with previous studies showing better preservation of brain structure in physically more active older adults (Colcombe et al., 2003; Erickson et al., 2015; Erickson et al., 2014; Erickson et al., 2011),

[3] The discussion was partially adopted from manuscript 1 in the Appendix.

possibly due to training-induced adaptations of the brains structural architecture (Draganski et al., 2004). Furthermore, according to the "Use-it-or-Lose-It"-hypothesis, the organism is thought to preserve brain regions that are needed in daily life against age-related changes, i.e. the premotor cortex in physically active people (Swaab et al., 2002). A correlation between higher alcohol consumption and decreased brain structure in premotor cortex has not been shown so far, but associations between alcohol consumption and the deterioration of functional motor networks has been reported (Parks et al., 2010; Zahr et al., 2011). In comparison to prior studies, the current study is the first to reveal differences in brain structure in premotor networks, which are only present in a combination of higher alcohol consumption and lower physical activity.

Third, variations in cortical folding index in right ventrolateral prefrontal cortex were mainly driven by social integration as revealed by the stepwise exclusion approach. The ventrolateral prefrontal cortex, a subregion of higher order cognitive networks, subserves cognitive processes needed for social interaction (Chester & Riva, 2016; Eisenberger, Lieberman, & Williams, 2003; E. K. Miller & Cohen, 2001). In terms of the "Use-it-or-Lose-It"-hypothesis (Swaab et al., 2002), it seems therefore likely that the pronounced recruitment of the ventrolateral prefrontal cortex in socially active older adults leads to better preservation of that brain region. In turn, better preservation of the prefrontal cortex may be particularly important in light of the pronounced vulnerability of the prefrontal cortex to aging (Raz et al., 2005).

All of these results were highly stable, observed in the main and sensitivity analyses, and even showed overlap with lifestyle-related differences in cortical thickness (see Supplementary material of manuscript one; Appendix).

Fourth, systematic alterations in RSFC were mainly influenced by smoking. These increases in RSFC may reflect reduced cognitive reserve related to smoking, as higher base levels of functional connectivity leave less room for further increases in activation during active tasks (Reuter-Lorenz & Cappell, 2008; Reuter-Lorenz & Park, 2010; Stern, 2012). They may as well reflect addiction-related functional adaptations of the brain (Janes et al., 2012; Vergara et al., 2017; Zhou et al., 2017). Hence, less smoking may be a convenient way to maintain efficient use of neuronal networks even up to older ages.

Furthermore, adjustment for general education, depressive symptomatology and genetic risk for smoking and alcohol consumption did not alter the general pattern of these results emphasizing the role of daily lifestyle habits, when it comes to healthy brain aging.

Finally, these observations resulted in subsequent research questions, which were to be addressed in the second study of this dissertation: Based on the literature, study one assumed the lifestyle behaviors to affect the brain in a certain direction, e.g. negative effects of smoking on the brain. Effects in the opposite direction, depending on the brain region examined, may still occur and should therefore be investigated in the second study. Additionally, some lifestyle behaviors may affect the brain in a non-linear manner (Mukamal et al., 2001). Further, as a comparison to the combined lifestyle risk score, it would be desirable to investigate all four individual lifestyle variables in one model.

In summary, a more protective lifestyle seems to contribute to brain reserve, i.e. the preservation of brain structure, and to cognitive reserve, i.e. the maintenance of efficient use of functional brain networks. Integrative concepts of lifestyle may be a strong instrument for advancing our understanding of risk and protective influences on aging in the general population and in patients suffering from neurodegenerative diseases, as well as for low-cost interventions preserving healthy aging.

When Your Brain Looks Older Than Expected: Combined Lifestyle Risk and BrainAGE[4]

The first study focused on the cortical folding index as a sensitive marker to measure age-related differences in brain structure and revealed higher combined lifestyle risk to be accompanied by stronger brain atrophy, i.e. a lower cortical folding index. This led to the subsequent question, whether individuals with a higher combined lifestyle risk would show accelerated structural brain aging in general as well. Here, BrainAGE, a recently developed imaging biomarker, was chosen to measure general structural brain aging in the second study. BrainAGE measures the gap between true chronological age of an individual and estimated age of the brain, as predicted from anatomical patterns in MRI (Franke, Ristow, Gaser, & Alzheimer's Disease Neuroimaging, 2014; Franke, Ziegler, Kloppel, Gaser, & Initiative, 2010). This estimation is done, separately for female and male participants, by machine learning algorithms, which are blind to the individuals true chronological age. A higher BrainAGE score indicates that a brain appears older from its anatomical pattern than expected from chronological age. It thus hints at *accelerated brain aging*, whereas lower BrainAGE scores reflect younger looking brains, thus hint at *decelerated brain aging* (Cole & Franke, 2017; Franke, Gaser, & Initiative, 2012; Franke et al., 2014; Gaser et al., 2013). BrainAGE hence measures the deviation between chronological age and the manifestation of that age in the brain (older versus younger looking brains despite the same chronological age) and aggregates the multidimensional pattern of aging into one value.

Additional to the main research question of this second study, whether individuals with a higher lifestyle risk would generally show accelerated structural brain aging, we

[4] Parts of this section were derived from manuscript 2 in the Appendix.

specifically addressed the questions, which resulted from study one: These were to test i) each individual lifestyle variable´s contribution to structural brain aging, ii) the expected directions of the variable´s effect and iii) non-linear effects of each variable. Based on recent literature on general differences between female and male brains (Ritchie et al., 2018; Ruigrok et al., 2014), as well as on different trajectories of aging patterns between the two sexes (Riedel, Thompson, & Brinton, 2016; Scheinost et al., 2015) sex differences in the association between lifestyle and BrainAGE were examined as well.

Analysis 4: Lifestyle Risk and the BrainAGE-Score

Similar to study 1, T1-wheighted anatomical MR scans (Caspers et al., 2014) were used to estimate each individuals BrainAGE. All T1-wheighted images were segmented into the tissue probability maps (GM, WM, CSF) using the SPM12 toolbox (The Wellcome Dept. of Imaging Neuroscience, London; www.fil.ion.ucl.ac.uk/spm) and the VBM8 package (http://dbm.neuo.uni-jena.de). Then, only the GM maps were included as input for BrainAGE estimation. The age estimation framework then estimates the brains age only from the anatomical patterns within these GM maps via machine learning algorithms (see manuscript two; Appendix). This estimated, anatomical age is then compared to chronological age:

$$\text{BrainAGE} = \text{estimated} - \text{chronological age}.$$

Thus, BrainAGE reflects whether a brain appears older (higher BrainAGE) or younger (lower BrainAGE) from its anatomical characteristics than expected at that chronological age (Figure 5). All analyses between lifestyle and BrainAGE were calculated in an older subsample of the 1000BRAINS study aged 55 to 85 years (n = 622, 272 females, 350 males) in IBM SPSS Statistics 23 (https://www.ibm.com/de-de/analytics/spss-statistics-software). The

operationalization of all four lifestyle variables and the combined lifestyle risk score was similar to study one (see manuscript two in the Appendix).

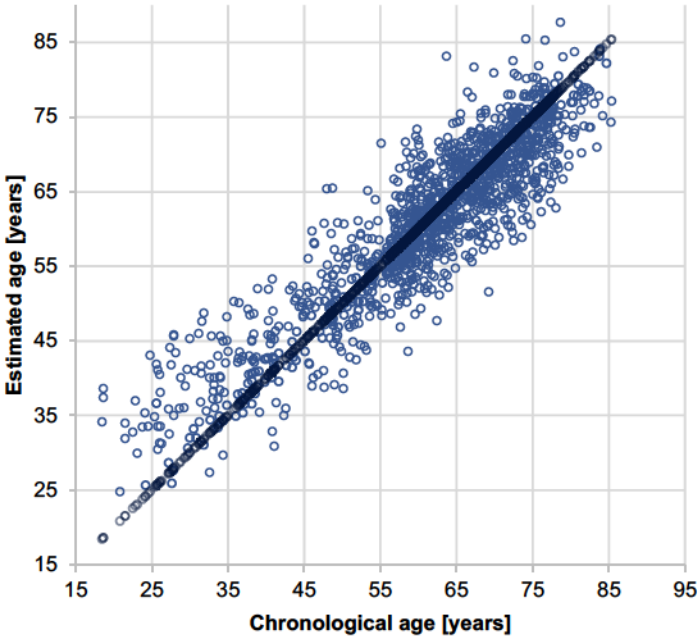

Figure 5. The correlation between chronological age and estimated age, as estimated by the machine learning algorithm. Dark blue dots represent a perfect correlation coefficient of $r = 1.0$. If estimated age is higher than chronological age, this results in a higher BrainAGE score and hints at accelerated structural brain aging. Figure adapted from manuscript two in the Appendix.

As BrainAGE aggregates the multidimensional aging pattern into one value, this enabled modeling the individual lifestyle variables using multiple linear regression to compare this approach to the combined lifestyle risk score. Based on the previously described literature and on the results of study one, study two hypothesized that higher combined lifestyle risk would be associated with higher BrainAGE scores as an indicator of accelerated structural brain aging. A general association between higher combined lifestyle risk and higher BrainAGE (Figure 6a) was found. This association was significant even after

outlier correction, as well as additional adjustment for general level of education, which was measured the same as in study one (UNESCO, 2003).

In addition to the combined lifestyle risk score, the relation between individual lifestyle variables and BrainAGE was examined. All four variables were simultaneously introduced as regressors in a multiple linear regression, correcting for age and sex, and using BrainAGE as dependent variable. Two-sided tests ($p < .05$) were employed to examine the expected direction of effects. As a result, higher pack-years (Figure 6b), reflecting a higher amount of lifetime smoking, as well as lower physical activity (Figure 6c), were associated to higher BrainAGE scores. Both associations were significant after outlier correction and additional adjustment for education, and Body Mass Index [for physical activity, Ho et al. (2011)]. Moreover, these effects were additionally quantified in terms of years: BrainAGE was estimated at 3.84 months older in addition to the effect of sex and age for one increase in the combined lifestyle risk score. The association between pack-years and BrainAGE was also dose-dependent with 0.36 month of additional BrainAGE per pack-year, such that brains of severe smokers were on average 1.6 years older than those of moderate smokers in a post hoc group comparison. In contrast, brains appeared 0.48 months younger with each increase in metabolic equivalent (MET) per week (with 4 MET reflecting one hour of 10mph bicycling), though this estimation was even higher when investigated in males only (brains appeared 0.6 months younger).

Next, sex-differences were tested by including an interaction term between sex and the combined lifestyle risk score or the respective lifestyle variable. There were no significant interactions between sex and lifestyle on BrainAGE. Nevertheless, stratifying for sex revealed that the association between lower physical activity and higher BrainAGE scores was no longer significant in female participants.

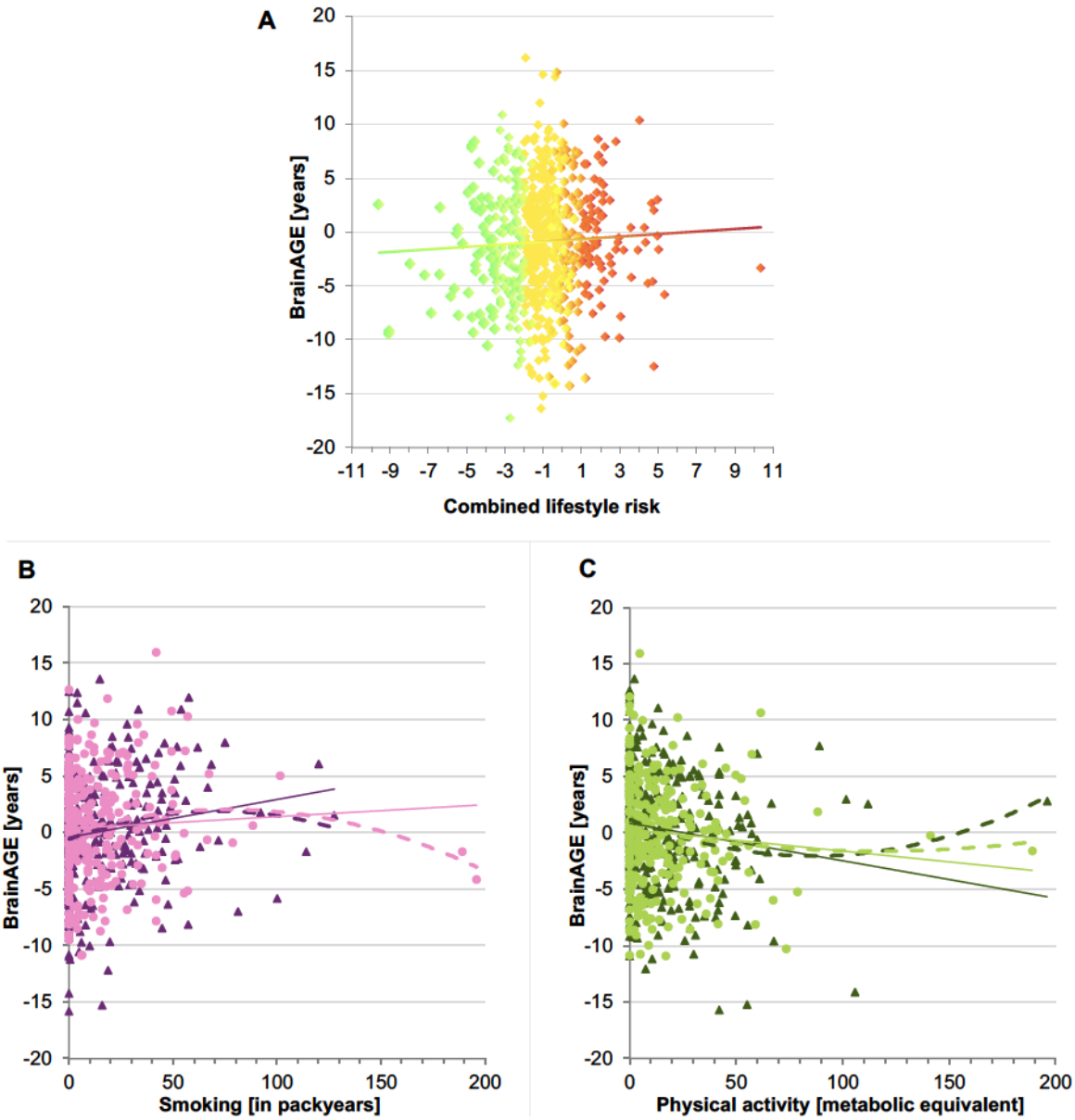

Figure 6. Associations between lifestyle and BrainAGE. (A) Higher combined lifestyle risk was related to higher BrainAGE. The color scale represents the transition from protective (green) to risky lifestyle behavior (pink). Smoking (B), as well as physical activity (C) explained the largest parts of variance in this association. Triangular shapes represent male participants, circular shapes represent female participants. Adapted from manuscript two in the Appendix.

Finally, non-linear associations, i.e. quadratic effects, between lifestyle and BrainAGE were examined. Adding quadratic effects of each individual lifestyle variable to the

regression models did not generally result in a better prediction of the data than testing linear effects only.

Discussion[5]

The second study shows that lifestyle differences contribute to the high inter-individual variance in structural brain aging of older adults. In contrast to the first study, differences in brain structure were investigated using BrainAGE as a meaningful imaging biomarker (Franke & Gaser, 2012; Gaser et al., 2013; Loewe, Gaser, Franke & ADNI, 2016; Franke et al., 2014). The analysis revealed smoking and physical activity to be the driving variables behind the association between combined lifestyle risk and accelerated brain aging, as indicated by BrainAGE.

Smoking has previously been related to lower GM volume, but most studies had small sample sizes (Almeida et al., 2008; Brody et al., 2004; Gallinat et al., 2006). For the first time, the current approach was able to quantify the deleterious effects of smoking: The association between pack-years and BrainAGE was shown to be dose-dependent with 0.36 month of additional BrainAGE per pack-year, such that brains of severe smokers were on average 1.6 years older than those of moderate smokers. Based on this analysis, conclusions about the biological mechanisms driving this association cannot be drawn, but the acceleration in brain aging patterns with higher smoking may be attributable to the direct toxic effects of tobacco onto the cerebro-vascular system, such as oxidative stress, which can result in cell death (Swan & Lessov-Schlaggar, 2007).

Further, the present results again support higher physical activity to be a fruitful target to promote brain health. The protective effect of physical activity has repeatedly been shown and discussed [for a recent review, see (Kramer & Colcombe, 2018)]. An important

[5] The discussion was partially adopted from manuscript 2 in the Appendix.

new aspect of the here applied approach was that physical activity was measured as a self-report measure in contrast to intervention studies (e.g. Colcombe et al., 2004; Erickson et al., 2011), where the kind and amount of activity is highly controlled. It is important to note, that self-report measures, as the hear employed one, can always be biased by social desirability and blurred recall. Still, physical activity contributed significantly to decelerated brain aging, even though the current self-report measure was not monitored and rather reflected the situation in real life. This again emphasizes the significance of protective lifestyle behavior during daily life of older adults when it comes to healthy aging. One potential mechanism how physical activity may act protectively on the aging brain may be increase of brain-derived-neurotropic factors, which affect synaptic efficacy, neuronal connectivity and use-dependent plasticity (Floel et al., 2010). Especially the latter may be particularly important, as the "lose-it-or-use-it"-hypothesis (Swaab et al., 2002) states that neurons needed and therefore stimulated in daily life are better preserved during the lifespan. By stimulation of involved brain regions higher physical activity may thus protect those regions against age-related structural atrophy, in turn leading to a perseveration of their structural integrity (Boyke, Driemeyer, Gaser, Buchel, & May, 2008; Churchill et al., 2002; Colcombe et al., 2003; Draganski et al., 2004; Erickson et al., 2011; Kramer & Erickson, 2007; Vaynman & Gomez-Pinilla, 2005; Vaynman, Ying, & Gomez-Pinilla, 2004).

Additionally, our results hint at sex differences in the association between lifestyle and BrainAGE that are often not explicitly considered. Interestingly, the association between physical activity and BrainAGE seems to be stronger in male than in female adults. Prior studies showed that the protective effect of physical activity in females may be dependent on hormonal influences (Erickson et al., 2007), such that the association between activity and brain health may be less stable in the general female population. In animal models it has

further been shown that levels of neurotrophins differ between male and female mice. If upregulation of neurotrophic factors drives the relationship between physical activity and brain structure (Floel et al, 2010; Ruscheweyh et al., 2011), sex-differences in the levels of neurotrophic factors may be another reason for the sex differences in the association between physical activity and brain structure reported in the current study. Possibly, the sexes also differ with respect to the specific kind of activity they engage in, as well as with respect to metabolism, which may lead to significantly different effects on the brain (Burd, Tang, Moore, & Phillips, 2009; Churchill et al., 2002; Colcombe & Kramer, 2003; Floel et al., 2010; Hayes, Hayes, Cadden, & Verfaellie, 2013; Kramer & Colcombe, 2018; Wu & O'Sullivan, 2011). However, biological mechanisms behind these sex differences remain to be elucidated for future studies considering the influence of the kind of activity, hormones, and the release of neurotrophic factors on the association between physical activity and the aged brain (de Melo Coelho et al., 2013; Venezia, Guth, Sapp, Spangenburg, & Roth, 2016).

General Discussion

By conducting four analyses, the current dissertation provides insights into the significant contribution of lifestyle to the high inter-individual variability in brain aging of older adults. The aim of the dissertation was (i) to develop an integrated approach to measure lifestyle as a combined concept rather than investigation of one factor and considering all other lifestyle behaviors as nuisance, and (ii) to describe its effects with emphasis on capturing the aged brain as a system by employing different methodologies and a multi-modal approach, examining GM volume (BrainAGE) versus cortical folding and its relation to functional connectivity, which is crucial with respect to understanding the complex nature of the aging brain.

The first paper therefore investigated the complexity between lifestyle and genetic factors, surface morphology (cortical folding index), GM of subcortical structures and functional connectivity. The second paper, in contrast, was dedicated to uncovering the relation between combined and individual effects of lifestyle, sex and BrainAGE, as a state-of-the-art imaging biomarker, which has been shown to validly predict future trajectories of brain aging (Cole & Franke, 2017; Franke et al., 2012; Gaser et al., 2013). Even though both studies examine the relation between lifestyle and the aged brain, they differ and complement each other with regard to the applied methods, the results and their interpretation.

The first study used a whole-brain approach, whereas the second study used an aggregated measurement, summarizing the multi-dimensional pattern of structural brain aging into one value – the BrainAGE score. Thus, the first study was able to make statements about regional specific differences in brain structure related to lifestyle risk, such as higher cortical folding index of the prefrontal cortex in relation to higher social integration. Here, the stepwise exclusion approach was applied, deducing the contribution of each lifestyle variable from its exclusion. This approach hinted at over-additive effects between the individual lifestyle variables, since only combinations showed associations to local brain atrophy as measured via the cortical folding index, but not the individual variables alone. Using this approach, we were able to deduce, that the combination of increasing alcohol consumption paired with decreasing physical activity seems to be risk behavior for advanced local brain atrophy. In the second study, the BrainAGE framework enabled investigation of the effects of the individual lifestyle variables in one multivariate model (multiple regression). BrainAGE particularly adds its meaningfulness when it comes to different trajectories of aging (Franke et al., 2012; Gaser et al., 2013). Whereas the first study

investigated differences in the aged brain *between* individuals, BrainAGE compares the individual anatomical aging pattern to the same individuals true chronological age. Even though this is not a longitudinal measurement of changes over time, it approximates estimation of intra- rather than inter-individual aging patterns: Each participant's image-based brain aging pattern is compared to his or her own chronologically expected brain aging in addition to the average brain aging pattern of the sample. The current approach therefore allowed identification of individuals whose brain anatomy hinted at accelerated brain aging: Those older individuals with a generally higher lifestyle risk (reflected in a higher combined lifestyle risk score) and with stronger lifetime smoking and lower physical activity.

Here, the employment of different methodologies revealed complementing insights into this association: The analysis of the cortical folding index, as well as BrainAGE (analysis one and four) revealed physical activity as one of the most important lifestyle behaviors regarding maintenance of structural brain health even in older ages. Analyzing BrainAGE further hinted at subtle sex differences within this association, not investigated in the first three analyses. This significantly adds to the current state of research as sex differences are rarely investigated in the association between lifestyle and brain aging, not only with regard to physical activity, but in general (Anaturk et al., 2018; Erickson et al., 2014; Karama et al., 2015; Mukamal et al., 2001). Shedding further light into sex as a possible influencing factor may facilitate tailoring interventions regarding lifestyle changes (higher physical activity, lower alcohol consumption) for the specific sex and may hence raise acceptance and maintenance of interventions in the community (Kramer & Colcombe, 2018). Together, the current dissertation showed that a more protective lifestyle (higher physical activity, higher

social integration) may enhance preservation of brain structure in the sense of the "Use-It-or-Lose-It-hypothesis"(Swaab et al., 2002), counteracting age-related structural decreases.

The results further hint at older adults with higher smoking behavior being at risk for accelerated structural brain aging, as revealed by higher BrainAGE (analysis one). In contrast, lifetime smoking was the only lifestyle variable that showed no systematic relation to differences in cortical folding index in the first analysis. It was the only lifestyle variable, which showed systematic variations to increases in RSFC (analysis two), though. Based on the analyses of cortical folding index and RSFC, the conclusion was drawn that smoking may be one lifestyle factor mainly affecting the functional, and not structural integrity of the aged brain. In light of the forth study´s results, this hypothesis needs to be reevaluated: Smoking seems to affect the regional blood flow, which is shown by the local increases in RSFC – and which may further hint at underlying changes in structural connectivity and WM – but smoking may also affect GM, when it comes to an aggregated measurement of brain aging (BrainAGE). Physical activity, in contrast, seems to be a lifestyle behavior affecting the *general* aging patterns of structural brain aging (BrainAGE) in GM, differences in cortical thickness (manuscript one; Appendix), as well as the complex characteristics of cortical folding index. In the first analysis, the effect of physical activity was only revealed when examined in combination with alcohol consumption. Thus, cessation of alcohol consumption and smoking, as well as engaging in physical activity may be fruitful targets to promote healthy brain aging and protect from increased risk for accelerated structural decrease. However, smoking cessation may also be an important target when it comes to maintenance of the brain *functional* efficiency in old ages, since higher smoking was also related to higher RSFC in the first study, hinting at lower cognitive reserve (Stern, 2009; 2012).

In contrast to the first study, the analysis of BrainAGE (Analysis 4) did not reveal an association between alcohol consumption or social integration and the aged brain. In contrast, social integration as a protective lifestyle behavior was related to preservation of brain structure as measured via the cortical folding index (analysis one) regarding the right prefrontal cortex and via greater hippocampal volume, two brain structures particularly vulnerable to age-related structural loss (Fjell et al., 2013; Raz et al., 2005). A potential reason for not replicating this effect in the analysis of BrainAGE (analysis four) may be that it was only found, when investigated in combination with additional lifestyle behaviors (analysis one). Here, the effect of social integration may only be detectable when it comes together with an accumulation of several lifestyle behaviors. One reason may be that the combination of individual lifestyle effects results in higher power to detect an effect, which would not be strong enough to be found when investigated alone. Another reason may be molecular biological interactions between the lifestyle behaviors, which are not directly measurable with statistics, which are covered by the combined lifestyle risk score. Another reason for not replicating this effect using BrainAGE, may be intrinsic to the complexity of the aged brain: The cortical folding index is thought to reflect the underlying regional complexity and connectivity of the brain (Hogstrom et al., 2013; Zilles et al., 2013). BrainAGE, in contrast, aggregates the multidimensional pattern of aging into one value. Prior studies, which reported a protective effect of social activities examined *regional* GM volumes (James et al., 2012) and cognition (Fratiglioni et al., 2004). Therefore, social integration may be more closely related to regional differences in the complex measure of cortical folding index, than to the aggregated aging pattern of GM.

However, similar to the present study, most measurements of social integration included activities with a high social and cognitive demand (Anaturk et al., 2018; Gow et al.,

2012; Hafsteinsdottir et al., 2012). Therefore, it would be desirable to deduce the cognitive and social components of social integration to make further statements about its effect on the aged brain in the future.

The observed effect sizes of lifestyle on the aged brain can be considered as small, though comparable to other studies (K. L. Miller et al., 2016). Still, the effects seem to be highly stable, since several subsequent analyses to test for the confounding influences of further variables were conducted. Here, the influences of several covariates including genetic risk variants, depressive symptomatology, general level of education, as well as non-linear effects, and sex differences were investigated (analysis three & four). The results remained stable and the effects were found independent of the measure, i.e. cortical folding index, RSFC or BrainAGE, used. This underlines the importance of daily lifestyle habits and their interplay for structural and functional brain health in older adults, beyond other influencing factors, such as genetics and education.

Some limitations of the current dissertation, as well as future questions resulting from the current results should be addressed. The present dissertation relied on a one-time measurement of lifestyle. Future studies are needed to investigate long-term measurements including change patterns of lifestyle behaviors. Further, both present studies applied correlational analyses, such that directionality of the effects cannot be inferred. It is e.g. impossible to disentangle whether enhanced social integration leads to a stronger regional cortical folding index, or whether higher initial brain reserve leads to higher social integration. Therefore, longitudinal studies are needed to test causality of the here reported effects. For future studies, it would also be desirable to analyze additional lifestyle behaviors, such as dietary (Gur et al., 2015) or sleep (Mander, Winer, & Walker, 2017) habits. The current dissertation provides insights into lifestyle influences on a range of parameters

characterizing age-related differences, but future work may extend these results onto WM and structural connectivity. As some associations between lifestyle and structural connectivity of the brain have been reported (Kohncke et al., 2016), this may shed further light into the complex relationship between lifestyle, brain structure and functional connectivity. Since some lifestyle variables contributed stronger to the present results than others, one could further deduce different weightings of the different lifestyle variables when entered into the combined score in future studies.

Together the four analyses add new evidence to the complex contribution of lifestyle to the variability in structural brain aging and functional connectivity and therefore give promising insights into why people age so differently. Such insights may help us to develop interventions to employ in daily life of older adults to promote healthy brain aging, maintenance of quality of life and autonomy even up to older ages. Applications of our combined concept further supported the notion that age-related neuronal differences are likely a manifestation of a cumulative effect of several factors and not only of individual influences, which needs to be further elucidated in future studies.

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Appendix

Manuscript 1 was published as:

Bittner, N., Jockwitz, C., Mühleisen, T. W., Hoffstaedter, F., Eickhoff, S. B., Moebus, S., Bayen, U. J., Cichon, S., Zilles, K., Amunts K., & Caspers, S. (2019). Combining lifestyle risks to disentangle brain structure and functional connectivity differences in older adults, *Nature Communications, 10* (1), 621-634.

Susanne Moebus, Katrin Amunts, and Svenja Caspers conceived and designed the general idea of the study. I selected the materials (lifestyle variables) with the support of Christiane Jockwitz and Svenja Caspers. I checked all lifestyle data for plausibility and quality and calculated all derived parameters. I did the preprocessing of all structural MR data with help from Kai Jannusch. Preprocessing of functional MR data was done by Felix Hoffstaedter. Thomas W. Mühleisen and Sven Cichon supported the design of the genetic analyses and provided genetic risk score data. I planned the statistical data analysis with the input of Christiane Jockwitz, Svenja Caspers, Felix Hoffstaedter, Simon B. Eickhoff, and Thomas W. Mühleisen. I performed all analyses. Christiane Jockwitz supported all aspects of data processing and analysis in terms of methods advice. I interpreted the results with support of all co-authors, where Ute J. Bayen provided advise on statistical methodology and data interpretation with regard to psychological relevance, Susanne Moebus provided advise on epidemiological data interpretation, Simon B. Eickhoff critically advised the methodological discussions and data interpretation, Katrin Amunts and Karl Zilles advised on interpretation of the study findings with particular focus on neuroanatomy and aging. I prepared the first draft of the manuscript, which was then iteratively revised by Christiane Jockwitz, Svenja Caspers, Ute J. Bayen, and myself. Thomas W. Mühleisen, Sven Cichon, Katrin Amunts, Karl

Zilles, Susanne Moebus, Simon B.Eickhoff, and Felix Hoffstaedter critically commented and revised on the pre-finalized version of the manuscript. I integrated these comments to create a final version, which was approved by all authors. Svenja Caspers and Ute J. Bayen supervised the whole study.

Manuscript 2 was submitted as:

Bittner , N., Jockwitz, C., Franke, K., Gaser, C., Moebus, S., Bayen, U. J., Amunts, K., & Caspers, S. *When your brain looks older than expected: Combined lifestyle risk and BrainAGE.* Manuscript submitted for publication to *Neuroimage*.

Christian Gaser, Svenja Caspers, and I conceived the general idea of the study. I selected the materials (lifestyle variables) with the support of Christiane Jockwitz and Svenja Caspers. I checked all lifestyle data for plausibility and quality and calculated all derived parameters. I did the segmentation of structural MR data with support from Felix Hoffstaedter. Katja Franke provided the age estimation framework and applied the machine learning algorithm to estimate brain age. I calculated the BrainAGE score and did the quality control, including the calculation of performance measures. I planned the statistical data analysis with the support of Katja Franke, Svenja Caspers, and Ute J. Bayen. Christiane Jockwitz supported all aspects of analysis in terms of methods advice. I conducted all analyses. I interpreted the results with support of all co-authors. Susanne Moebus provided advise on epidemiological data interpretation. Here, Ute J. Bayen provided advise on statistical methodology, as well as data interpretation with regard to psychological relevance. Katrin Amunts advised on interpretation of the study findings with particular focus on neuroanatomy and aging. Christian Gaser critically advised the methodological discussion. I prepared the first draft of

the manuscript, which underwent several revisions by Christiane Jockwitz, Svenja Caspers, Ute J. Bayen, and myself. Katja Franke, Christian Gaser, Katrin Amunts, and Susanne Moebus critically commented and revised on the resulting manuscript. I addressed these comments and methodological considerations in a second version. This second version was revised and approved by all authors for submission. Svenja Caspers and Ute J. Bayen supervised the whole study.

Die obigen Angaben bezüglich der Beiträge der Doktorandin zu den genannten Manuskripten sind zutreffend:

Prof. Ute J. Bayen, Ph.D.

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Combining lifestyle risks to disentangle brain structure and functional connectivity differences in older adults

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Lifestyle contributes to inter-individual variability in brain aging, but previous studies focused on the effects of single lifestyle variables. Here, we studied the combined and individual contributions of four lifestyle variables - alcohol consumption, smoking, physical activity, and social integration - to brain structure and functional connectivity in a population-based cohort of 549 older adults. A combined lifestyle risk score was associated with decreased gyrification in left premotor and right prefrontal cortex, and higher functional connectivity to sensorimotor and prefrontal cortex. While structural differences were driven by alcohol consumption, physical activity, and social integration, higher functional connectivity was driven by smoking. Results suggest that combining differentially contributing lifestyle variables may be more than the sum of its parts. Associations generally were neither altered by adjustment for genetic risk, nor by depressive symptomatology or education, underlining the relevance of daily habits for brain health.

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Structural decline and functional reorganization are major hallmarks of brain aging. Both show high inter-individual variability, especially in later decades of life. Here, lifestyle habits came into focus possibly influencing this variability[1]. While some lifestyle habits may pose serious risks to brain health, others may be protective. Physically more active older adults show less volume loss[2,3], and better performance in cognitive tasks[4] along with higher task-related activity in attentional networks[5]. Likewise, stronger social integration of older adults is associated with reduced cognitive decline[1], reduced risk of dementia[1] and Alzheimer's disease[6], and higher regional and overall gray matter (GM) volumes[7,8]. Both lifestyle habits therefore seem to promote cognitive or neural reserve capacity[9,10], that is, the ability to tolerate age or disease load without functional impairments. In contrast, other lifestyle habits may pose serious risks to healthy brain aging. Studies reported associations between heavy smoking and cortical thinning[11] or lower GM density[12]. In addition, smokers, compared to non-smokers, showed reduced resting-state functional connectivity (RSFC)[13], as a correlate of generally altered functional brain architecture[14], between the insula and prefrontal cortex. Similarly, chronic alcohol dependence can lead to severe neurological diseases, for example, Korsakoff syndrome[15]. GM loss, however, has also been reported in healthy older adults with non-dependent alcohol consumption[16]. In alcohol-dependent patients[17], regional GM loss was found particularly in the frontal cortex, whereas white matter (WM) loss is more pronounced in corpus callosum and cerebellum[17]. Further, RSFC as well as performance in a simple motor task and associated brain activation were found to be decreased in alcohol-dependent patients[18]. Hence, alcohol consumption and smoking may both be variables accelerating brain aging and reducing brain reserve.

Previous studies mainly investigated effects of single lifestyle variables in isolation. However, individuals rather show a combination of lifestyle habits that could all possibly influence brain reserve, for example, being a smoker (risk) and socially and physically active person (protective) versus being a smoker and an inactive person. Yet, studies examining combinations of lifestyle variables are rare. One study found pronouncedly different RSFC, particularly when participants who both smoked and consumed alcohol were compared to participants with only one of these risk variables[19]. This underlines the notion that individual lifestyle variables may have intermingling effects on the aging brain. It is therefore essential to examine combinations of lifestyle habits to understand the high inter-individual variability in the reserve capacity to tolerate age-related differences.

Consequently, we developed a combined lifestyle risk score to investigate the relation between lifestyle risk as a combined concept and brain aging. Based on the literature described above, physical activity[2–5] and social integration[1,6–8] were classified as protective variables, and alcohol consumption[15–18] and smoking[11–13] as risk variables. Data on all four lifestyle variables were assessed via self-report in 549 older participants (248 female) aged 55 to 85 years from the population-based 1000BRAINS cohort study[20]. Physical activity was examined as metabolic equivalent[21], alcohol consumption in grams of consumed alcohol per week, smoking as pack years, and social integration as the social integration index[22]. Similar to the concept of genetic risk scores, we combined these four lifestyle variables into one risk score indicating combined lifestyle risk (Fig. 1). Negative values indicated a rather protective lifestyle (e.g., high levels of physical activity and social integration, plus low alcohol consumption and no smoking), and positive values a combination of more risky behaviors. Further details can be found in the Methods section.

To investigate the relation between lifestyle and brain structure and function, we tested not only this combined risk score but also investigated the contributions of each lifestyle variable. To this end, we successively excluded each lifestyle variable from the combined lifestyle risk score resulting in eight risk score models with different combinations of single lifestyle variables (see Methods and Supplementary Table 1): Four risk score models included three single variables and another four included two single variables, where always one protective and one risk variable were combined to examine whether they canceled each other out[19]. We used this epidemiologically motivated comprehensive operationalization of lifestyle in three consecutive analyses to understand its relation to reorganization during brain aging: Analysis of (1) brain structure regarding (a) gyrification and (b)

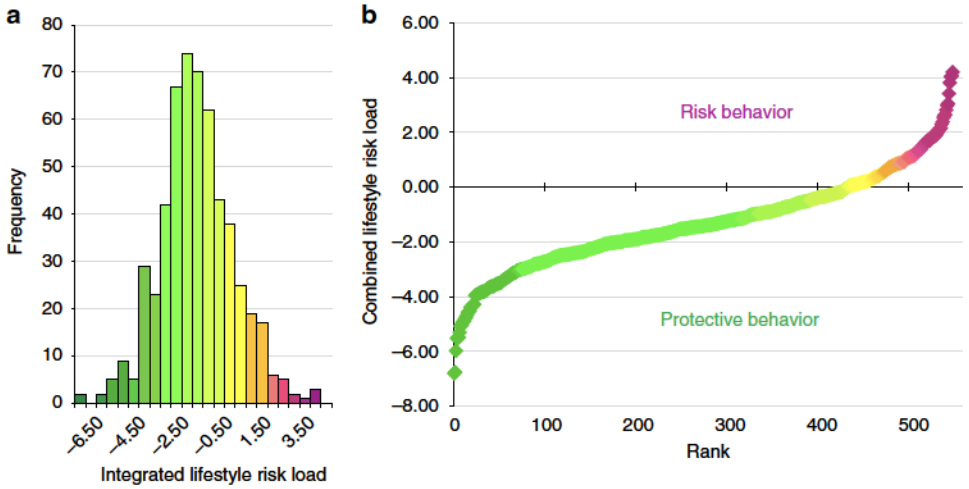

Fig. 1 Combined lifestyle risk measured with the developed combined lifestyle risk score. Lifestyle related data, that is, alcohol consumption (g/week), smoking (pack years), social integration (social integration index[23]), and physical activity (metabolic equivalent per week[22]) were *z*-transformed to obtain uniform measure units. *Z*-transformed data on the protective variables physical activity and social integration were first multiplied with (−1) and then summed up with *z*-transformed data on the risk variables of alcohol consumption and lifetime smoking. This resulted in an individual score of combined lifestyle risk. **a** The combined lifestyle risk score shows a nearly normal distribution. **b** Rank-sorted data of the combined lifestyle risk score: The participant with the lowest score gets rank 1 and the highest rank 549. Green colors indicate more protective lifestyle behavior and magenta colors indicate more risky lifestyle behavior

subcortical volumes, (2) RSFC, and (3) both analyses while additionally accounting for genetic risk.

Recent studies identified the local gyrification index (LGI)[23], the degree of cortical folding, as a sensitive surface-based measure[24,25] for studying age-related differences in local brain structure. Differences in arealization and gyrification seem to be more closely related to age than differences in cortical thickness (CT)[24]. Higher gyrification is supposed to promote functional development and brain connectivity more efficiently than increasing cortical GM[24]. In turn, lower gyrification would imply lower brain reserve capacity. We therefore hypothesized (1a) that higher combined lifestyle risk would be associated with lower local gyrification. We tested this hypothesis in FreeSurfer[26,27] based on T1-weighted structural magnetic resonance (MR) images. To complement this surface-based approach, we tested for an association between combined lifestyle risk and volume of subcortical structures (1b), as well as (1c) CT as a second surface parameter[28] adding the dimension of cortical GM volume in supplementary analyses. Our second analysis examined whether the identified lifestyle-related variations in brain structure would be accompanied by variations in functional connectivity (2). Here, we tested for decreases in RSFC, as well as RSFC increases that have been repeatedly reported in pathological conditions[14]. RSFC increases that already occur during rest may reflect higher base levels that leave no room for additional increases in brain activity during active tasks to boost performance[9,28]. Therefore, higher base levels supposedly reflect lower cognitive reserve[29,30] and would be expected in individuals with higher combined lifestyle risk. We hence hypothesized that those regions that showed lifestyle-related variations in cortical folding would also show variations in RSFC. Finally, evidence for influences of genetic susceptibility on variability in behavior[31–34] and brain organization[35] has recently been provided. To account for individual genetic susceptibility (3), we constructed a polygenetic risk score (PRS) from genome-wide association studies (GWAS, see Methods) of smoking and alcohol consumption.

The current study shows that combined lifestyle risk is associated with decreased gyrification in left premotor and right prefrontal cortex, and related higher functional connectivity with sensorimotor and prefrontal cortex. While decreased gyrification was driven by alcohol consumption, physical activity, and social integration, higher functional connectivity was driven by smoking. Neither genetic influences nor a set of non-lifestyle variables (depressive symptomatology, Beck Depression Inventory-II (BDI-II)[36] and education level as measured with the international standard classification of education (ISCED)[37]) modulated the relation between combined lifestyle risk and the brain phenotypes. Daily lifestyle habits and their interplay therefore seem to influence structural and functional brain health in older adults, beyond other influencing factors.

Results

Sample characteristics. Mean age of the final study sample ($n = 549$) was 67.4 years (SE = 0.28, Table 1). Mean combined lifestyle risk (summed-up z-scores) was −1.30 (SE = 0.07) and its distribution did not significantly deviate from normality (Kolmogorov–Smirnov test: $p = 0.226$, Fig. 1). Data on all four single lifestyle variables (Table 1) were considerably skewed due to a substantial number of participants not engaging in the specific lifestyle behavior, for example, 216 (136 female) participants did not consume alcohol. Among these 216 participants, 16 never consumed alcohol and no participant reported abstinence due to former alcohol dependence. Similarly, 255 (139 female) participants never smoked. We used subgroups of the study sample for the analysis of RSFC ($n = 501$) and for the PRS analysis ($n = 488$), since RSFC and PRS were not available for all participants.

Table 1 Descriptive statistics of lifestyle variables

Lifestyle variable	Mean	SE	Min	Max
Alcohol consumption (in g/week)	70.00	4.41	0.00	952.77
Smoking (in pack years)	12.77	0.80	0.00	120.00
Social integration (as social integration index)	12.72	0.25	2.00	43.00
Physical activity (metabolic equivalent per week)	40.75	1.71	0.00	257.75
Combined risk score (combined z-scores)	−1.30	0.07	−6.81	4.20

Combined lifestyle risk and gyrification. All reported analyses were statistically corrected for age and gender as covariates and corrected for multiple comparisons using Monte Carlo simulations at $\alpha = 0.05$ with a cluster-wise p value (cwp) < 0.01 (two-sided test, for uncorrected results, see Supplementary Fig. 1). A higher combined lifestyle risk score was associated with lower local gyrification in two distinct cortical areas, namely left dorsal premotor cortex (dPMC, cwp = 0.0001, Fig. 2a) and ventrolateral prefrontal cortex (vlPFC, cwp = 0.0001, Fig. 2a) extending from the frontal pole to middle frontal gyrus and to posterior inferior frontal gyrus and sulcus.

Stepwise exclusion (Fig. 2) of one or more lifestyle variables from the combined score and examining the associated differences in gyrification revealed a consistent pattern: (i) Higher lifestyle risk in all risk score models including alcohol consumption and/or physical activity (Fig. 2a–h) was associated with decreased cortical folding in left dPMC. This association disappeared, when excluding both alcohol consumption and physical activity (Fig. 2i). This hinted at alcohol consumption and physical activity being the driving behaviors behind this association. (ii) Lower lifestyle risk in all risk score models that included social integration (Fig. 2a, c, d, e, g, i) was consistently associated with decreased cortical folding in right vlPFC. Since this association vanished when social integration was excluded (Fig. 2b, f, h), this hinted at social integration as the main driving behavior for this association. To further test this association, we extracted cortical folding values of the vlPFC region and used them as dependent variable in a post-hoc multiple linear regression including all four single lifestyle variables, age, and gender as regressors. With $\beta = 0.11$, social integration was the strongest predictor of cortical folding in vlPFC (F test, $F(1,542) = 3.95$, $p = 0.0007$; for details, see Supplementary Fig. 17).

We did not find any significant association between single lifestyle variables and cortical folding using permutation-based inference. Uncorrected results are shown in Supplementary Fig. 2. Analyses including additional covariates and measure (CT) can be found in Supplementary Figs. 11–16.

GM volume of subcortical areas. The strongest association was found between enhanced social integration alone and greater GM volume of left hippocampus (partial Spearman's $\rho = 0.15$, $p = 0.0017$). This was the only correlation surviving a post-hoc Bonferroni correction using a threshold of $\alpha_{corr} = 0.05/20 = 0.0025$ with 20 subcortical structures tested (Table 2).

Combined lifestyle risk and RSFC. We used regions showing variations in cortical folding in relation to the combined lifestyle risk score as seed regions (dPMC and vlPFC) to test for variations in RSFC to all other GM voxels. All reported associations were significant at $\alpha = 0.05$ (cluster level corrected, cluster-forming threshold $\alpha < 0.001$, two-sided) and corrected for age and gender. Decreased RSFC between the seeds and other regions showed no systematic differences (see Supplementary Figs. 3, 4).

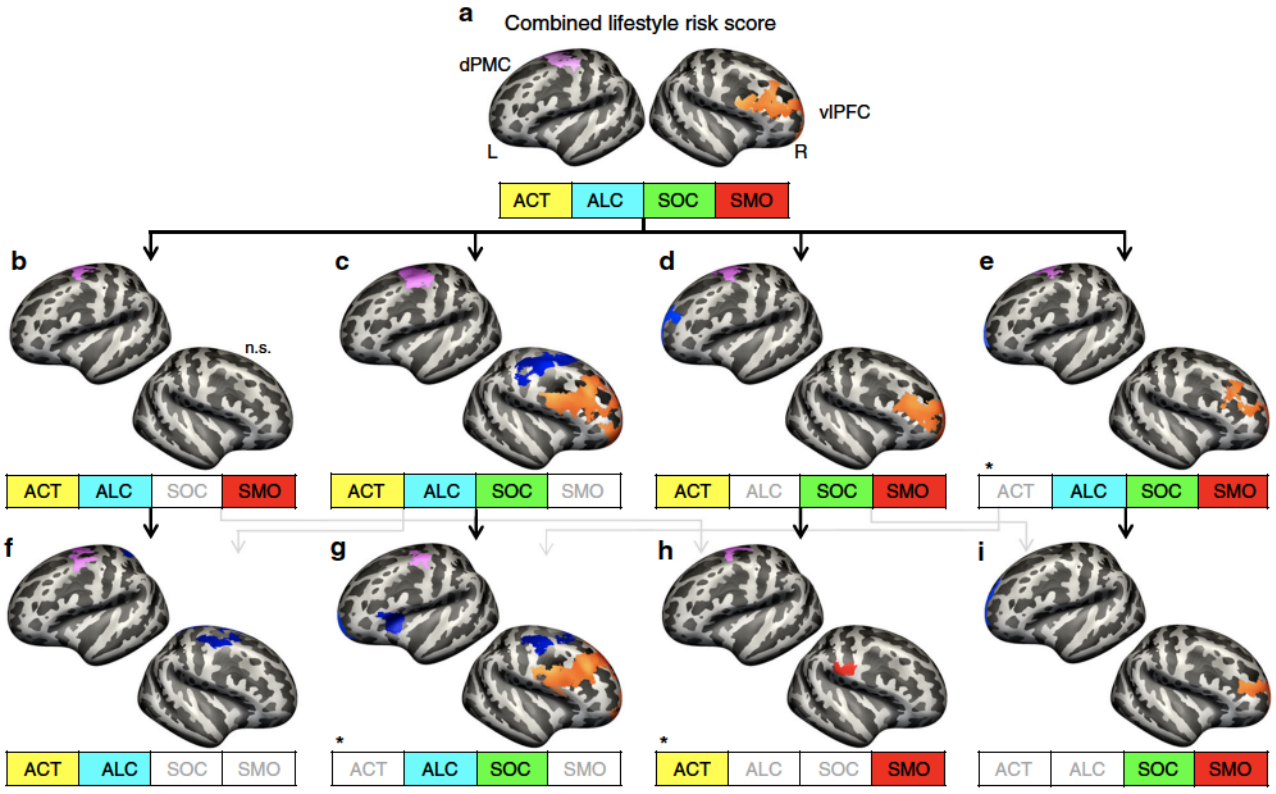

Fig. 2 Differences in cortical folding associated with lifestyle risk in different risk score models. **a–i** Small boxes represent different risk score models derived from the stepwise exclusion approach with excluded single lifestyle variables grayed out. Associations between cortical folding and the combined lifestyle risk score, or the different risk score models, respectively, are depicted on the inflated surfaces of the fsaverage brain. Arrows indicate the work flow of the stepwise exclusion. Negative associations are represented in blue, and positive associations in red. The recurrent negative associations between higher lifestyle risk and reduced cortical folding in left dorsal premotor cortex (dPMC) and right ventro-lateral prefrontal cortex (vlPFC) are colored in pink and orange, respectively. All results are corrected for age and gender and corrected for multiple comparisons using Monte Carlo Z simulations with $\alpha = 0.05$. Some of the associated regions in specific risk score models marked with an asterisk did not pass an additional correction for multiple comparisons at $\alpha = 0.05/13 = 0.0033$. For detailed information see Supplementary Table 2. n.s. = not significant, ACT = physical activity, ALC = alcohol consumption, SOC = social integration, SMO = pack years of smoking, L = left hemisphere, R = right hemisphere

Regarding the seed region of left dPMC, higher combined lifestyle risk scores were associated with increased RSFC between left dPMC seed and bilateral primary motor, left somatosensory, left entorhinal, and left higher visual cortex.

Again, we applied our stepwise exclusion approach to test for individual contributions of single lifestyle variables. Excluding social integration, alcohol consumption, or physical activity from the risk score left the overall association between left dPMC and reported regions unaltered (Fig. 3b, d, e, h, l). Contrarily, when excluding smoking from the risk score models, increases in RSFC were no longer found (Fig. 3c, f, g). Further, smoking as a single variable revealed higher RSFC between left dPMC and bilateral primary motor and somatosensory cortex, left superior frontal gyrus, left dorsal and right lateral occipital cortex, that is, clusters that were similarly found in the respective risk score models that included smoking (compare Fig. 3a, b, d, e, h with Fig. 3l). Thus, RSFC was only increased when smoking was considered, either incorporated in a risk score model or as single variable. Besides this general pattern, we found an additional systematic change in RSFC of left dPMC (Supplementary Table 3): All risk score models that included both physical activity and smoking, or smoking as a single variable, were significantly related to higher RSFC between left dPMC and dorsal visual cortex (Fig. 3a, c, d, h, l).

For single lifestyle variables, we found additional associations (Supplementary Table 3): Higher RSFC between dPMC and (i) left rostral superior frontal gyrus (for smoking), (ii) left temporo-parietal junction (for social integration, Fig. 3m), and (iii) left cerebellum (for alcohol consumption, Fig. 3k).

Now analyzing RSFC of the vlPFC, this seed showed increased RSFC to right anterior superior frontal gyrus (SFG, Fig. 4a, Supplementary Table 4) in relation to higher combined lifestyle risk.

Again, we applied our stepwise exclusion approach for the seed in right vlPFC. The cluster within right SFG was repeatedly found in all risk score models containing smoking and not found when smoking was excluded (Fig. 4c, f, g). Additionally, this cluster showed higher RSFC in relation to smoking as a single variable (Fig. 4l), as well as clusters in its left homolog and thalamus. Results remained unchanged when removing any other lifestyle variable (Fig. 4b–i).

Two other single variables were associated with increased RSFC to the right vlPFC seed, that is, to right caudate nucleus, and right rostral inferior frontal gyrus (social integration, Fig. 4m); and to left inferior frontal gyrus triangular part, left post-central gyrus, and right middle temporal gyrus (physical activity, Fig. 4j).

Additional adjustment for polygenic risk. Entering the polygenic risk score (PRS, composed of genetic risk for alcohol consumption and smoking, see Table 3 in Methods) as a covariate did not change the general pattern of results, but revealed an

Table 2 Correlations between lifestyle risk score models and subcortical structures

Risk score model	Hemisphere	Subcortical structure	r (correlation)	p value
Combined risk score, ACT, ALC, SMO, SOC	Right	Amygdala	−0.086	0.044
		Nc. accumbens	−0.100	0.019
ACT, ALC, SMO	Left	Thalamus	0.089	0.037
ACT, ALC, SOC	Right	Amygdala	−0.087	0.042
ACT, SOC, SMO	Left	Hippocampus	−0.089	0.037
		Putamen	−0.092	0.030
		Globus pallidus	−0.089	0.037
	Right	Nc. accumbens	−0.084	0.049
		Hippocampus	−0.091	0.033
		Putamen	−0.089	0.036
ALC, SOC, SMO	Left	Hippocampus	−0.091	0.033
	Right	Nc accumbens	−0.129	0.003
		Putamen	−0.088	0.040
ACT, ALC	Bilateral	Mid anterior cingulum	−0.105	0.013
ALC, SOC	Left	Hippocampus	−0.105	0.014
		Amygdala	−0.105	0.014
SOC, SMO	Left	Hippocampus	−0.107	0.012
		Globus pallidus	−0.096	0.024
	Right	Putamen	−0.098	0.022
		Nc. accumbens	−0.108	0.012
ACT as single variable	Bilateral	Anterior cingulum	0.107	0.012
SMO as single variable	Right	Nc. accumbens	−0.101	0.018
SOC as single variable	Left	Hippocampus[a]	0.134[a]	0.002[a]
		Amygdala	0.088	0.039
	Right	Hippocampus	0.101	0.018
		Nc. caudatus	0.12	0.005

Table shows significant partial correlations between lifestyle risk score models and subcortical structures, corrected for age, gender, and total intracranial volume, $n = 549$
Nc: nucleus, r: Spearman's correlation coefficient
[a]The partial correlation between social integration and the left hippocampus was the only one being significant at an α-level corrected for multiple comparisons $\alpha_{corr} = 0.05/20 = 0.0025$. The complete correlation matrix can be found in Supplementary Data 1

additional effect in right precuneus as can be seen in Fig. 5 (see Supplementary Fig. 5 and Supplementary Table 7). Results of the RSFC analyses did not change either when adjusting for PRS (Supplementary Figs. 6 and 7, Supplementary Tables 8 and 9).

Discussion

This study examined the relation between combined lifestyle risk and cortical folding and RSFC of the aged brain. Four core findings emerged: First, the combined lifestyle risk score showed significant systematic associations to regional cortical folding, whereas single lifestyle variables did not. Second, physical activity and alcohol consumption were the main variables contributing to reduced cortical folding in left dPMC, and third, social integration was the main variable contributing to higher cortical folding in right vlPFC as revealed by the stepwise exclusion approach. These patterns were highly stable, observed in the main as well as in all additional and sensitivity analyses, as often used in epidemiological research (see Supplementary Methods). Fourth, systematic alterations in RSFC were mainly influenced by smoking. Finally, adjustment for genetic risk for smoking and alcohol consumption did not alter the general pattern of results.

Our first finding that higher combined lifestyle risk scores showed significant systematic associations to lower regional cortical folding, whereas single lifestyle variables did not, may be explained by additivity: Many different influencing factors contribute to variations in older adults' brains[38], with each variable explaining only a small amount of variance. Hence, single effects of each lifestyle variable on cortical folding might be too small to reach significance. By combining different lifestyle variables, their effects on the aged brain might either add up or integrate—becoming strong enough to reach significance. Another reason for finding effects for the combined, but not for single lifestyle variables might be that the combined lifestyle risk score reduces noise by integrating underlying information from different single lifestyle variables. Reasons explaining why we did not replicate previously reported results on single lifestyle variables might be manifold, for example, the population-based and therefore heterogeneous nature of our sample in contrast to intervention studies often having very homogenous samples[8]. Further, we did not investigate lifestyle behavior in patients[6,17], but in healthy older adults, where pathological differences may be subtler.

Our second core finding that higher combined lifestyle risk was associated with lower cortical folding in left dPMC is significant, but only when alcohol consumption and/or physical activity were included into the risk score models. Importantly, neither physical activity nor alcohol consumption as single variables were significantly related to cortical folding. Thus, both seem to drive the association between lifestyle risk and cortical folding only in combination with other lifestyle risk variables. Interestingly, we found the same association between physical activity and alcohol consumption and decreases in brain structure also with respect to decreased CT (Supplementary Fig. 14). Physical activity has repeatedly been linked to better cognitive performance[4] and reduced age-related GM loss[3]. It has been discussed that physical activity promotes increase or preservation of brain structure[3,4], analogous to "activity"-induced or "training"-induced structural adaptations of neuronal tissue described in animals[39] and humans[40]. This increase in GM was replicated in older adults[41], suggesting that structural adaptations are even possible in later life. A similar relation has also been described in the "use it or lose it" hypothesis[42] regarding cognitive or motor abilities. According to this hypothesis, the more particular abilities are used during daily life, the better they are preserved in the course of aging. Consequently, often engaged brain regions show structural adaptation[40,43] and are better preserved[42] also in late life[44], which contradict the theory of often used brain regions suffering from faster decline[42]. Consistent with the current study, structural correlates of physical activity have been found in PMC[44,45]. The dPMC is involved in a variety of processes needed for daily physical activities and sports, such as movement planning, control, and learning (e.g., dancing), sensorimotor transformations, and action selection[46,47]. Possibly, older adults more engaged in sports and physical activity in daily life recruit the resources of PMC more often. This could lead to better preservation of brain tissue in the very same area, such that an age-related decrease[2] would be less pronounced in physically more engaged older adults — similar to activity-induced structural adaptations.

The other variable contributing to cortical folding in dPMC in the healthy older adults of the present study was alcohol consumption. Chronic alcoholism has repeatedly been related to neurodegeneration, for example, of frontal GM[16], cerebellar Purkinje cells[48], and motor performance impairment[18]. However, a direct association between alcohol abuse and degeneration of PMC was not shown previously. A recent study, though, did find

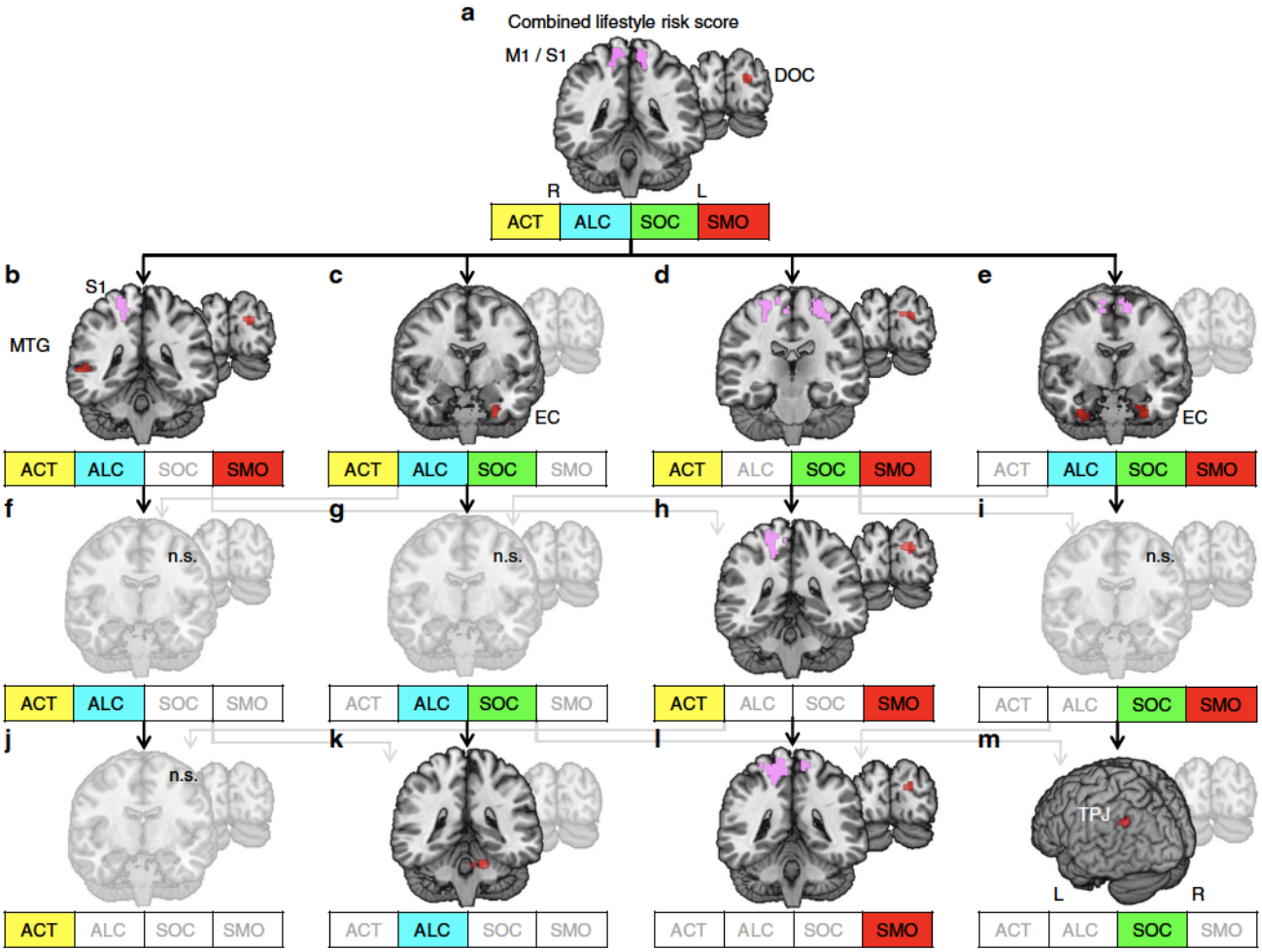

Fig. 3 Lifestyle risk-associated increases in resting-state functional connectivity (RSFC) to the seed in the left dorsal premotor cortex (dPMC). **a**–**m** Abbreviations in the small boxes refer to the same variables as in Fig. 2. Coronar sections show brain regions exhibiting increases in RSFC depicted in red. The recurrent pattern of higher RSFC between the dPMC and the somato-motor cortex is highlighted in pink. Arrows indicate the work flow of the stepwise exclusion. Transparent sections represent not significant (n.s.) results. This analysis included a subsample of the study population ($n = 501$). Results were significant at $\alpha = 0.05$ (cluster level corrected, cluster-forming threshold $\alpha = 0.001$). S1 = primary somatosensory cortex, MTG = middle temporal gyrus, M1 = primary motor cortex, DOC = dorsal occipital cortex, EC = entorhinal cortex, TPJ = temporo-parietal junction, L = left hemisphere, R = right hemisphere

decreases in RSFC of motor networks in smoking alcohol consumers[19]. In the present study, older adults with stronger drinking habits showed lower cortical folding in dPMC and exhibited increased RSFC between dPMC and cerebellum (Fig. 3l). In our study, we found increased alcohol-related RSFC in a fronto-cerebellar network already during rest. As previously described, increases in RSFC may hint at lower cognitive reserve[9] capacity, leaving no room for compensatory increases in brain activity during task performance (compensation-related utilization of neural circuit's hypothesis[29], CRUNCH). Altogether, this hints at impairment of (pre-) motor system organization in older adults with stronger drinking habits. This may also explain why alcohol consumers, compared to controls, need to recruit additional brain regions to perform simple motor tasks[18]. Future studies on the interplay between alcohol consumption and motor networks in older adults may possibly provide new insights into how "normal" alcohol consumption influences the aged brain. Interestingly, the association between physical activity, alcohol consumption, and left dPMC was not as stable as the association between social integration and right vlPFC when additionally correcting for depressive symptomatology and education (Supplementary Figs. 11–13), even though the general association between lifestyle risk and cortical folding did not change (Fig. 6). Hence, depressive symptomatology and education may additionally contribute to this more complex association.

The third core finding was the association between lower combined lifestyle risk and reduced decreases in cortical folding in right vlPFC, mainly driven by enhanced social integration. A post-hoc multiple linear regression further confirmed social integration as the strongest explanatory variable of cortical folding within vlPFC (see Supplementary Methods). Consistent with this finding, larger brain volume was found in more socially engaged older adults[7] and after a social activity intervention for older adults[8]. Several mechanisms might drive this association: Social integration may attenuate age-related neuronal loss[7], leading to greater brain volume, which is generally considered a factor contributing to brain reserve[9,10]. Animal studies on enriched environment including possibilities for physical and social activities, have indeed shown that socially integrated animals exhibited greater neurogenesis and better spatial learning than isolated animals[49]. Similarly, experimentally enlarged social network size in macaques increased GM volume within temporal and prefrontal cortex and concurrently lead to increased functional connectivity[50]. Social integration may also contribute to

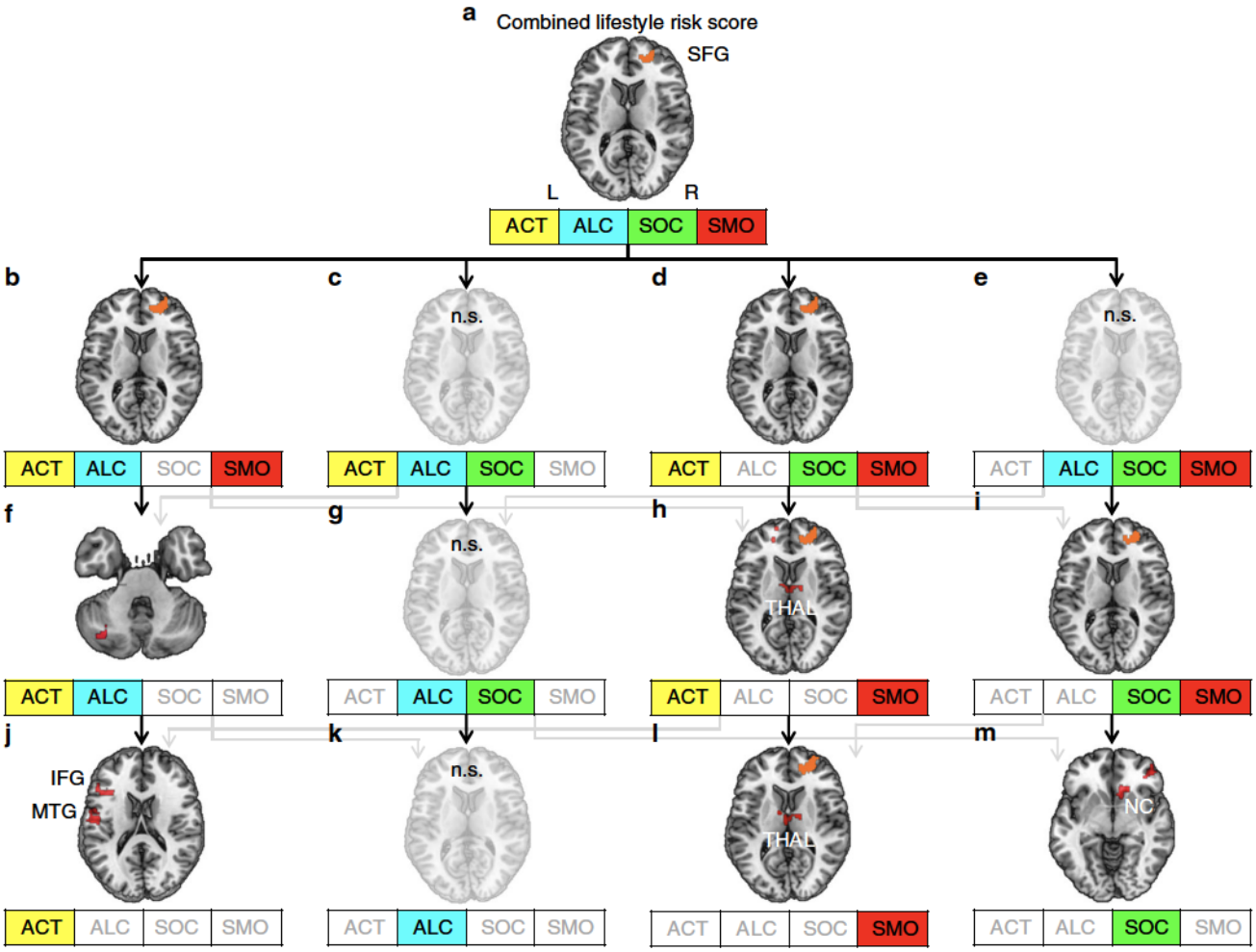

Fig. 4 Lifestyle risk-associated increases in resting-state functional connectivity (RSFC) to the seed in right ventro-lateral prefrontal cortex (vlPFC). **a–m** Transversal sections show brain regions exhibiting increases in RSFC depicted in red. The recurrent pattern of higher RSFC between right vlPFC and superior frontal gyrus is outlined in orange. Arrows indicate the work flow of the stepwise exclusion. This analysis included a subsample of the study population ($n = 501$). For other conventions, see Fig. 2. Results were significant at $\alpha = 0.05$ (cluster level corrected, cluster-forming threshold $\alpha = 0.001$). SFG = superior frontal gyrus, MTG = middle temporal gyrus, THAL = thalamus, NC = caudate nucleus, n.s. = not significant, L = left hemisphere, R = right hemisphere

Table 3 Selected SNPs from GWAS of smoking and alcohol consumption

Trait	Study	SNP	Effect size	*p* overall	Min	Maj	Effect	Gene	CHR
SMO	Liu et al.[33]	rs1051730	−0.08	$1.71E^{-66}$	A	G	G	*CHRNA5/3*	4
		rs6495308	0.07	$5.82E^{-44}$	C	T	T	*CHRNA3*	4
	Thorgeirsson et al.[32]	rs13280604	0.31	$1.3E^{-8}$	G	A	A	*CHRNB3*	
		rs4105144	0.39	$2.2E^{-12}$	T	C	C	*CYP2A6*	8
		rs7937	0.24	$2.4E^{-09}$	C	T	T	*RAB4B*	10
		rs7260329	0.20	$5.5E^{-06}$	A	G	G	*CYP2B6*	10
	Tobacco and Genetics Consortium[33]	rs1329650	−0.37	$5.67E^{-10}$	T	G	G	*LOC100188947*	15
		rs1028936	−0.45	$1.29E^{-09}$	C	A	A	*LOC100188947*	15
		rs3733829	0.33	$1.04E^{-08}$	G	A	G	*EGLN2*	15
ALC	Clarke et al.[35]	rs1260326	−0.03	$1.34E^{-21}$	T	C	T	*GCKR*	2
		rs9841829	0.02	$3.36E^{-10}$	G	T	G	*CADM2*	3
		rs11940694	−0.03	$8.4E^{-19}$	A	G	A	*KLB*	4
		rs145452708	−0.03	$1.15E^{-30}$	C	G	C	*ADH1B/c*	4q23
		rs29001570	−0.03	$9.58E^{-19}$	C	T	C	*ADH5*	4q23
		rs35081954	0.02	$2.14E^{-10}$	CTG	C	CTG	*ADH1c*	4q23
		rs193099203	−0.03	$3.79E^{-25}$	T	C	T	Intergenic	4

SMO: smoking (cigarettes per day), SNP: single-nucleotide polymorphism, GWAS: genome-wide association studies, ALC: alcohol consumption (g/day), Min: minor frequency allele, Maj: major frequency allele, CHR: chromosome

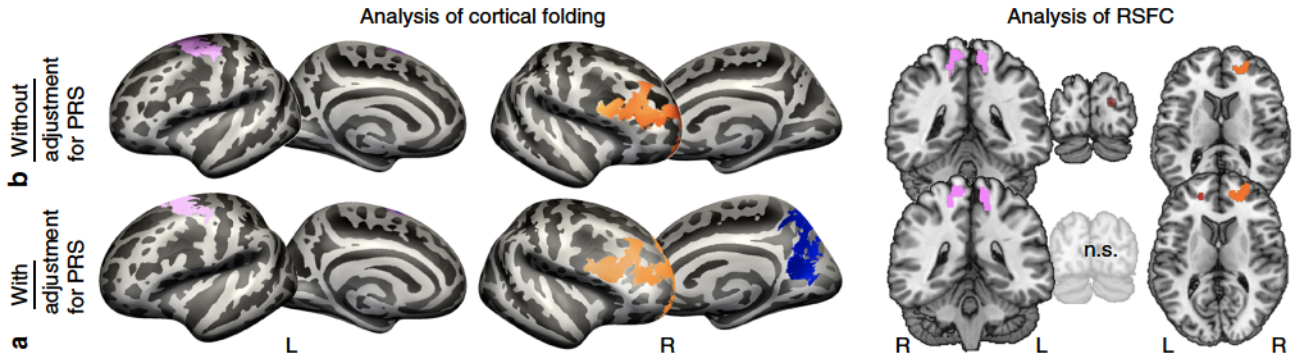

Fig. 5 Comparison of analyses without adjustment for polygenetic risk scores (GRS) and with adjustment for polygenetic risk score (PRS). **a** The lower row shows the results of the analyses of cortical folding and resting-state functional connectivity (RSFC) with adjustment for PRS. **b** The upper row shows the results of the analyses of cortical folding and RSFC with adjustment for PRS. Whereas the general pattern of the results did not change after adjustment for PRS, an additional decrease in cortical folding in right precuneus was observed. n.s. = not significant, L = left hemisphere, R = right hemisphere

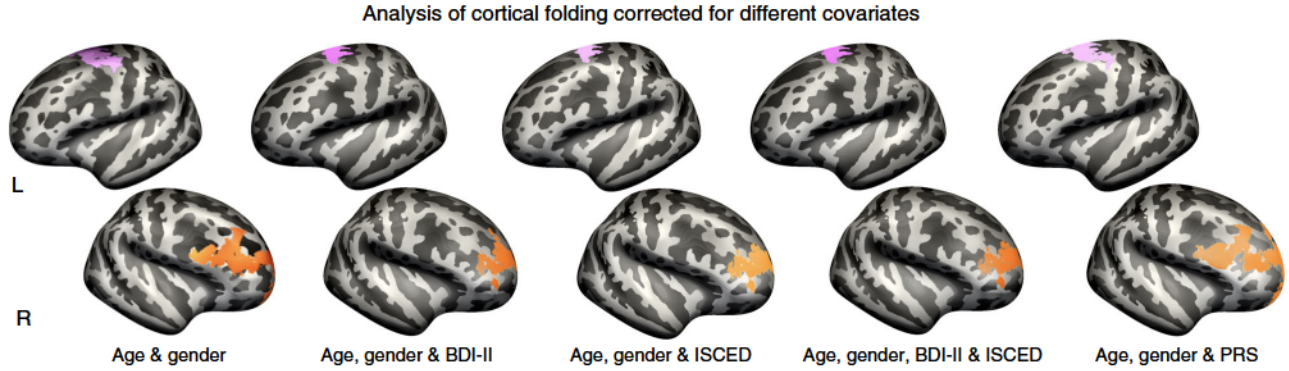

Fig. 6 Analyses of cortical folding with additional adjustment for a set of non-lifestyle covariates. The illustrations show the associations between the combined lifestyle risk score and cortical folding when correcting for a set of non-lifestyle covariates. The additional adjustments did not change the general associations between the combined lifestyle risk and decreased cortical folding within the left dorsal premotor cortex (dPMC) (pink) and the right ventro-lateral prefrontal cortex (vlPFC) (orange). BDI-II = depressive symptomatology, as measured with Beck's depression inventory-II[36], ISCED = educational level as measured with international classification of education[37], PRS = polygenic risk score

brain reserve more indirectly by providing an enriched and cognitively stimulating environment, for example, via cognitive stimulation provided by social activities, better health support, or less depression due to emotional support[1].

In the current study, a negative correlation between combined lifestyle risk and gyrification was found in right vlPFC, which comprises regions associated with executive networks[51], attentional control, decision making, and prediction of future outcomes[52]. All of these abilities play a crucial role in successful participation in social situations, for example, requiring to update information on the dialog partner and the group[53]. Furthermore, social interaction also demands inhibition of inadequate behavior, which in turn is based on goal-directed thinking[52] and prediction of future outcomes to establish long-term, rather than short-term relationships. Similarly, social interactions require emotional self-regulation through top-down control, a process that also has repeatedly been linked to (particularly right) vlPFC[54]. These social activity related abilities pose a high cognitive load and require executive and integration skills. Since these highly complex abilities are precisely subserved by vlPFC, this may explain why the current study found social integration associated with brain structure of the vlPFC, rather than brain structures more typically associated with the social brain. Similar to the activity-induced structural adaptation of brain regions to skill learning and (exercise) training, particularly those brain regions subserving high cognitive, integrative, and emotional load needed for social interactions, may suffer less from age-related decline due to their reoccurring recruitment. This could be referred to as a "use it—or lose it" hypothesis in aging[41], and is of special interest since the PFC is particularly vulnerable to aging[55].

Such activity-induced structural adaptation may also explain the correlation between enhanced social integration and reduced decrease in GM volume in left hippocampus. Socially enriched environmental conditions have been linked to hippocampal neurogenesis in animals[49]. Additionally, the hippocampus is involved in memory and flexible provision of knowledge in complex human social situations[52]. Thus, stimulating environments and memory demands of social activity may slow down its age-related decline. These findings are complemented by a positive correlation between social integration and increased RSFC between dPMC and left temporo-parietal junction (Fig. 3m), a region involved in social cognition and theory of mind[56].

The general pattern of increases in RSFC associated with higher lifestyle risk, though, was mainly driven by smoking—as revealed by the stepwise exclusion approach. Similar to the increases in RSFC associated with alcohol consumption, the current study observed smoking-related increases in RSFC already during rest. As a result of these higher base levels, older adults who smoke may reach their limits faster during an active task possibly performing worse than non-smokers[9,29], as their neural reserve might be exhausted. Therefore, smoking and the here observed associated increase in RSFC may reflect less neural

reserve capacity and less potential for compensation during active tasks. Since such higher base levels are one major hallmark in brain aging[29], they may even hint at accelerated aging in smokers. Other mechanisms that may lead to enhancement of functional networks in the brains of smokers may more resemble addictive mechanisms: Complex alterations have previously been found in executive networks[13], comprising vlPFC and SFG—structures that exhibited higher smoking-associated RSFC also in the current study. These alterations have been suggested to reflect a shift from more endogenous to more exogenous processing of addiction-related cues in smokers[13]. Smoking-related alterations in functional connectivity, however, seem not to be limited to frontal brain networks: Consistent with the altered RSFC between PMC and motor networks in the current study, disruptions in motor networks have previously been observed in smokers[19], together with decreased GM density[12]. Smoking thus seems to be accompanied by a complex pattern of increases and decreases in functional connectivity affecting frontal and motor networks. Importantly, most of the here reported studies investigated smoking in young to middle-aged adults. Within the aged population, increase and dedifferentiation in RSFC seems to be one hallmark[29]. Smoking-related alterations in RSFC may therefore rather shift to systematic increases in RSFC than to decreases. Interestingly, while decreased functional coupling within motor networks has been previously observed in smokers in comparison to non-smokers[19], RSFC amongst smokers was positively correlated with smoking severity. We also investigated smoking in a dose-dependent manner (the more pack years, the more RSFC), which may explain why we only found RSFC increases related to smoking and no systematic decreases (see Supplementary Figs. 1 and 2). Further, we found a systematic relation between smoking and dorsal occipital cortex (dOC, Fig. 3a, c, d, h, i), which is involved, for example, in visuospatial attention[57]. Influences of smoking on visuospatial attention have been circumscribed in terms of smoking facilitating visual attention by enhancing the blood oxygen-level-dependent (BOLD) signal in dorsal visual stream[58] and higher coupling between visual processing regions[19]. These alterations in visual processing during acute nicotine withdrawal may shift towards altered RSFC patterns when abstaining from smoking. The increases were observed in networks related to sensory awareness and attention (dPMC and dOC), which may reflect a more general shift of sensory and attention systems towards addiction-related processing. Importantly, smoking was not related to structural decline in the current study, but was the only variable showing systematic associations to increased RSFC of those regions that showed decreased brain structure.

We hypothesized that genetic susceptibility may modify the relationship between lifestyle and the aged brain. Adjustment for genetic risk revealed an extra effect in the precuneus, indicating enhanced sensitivity of these analyses, while the general result pattern remained unchanged. Thus, genetic susceptibility seems to be a non-negligible, but not a strong contributor when it comes to the relationship between lifestyle and brain aging. Our approach shows that accounting for different concurrent influences may help to identify small effects. In future studies, it would be desirable to also include potential genetic influences of the other two lifestyle variables (social integration and physical activity), for which genetic factors had not been consistently identified at the time of our analysis.

Some further limitations of the current study should be addressed. First, the questionnaire items that were used to measure lifestyle variables concerned different time windows (e.g., physical activity within the last 4 weeks, smoking as number of cigarettes smoked over the whole lifetime). Each lifestyle variable was operationalized to be as representative as possible for the long-term lifestyle behavior of each person[59]. Epidemiological research has shown that assessments specifically regarding defined short time frames (e.g., a month, a week) are more reliable indicators of long-term behavior than self-reports regarding longer time frames (e.g., a whole year[60]). Future studies are warranted to additionally study the influence of changing lifestyle habits.

Additionally, all lifestyle habits were assessed using self-report, which may be influenced by memory effects or social desirability bias. However, self-report measurements have been shown to have high validity and reliability[60] and are thus suitable in such an epidemiological population-based cohort setting.

Our cross-sectional and correlational design makes it impossible to determine causal directionality of effects or to rule out cohort effects. It is, for example, impossible to disentangle whether enhanced social integration leads to stronger cortical folding, or whether higher initial neuronal reserve leads to higher social integration. Further, it might be interesting to also include non-linear or differentially weighted effects when analyzing lifestyle variables individually or in combination. However, based on the current state of research, assumptions regarding a specific weighting of different lifestyle variables would be speculative. Future studies could use simulations to evaluate the potentially non-equal contributions of different lifestyle variables by simulating different weightings and examine their association to brain structure and function. This needs to be evaluated in future studies.

Based on the literature, we assumed the lifestyle behaviors to affect the brain in a certain direction, for example, negative effects of alcohol consumption on the brain. Effects in the opposite direction, depending on the brain region examined, may still occur. Importantly, the post-hoc multiple regressions using extracted cortical folding values confirmed our assumption of effect direction for the examined regions (Supplementary Figs. 8–9). Additionally, although our concept of social integration is widely used in epidemiological studies[22], it covers mostly quantitative, summed-up measures of social network (visits of friends, relatives, or children). According to socio-emotional selectivity theory[61], however, older adults select their companions based on relationship quality, leading to a small quantity of close friends rather than a large number of superficial acquaintances without feeling less emotionally supported. Future research could explore how the quality of social contacts contributes to brain aging.

Besides the known beneficial effects of physical activity[2–5], our results emphasize the significance of low alcohol consumption and high social integration as protective factors in aging. Whereas interventions to preserve brain health based on physical activity are quite common[3], research lacks randomized intervention trials of social integration of older adults, although both might be easily combined[8]. Further, other lifestyle variables such as dietary or sleeping habits should be examined in future studies.

In summary, our results provide insights into the complexity between environmental and genetic factors, brain structure, and functional connectivity of the aged brain. Our newly developed combined approach enabled us to examine this complexity and revealed that older individuals carrying higher lifestyle risk load seem to be at higher risk to suffer from structural brain atrophy. While these variations in structural decline were mainly explained by levels of alcohol consumption, physical activity, or social integration, smoking was the main variable driving the associations between higher lifestyle risk and increased RSFC. The increases in RSFC, though, may further reflect reduced cognitive reserve and accelerated aging related to smoking, as well as addiction-related functional adaptations of the brain. In consequence, a more protective lifestyle may contribute to brain

reserve, that is, the preservation of brain structure, and to cognitive reserve, that is, the more efficient use of functional brain networks. Our study therefore shows that integrative concepts of lifestyle may be a strong instrument for advancing our understanding of risk and protective influences on aging in the general population and in patients suffering from neurodegenerative diseases, as well as for low-cost interventions preserving healthy aging.

Methods

Sample characteristics. Seven hundred and fifteen older adults from the population-based cohort of the 1000BRAINS study[21], recruited from the Heinz Nixdorf Recall study[60], were available for the current study. Two participants had to be excluded from analyses due to incidental findings. Seventy MR data sets could not be used for analyses of cortical characteristics due to poor quality of WM/GM segmentation (see MRI processing), surface reconstruction, or registration. Due to missing values in behavioral data 63 participants had to be excluded. After calculating the combined lifestyle risk scores (see Construction of lifestyle-related risk scores), we excluded another 31 participants as outliers (±3 SD from the mean) to ensure that these extreme values would not bias the overall outcome. Finally, 549 older adults (248 female) were included in the analyses. All participants gave written informed consent in agreement with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the University of Essen, Germany. An overview of demographic data is given in Table 1.

Materials. Lifestyle measures: Lifestyle data were retrieved from the database of the Heinz Nixdorf Recall study[59].

Single lifestyle variables: Alcohol drinking behavior was assessed via a self-report questionnaire asking about average consumption of different beverages (beer, red wine, white wine, spirits, and cocktails) within the last 4 weeks. The proportion of pure alcohol per beverage was then multiplied with the frequency of drinking. Next, all beverages per person were summed up, resulting in the amount of total consumption of pure alcohol in grams per month (g/month).

The degree of lifetime exposure to tobacco smoking was assessed in pack years, calculated by multiplying the years of smoking with the self-reported number of smoked cigarettes per day (CPD).

Social integration was assessed using an adapted version of the social integration index developed by Berkman[22]. The present social integration index comprised three domains: The first domain represented "marital status." Married or cohabitating participants were scored a 2; single, never married, widowed, or divorced participants were scored a 0. The second domain was "close ties" and represented a sum score of the number of children, close relatives, and friends reported by the participants. The third domain was "membership in organizations". This domain represented a sum score of the number of organizations participants were members in and participated in at least once a month. Organizations included were: sport clubs, regional clubs, hunting clubs, choirs, theater clubs, music clubs, occupational or labor unions, political clubs or parties, congregations, and self-help groups. The scores of all three domains were summed up into the social integration score.

Physical effort was measured using the metabolic equivalent of task (MET[21]), a measurement for the energy expenditure of a given activity compared to rest. The compendium of physical activities[21] provides a mean energy expenditure value per hour of each activity. It is based on several studies measuring energy expenditure of heterogeneous activities and lists activities, which include willful physical exercise, but also several physically stressful activities not intending exercise, like cleaning or home carpentry. Participants were asked to report up to four different sportive (e.g., running) and up to four different physical activities (e.g., gardening), carried out within the last month. Based on the MET values assigned to the activities listed in the compendium, MET values were assigned to each of the activities reported by the participants and multiplied with the duration in hours (per month). Finally, a sum score of all activities was built.

Construction of the combined lifestyle risk score: Based on the literature, we classified cigarette smoking[11,13,19] and alcohol consumption[15–18] as risk variables for brain atrophy. Social integration[1,6,7,50] and physical activity[2–5] in contrast were classified as protective variables. Raw data on each single lifestyle variable were transformed into z-scores to obtain uniform measure units. The first aim was to obtain a risk score that indicated higher risk in higher values. Therefore, we reversed signs of the protective variables (social integration and physical activity), such that negative values reflected higher protection. The second aim was to obtain a risk score where a value of zero would indicate a mathematical balance of negative and protective behavior. Hence, we applied an additional linear transformation on the z-transformed lifestyle variables before summing them up: The protective variables were linearly transformed into negative values (by subtracting the maximum value from each value). Analogously, the risk variables were linearly transformed into positive values (by adding the minimum value to each value). Hence, all values for risk variables were positive. Finally, the linearly transformed values of all lifestyle variables were summed up into one combined lifestyle risk score, which indicated protection as negative values and risk as positive values.

Stepwise exclusion of single lifestyle variables: To examine the contributions of each lifestyle variable to the combined lifestyle risk score, we used a stepwise approach to exclude single lifestyle variables from the risk score. This resulted in four risk score models integrating three single lifestyle variables, and another four risk score models including only two single variables (Supplementary Table 1). The first possible exclusions are exemplarily described here: For example, we first excluded social integration from the combined lifestyle risk score, resulting in a model including physical activity, alcohol consumption, and smoking (Fig. 2b). In the next step, we additionally excluded smoking from the risk score, which now included only physical activity and alcohol intake (Fig. 2f).

Genetic data: Lymphocyte DNA from participants was isolated from ethylenediaminetetraacetic acid-coagulated venous blood by a Chemagic Magnetic Separation Module I (Chemagen, Baesweiler, Germany). DNA samples were genome-wide genotyped using Infinium assays (Illumina, San Diego, CA, USA) for BeadChips HumanOmniExpress, HumanOmni1-Quad, or HumanCoreExome. Quality control of raw genotype data comprised an exclusion of single-nucleotide polymorphisms (SNPs) (deviation from Hardy–Weinberg equilibrium (HWE): $p \leq 1 \times 10^{-4}$; genotyping call rate: ≤95%; minor allele frequency: MAF ≤ 3%) and participants (SNP-based principal component analysis: >8 SD from the mean in one of the first ten principal components; mismatch between self-reported and X-chromosomal-derived gender). To increase the number of available SNPs and decrease the number of missing genotype calls, dosage data were generated for all participants using IMPUTE (version 2.3.1) as tool and phased haplotypes from The 1000Genomes Project (ALL macGT1 reference panel, phase 1, release 3, March 2012) as reference.

The next step was the identification of phenotypically relevant SNPs that would be included into the PRS. To further test whether genetic factors modify the association between lifestyle risk and variations in cortical folding as well as functional connectivity of older adults, we reviewed recent literature on common genetic factors for lifestyle risk in European ancestry populations. Study selection was based on the NHGRI-EBI Catalog of published GWAS as of 23th May 2018, provided by the National Human Genome Research Institute (NHGRI) of the United Kingdom and the European Bioinformatics Institute (EMBL-EBI) available at www.ebi.ac.uk/gwas.

First, we identified studies that examined quantitative traits matching those we investigated in the current study, namely smoking and alcohol consumption as risk variables, and physical activity and social integration as protective variables. Concerning physical activity and social integration, there were no GWAS examining a matching phenotype in European ancestry populations. Further, there were no studies available investigating the genetic basis of lifetime smoking operationalized as pack years, but as in CPD, which was the closely related phenotype found. Concerning the risk variables of alcohol consumption, due to the population-based nature of our study, we found studies investigating alcohol consumption in population-based cohorts and not in clinical cohorts. Secondly, from those studies investigating one of the two phenotypes, we chose only those that found significant association at genome-wide significance (p value $<5 \times 10^{-8}$). Additionally, we chose only those for which results had been replicated in a second sample of European ancestry. By doing so, we identified three major studies of interest for the phenotype "smoking" (in CPD)[31–33], and one study for "alcohol consumption"[34]. Within the three publications relevant to smoking, the SNP rs1051730 was identified as the top SNP for smoking quantity in CPD; ten additional SNPs also showed significant association with CDP. For alcohol, the number of candidate SNPs was 14. From the overall set of 25 SNPs, we included those meeting the following conditions: Small difference of MAF (≤3%) between the reference population (1000Genomes CEU) and the current 1000BRAINS study, no strong linkage disequilibrium (<0.8) to other selected SNPs of this phenotype, and high imputation quality (median info score = 99.5%), as well as no deviation from HWE ($p > 0.05$) in the subsample of 1000BRAINS. Finally, this resulted in nine SNPs for the phenotype "smoking" and seven SNPs regarding the phenotype "alcohol consumption" (Table 3). In the next step, genetic information provided by the quality controlled 16 SNPs was transformed into a PRS.

PRS was calculated using the weighted allelic scoring routine by PLINK (v1.9). In particular, effect alleles and effect sizes (R^2) were used as defined by the original studies (Table 3). The individual GRS value was then calculated as the mean of the summarized effects in an SNP set that is for the SNP set of alcohol consumption and for the SNP set of CPD. This resulted in individualized values of combined genetic risk for smoking and alcohol consumption for each participant of the current study.

Acquisition and processing of structural MR images: T1-weighted anatomical three-dimensional (3D) images were collected with a 3 T Tim-TRIO MR scanner (Siemens Medical System, Erlangen, Germany). The following scan parameters were used: repetition time = 2.25 s, echo time = 3.03 ms, inversion time = 900 ms, field of view = 256 × 256mm2, flip angle = 9°, voxel resolution = 1 × 1 × 1 mm^3, 176 axial slices. A detailed description of the 1000BRAINS study protocol can be found in Caspers et al.[20].

3D images were processed using the automated surface-based pipeline of the FreeSurfer Software package (version 5.3.0, Athinoula A. Martinos Center for Biomedical Imaging). A detailed description of all steps included in the streamline was provided by Dale et al.[27] and Fischl et al.[62] and in the FreeSurfer documentation at http://surfer.nmr.mgh.harvard.edu. Processing includes motion correction, intensity normalization, removing of extra-cerebral voxels (non-brain

tissue) using SPM12 (The Wellcome Dept. of Imaging Neuroscience, London; www.fil.ion.ucl.ac.uk/spm), spatial normalization, volumetric segmentation[62], and cortical surface reconstruction[27,63]. To reconstruct the cortical surface, first the so-called white surface is generated at the interface of WM and GM. Then, the pial surface is created at the interface between GM and the cerebrospinal fluid (CSF). The final mesh model of the pial surface is tessellated into triangles and consists of about 120,000 vertices per hemisphere with an average surface area of 0.5 mm^2.

Vertex-wise LGI was calculated using the surface-based approach as implemented in FreeSurfer[26], which is the 3D extension of the work by Zilles et al.[23]: First, an outer hull of the pial surface (outer smoothed surface) is created. This is obtained by a morphological closing operation and follows the exposed (visible) surface along the gyri but does not reach into the segments buried within the sulci. Then, for each 100th vertex on the outer surface the ratio between the area on the pial surface and the corresponding area on the outer smoothed surface (both defined as a circular region with 25 mm radius around the vertex[26]) was calculated. Using a weighted average, finally local gyrification indices were calculated at each vertex.

CT was also extracted using FreeSurfer[28]. First, the boundary between GM and WM was identified. CT was then measured by finding the shortest distance between a given point on the reconstructed pial surface and the GM/WM boundary surface and vice versa[28]. Finally, averaging both values resulted in about 150,000 CT values per hemisphere.

Subcortical structures were segmented using the automatic segmentation provided by FreeSurfer[63]. Here, subcortical GM is automatically segmented into different volumes. Then, a neuroanatomical label is assigned to each volume based on probabilistic information estimated from a manually labeled data set.

Acquisition and processing of functional MR images: We investigated functional connectivity as measured by resting-state functional MRI. BOLD signal time series were acquired using gradient-echo echo planar imaging (EPI) pulse sequences (300 images, TR 2.2 s, 36 axial slices[20]). The first four images were discarded and the remaining images were processed using SPM12. Head motion correction was done by affine registration using a two-pass procedure registering all images to the individual mean of the respective participant. This mean image was spatially normalized to MNI152 using the unified segmentation procedure[64]. We then applied the resulting deformation to the individual EPI volumes. Residual anatomical variations were compensated for by smoothing with a Gaussian kernel of 5 mm full width at half maximum, which additionally approximates requirements of normal distribution of the residuals for Gaussian random field inference to correct for multiple comparisons[65]. Variance that could be explained by first- or second-order effects of the following covariates was removed for each voxel's time series: (i) the six motion parameters derived from the image realignment; (ii) their first derivative; (iii) mean GM and WM as well as CSF signal intensity. The first three covariates (i–iii) entered the model as first- and second-order terms, which was shown to increase specificity and sensitivity of the FC analyses[65]. Finally, the data were band pass filtered between 0.01 and 0.08 Hz to keep only those frequencies most relevant for studying neural signal fluctuations in the brain[66].

For analyses of differences in RSFC, the maximum vertices of the structurally localized regions in dPMC and vlPFC were determined and transformed into MNI space. A sphere of 5 mm was then drawn around the coordinates and used as seed volumes of interest (VOIs). The time courses of these VOIs were extracted for each participant as the first eigenvariate of all GM voxels according to segmentation within the respective VOI, mainly since this eigenvariate is robust against inter-individual variance in anatomical localization and distribution of voxels mainly driving the functionally relevant and representative VOI time series used for FC analysis to a certain extent[65].

Statistical analyses of surface-based measures: All statistical analyses regarding the association between combined lifestyle risk and local gyrification, as well as CT were carried out using Qdec, a graphical user interface implemented in the FreeSurfer software package (http://surfer.nmr.mgh.harvard.edu) and IBM SPSS Statistics 20.0. General linear models (GLMs), as implemented in Qdec, were used to evaluate the association between lifestyle risk and vertex-wise LGI, as well as CT, respectively. Qdec allows whole-brain analyses of surface morphology, thus no specific brain region needs to be defined a priori to test for an effect of behavioral data. Instead, each vertex on the cortical surface is tested for an association between the parameter of interest (cortical folding, CT) and the behavior of interest (lifestyle). Here, we calculated a linear regression using the risk score as explanatory variable and LGI and CT as dependent variables. Gender and age were used as covariates that were statistically controlled for. Two-tailed F tests were used to test whether lifestyle risk would be associated with higher or lower LGI and CT. Corrections for multiple comparisons were performed by testing results against a simulated null distribution of maximum cluster size across 10,000 iterations using Monte Carlo Z simulation as implemented in Qdec[67] using a cluster-forming threshold of $\alpha = 0.05$. This analysis was then recalculated with the inclusion of the PRS as a third covariate, as well as with depressive symptomatology, as measured with the BDI-II[36], ISCED[37].

To specifically describe the association between combined lifestyle risk and variations in local cortical folding, we extracted LGI values post hoc at the cluster's maximum vertex of those regions that showed variations in cortical folding associated with combined lifestyle risk in the main analyses. To estimate the contributions of the single lifestyle variables to cortical folding of these regions with a different technique than the stepwise exclusion procedure, these LGI values were imported into IBM SPSS Statistics 20.0 and submitted as dependent variable into a multiple linear regression using the remove method. All four single lifestyle variables, age, and gender were submitted as explanatory variables to the first model. Then, single lifestyle variables were removed step by step from this model, while changes in F and R^2 were measured for each step. When submitting the extracted cortical folding values of the dPMC as dependent variable to the first model, we excluded physical activity in the first step and alcohol consumption in the second. This was done because the stepwise exclusion procedure in our main analysis hinted at these two variables as the main contributors to cortical folding in this region. Third, social integration and fourth, pack years were excluded. Regarding the vlPFC, the stepwise exclusion procedure hinted at social integration as the main contributor to differences in cortical folding. Therefore, we excluded social integration in the first step when using the extracted cortical folding values of the vlPFC as dependent variable. Second physical activity, third alcohol consumption, and fourth pack years were excluded.

We then applied our stepwise exclusion procedure. All models derived from the stepwise exclusion procedure were tested in the same manner as the combined lifestyle risk score using linear regression models with gender and age as covariates that were statistically controlled for. Again, all results were corrected for multiple comparisons using Monte Carlo Z simulation with $\alpha = 0.05$ and a cluster-wise p-value <0.01.

We examined the effects of single lifestyle variables on LGI and CT, using a series of GLMs: For each linear model, z-transformed data of one lifestyle variable (alcohol consumption, smoking, social integration, physical activity) was taken as the predictor. Comparable to the overall lifestyle risk score analysis, we included age and gender as covariates that were statistically controlled for and tested two-sided F tests. These GLMs were corrected for multiple comparisons by testing results against permuted data in 10,000 iterations at $\alpha < 0.05$ and a cluster-wise p-value <0.01, as implemented in FreeSurfer. We chose to use permutation testing, because data of single lifestyle variables showed a highly skewed distribution and permutation-based inference as an exact non-parametric statistical test is applicable to skewed data[68].

Statistical analyses of subcortical structures: To examine the association between GM volume of subcortical structures and lifestyle, we imported individual volumes of 20 subcortical structures into SPSS and calculated partial Spearman's correlations between the combined lifestyle risk score, the different risk score models and single lifestyle variables, controlling for age, gender, and total intracranial as covariates of non-interest. We additionally applied a post-hoc Bonferroni correction using a threshold of $\alpha_{corr} = 0.05/20 = 0.0025$ with 20 subcortical structures (Table 2) tested.

Statistical analyses of RSFC: To assess the association between combined lifestyle risk and RSFC we computed linear (Pearson's) correlation coefficients between the extracted time courses of the seed regions derived from the analysis of differences in local brain structure, namely the dPMC and the vlPFC, and the time series of all other GM voxels in the brain. The voxel-wise correlation coefficients were then transformed into Fisher's Z-scores, and tested for consistency across subjects by a second-level multivariate analysis of variance (including appropriate non-sphericity correction) with the combined lifestyle risk score as explanatory variable (linear regression model). Results are reported at $\alpha = 0.05$ and tested two-sided for increases, as well as decreases in RSFC (cluster level corrected, cluster-forming threshold $\alpha = 0.001$).

Comparable to the surface-based analyses all models derived from the stepwise exclusion procedure were tested for associations between the two seeds and RSFC of all GM voxels in the same manner as the combined lifestyle risk score using linear regression models while statistically controlling for gender and age as covariates. Again, all results were thresholded at an uncorrected α value of $\alpha = 0.001$ (cluster-forming threshold) and corrected at the cluster level at $\alpha = 0.05$.

The same analyses conducted for the combined lifestyle risk score, as well as the different risk score models derived from the stepwise exclusion procedure, were also applied to investigate the association between single lifestyle variables and RSFC of the two seeds. Here, always one single lifestyle variables was investigated at one time.

Additional adjustment for polygenic risk: To see whether genetic influences would modulate the relation between combined lifestyle risk scores and the aging brain, we calculated the analyses of gyrification, CT and the analyses of RSFC twice: (i) Without further adjustment for GRS and (ii) with additional adjustment for GRS. Please note that all analyses were corrected for age and gender.

Sensitivity analyses: Subsequent to the main analysis, we conducted additional sensitivity analyses as often used in epidemiological research to confirm our results. From each single lifestyle variable we calculated residuals, corrected for the three other single lifestyle variables: for example, we calculated residuals for social integration by correcting for physical activity, alcohol consumption, and smoking using partial correlations. The purpose was to clean each lifestyle variable from any variance introduced by the other three lifestyle variables and to test whether the results of the main analyses could be replicated. We then used these residuals to again calculate the combined lifestyle risk score. Further, we repeated the stepwise exclusion in the same manner as in the main analyses.

Anatomical allocation of significant findings: Significant clusters resulting from the surface-based as well as the RSFC analysis were anatomically interpreted using the JuBrain Cytoarchitectonic Atlas[69]. Concerning the surface-based analysis, we

converted coordinates of significant surface-based clusters from Talairach to MNI space using the transformation tool "mri_surf2vol" as provided by FreeSurfer. Concerning the RSFC analysis, the thresholded statistical parametric maps resulting from the analyses on the different risk scores were used. Overlap between significant clusters and cytoarchitectonically defined areas was determined using the SPM Anatomy toolbox 2.2c[70] available at http://www.fz-juelich.de/inm/inm1/DE/Forschung/_docs/SPMAnatomyToolbox/SPMAnatomyToolbox_node.html. This was done using SPM12 (The Wellcome Dept. of Imaging Neuroscience, London; www.fil.ion.ucl.ac.uk/spm) within the environment of Matlab (The MathWorks Inc., Natick, MA, USA). Localization of significant clusters is therefore given on a macroanatomical and a cytoarchitectonic level where available (see Supplementary Tables 2–12).

Data availability

The data sets generated and/or analyzed during the current study will be made available from the corresponding author to other scientists on request in anonymized format and according to data protection policy in the ethics agreement.

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Author contributions

N.B. processed all data, conducted all analyses, interpreted the results, and wrote the manuscript. C.J. supported all aspects of data processing and analysis in terms of methods advice and neuropsychological expertise. F.H. and S.B.E. contributed to processing of functional MRI data. T.W.M. and S.Ci. co-designed the genetic analyses and provided genetic risk score data. S.M., K.A. and S.Ca. conceived and designed the study. S.M. provided advise on epidemiological data interpretation. S.B.E. critically advised and contributed to methodological discussions and data interpretation. K.A. and K.Z. advised on interpretation of the study findings, with particular focus on neuroanatomy and aging. S.Ca. and U.B. supervised the whole study. U.B. provided advise on statistical methodology, as well as data interpretation with regard to psychological relevance. N.B., C.J. and S.Ca. wrote the paper. T.W.M., S.Ci., K.A., K.Z., S.M., S.B.E., F.H. and U.B. critically revised the paper.

Additional information

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Supplementary Information

Bittner et al. "Combining lifestyle risks to disentangle brain structure and functional connectivity differences in older adults"

Supplementary Methods

Associations between lifestyle risk and cortical folding: Post-hoc multiple linear regression within IBM SPSS Statistics 20.0.

Associations between lifestyle risk and cortical folding. Higher combined lifestyle risk was associated with lower cortical folding in two distinct cortical areas (Figure 2a): First, left dorsal premotor cortex (dPMC, $p = 0.0001$) and second, ventro-lateral prefrontal cortex (vlPFC, $p = 0.0001$) extending from the frontal pole to the middle frontal sulcus and to the posterior portions of the inferior frontal gyrus and sulcus. The stepwise exclusion approach hinted at alcohol consumption and physical activity being the driving variables behind this association as it disappeared when excluding both, alcohol consumption and physical activity (Figure 2i) from the risk score models.

To confirm this, we extracted cortical folding values within the dPMC and imported them to IBM SPSS Statistics 20.0 and submitted them as dependent variable in a multiple linear regression using the "remove" method. All four single lifestyle variables, age, and gender were submitted as explanatory variables to the first model. We used the remove method such that removing specific variables would reveal the individual contribution to the specific pattern, while change in F and r^2 for each step was measured.

Regarding the extracted cortical folding of the dPMC, the first model explained 2.9% of the variance in cortical folding of the dPMC, $F\ (1,542) = 3.69$, $R^2 = 0.029$, $p = 0.0013$. Excluding physical activity reduced the explained variance to 2% and resulted in a significantly lower model fit, $F(1,542) = 3.37$, R2 = 0.02, F-change = 5.64, $p = 0.018$. Excluding alcohol consumption in the second step did not result in a significantly different model fit ($R^2 = 0.017$, F-change = 2.63, $p = 0.106$), while

the overall model was still significant ($F(1,543) = 3.42$, $p = 0.009$). In the next step social integration was removed, which didn´t lead to a significantly different model fit ($R^2 = 0.015$, $p = 0.133$, F-change = 2.27), while the model was not significant anymore ($F(1,544) = 3.79$, $p = 0.103$). When last excluding pack years, the model explained significantly less variance ($R^2 = 0.008$, $p = 0.027$, F-change = 4.95), but was significant ($F(1,545) = 3.19$, $p = 0.042$). Hence, physical activity indeed explained the greatest amount of variance amongst the lifestyle variables as suggested by the stepwise exclusion procedure in the main analysis. Here, exclusion of alcohol consumption did not change the amount of explained variance significantly in contrast to the stepwise exclusion procedure.

Submitting the extracted cortical folding values of the vlPFC as dependent variable, the first model explained 3.1%, $F(1,542) = 3.95$ ($R^2 = 0.031$, $p = 0.0007$). Excluding social integration reduced the explained variance to 2% and resulted in a significantly lower model fit ($F(1,542) = 3.25$ ($R^2 =$ 0.02, F-change = 7.26, $p = 0.007$). Excluding physical activity in the second step did not result in a significantly lower model fit ($R^2 = 0.018$, F-change = 1.996, $p = 0.158$,), while the overall model was still significant ($F(1,543) = 3.555$, $p = 0.007$). In the next step, alcohol consumption was removed, which didn´t lead to a significantly different model fit fit ($R^2 = 0.02$, $p = 0.835$, F-change = 0.044), while the model was still significant, $F(1,544) = 4.733$, p = 0.003). When last excluding pack years, the model explained significantly less variance ($R^2 = 0.013$, $p = 0.024$, F-change = 5.16). In summary, this multiple linear regression revealed social integration as the most contributing variable to the differences in cortical folding in vlPFC. Beta weights for each single lifestyle variable as estimated for the first model including sex, age, and all four single lifestyle variables are shown in Supplementary Figure 9.

Associations between lifestyle risk and cortical folding: Additional systematic reductions in cortical folding observed in the stepwise exclusion procedure. The stepwise exclusion models yielded additional systematic reductions in cortical folding (Fig. 2, Suppl. Table 2): Decreased cortical folding in right dPMC, expanding to primary motor cortex, was associated with higher lifestyle risk in the models that included a combination from physical activity, alcohol consumption, or social integration (Fig. 2c, 2f, 2g). Decreased cortical folding in left frontal pole was found for the risk score models that included different combinations of social integration (Fig. 2d, 2g, 2i).

Sensitivity analyses

As sensitivity analysis, we calculated residuals from each single lifestyle variable, corrected for the three other single lifestyle variables (see methods) to clean each lifestyle variable from any variance influenced by the other three lifestyle variables. We calculated the combined lifestyle risk score from these residuals again and repeated the stepwise exclusion similar to the main analyses for replication purposes. Results of the sensitivity analyses reproduced almost entirely the result pattern of the main analyses of the association combined lifestyle risk, cortical folding and RSFC. An additional association was only found between the combined lifestyle risk score and cortical folding in the right dorsal premotor cortex (dPMC, Suppl. Fig. 6a). Compared to the main analyses, the clusters found in the right homologue of the dPMC were larger and expanding more into posterior parts of the precentral gyrus (Suppl. Fig. 6c, f, g). Exact anatomical localization is given in suppl. Table 10. Regarding RSFC, we still found the same pattern in the results: Risk score models including smoking showed higher RSFC between the dPMC and the sensorimotor cortex

and between the vlPFC and the superior frontal gyrus, respectively. The sensitivity analyses, though, revealed additional significant associations: The risk score model composed of physical activity, alcohol consumption and social integration, as well as the risk score model including alcohol consumption and social integration, showed higher RSFC between the dPMC and the left hippocampus (Suppl. Fig. 7c & g). Further, alcohol consumption as a single variable showed additional associations between the dPMC and large clusters in the inferior temporal lobe and several subcortical nuclei (Figure 7k). Compared to the main analysis, the combined lifestyle risk score showed no significant association to increases in RSFC of the vlPFC. Still, the main pattern of risk score models including smoking being significantly associated to increased RSFC of the vlPFC was found (Suppl. Fig. 8).

Exact anatomical localization is given in suppl. Table 11 and Table 12.

Associations between lifestyle risk and cortical folding: Additional adjustment for non-lifestyle variables. Additionally, adding BDI-II[1], ISCED[2], or both, respectively, as covariates did not change the general association between combined lifestyle risk and cortical folding (Figure 6). However, in the stepwise exclusion approach some of the risk score models did not reach significance - particularly those risk score models, which were associated with left dPMC, while those associated with right vlPFC remained largely unaltered when applying different sets of covariates (Suppl. Fig. 11 – 13). It is particularly interesting that this association with social integration and right VLPFC survived even if depressive symptomatology is now regressed out. This further supports the strong association which was already consistently found in our original analysis. Contrarily, the association to left dPMC was not that stable, as already evident from our

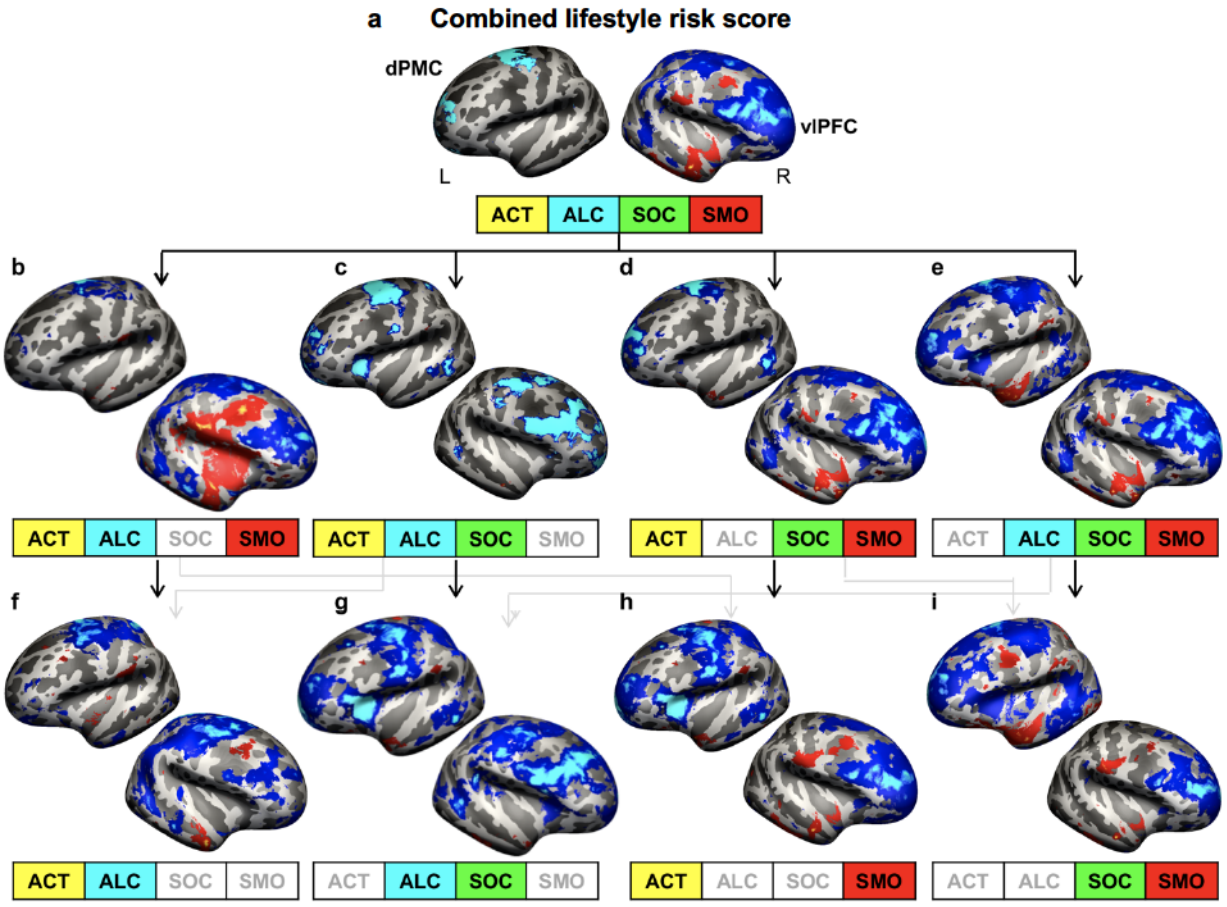
original analysis. Since it was only found in risk score models including both alcohol consumption and physical activity, depressive symptomatology seemed to additionally contribute to this more complex association.

As there were only changes within the extend, but not the location of the brain regions associated to lifestyle risk when additionally correcting for depressive symptomatology and / or depressive symptomatology, respectively, we did not provide additional Tables presenting the associated cytoarchitectonically defined areas.

Supplementary analysis of associations between lifestyle risk and cortical thickness: To complement our analysis of lifestyle risk and cortical folding, we performed supplementary analyses of an association between combined lifestyle risk and vertex-wise cortical thickness as an additional dimension in surface-based analyses[3].

Within the analysis of cortical thickness, we again found a reoccurring association between those risk score models integrating alcohol consumption and physical activity and the left dPMC (Suppl. Fig. 14a, b, c, e & f). Further, risk score models including alcohol consumption, and / or smoking were associated with decreased CT in left inferior and superior parietal lobule. Uncorrected results can be found in Suppl. Fig. 15 & 16. No association between lifestyle risk and CT of right vlPFC could be found. It might thus be assumed that the so-far strong association between lifestyle risk and brain structure and right vlPFC, which is also highly robust against confounders, might be attributable to other mechanisms involving curvature and cortical folding as compared to the effects on CT.

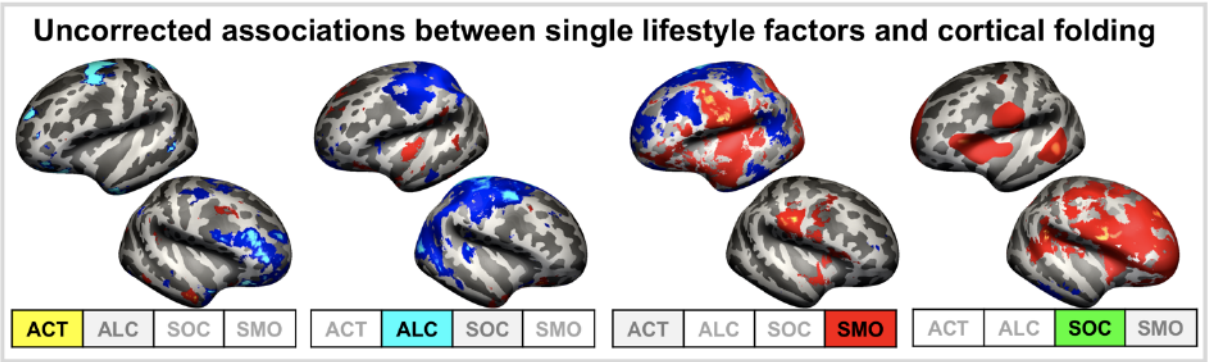
Supplementary Figures



Supplementary Figure 1: Brain regions showing alterations in cortical folding associated with lifestyle risk without correction for multiple comparisons.

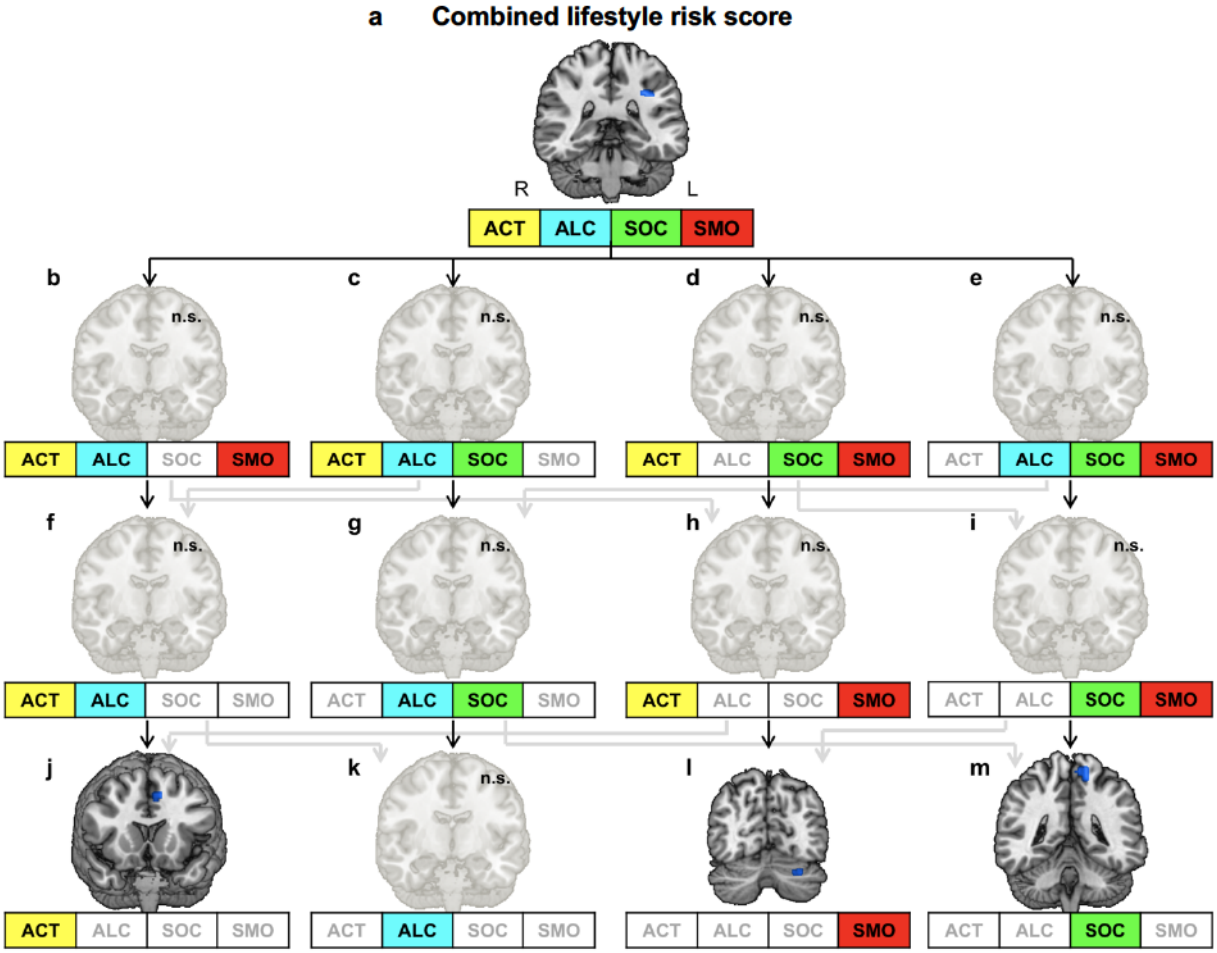
Associations between different risk score models and cortical folding without any correction for multiple comparisons depicted on the inflated surfaces of the fsaverage brain. Red colours indicate a positive association, while blue colours indicate a negative association between lifestyle risk and cortical folding.

Abbreviations: ACT = physical activity, ALC = alcohol consumption, SOC = social integration, SMO = pack years of smoking, L = left hemisphere, R = right hemisphere.



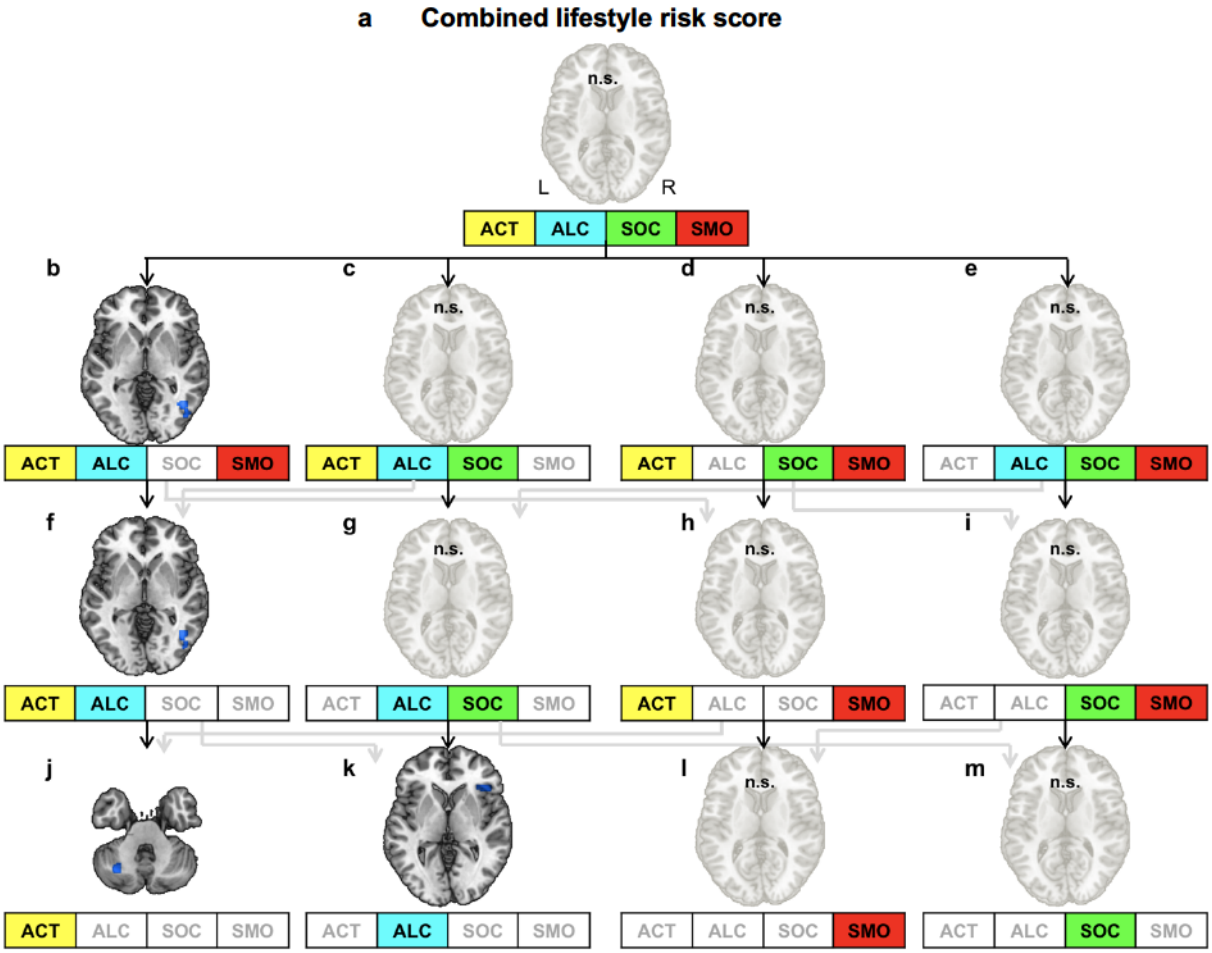
Supplementary Figure 2: Brain regions showing alterations in cortical folding associated with all four single lifestyle variables without correction for multiple comparisons.

Associations between single lifestyle variables and cortical folding without any correction for multiple comparisons depicted on the inflated surfaces of the fsaverage brain. For further conventions and abbreviations please see Suppl. Fig. 1.

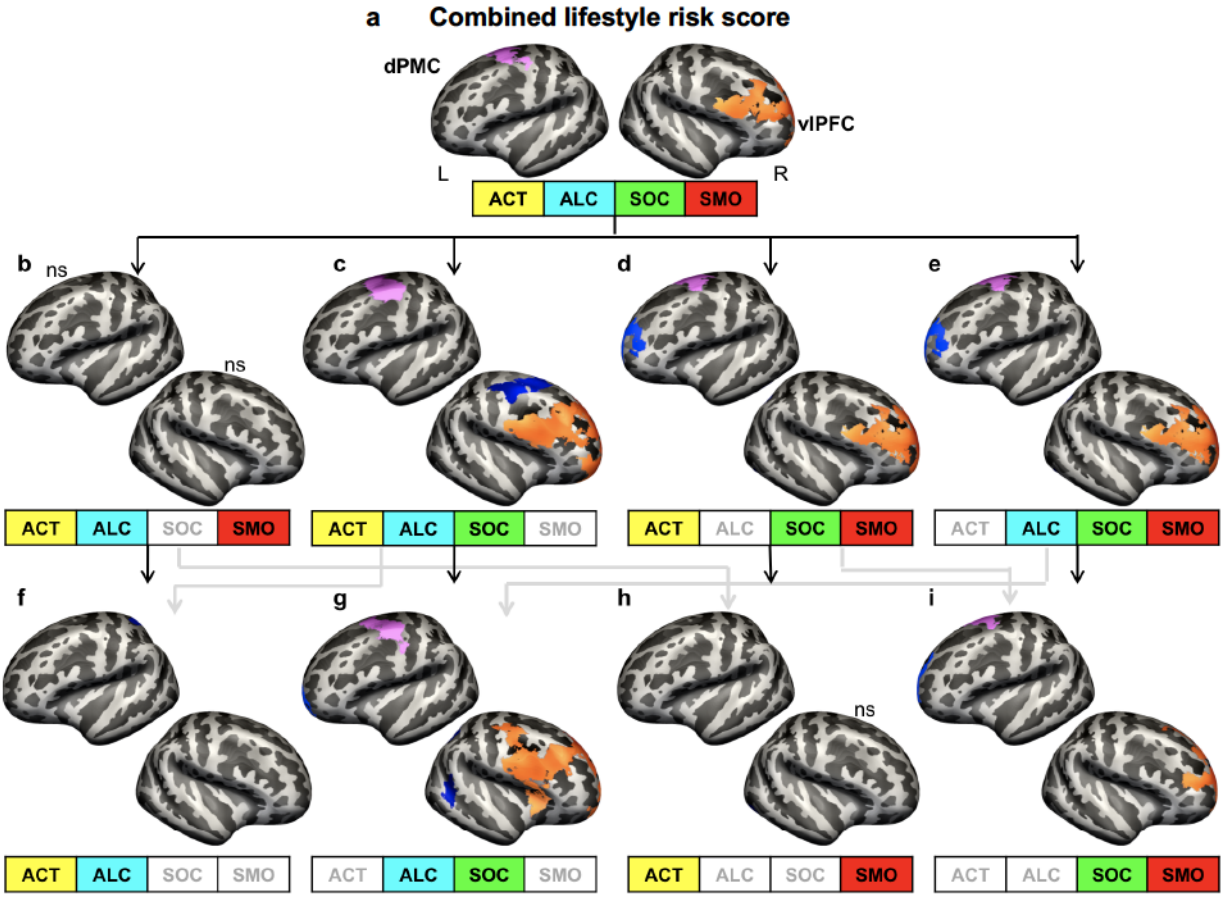


Supplementary Figure 3: Brain regions showing a lifestyle risk associated decrease in RSFC to the seed in the left dPMC.

Abbreviations in the small boxes refer to the same meaning as in Figure 2. Coronar sections show decreases in RSFC depicted in blue. n.s. = not significant, dPMC = dorsal PMC, vlPFC = ventro-lateral prefrontal cortex.

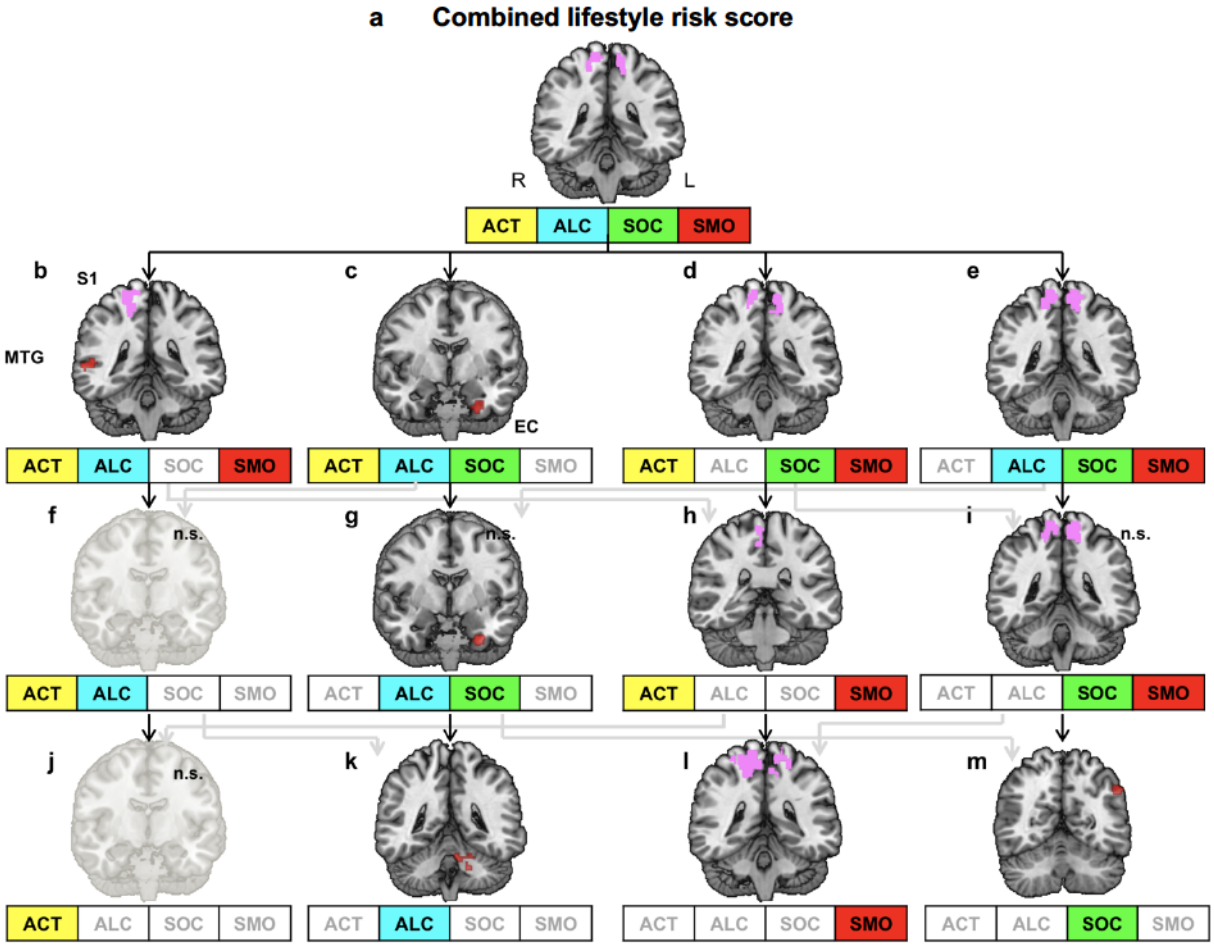


Supplementary Figure 4: Brain regions showing a lifestyle risk associated decrease in RSFC to the seed in the right vlPFC. Abbreviations in the small boxes refer to the same meaning as in Figure 2. Transversal sections show decreases in RSFC depicted in blue. n.s. = not significant, dPMC = dorsal PMC, vlPFC = ventro-lateral prefrontal cortex.



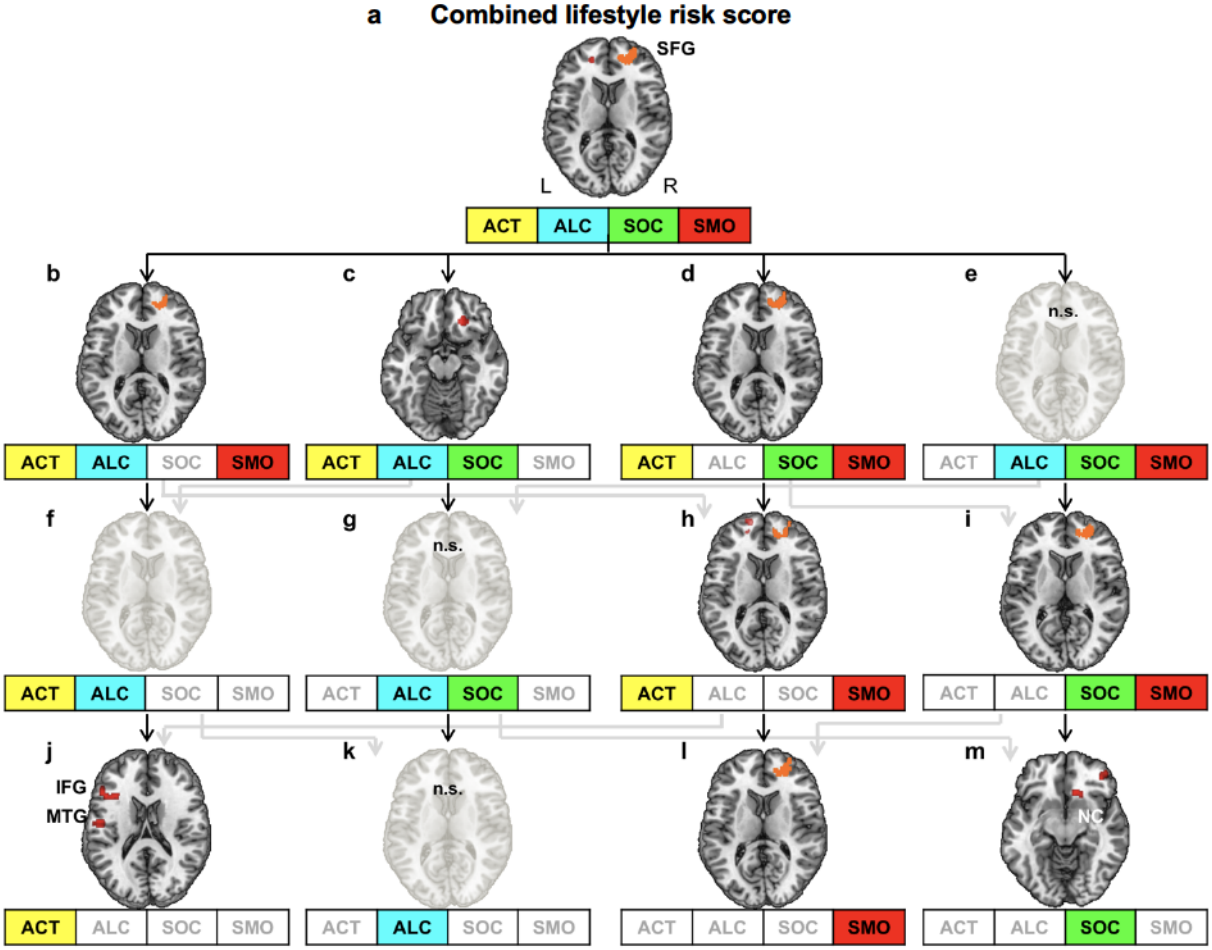
Supplementary Figure 5: Brain regions showing alterations in cortical folding associated with lifestyle risk when additionally adjusting for polygenic risk.

Associations between different risk score models and cortical folding when additionally adjusting for genetic risk as measured with the polygenic risk score (PRS). All results are depicted on the inflated surfaces of fsaverage. The recurrent associations between higher lifestyle risk and reduced cortical folding in the left dorsal premotor Cortex (PMC) and the right ventro-lateral prefrontal Cortex (PFC) are highlighted in rose and orange, respectively. n.s. = not significant, dPMC = dorsal PMC, vlPFC = ventro-lateral prefrontal cortex.



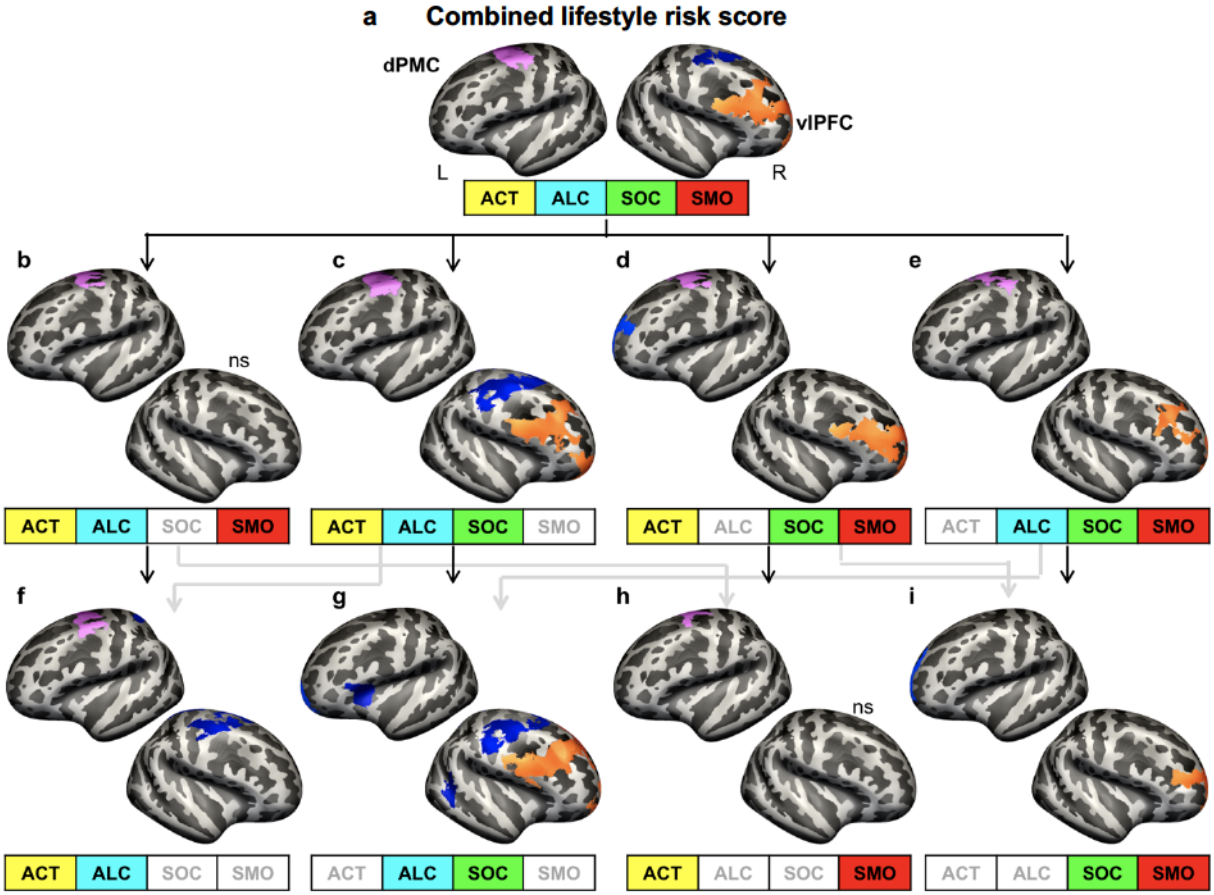
Supplementary Figure 6: Brain regions showing a lifestyle risk associated increase in RSFC to the seed in the right dPMC when additional adjusting for polygenic risk.

Figure depicts the associations between different risk score models and brain regions showing increased RSFC to the seed in dPMC in relation to higher lifestyle risk when additionally adjusting for genetic risk as measured with the combined genetic risk score (GRS). Abbreviations are explained in Supplementary Figure 1.



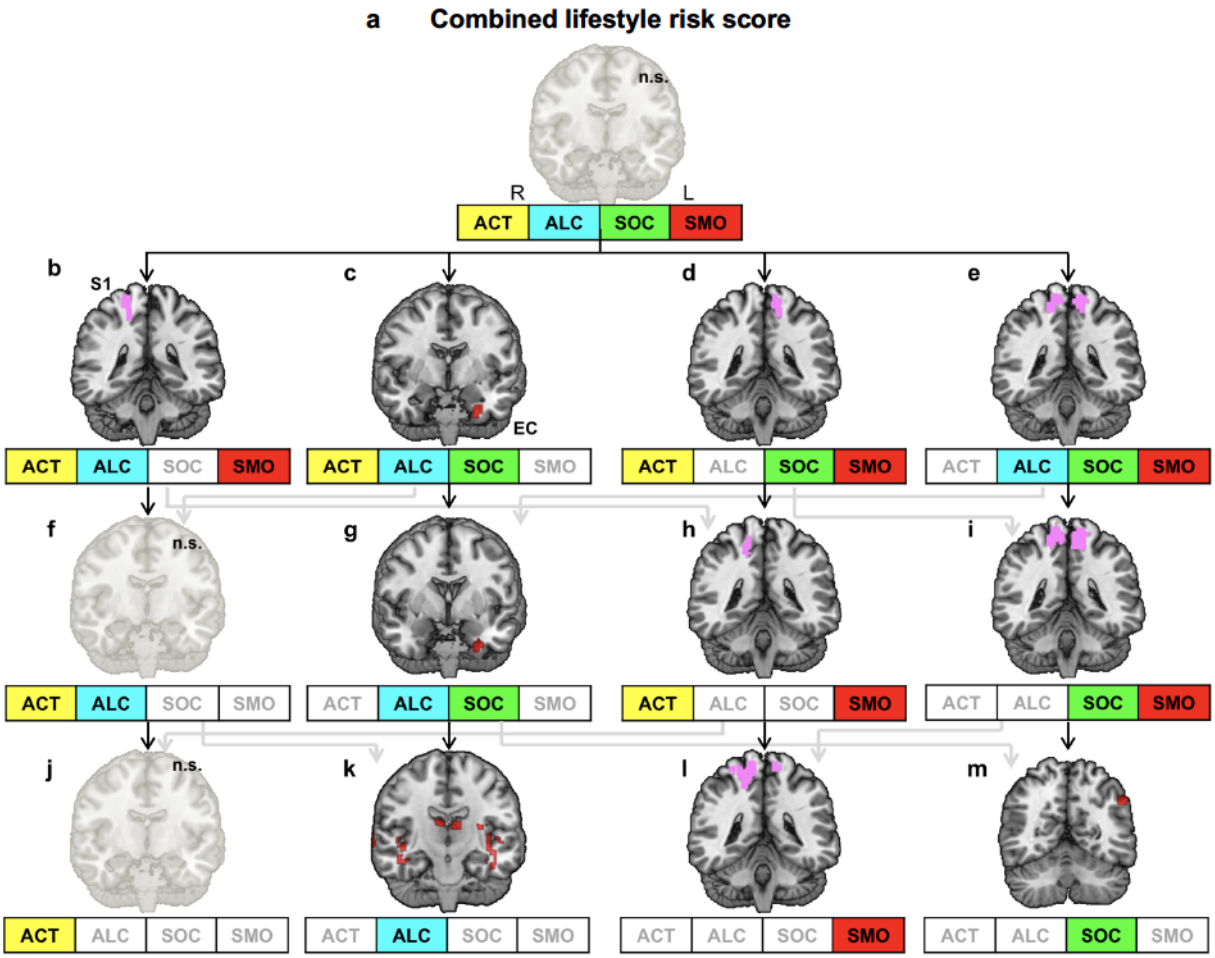
Supplementary Figure 7: Brain regions showing a lifestyle risk associated increase in RSFC to the seed in the right vlPFC when additional adjusting for polygenic risk.

Associations between different risk score models and regions showing increased RSFC to the seed in vlPFC in relation to higher lifestyle risk when additionally adjusting for genetic risk as measured with the combined genetic risk score (PRS) are shown. Explanations of abbreviations can be found in Supplementary Figure 1.

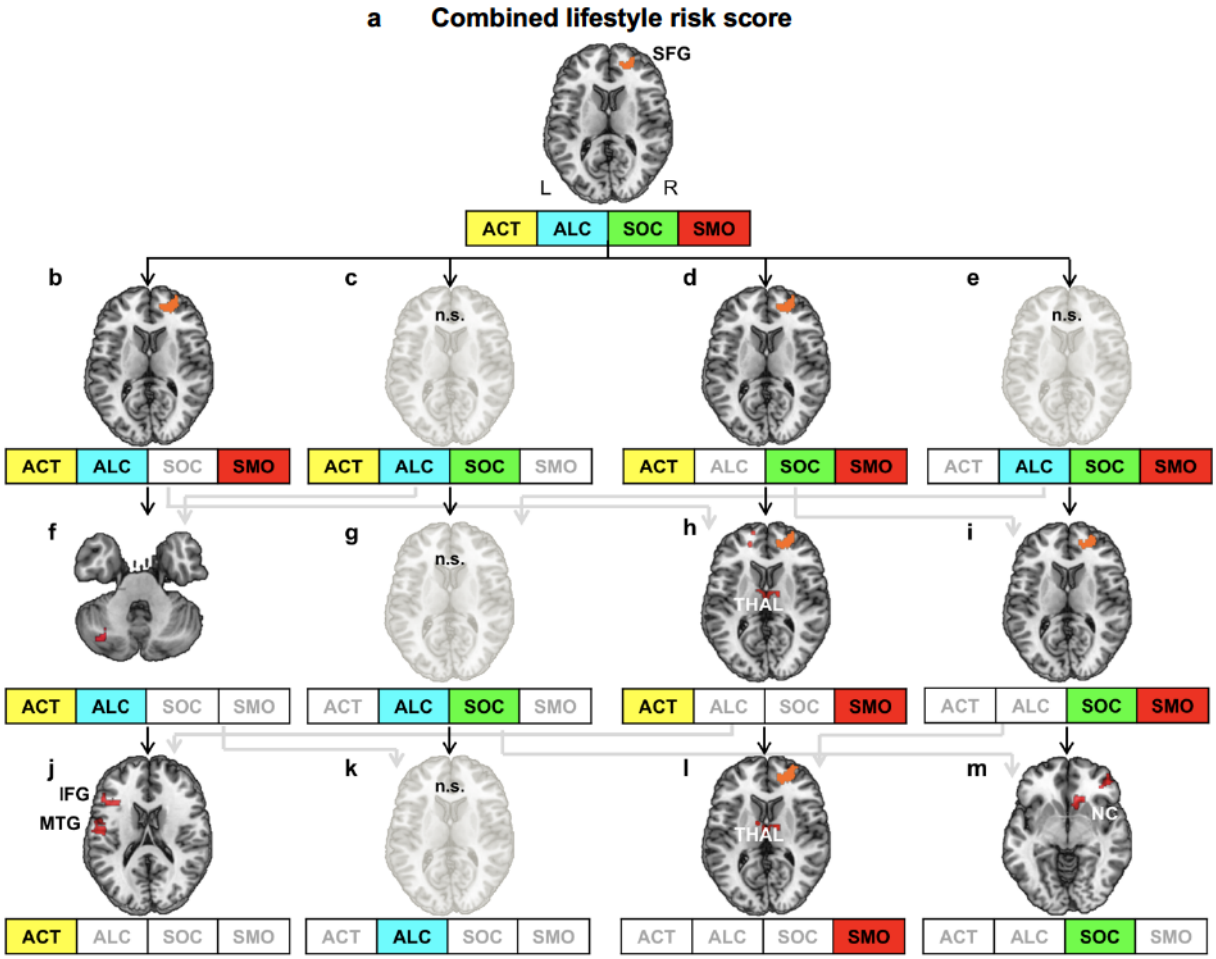


Supplementary Figure 8: Sensitivity analysis for the association between lifestyle risk and cortical folding.

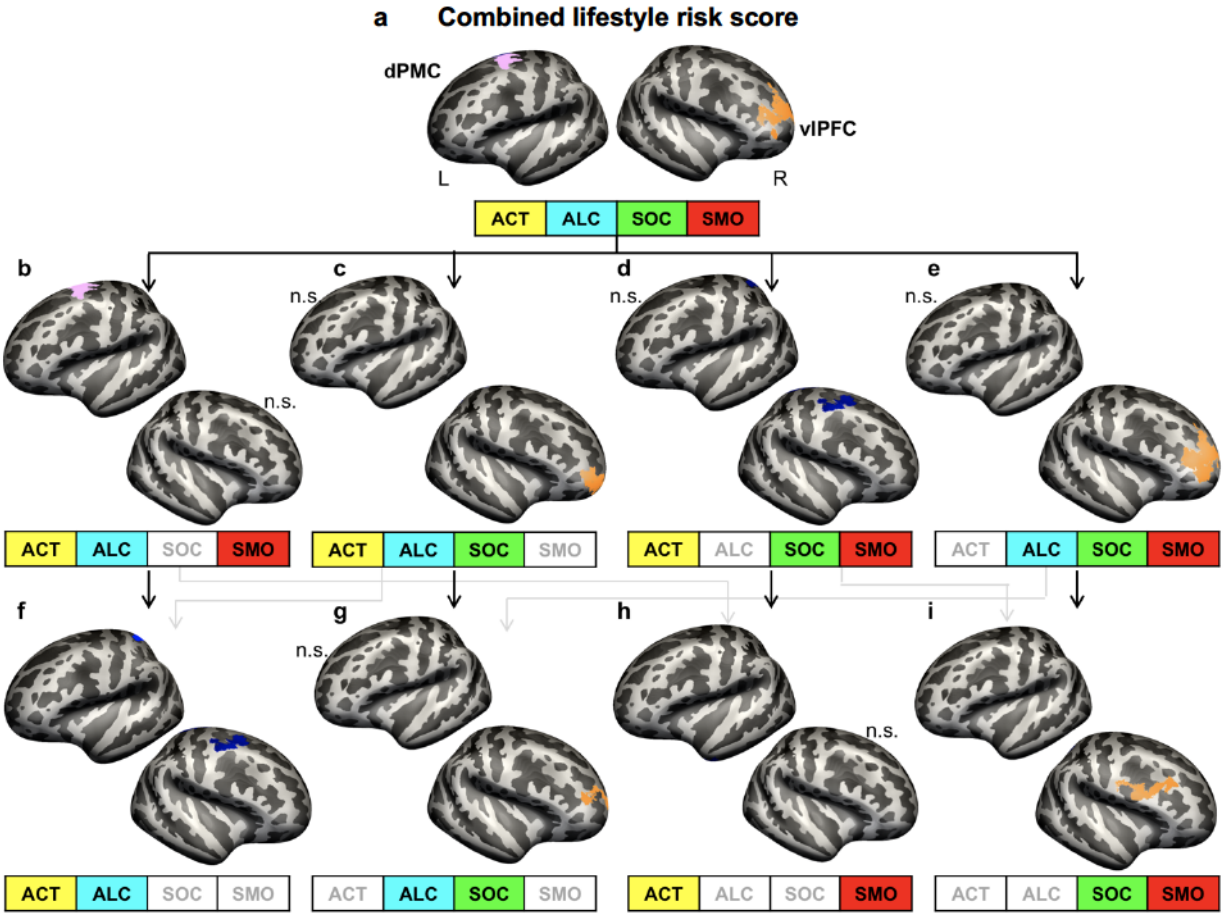
The figure depicts results of the analysis of cortical folding in relation to lifestyle risk after correcting the effect of each single lifestyle variable out of the other lifestyle variables using partial correlations. The recurrent associations between higher lifestyle risk and reduced cortical folding in the left dorsal premotor Cortex (PMC) and the right ventro-lateral prefrontal Cortex (PFC) are highlighted in rose and orange, respectively. Results shown in this figure are not adjusted for the combined genetic risk score (GRS). Explanations of abbreviations can be found in Supplementary Figure 1. Abbreviations: n.s. = not significant.



Supplementary Figure 9: Sensitivity analysis for the association between lifestyle risk and RSFC of the seed in dPMC. Figure represents regions showing increased RSFC to the seed in dPMC in relation to increased lifestyle risk after correcting the influence of each single lifestyle variable out of the other lifestyle variables using partial correlations. Explanations of abbreviations can be found in Supplementary Figure 1.

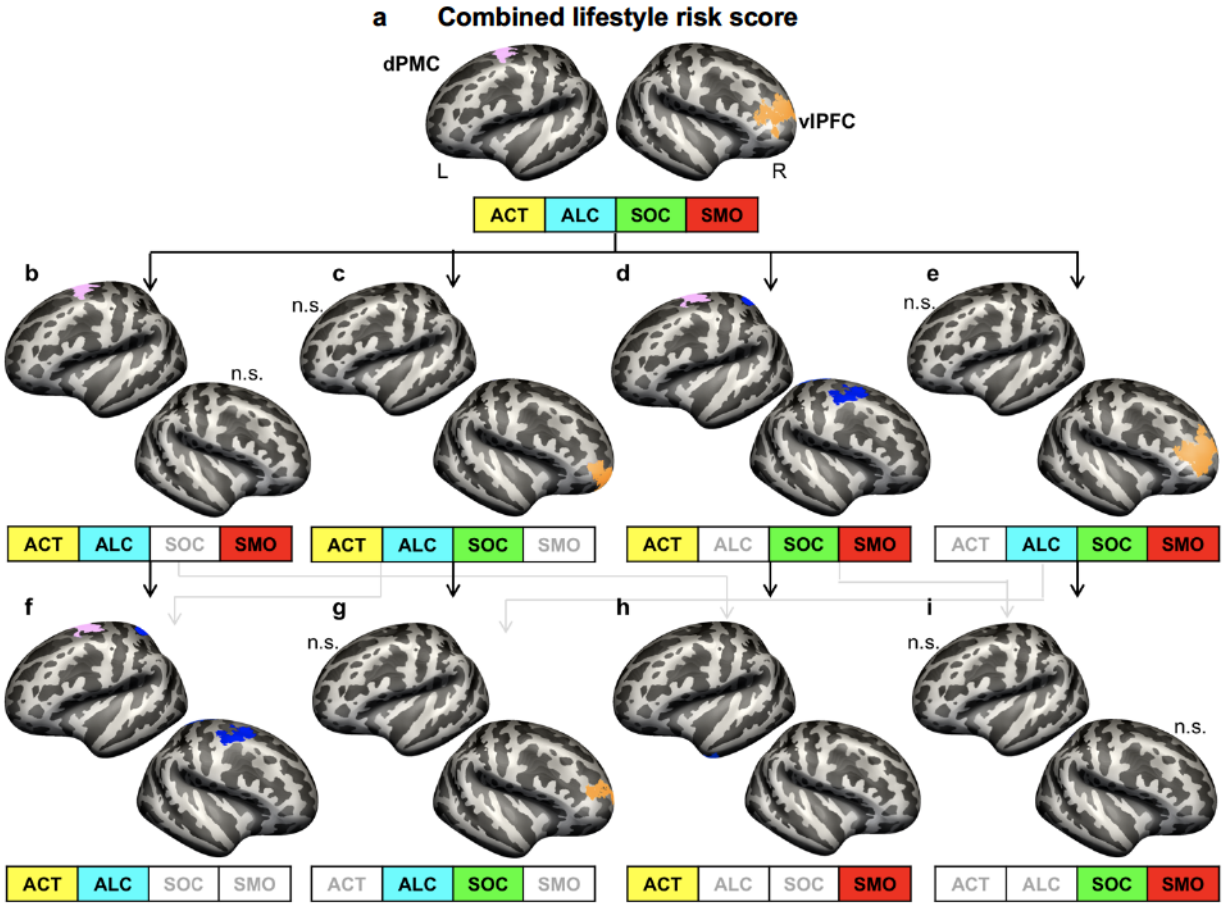


Supplementary Figure 10: Sensitivity analysis for the association between lifestyle risk and RSFC of the seed in vlPFC. Figure represents GM voxels showing increased RSFC to the seed in vlPFC in relation to increased lifestyle risk after correcting the influence of each single lifestyle variable out of the other lifestyle variables using partial correlations. Explanations of abbreviations can be found in Supplementary Figure 1.



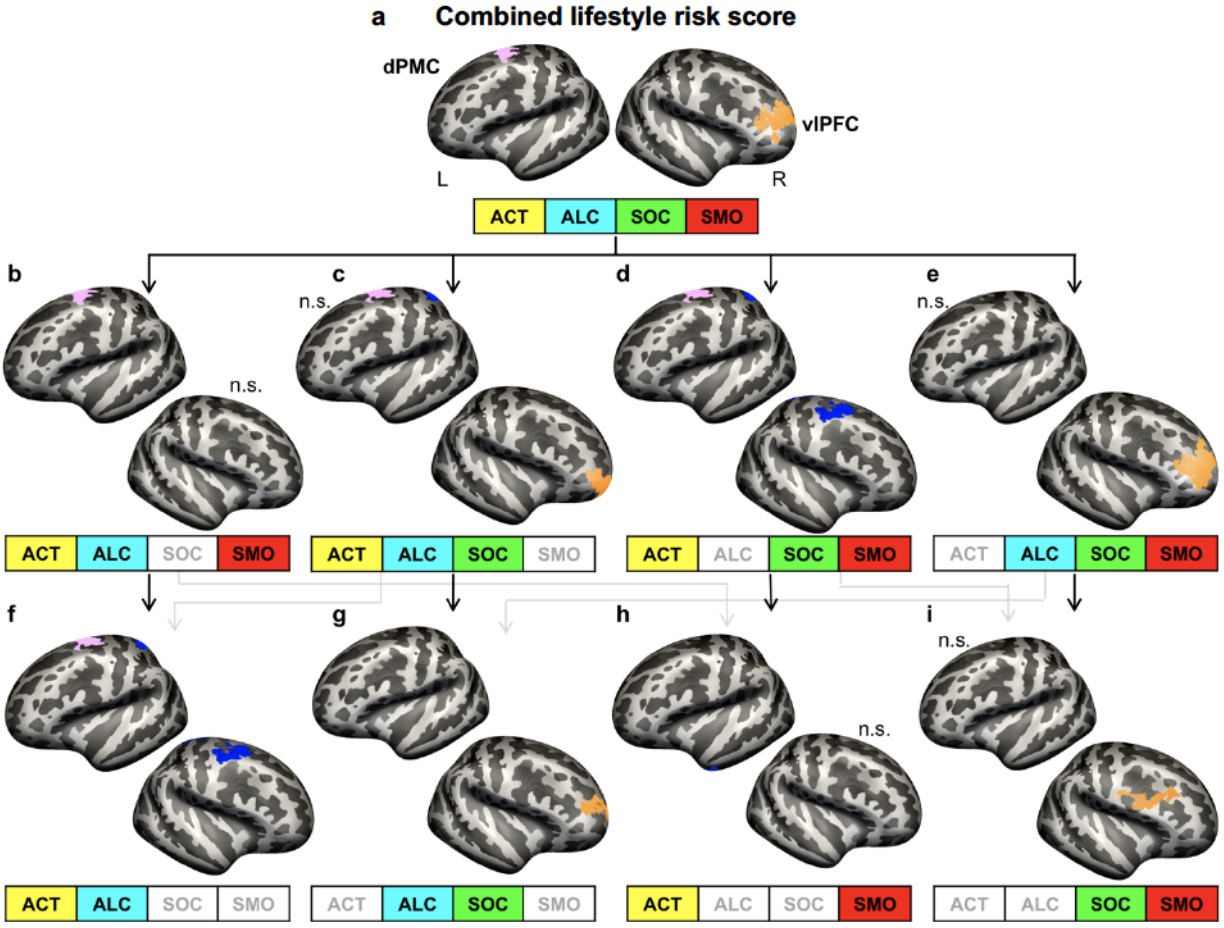
Supplementary Figure 11: Brain regions showing alterations in cortical folding associated with lifestyle risk when additionally adjusting for depressive symptomatology.

Associations between different risk score models and cortical folding when additionally adjusting for depressive symptomatology as measured with the Beck´s depression inventory score (BDI-II). All results are depicted on the inflated surfaces of fsaverage. The recurrent associations between higher lifestyle risk and reduced cortical folding in the left dorsal premotor Cortex (PMC) and the right ventro-lateral prefrontal Cortex (PFC) are highlighted in pink and orange, respectively. Other negative associations are depicted in blue. n.s. = not significant, dPMC = dorsal PMC, vlPFC = ventro-lateral prefrontal cortex.



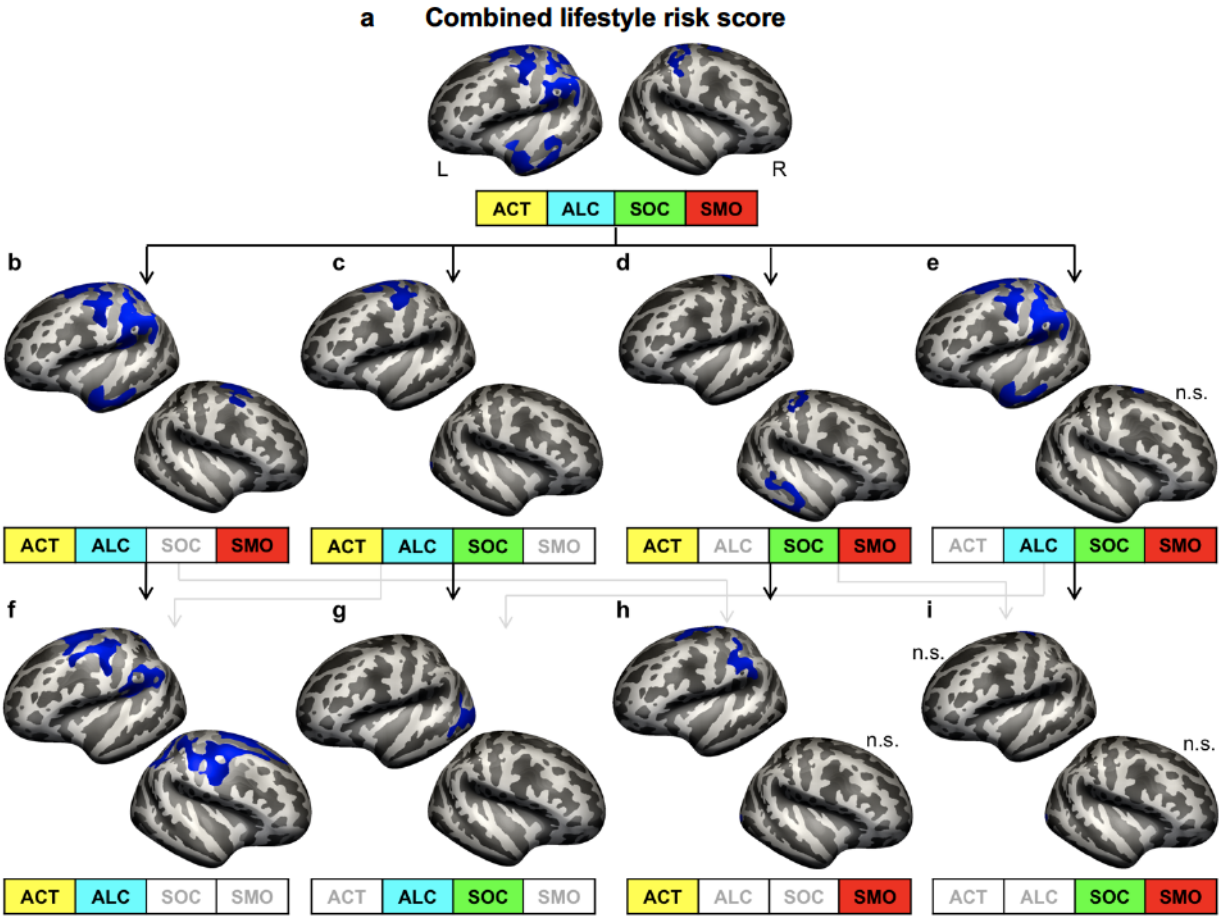
Supplementary Figure 12: Brain regions showing alterations in cortical folding associated with lifestyle risk when additionally adjusting for education.

Associations between different risk score models and cortical folding when additionally adjusting for education as measured with the international standard classification for education (ISCED, Unesco, 2011). All results are depicted on the inflated surfaces of fsaverage. The premotor Cortex (PMC) and the right ventro-lateral prefrontal Cortex (PFC) are highlighted in pink and orange, respectively. Other negative associations are depicted in blue. n.s. = not significant, dPMC = dorsal PMC, vlPFC = ventro-lateral prefrontal cortex.

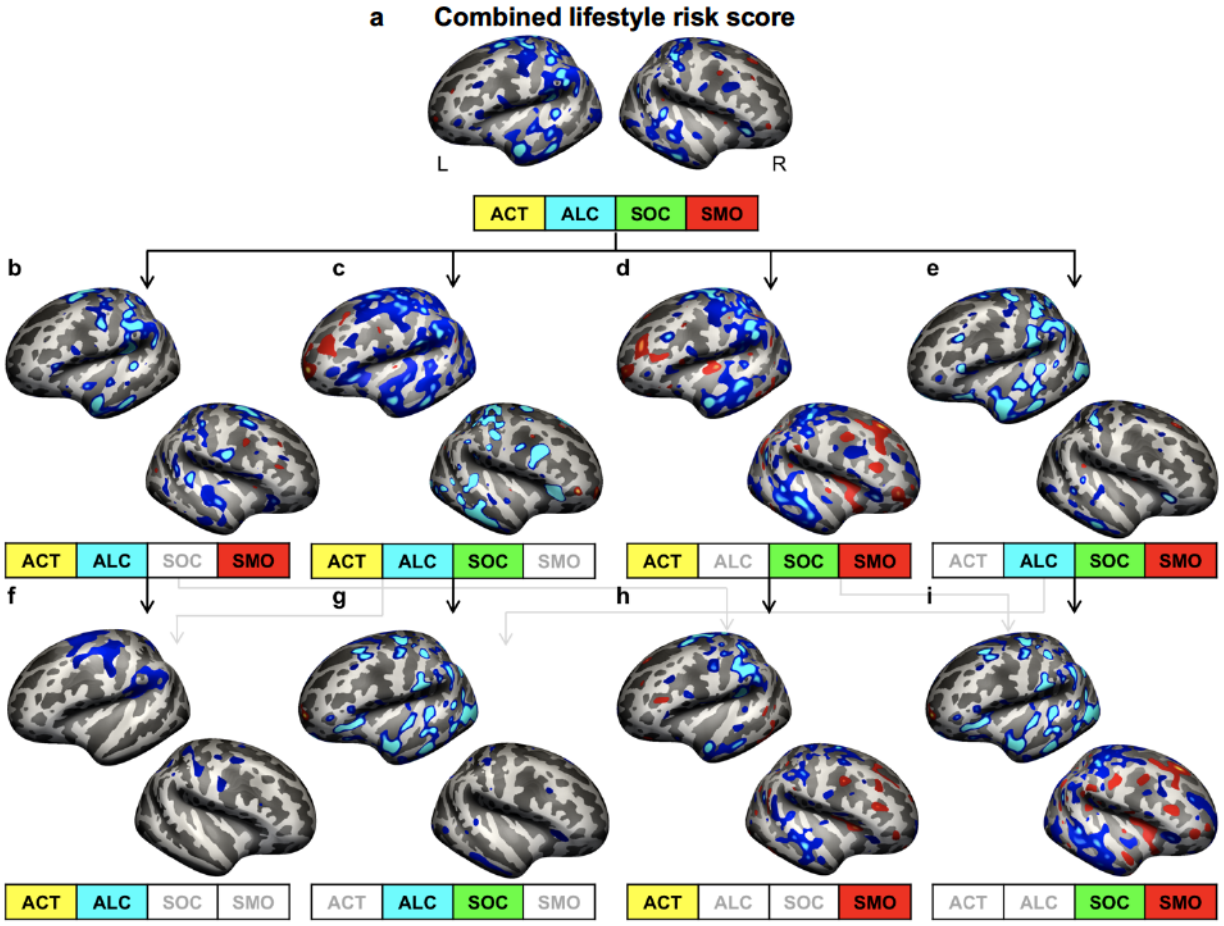


Supplementary Figure 13: Brain regions showing alterations in cortical folding associated with lifestyle risk when additionally adjusting for depressive symptomatology and education.

Associations between different risk score models and cortical folding when additionally adjusting for education as measured with the international standard classification for education (ISCED, Unesco, 2011) and depressive symptomatology as measured with Beck´s depression inventory (BDI-II). All results are depicted on the inflated surfaces of fsaverage. The recurrent associations between higher lifestyle risk and reduced cortical folding in the left dorsal premotor Cortex (PMC) and the right ventro-lateral prefrontal Cortex (PFC) are highlighted in rose and orange, respectively. n.s. = not significant, dPMC = dorsal PMC, vlPFC = ventro-lateral prefrontal cortex.

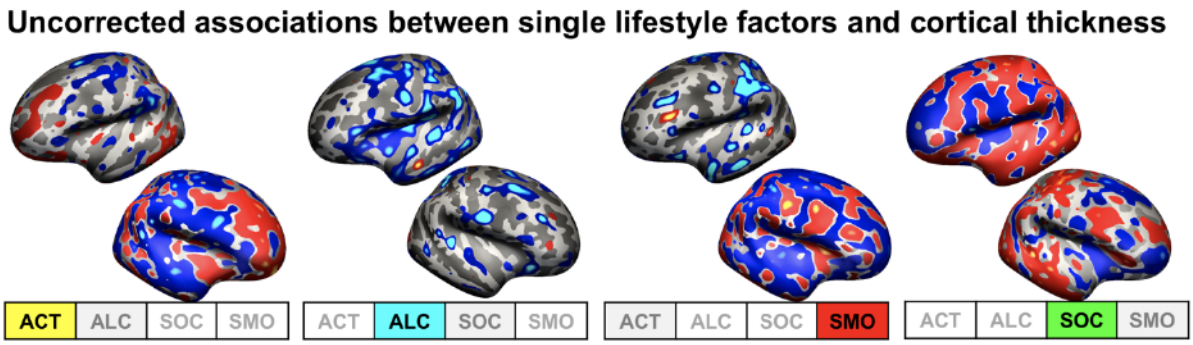


Supplementary Figure 14. Brain regions showing alterations in cortical thickness associated with lifestyle risk in different risk score models (with stepwise exclusion). All results are corrected for age and gender and corrected for multiple comparisons using Monte Carlo Z simulations with $\alpha = 0.05$. Positive associations between risk score models and cortical thickness were not found.



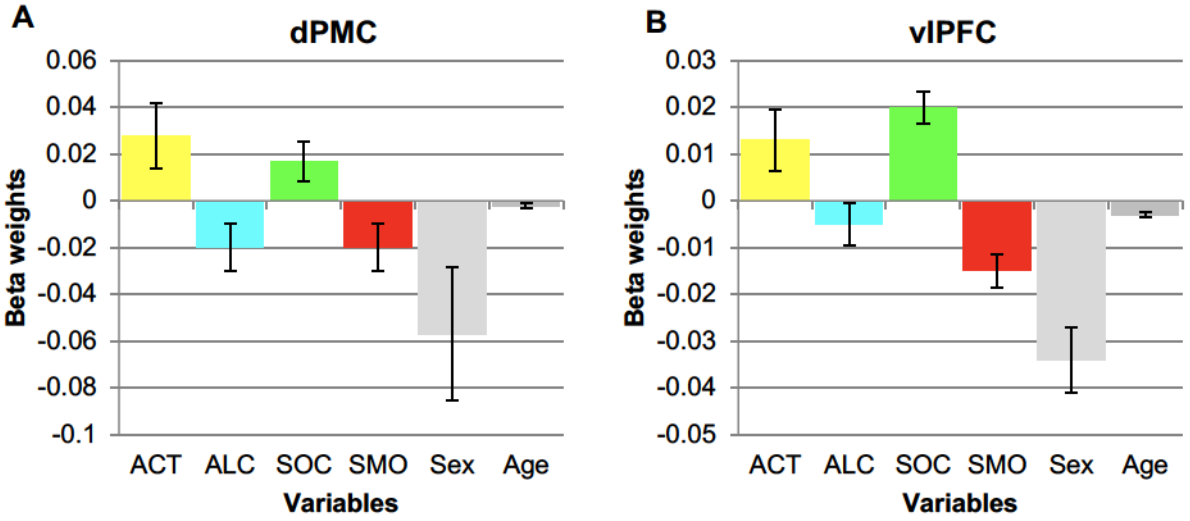
Supplementary Figure 15: Associations between lifestyle risk and differences in cortical thickness without correction for multiple comparisons.

Associations between different risk score models and cortical thickness without any correction for multiple comparisons. For further conventions and abbreviations please see Suppl. Fig. 1.



Supplementary Figure 16: Brain regions showing alterations in cortical thickness associated with lifestyle risk without correction for multiple comparisons.

Associations between single lifestyle variables and cortical thickness without any correction for multiple comparisons depicted on the inflated surfaces of the fsaverage brain. For further conventions and abbreviations please see Suppl. Fig. 1.



Supplementary Figure 17: Beta weights of the multiple linear regression using extracted cortical folding values of the dPMC and vlPFC. Plots show beta weights of each single lifestyle factor, sex and age as estimated in the first model of the multiple linear regression calculated in SPSS using extracted cortical folding values of the dPMC (A) and vlPFC (B) as dependent variable. Error bars refer to standard errors of the individual beta weights.

Supplementary Table 1. All possible combinations of lifestyle risk score models.

Risk score model	Included lifestyle variables
ACT, ALC, SOC, SMO	The combined lifestyle risk score: Physical activity, alcohol consumption, social integration, smoking
ACT, ALC, SMO	Physical activity, alcohol consumption, smoking
ACT, ALC, SOC	Physical activity, alcohol consumption, social integration
ACT, SOC, SMO	Physical activity, social integration, smoking
ALC, SOC, SMO	Alcohol consumption, social integration, smoking
ACT, ALC	Physical activity, alcohol consumption
ALC, SOC	Alcohol consumption, social integration
ACT, SMO	Physical activity, smoking
SOC, SMO	Social integration, smoking

Supplementary Table 2: Overlap between probability maps of the JuBrain atlas[4-7] and regions showing significantly decreased cortical folding with higher combined lifestyle risk.

Risk score model	Hemis-phere	Anatomical landmark	Cytoarchitectonically defined region[4-27]	*Cwp*-value
Combined risk score **ACT, ALC, SMO, SOC**	left	Premotor cortex (PMC)	-	0.0001
	right	Frontal pole extending to inferior frontal gyrus (IFG)	Fp1, Fp2, Area 45	0.0001
ACT, ALC, SMO	left	PMC	-	0.0029
	right	n.s.	-	
ACT, ALC, SOC	left	PMC	-	0.0001
	right	Frontal pole, middle orbital gyrus	Fp1, Fp2, Fo2	0.0001
		IFG	Area 44, 45	0.0001
		PMC	-	0.0001
		Superior parietal lobule (SPL), extending to intra-parietal sulcus (IPS) and superior occipital gyrus	7A, 7M, 7P, 7PC, hIP3 [IPS], hoc4d (V3A), hoc3d (V3d)	0.0001
ACT, SMO, SOC	left	Frontal pole	Fp1, Fp2	0.0007
		PMC	-	0.0001
		Fusiform gyrus	FG3, FG4	0.0079
	right	Frontal pole, extending to IFG	Fp1, Fp2, Area 44, 45	0.0001
		Fusiform gyrus	FG4	0.0069
ALC, SOC, SMO	left	Frontal pole	Fp1, Fp2	0.0090
		PMC	-	0.0142
	right	Frontal pole	Fp1, Fp2	0.0008
		SFG, MFG	-	0.0001
ACT, ALC	left	PMC	-	0.0002
		SPL, extending to sensori-motor region	5A, 5M, 7A, 7PC Area 4a, Area 2	0.0015
	right	Premotor to sensori-motor cortex	Area 1, 2, 3b, 4a	0.0001
		SPL	5L	0.0001
ALC, SOC	left	Frontal Pole	Fp1, Fp2	0.0009
		IFG	Area 45	0.0042
		PMC	-	0.0236
	right	Frontal pole	Fp1, Fp2	0.0019
		IFG	Areas 44, 45	0.0020
		PMC, M1	Area 4p	0.0001
		SPL, extending to IPS and superior occipital gyrus	7A, 7M, 7P, hIP3 [IPS], hOC4d (V3A)	0.0001
ACT, SMO	left	PMC	-	0.0080
		Parietal operculum	OP4	0.0093
SMO, SOC	left	Frontal pole	Fp1, Fp2	0.0001
	right	Prefrontal cortex	Fp1, Fp2	0.0001

Note. This table summarizes the overlap between the macroanatomical localization of the reported effects with cytoarchitectonical maps of the JuBrain atlas[1-24]. Abbreviations: n.s. = not significant.

Supplementary Table 3: Overlap between probability maps of the JuBrain atlas with brain regions showing significantly increased RSFC to the seed in left dPMC.

Risk score model	Hemi sphere	Anatomical landmark	Cytoarchitectonically defined region[4-27]	Voxel size
Combined risk score **ACT, ALC, SMO, SOC**	left	Entorhinal cortex	-	72
		S1 / M1	Areas 3a, 4a	60
		V3 (dorsal occipital cortex)	Area hOc4d [V3A]	41
		M1	Area 4a	37
	right	M1	Area 4a	83
		M1	Area 4a	76
ACT, ALC, SOC	left	Enthorinal cortex	-	33
ACT, ALC, SMO	left	V3 (dorsal occipital cortex)	Area hOc4d [V3A]	71
	right	M1	Area 4a	110
		S1 / M1	Areas 3b, 4a	107
		Middle temporal gyrus	-	45
ACT, SMO, SOC	left	M1	-	114
		S1	Area 3a	69
		V3 (dorsal occipital cortex)	Area hOc4d [V3A]	51
		Medial frontal gyrus	-	49
	right	M1	Area 4a	87
		M1 / S1	Areas 4a, 3b	79
		M1 (precentral gyrus)	-	45
		Medial frontal gyrus	-	41
ALC, SMO, SOC	left	paracentral region	-	128
		M1 (precentral gyrus)	Area 4a	99
		Entorhinal cortex	Entorhinal cortex, Subiculum	89
		S1, extending to SPL	Area 3a, 5M (SPL)	74
	right	Paracentral region	4a, 3b	467
		Entorhinal cortex	entorhinal cortex	62
ACT, SMO	left	V3 (dorsal occipital cortex)	Area hOc4d [V3A]	106
		M1	Area 4p	50
	right	M1	Area 4a	159
		S1 / M1	Areas 3b, 4a	154
SOC (as a single variable)	left	Temporo-parietal junction	Areas PGa (IPL), PFm (IPL)	59
SMO (as a single variable)	left	M1	Area 4a	255
		S1	Area 3a	64
		V3 (dorsal occipital cortex)	Area hOc4d [V3A]	53
		Superior frontal gyrus		37
	right	M1	Area 4a	945
		M1	-	54
		S1	Area 2	37
		V3 (dorsal occipital cortex)	Area hOc4la [V3A]	32
ALC (as a single variable)	left	Cerebellum	-	118
		Brainstem	-	57
	right	Cerebellum	-	56
		Hippocampus	-	35

Note. Abbreviations: n.s. = not significant.

Supplementary Table 4: Overlap between probability maps of the JuBrain atlas with brain regions showing significantly increased RSFC to the seed in right vlPFC.

Risk score model	Hemi sphere	Anatomical landmark	Cytoarchitectonically defined region[4-27]	Voxel size
Combined risk score **ACT, ALC, SMO, SOC**	right	Superior frontal gyrus	Fp1	64
ACT, SMO, SOC	right	Superior frontal gyrus	Fp1	58
		Superior medial gyrus	Fp2	51
ACT, ALC, SMO	right	Super frontal gyrus	Fp1	69
		Anterior cingulum	-	35
ACT, ALC, SOC	right	Orbitofrontal gyrus	-	
ALC, SOC, SMO	right	Superior frontal gyrus	Fp1	171
		Temporo-parietal junction	Areas PGa (IPL), PFm (IPL)	34
ACT, ALC	left	Cerebellum		
ACT, SMO	left	Superior frontal gyrus	Fp1	80
ACT, SMO	right	Superior frontal gyrus	Fp1	274
	bilateral	Thalamus	-	61
	right	Middle frontal gyrus	-	33
SMO, SOC	right	Superior frontal gyrus	Fp1	117
SMO (as a single variable)	left	Middle frontal gyrus	-	94
		Superior frontal gyrus	-	83
	right	Superior frontal gyrus	Fp1	389
	bilateral	Thalamus	-	60
SOC (as a single variable)	right	Caudate nucleus	-	112
		Inferior frontal gyrus, p. orbitalis	-	53
ACT (as a single variable)	left	Inferior frontal gyrus, p. triangularis	Area 45	109
		Postcentral gyrus	-	93
	right	Middle temporal gyrus	hOc4la	88

Note. Abbreviations: n.s. = not significant.

Supplementary Table 5: Overlap between probability maps of the JuBrain atlas with brain regions showing significantly decreased RSFC to the seed in left PMC.

Risk score model	Hemi sphere	Anatomical landmark	Cytoarchitectonically defined region[4-27]	Voxel size
Combined risk score **ACT, ALC, SMO, SOC**	right	Precentral sulcus	-	45
SMO (as a single variable)	left	Cerebellum	-	33
SOC (as a single variable)	left	M1	Area 4a	42
ACT (as a single variable)	left	Paracingulate sulcus	-	37

Supplementary Table 6: Overlap between probability maps of the JuBrain atlas with brain regions showing significantly decreased RSFC to the seed in right vlPFC.

Risk score model	Hemi sphere	Anatomical landmark	Cytoarchitectonically defined region[4-27]	Voxel size
ACT, ALC, SMO	right	Dorsal occipital cortex	-	43
ACT, ALC	right	Dorsal occipital cortex	-	70
ALC (as a single variable)	left	Inferior frontal gyrus	Area 44	53
ACT (as a single variable)	right	Cerebellum	-	40

Supplementary Table 7: Regions showing significantly decreased cortical folding with higher integrated lifestyle risk when additionally adjusting for polygenic risk.

Risk score model	Hemis-phere	Anatomical landmark	Cytoarchitectonically defined region[4-27]	*Cwp*-value
Combined risk score **ACT, ALC, SMO, SOC**	left	Premotor cortex (PMC)	-	0.00010
	right	Frontal pole, extending to inferior frontal gyrus (IFG)	Fp1, Fp2, Area 45	0.00010
		Precuneus	7A, 7P	0.00010
ACT, ALC, SMO	left	n.s.	-	-
	right	n.s.	-	-
ACT, ALC, SOC	left	PMC	-	0.00010
	right	Frontal pole, middle orbital gyrus	Fp1, Fp2, Fo2	0.00010
		IFG	Area 44, 45	0.00010
		Precentral gyrus	4a, 4p	0.00010
		Precuneus	7A, 7P, hOc4d [V3A]	0.00010
ACT, SMO, SOC	left	PMC	-	0.00070
		Frontal Pole	Fp1, Fp2	0.00010
		Fusiform gyrus	FG3, FG4	0.00010
	right	Frontal pole, extending to IFG	Fp1, Fp2, Area 45	0.00010
		Precuneus, extending to fusiform gyrus	FG3, FG4	0.00010
ALC, SOC, SMO	left	Frontal pole	Fp1, Fp2	0.00010
		PMC	-	0.00010
		Fusiform gyrus	FG3, FG4	0.00010
	right	Frontal pole, extending to IFG	Fp1, Fp2, Area 44	0.00010
		SPL, extending to intra-parietal sulcus	7A, 7M (SPL), hIP3 (IPS]	0.00010
		Precuneus, extending to calcarine sulcus and fusiform gyrus	hOc1 [V1], hOc2 [V2], FG3, FG4	0.00010
ACT, ALC	left	SPL	5L, 7A	0.00480
	right	Precentral gyrus	4a	0.00287
ALC, SOC	left	Frontal Pole	Fp1, Fp2	0.00200
		IFG	Area 45	0.03190
		PMC	-	0.00010
	right	Frontal pole, extending to IFG	Fp1, Fp2, Areas 44, 45	0.00010
		PMC	-	0.00010
		Superior temporal sulcus	-	0.00180
ACT, SMO	left	n.s.	-	-
	right	Fusiform gyrus, extending to lateral occipital cortex	FG3, FG4, hOc4v [V4 (v)]	0.00010
SMO, SOC	left	Frontal pole	Fp1, Fp2	0.00010
		Premotor cortex	-	0.00100
		Fusiform gyrus	FG3, FG4	0.00010
	right	Frontal Pole, extending to superior frontal gyrus	Fp1, Fp2	0.00010

	Superior parietal lobule, extending to intra-parietal sulcus	7A [SPL], hIP3 [SPL]	0.00010

Note. Abbreviations: n.s. = not significant.

Supplementary Table 8: Overlap between probability maps of the JuBrain atlas with brain regions showing significantly increased RSFC to the seed in left dPMC when additionally adjusting for polygenic risk.

Risk score model	Hemi sphere	Anatomical landmark	Cytoarchitectonically defined region[4-27]	Voxel size
Combined risk score **ACT, ALC, SMO, SOC**	left	Entorhinal cortex	Entorhinal cortex, Subiculum	76
		S1 / M1	Areas 3a, 4a, 5L (SPL)	64
		M1	Area 4a	46
	right	M1	Area 4a	79
		M1	Area 4a, 3b, 5L (SPL)	72
	bilateral	Brainstem	-	33
ACT, ALC, SOC	left	Entorhinal cortex	Entorhinal cortex	47
ACT, ALC, SMO	left	V3 (superior occipital gyrus)	Area hOc4d [V3A]	50
	right	M1	Area 4a, 3b	133
		M1	Area 4a	125
		Middle temporal gyrus	-	36
ACT, SMO, SOC	left	M1	4a	99
		S1, extending to SPL	3a, 5M	84
		Superior occipital gyrus	hOc4d [V3A]	43
		Medial frontal gyrus	-	42
		Medial frontal gyrus	-	38
	right	M1 / S1	Area 4a, 3b	73
		M1	Area 4a	65
ALC, SMO, SOC	left	M1	Area 4a	121
		M1 / S1, extending to SPL	4a, 3a, 5M	75
		Medial frontal gyrus	-	74
		Entorhinal cortex	Entorhinal cortex	71
		Paracentral cortex	Entorhinal cortex	47
	right	M1	Area 4a	416
ACT, SMO	right	M1	Area 4a	153
		M1	Area 4a, 3b	145
	left	V3 (superior occipital gyrus)	Area hOc4d [V3A]	106
		M1	4p, 4a	53
SOC, SMO	left	M1	Area 4a	192
		Paracentral cortex	Areas 4a, 3a, 5M	128
		Medial frontal gyrus	-	101
	right	Paracentral cortex	Area 4a, 3b, 5M	549
		Medial frontal gyrus	-	41
		M1	-	38
		SMA	-	37
SOC (as a single variable)	left	Temporo-parietal junction	Areas PGa (IPL), PFm (IPL)	56
SMO (as a single variable)	left	M1	Area 4a	229
		S1	Area 3a	54
		V3 (superior occipital gyrus)	Area hOc4d [V3A]	51
		M1	Area 4a	49
	right	M1	Area 4a, 3b, 5L	967
		Medial frontal gyrus	-	46
		S1	Area 2	42
		Superior frontal gyrus	-	35

	Posterior middle temporal gyrus	Area hOc4la	31

Supplementary Table 9: Overlap between probability maps of the JuBrain atlas with brain regions showing significantly increased RSFC to the seed in right vlPFC when additionally adjusting for polygenic risk.

Risk score model	Hemi sphere	Anatomical landmark	Cytoarchitectonically defined region[4-27]	Voxel size
Combined risk score **ACT, ALC, SMO, SOC**	right	Superior frontal gyrus	Fp1	68
ACT, ALC, SMO	right	Superior frontal gyrus	Fp1	65
ACT, SMO, SOC	right	Superior frontal gyrus	Fp1	171
		Supramaginal gyrus	-	34
ACT, SMO	left	Superior frontal gyrus	Fp1	54
	right	Superior frontal gyrus	Fp1	259
		Middle frontal gyrus	Fp1	37
SMO, SOC	right	Superior frontal gyrus	Fp1	59
		Superior medial gyrus	-	50
		Insula	-	37
SMO (as a single variable)	left	Superior frontal gyrus	-	81
		Middle frontal gyrus	-	85
	right	Superior frontal gyrus	Fp1	311
SOC (as a single variable)	right	Caudate nucleus	-	53
		Inferior frontal gyrus, orbital part	-	51
ACT (as a single variable)	left	Inferior frontal gyrus, triangular part	Area 45	76
		Postcentral Gyrus	OP4	52
	right	V3 (dorsal occipital cortex)	Area hOc4la	66

Supplementary Table 10: Regions showing significantly decreased cortical folding with higher combined lifestyle risk in the sensitivity analysis.

Risk score model	Hemis-phere	Anatomical landmark	Cytoarchitectonically defined region[4-27]	Cwp-value
Combined risk score **ACT, ALC, SMO, SOC**	left	Premotor cortex (PMC)	-	0.00010
	right	Frontal pole, extending to Inferior frontal gyrus (IFG)	Fp1, Fp2, Areas 45	0.00010
		PMC	-	0.00020
ACT, ALC, SMO	left	PMC	-	0.00020
	right	n.s.	-	-
ACT, ALC, SOC	left	PMC	-	0.00010
	right	Frontal pole, middle orbital gyrus	Fp1, Fp2, Fo2	0.00010
		IFG	Area 44, 45	0.00010
		PMC, extending to M1	4a, 4p	0.00010
		SPL, extending to cuneus	7A, 7P, hOc4d [V3A]	0.00010
ACT, SMO, SOC	left	Frontal pole	Fp1, Fp2	0.00020
		PMC	-	0.00010
	right	Frontal pole, extending to IFG	Fp1, Fp2, Area 44	0.00010
		PMC	-	0.00010
	right	Frontal pole	Fp1, Fp2	0.00010
		PMC	-	0.00170
ACT, ALC	left	PMC	-	
		SPL	5L	0.00140
	right	Premotor cortex	-	0.00010
		Sensory-motor cortex, extending to SPL	Area 4, 2, 5L (SPL)	0.00010
ALC, SOC	left	Frontal Pole	Fp1, Fp2	0.00390
		IFG	Area 45	0.00730
	right	Frontal pole, extending to IFG	Fp1, Fp2, Areas 44, 45	0.00010
		PMC, extending to inferior M1	3b	0.00590
		Superior M1	4a	0.00180
		SPL, Precuneus	7A, 7P (SPL)	0.00010
		Middle temporal gyrus	-	0.00210
ACT, SMO	left	PMC	-	0.00680
	right	n.s.	-	
SMO, SOC	left	Frontal pole	Fp1, Fp2	0.00010
	right	Frontal Pole, extending to middle frontal gyrus	Fp1, Fp2	0.00010

Supplementary Table 11: Overlap between probability maps of the JuBrain atlas with brain regions showing significantly increased RSFC to the seed in left dPMC in the sensitivity analyses.

Risk score model	hemi sphere	anatomical landmark	cytoarchitectonically defined region[4-27]	voxel size
Combined risk score **ACT, ALC, SMO, SOC**	left	Entorhinal cortex	Entorhinal cortex	67
ACT, ALC, SMO	right	S1 / M1	Areas 3b , 4a	52
		M1	4a	37
ACT, ALC, SOC	left	Entorhinal cortex	Entorhinal cortex	37
ACT, SMO, SOC	left	M1	-	114
		S1	Area 3a	69
		V3 (superior occipital gyrus)	Area hOc4d [V3A]	51
		Medial frontal gyrus	-	49
	right	M1	Area 4a	87
		M1 / S1	Areas 4a, 3b	79
		M1 (precentral gyrus)	-	45
		Medial frontal gyrus	-	41
ALC, SMO, SOC	left	Paracentral lobule	-	128
		M1 (precentral gyrus)	Area 4a	99
		Entorhinal cortex	-	89
		SPL (precuneus)	Area 5M (SPL)	74
	right	M1	Area 4a	467
		Entorhinal cortex	-	62
ACT, SMO	left	V3 (dorsal occipital cortex)	Area hOc4d [V3A]	77
		M1	Area 4p	32
	right	M1	Area 4a	80
		S1 / M1	Areas 3b, 4a	92
SOC (as a single variable)	left	Temporo-parietal junction	Areas PGa (IPL), PFm (IPL)	59
SMO (as a single variable)	left	M1	Area 4a	255
		S1	Area 3a	64
		V3 (superior occipital gyrus)	Area hOc4d [V3A]	53
		Superior frontal gyrus		37
	right	M1	Area 4a	945
		M1	-	54
		S1	Area 2	37
		V3 (dorsal occipital cortex)	Area hOc4la	32

Supplementary Table 12: Exact anatomical localization of brain regions showing significantly increased RSFC to the seed in VLPFC in the sensitivity analyses.

Risk score model	Hemisphere	Anatomical landmark	Cytoarchitectonically defined region[4-27]	Voxel size
Combined risk score **ACT, ALC, SMO, SOC**	right	Superior frontal gyrus	Fp1	64
ACT, SMO, SOC	right	Superior frontal gyrus	Fp1	126
		Superior medial gyrus	Fp2	42
ACT, SMO	left	Superior frontal gyrus	Fp1	80
ACT, SMO	left	Superior frontal gyrus	-	62
	right	Superior frontal gyrus	Fp1	237
		Middle frontal gyrus	-	45
SMO, SOC	right	Superior frontal gyrus	Fp1	117
SMO (as a single variable)	left	Middle frontal gyrus	-	94
		Superior frontal gyrus	-	83
	right	Superior frontal gyrus	Fp1	389
	bilateral	Thalamus	-	60
SOC (as a single variable)	right	Caudate nucleus	-	112
		Inferior frontal gyrus, p. orbitalis	-	53
ACT (as a single variable)	left	Inferior frontal gyrus, p. triangularis	Area 45	109
		Postcentral gyrus	-	93
	right	Middle temporal gyrus	hOc4la	88

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When Your Brain Looks Older Than Expected:

Combined Lifestyle Risk and BrainAGE

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Abstract

There is great interindividual variability in age-related decline of brain structure. Lifestyle may be one source of unexplained variance: While physical and social engagement are protective against structural decline, other lifestyle behaviors may be accelerating factors. We examined whether there may be a lifestyle-dependent deceleration or acceleration of structural brain aging using the BrainAGE score in a sample of 622 older adults from the population-based 1000BRAINS cohort. Lifestyle was measured using a combined lifestyle risk score, composed of risk (smoking, alcohol intake) and protective variables (social integration, physical activity). Individual BrainAGE was estimated from T1-weighted MRI data indicating accelerated brain atrophy (brains looking older than expected by chronological age) by higher values. The effect of combined lifestyle risk and individual lifestyle variables on BrainAGE was calculated using linear regressions. One unit increase in combined lifestyle risk was associated with 3.84 months of additional BrainAGE. This was driven by two individual lifestyle behaviors, i.e. smoking with 0.36 additional months of BrainAGE per packyear and physical activity with a decrease of 0.48 months in BrainAGE per metabolic equivalent. Post-hoc stratification by sex revealed a stronger association between physical activity and BrainAGE in males (0.6 months less BrainAGE) compared to females (0.48 months less BrainAGE). Thus, riskier lifestyle seems to be one contributor to accelerated rates of brain aging. Especially the differential relation between physical activity and BrainAGE between the sexes underline the need for sex tailored lifestyle interventions. These observations may be particularly helpful with regard to the development of interventions that slow or delay age-related changes in brain structure.

Keywords: Lifestyle, BrainAGE, smoking, physical activity, sex differences, inter-individual variability

1. Introduction

Structural brain changes during normal aging comprise decreases in gray matter (GM) and white matter (WM; Fjell & Walhovd, 2010). Interestingly, some older individuals experience strong and early manifestations (accelerated brain aging), while others of comparable age spared affections expected at that age (decelerated brain aging; Bartrés-Faz & Arenaza-Urquijo, 2011; Ziegler et al., 2012). As this high interindividual variability cannot be fully explained by chronological age (Jockwitz et al., 2017; Stern, 2002; Stern 2009), other factors that provide potential explanatory insight have come into focus, one of them being lifestyle.

Indeed, some lifestyle behaviors such as smoking and alcohol consumption may pose a serious risk to brain health, whereas others show promising beneficial effects, e.g. physical activity and social integration (Anatürk et al., 2018; Arenaza-Urquijo et al., 2015; Fratiglioni et al., 2004). Socially integrated Alzheimer's disease patients show higher cognitive stability compared to not integrated patients, even when suffering from a similar degree of pathology (Bennet et al., 2006). Further, social network size correlates positively with amygdala volume in humans (Bickart et al., 2011). Likewise, higher physical activity, especially in older adults, has repeatedly been associated with better cognitive performance (Colcombe & Kramer, 2003; Hughes & Ganguli, 2009; Kramer et al., 1999, Kramer et al., 2003; Kramer & Erickson, 2007; Voelcker-Rehage et al., 2010) and preservation of GM volume (Colcombe et al., 2003, Erickson et al., 2014). Older adults engaging in physical activity training showed increased hippocampal volume (Erickson et al., 2011) and more efficient use of functional brain networks (Colcombe et al., 2004; Voelcker-Rehage et al., 2011). In contrast, smoking seems to be associated with cortical thinning in prefrontal and temporal regions (Karama et al., 2015) and decreased GM density within cingulum, precuneus, thalamus, and precentral gyrus

(Almeida et al., 2008). Additionally, excessive alcohol consumption can lead to serious neurological diseases, e.g. Korsakow syndrome (de la Monte & Kril, 2014) and is associated with reduced GM and WM volume and density (Paul et al., 2008; Topiwala et al., 2017; Pfefferbaum et al., 1995) in alcohol-dependent as well as non-dependent individuals (Mukamal et al., 2001).

Most previous studies focused on specific effects of a single lifestyle variable on brain structure and function. In real life, however, individuals engage in a combination of lifestyle behaviors, e.g. physical exercise and afterwards meeting friends (social integration) while drinking a beer (alcohol consumption). Only few studies investigated the effects of lifestyle on brain structure and function or on cognition in a multidimensional way. For example, Flöel et al. (2008) found that the combination of exercise, dietary habits, BMI, smoking and alcohol intake was a better predictor for memory performance than the individual lifestyle behaviors. In a previous study, we developed a combined lifestyle risk score reflecting individual combinations of the above described daily lifestyle behaviors, with higher values reflecting more risky behavior (e.g. high smoking and alcohol consumption, low social integration and physical activity), whereas lower values indicate protective combinations (Bittner et al., 2019). We showed that higher combined lifestyle risk was associated with brain atrophy, e.g. more alcohol consumption combined with low physical activity was associated with structural decreases in the premotor region. Based on these findings it might thus be assumed that combined risky lifestyle leads to accelerated brain aging, accompanied by decreases in cognitive performance. Non-linear effects and covariates such as sex or education as these affect not only brain phenotypes, but also lifestyle habits and the association between both are of additional relevance (Cullen et al., 2011; Fratiglioni et al.,

2004; Gur & Gur, 2017; Kramer & Colcombe, 2018; McKenna et al., 2003; Mukamal et al., 2001).

To measure brain aging patterns, the BrainAGE framework was developed (Franke et al., 2010), which multidimensionally aggregates voxel-wise GM alteration patterns of brain aging into one single value, the estimated brain age. The BrainAGE score is the difference between brain age as estimated from MR images and true chronological age (Franke et al., 2010). BrainAGE is positive if aging patterns observed via MRI appear older than expected based on chronological age (accelerated brain aging), and negative if they appear younger (decelerated brain aging). BrainAGE was shown to be a meaningful imaging biomarker for predicting the conversion from mild cognitive impairment to Alzheimer´s disease (Gaser et al., 2013), as well as for cognitive decline and disease severity (Franke & Gaser, 2012). Further, higher BrainAGE seems to be related to several physiological markers, such as higher BMI and markers of liver and kidney function (Franke et al., 2014).

The current study aimed at examining whether combined lifestyle risk contributes to deceleration or acceleration of brain aging reflected in BrainAGE in the population-based 1000BRAINS cohort of older adults (Caspers et al., 2014). First, we examined the relation between our newly developed combined lifestyle risk score (Bittner et al., 2019) and BrainAGE. We hypothesized that higher combined lifestyle risk would generally be associated with accelerated brain aging, i.e. higher BrainAGE scores. Second, we investigated the association between each individual lifestyle variable and BrainAGE to further elucidate contributions of single lifestyle variables to this general association. All analyses were run for the whole sample as well as for males and females separately (based on separate BrainAGE estimations) to account for sex-specific differences.

2. Materials and Methods

2.1 Participants

1,316 participants with an age range from 18.5 to 87.0 years were available from the population-based 1000BRAINS study (Caspers et al., 2014). Due to the population-based nature of the study, the only exclusion criteria for 1000BRAINS were contraindications for the MR session (Caspers et al., 2014). From the overall cohort sample, 87 participants were exluded due to missing MR scans, methodological failure during data processing (see MRI preprocessing) or missing BrainAGE estimation. Hence, 1,229 MR datasets were available as training sample for brain age estimation (see section 2.5, "Age Estimation Framework").

For the lifestyle analyses, we used data of those participants aged older than 55 years recruited from the Heinz Nixdorf Recall study (Schmermund et al., 2002). From those 1,229 participants with available MR data, $n = 666$ were within the selected age range. Two participants had to be excluded from the lifestyle analyses due to incidental findings, and 42 participants due to missing values in behavioral data. Finally, the older subsample for the lifestyle analyses consisted of 622 participants (272 females, 350 males) with an age range of 56 to 85 years (*mean* = 67.5 years, $SD = 6.7$). The study protocol of 1000BRAINS was approved by the Ethics Committee of the University of Essen (Germany). All participants gave written informed consent in agreement with the declaration of Helsinki.

2.2 Lifestyle Measures

Lifestyle data were retrieved from the database of the third examination (10-year follow up) of the Heinz Nixdorf Recall study that commenced in June 2011 (Caspers et al. 2014; Schmermund et al., 2002).

2.2.1 Alcohol consumption

Average consumption of different alcoholic beverages (beer as 0.2 liter, red and white wine as one glass of 0.2 liter, and spirits as 0.02 liter) within the last four weeks was assessed via a self-report questionnaire (Schmermund et al., 2002). The proportion of pure alcohol within the specific beverage was then multiplied with the frequency of drinking. Next, all beverages per person were summed up, resulting in the total amount of pure alcohol consumption in grams per month (g/month). Alcohol consumption as assessed via self-report questionnaires has been shown to highly correlate with blood indices of alcohol consumption (Giovannucci et al., 1991), multiple weekly self-report diet records (Giovannucci et al., 1991) and transdermal alcohol use assessment (Simons et al., 2015), thus providing adequate reliability and validity for most research purposes (Del Boca & Darkes, 2003).

2.2.2 Smoking

The degree of lifetime exposure to tobacco smoking was assessed as packyears (Duriez et al., 2014; Franklin et al., 2014; Karama et al., 2015), calculated by multiplying the years of smoking with the self-reported number of smoked cigarettes per day.

2.2.3 Social integration

Social integration was assessed using an adapted version of the social integration index (Berkman et al., 2004). The present social integration index comprised the domains "Marital status" (married or cohabitating participants were scored a 2; single, never married, widowed, or divorced participants were scored a 0), "close ties" (sum score derived from the number of children, close relatives, and friends) and "membership in organizations" (sum score of the number of organizations participants were members in and participated in at least once a month). Organizations included were: sport clubs, regional clubs, hunting clubs, choirs, theater clubs, music clubs, occupational or labor unions, political clubs or parties,

congregations, and self-help groups. The scores of all three domains were summed up into the social integration score.

2.2.4 Physical activity

To measure physical activity, we used the metabolic equivalent of task (MET, Ainsworth et al., 2000) measuring the energy expenditure of a given activity compared to rest. The compendium of physical activities (Ainsworth et al., 2000) provides a mean energy expenditure value per hour of each activity. Participants were asked to report up to four different sportive activities, carried out within the last month. Based on the MET values assigned to the activities listed in the compendium, MET values were assigned to each of the activities reported by the participants and multiplied by the duration in hours (per month). Finally, a sum score of all activities was calculated. Additionally, body-mass-index (BMI) was measured, since it has been shown to affect the association between physical activity and brain volume (Ho et al., 2011).

2.2.5 Construction of the combined lifestyle risk score

The combined lifestyle risk score was constructed as used in a previous study (Bittner et al., 2019). That is, to standardize the measurements of the four lifestyle variables, we first transformed the raw score on each individual lifestyle variable into a *z*-score. Next, in order to obtain a risk score that indicated higher risk with higher values, we reversed signs of the protective behaviors (social integration and physical activity). To obtain a risk score where a value of zero would indicate a mathematical balance of negative and protective behaviors, an additional linear transformation of the *z*-transformed lifestyle behaviors was applied: The protective variables were linearly transformed into negative scores by subtracting the maximum value from each individual measurement, whereas risk behaviors were analogously transformed into positive scores by adding the minimum value to each

individual measurement. Hence, all values for risk behaviors were positive. Finally, the linearly transformed values of all individual lifestyle variables were summed up into one combined lifestyle risk score.

2.3 Covariates

As the brains of women and men show some differences with respect to their structural architecture, e.g. in the proportion of gray matter and the thickness of the cortex (Ritchie et al., 2018) as well as aging trajectories (Cosgrove et al., 2007; Franke et al., 2014; Gur & Gur, 2017; Ruigrok et al, 2014; Wierenga et al., 2018), we considered sex as a covariate in the statistical models, and separately examined sex differences in performance measures. Generally, all statistical models were adjusted for chronological age to investigate the residual variability in brain aging. In addition, higher education is associated with higher cognitive performance (Elias et al., 1997) and is generally considered a proxy for brain reserve, the ability to better tolerate age-related neuronal loss (Bartés-Faz & Arenaza-Urquijo, 2011; Christensen et al., 2008; Stern, 2012). Further, there may be associations between education, intelligence, and lifestyle behavior (Cullen et al., 2011; Fratiglioni et al., 2004), where less smoking has been found in more educated individuals (McKenna et al., 2003). Hence, we hypothesized general educational level as a possible confounding factor and added education as a covariate into the statistical model. General education was measured using the international classification of education (ISCED, Unesco, 1997), a standard classification system with 10 levels, where higher levels indicate higher education.

2.4 MRI Preprocessing

T1-weighted anatomical 3D images were collected with a 3T Tim-TRIO MR scanner (Siemens Medical System, Erlangen, Germany). The following scan parameters were used: TR = 2.25 s, TE = 3.03 ms, TI = 900ms, FoV = 256 × 256mm2, flip angle = 9°, voxel resolution =

1 × 1 ×1mm³, 176 axial slices. A detailed description of the 1000BRAINS study protocol can be found in Caspers et al. (2014). MRI preprocessing was done using the SPM12 toolbox (The Wellcome Dept. of Imaging Neuroscience, London; www.fil.ion.ucl.ac.uk/spm) and the VBM8 package (http://dbm.neuo.uni-jena.de) running under Matlab (The MathWorks Inc., Natick, MA, USA). First, T1-weighted images were corrected for bias field inhomogenities and spatially normalized. Then, images were segmented (Ashburner & Friston, 2005) into gray matter (GM), white matter (WM) and cerebro-spinal fluid (CSF) using an approach additionally accounting for partial volume effects (Tohka et al., 2004) by applying adaptive maximum a posteriori estimations (Rajapaske et al., 1997) and a hidden Markov Random Field Model (Cuarda et al., 2005), as described in Franke et al. (2010). From these segmentation maps, only GM maps were used for the BrainAGE estimation framework. GM maps were registered using an affine registration and further smoothed with an 8-mm full-width-at-half-maximum (FWHM) kernel, while resampling the volumes at 8mm spatial resolution. Next, since neighbouring voxels are spatially correlated and therefore contain redundant information, principal component analysis (PCA) was conducted to reduce data dimensions using the "Matlab toolbox for Dimensionality Reduction" (version 0.7b; van der Maaten, 2007; Van der Maaten et al., 2007).

2.5 Age Estimation Framework

2.5.1 Training data

The BrainAGE framework is based on a support vector machine (Tipping, 2001) that transforms training data into a high-dimensional space (Bennett & Campbell; 2000) and translates features learned from a training sample onto an unkown test sample. To train and test the BrainAGE framework with respect to prediction accuracy and reliability, we used a 'leave 10% out'-procedure within the full cohort of 1000BRAINS, which spans 1,229 MR

datasets with an age range from 18.5 to 87.0 years (M = 60.7, SD = 13.4; M_{male} = 60.7, SD_{male} = 14.0; M_{female} = 60.9, SD_{female} = 12.5). In the training stage, the input data (90 percent of the whole sex-split sample) were used to train the BrainAGE framework using chronological age and the GM tissue probability maps (Franke et al., 2010), with separate age estimation training for female and male participants. Blind to their true chronological age, the patterns learned by the support vector regression were then transferred to the unknown 10 percent test participants, such that estimated age was based only on their anatomical patterns within the GM maps. For each participant, true chronological age was then subtracted from estimated age:

$$BrainAGE = estimated\ age - chronological\ age$$

Positive BrainAGE scores reflect accelerated aging, i.e., the estimated age is higher than the chronological age. In contrast, negative BrainAGE scores reflect decelerated aging, i.e. the estimated age is lower than the chronological age. Finally, a correction for a quadratic age trend, which is identifiable in Figure 1A, was applied to the resulting BrainAGE values using spm_detrend (SPM12, The Wellcome Dept. of Imaging Neuroscience, London; www.fil.ion.ucl.ac.uk/spm).

2.6 Statistical Analysis

In the first step, we explored performance measures of the BrainAGE framework. In a second step, we investigated the influence of possible covariates (sex and education) on BrainAGE to examine whether we need to consider their influences in the lifestyle analyses. In the main analyses, we then examined the assocations between combined lifestyle risk, as well as the individual lifestyle behaviors and BrainAGE. All statistical analyses were carried out using IBM SPSS Statistics 23.0.

2.6.1 Performance measures for the BrainAGE estimation

Following recent recommendations on age estimation models (Cole & Franke, 2017), we provide study sample specific performance measures for the age estimation framework applied within this study. To test accuracy of our model, we took the mathematical absolute value of each BrainAGE value and calculated the sample mean, which should then reflect the mean absolute error (MAE) of the brain age estimation. The smaller the MAE, the better the performance of the estimation (Cole & Frane, 2017). The idea behind is that each individual BrainAGE value can also be interpreted as the error of the age estimation model (i.e., the extent to which the estimation deviates from the true age). In addition, we examined Pearson correlations between *estimated age*, *chronological age*, and *BrainAGE*.

2.6.2 Relation between covariates and BrainAGE

We calculated a between-subjects MANCOVA with *sex* as independent factor (male vs female), educational level as covariate and *chronological age*, *estimated age*, and *BrainAGE* as dependent variables. Sex differences in educational level were examined using a between-subjects ANCOVA with independent factor *sex* (male vs female), covariate *chronological age* and *education* as dependent variable. Additionally, we calculated Spearman correlations (method of choice for ordinally scaled variables, such as the ISCED) between education and BrainAGE.

2.6.3 Lifestyle and BrainAGE

In our main analyses, we investigated the associations between lifestyle and BrainAGE using a twofold approach. First, we examined the association between BrainAGE and our combined lifestyle risk score. Second, we examined the association between BrainAGE and each individual lifestyle variable.

To investigate the linear effect of combining the four lifestyle variables into one score, we calculated multiple linear regressions (with IBM SPSS Statistics 20.0). In the analysis of

combined lifestyle risk, we first used the combined lifestyle risk score, age and sex as explanatory variables and BrainAGE as dependent variable. In the second analysis, we introduced age, sex and the individual lifestyle behaviors (physical activity, social integration, alcohol consumption, packyears of cigarettes) as explanatory variables to predict BrainAGE. In both analyses, we did a post hoc outlier exclusion (values > 3 SD) for each variable that showed a significant effect on BrainAGE. Afterwards, we added education as additional explanatory variable. To assess whether the combined lifestyle risk score explaines more variance than the individual lifestyle variables, we compared the explained variance in R^2 of both approaches.

2.6.3.1 Sex differences in the association between lifestyle and BrainAGE

We wanted to know whether sex changes the association between lifestyle and BrainAGE. We calculated a between-subjects ANCOVA using *sex* as independent between-subjects factor, *chronological age*, *education* and *the combined lifestyle risk score* as covariates and BrainAGE as dependent variable, while introducing an interaction term between sex and the combined lifestyle risk score to test for a moderating effect of sex on the association between lifestyle risk and BrainAGE. To test for moderating effects of sex on the association between the individual lifestyle behaviors and BrainAGE, we calculated a between-subjects MANCOVA using the same independent factor, but using chronological age, education and social integration, physical activity, alcohol consumption, and smoking as covariates, while introducing interaction terms between sex and each respective lifestyle variable.

Next, we performed separate multiple linear regression analyses for the two sexes first introducing only age as covariate and then adding education.

To further test for quadratic effects of the combined lifestyle risk score, as well as individual lifestyle behaviors on BrainAGE, we used curve fitting to compare whether

quadratic functions result in a better fit for any lifestyle variable and BrainAGE by first regressing the linear effect of the three covariates age, sex and education out and then using the residuals of the respective lifestyle variable as input. The linear function was defined as

$$BrainAGE = intercept + b_{1l} \times lifestyle + \varepsilon$$

whereas the quadratic function was defined as

$$BrainAGE = intercept + b_{1q} \times lifestyle + b_{2q} \times lifestyle^2 + \varepsilon$$

Subscript "l" indicates that the regressor belongs to the linear model, whereas subscript "q" indicates that the regressors to belong to the quadratic function. In the final step, linear and quadratic functions were compared with regard to the explained variance R^2.

2.6.3.2 Quantification of lifestyle effects

To quantify the effect of the combined lifestyle risk score and those individual lifestyle variables that showed a significant effect on BrainAGE, we estimated the slopes of the linear regression line for each explanatory factor using the linear equation:

$$BrainAGE = intercept + b_1 \times sex + b_2 \times age + b_3 \times lifestyle + \varepsilon$$

We used the intercept and unstandardized regression coefficients as calculated in the multiple linear regression as input for this equation, while the respective lifestyle variable (i.e., the combined lifestyle risk score or one individual lifestyle variable) was set to 1. We then multiplied the parameter b_3 with 12 months reflecting the increase in months of BrainAGE with one increase in the explanatory variable.

We defined groups to further analyze the association between smoking and BrainAGE and matched never (packyears = 0, 82 male, 82 female) to moderate (packyears < 20, 96 males, 68 female) to severe smokers (packyears ≥ 20, 55 females, 109 males) for age. We then calculated an ANCOVA using the factors group (never vs. moderate vs. severe) and sex, and the covariates age and education on the dependent variable BrainAGE.

2.6.3.3 Power analysis

As the effects of lifestyle on the brain are rather small (e.g. Miller et al., 2016), post-hoc power-analyses using GPower (Faul et al., 2009; http://www.gpower.hhu.de/) were calculated for the applied linear regression models as recommended for studies with a given sample size (Faul et al., 2009). Power reflects the probability of rejecting false null hypotheses, in our case rejecting an association between lifestyle and BrainAGE which is truly not there. Here, type-I error level α, the respective sample size (n = 622 for the whole subsample, n = 272 for female and n = 350 for male participants) and the fully adjusted model number of predictors was used.

2.6.5 Data availability

The datasets generated and / or analyzed during the current study will be made available from the corresponding author to other scientists on request in anonymized format and according to data protection policy in the ethics agreement.

3. Results

3.1 Performance of the BrainAGE estimation framework

Descriptive statistics of the training data set, comprising the whole available sample of 1000BRAINS (n = 1,229), are shown in Table 1. Mean BrainAGE was 0.00 (SD = 5.04). The mean absolute error (MAE) between chronological and estimated age was low with 4.62 years (SD = 3.67), respective was the correlation very high (r = .90, p < .001, Table 2, Figure 1A). The regression of estimated age on chronological age explained up to 83% of the variance (Table 1).

Within the older subsample (n = 622), which we used for our main analysis of the association between lifestyle and BrainAGE, mean BrainAGE was 0.23 years (SD = 4.96) with a maximum positive deviation between chronological and estimated age of + 15.92 years (brains appearing older compared to their chronological age) and a maximum negative deviation of – 15.67 years (brains appearing younger compared to their chronological age). MAE was 3.97 (SD = 2.99) years, and did not differ between the two sexes [T (2, 620) = 0.20, p = 0.839]. The regression of estimated age on chronological age explained up to 52% of the variance (Table 1). The correlation between chronological and estimated age was r = 0.714 (p = 0.0001, Table 2, Figure 2a), whereas the correlation between chronological age and BrainAGE was r = - 0.10 (p = 0.025, Figure 2b). Hence, the older the participants, the lower were the BrainAGE scores.

3.2 Relation between covariates and BrainAGE in the older adult sample

In the between-subjects MANCOVA, there was no significant difference between female and male participants in chronological age, $F(1,621) = 1.55$, $p = 0.213$, $\eta^2 = 0.002$, nor in BrainAGE, $F(1, 621) = 1.35$, $p = 0.246$, $\eta^2 = 0.002$. Mean estimated age was 1.28 years lower for females than for males with $F(1,621) = 3.98$, $p = 0.046$, $\eta^2 = 0.005$ (Table 1). In the between-

subjects ANCOVA, males showed higher educational level than females [$F(1,621) = 39.50$, $p < .0001$, $\eta^2 = 0.06$]. No correlation between BrainAGE and education was found ($p = 0.937$), even when stratifying the analyses for the two sexes (Table 3).

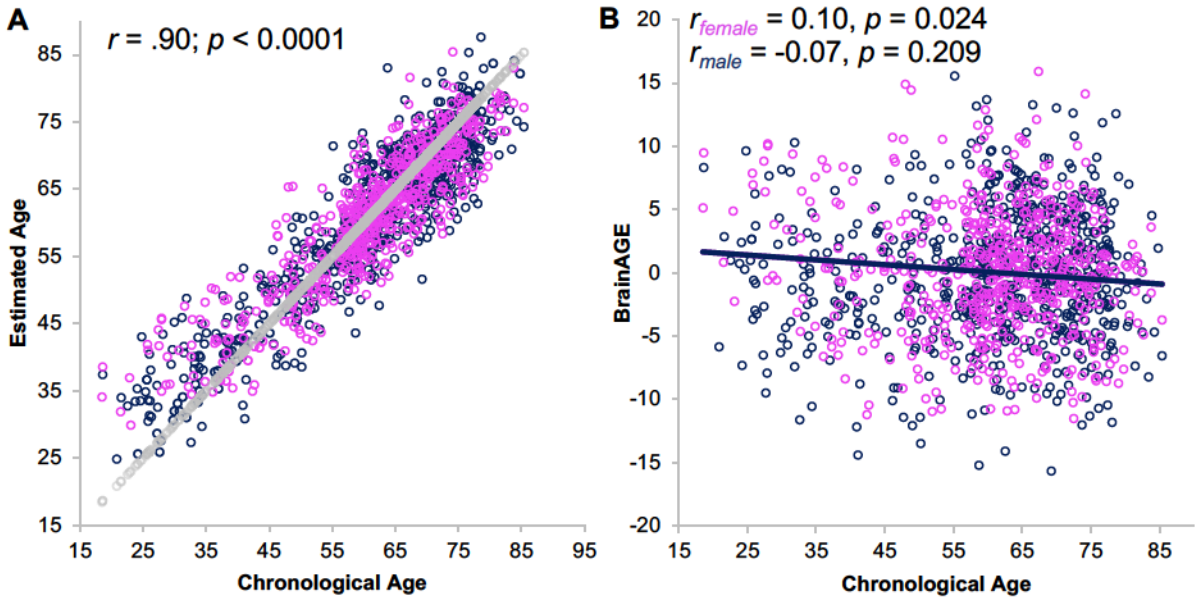

Figure 1. Scatter plots for the whole sample of 1000BRAINS, n = 1,229. **A.** Correlation between estimated and chronological age. Grey dots represent a regression line fitted to a simulated perfect correlation between estimated and chronological age of r = 1.0. **B.** Correlation between chronological age and BrainAGE. The correlation was significant for female (pink), but not for male participants (blue).

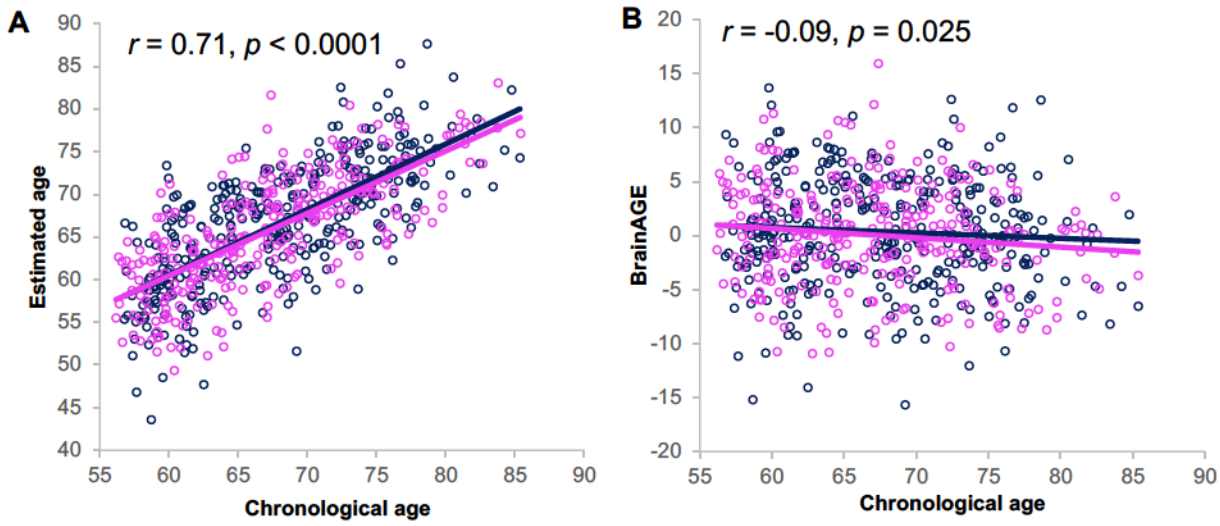

Figure 2. Scatter plots for the older subsample of $n = 622$ used in the lifestyle analyses. **A.** Correlation between estimated and chronological age was significant with $r = 0.71$ ($p < 0.0001$). **B.** Correlation between chronological age and BrainAGE was $r = -0.09$ ($p = 0.025$). Blue dots represent men, pink plots represent women. Sex-specific correlation values can be found in Table 2.

Table 1

Performance of the BrainAGE estimation framework

Whole sample of 1000BRAINS, $n = 1,229$, age range 18.5 – 85.4

	Mean chronological age	*Mean* estimated age	*Mean* BrainAGE	*MAE*	R^2
Whole group	60.8 (13.4)	60.7 (11.6)	0.0 (5.0)	4.6 (3.7)	0.81
Male ($n = 680$)	60.7 (14.0)	60.8 (10.5)	0.0 (5.1)	4.6 (3.6)	0.78
Female ($n = 549$)	60.9 (12.5)	60.6 (12.4)	-0.1 (4.9)	4.6 (3.7)	0.83

Older subsample used in analyses of lifestyle, $n = 622$, age range 56.2 – 85.4

Whole group	67.5 (6.7)	66.8 (7.5)	-0.2 (5.0)	4.0 (3.0)	0.51
Male ($n = 350$)	67.8 (6.7)	66.5 (7.2)	-0.4 (5.1)	4.1 (3.1)	0.51
Female ($n = 272$)	67.1 (6.7)	65.7 (6.9)	0.1 (4.8)	3.8 (2.9)	0.52

Note. Descriptive statistics and performance measures for the whole training sample, as well as the older subsample for lifestyle analyses. Age is given in years. Standard deviation is given in parentheses. *MAE* = mean absolute error between estimated and chronological age. R^2= explained variance drawn from linear regressions of estimated age on chronological age.

Table 2

Correlations between chronological age and estimated age and BrainAGE

Whole sample of 1000BRAINS, *n* = 1,229			
		Estimated age	BrainAGE
Chronological age	Whole group	r = -0.90, p < 0.001	r = 0.00, p = 0.999
	Male (n = 650)	r = 0.92, p < 0.001	r = -0.07, p = 0.083
	Female (n = 549)	r = 0.88, p < 0.001	r = 0.10, p = 0.024
Older subsample used in analyses of lifestyle, *n* = 622			
Chronological age	Whole group	r = 0.71, p < 0.001	r = -0.10, p = 0.025
	Male (n = 350)	r = 0.71, p < 0.001	r = 0.07, p = 0.209
	Female (n = 272)	r = 0.72, p < 0.001	r = - 0.12, p = 0.047

Table 3

Correlations between ISCED and chronological age, estimated age and BrainAGE

Spearman Correlations		Chronological age	Estimated Age	BrainAGE
ISCED	Whole subsample (n = 622)	ρ = -0.14, p < 0.001	ρ = 0.13, p = 0.001	ρ = -0.03, p = 0.937
	Male (n = 350)	ρ = -0.11, p = 0.040	ρ = 0.12, p = 0.028	ρ = -0.06, p = 0.251
	Female (n = 272)	ρ = -0.16, p = 0.008	ρ = - 0.13, p = 0.029	ρ = 0.02, p = 0.705

Note. Spearman correlations between general level of education as measured by ISCED with chronological age, estimated age, and BrainAGE for the older subsample of lifestyle analyses. ρ = Spearman correlation coefficient.

3.3 Main analyses of lifestyle and BrainAGE

3.3.1 Combined lifestyle risk

In our first analysis of lifestyle, we investigated the association between combined lifestyle risk and BrainAGE. Table 4 shows descriptive statistics for all individual lifestyle variables as well as for the combined lifestyle risk score. Mean combined lifestyle risk was -1.02 (SD = 2.1), reflecting a rather protective behavior within the selected older subsample of 1000BRAINS. The first multiple linear regression using combined lifestyle risk as

independent variable and age and sex as covariates showed a significant effect on BrainAGE [$F(3, 617) = 8.77$, $p < 0.001$, $R^2 = 0.04$)]. Here, higher combined lifestyle risk was significantly associated with higher BrainAGE, with a regression coefficient of $\beta = 0.18$, $T = 4.54$, $p < 0.001$. These results remained stable, even after outlier exclusion ($\beta = 0.17$, $T = 4.28$, $p < 0.001$, Figure 3; outlier marked with diamond shapes) and introducing education as a third covariate ($\beta = 0.171$, $T = 4.18$, $p < 0.001$).

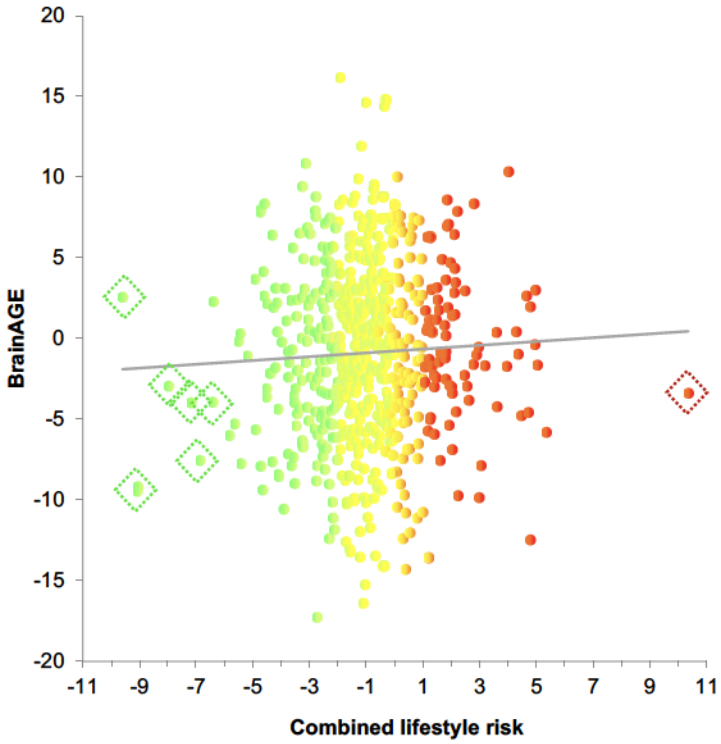

Figure 3. Correlation between combined lifestyle risk and BrainAGE. Higher combined lifestyle risk was associated with higher BrainAGE. The color spectrum depicts the increase in lifestyle risk from protective (green) to balanced (yellow) to more risky (red) behaviour.

3.3.2 Individual lifestyle variables

In the next step, we examined how each individual lifestyle variable was related to BrainAGE by including all four individual lifestyle variables into a multiple linear regression as explanatory variables, while correcting for age and sex. Overall, the model significantly predicted BrainAGE, $F(6,615) = 5.14$, $p < 0.001$, $R^2 = 0.05$. We found a significant association

between higher amount of smoking and higher BrainAGE, $\beta = 0.14$, $T = 3.41$, $p = 0.009$, even after outlier correction ($\beta = 0.17$, $T = 4.24$, $p < 0.0001$; Figure 4; 10 outlier marked with diamond shapes) and adding educational level as covariate ($\beta = 0.17$, $T = 4.17$, $p < 0.0001$). Additionally, higher physical activity was associated with lower BrainAGE, $\beta = -0.11$, $T = -2.75$, $p = 0.006$. This assocation remained significant even after exclusion of 9 outliers (Figure 5, marked with diamond shapes, $\beta = -0.15$, $T = -3.72$, $p < 0.001$) and adding educational level as covariate ($\beta = -0.15$, $T = -3.72$, $p < 0.001$). We additionally corrected this association for body mass index (BMI), as BMI has been shown to influence the association between physical activity and brain structure (Ho et al., 2011) and memory performance (Flöel et al., 2008). Higher physical activity remained significantly associated with lower BrainAGE ($\beta = -0.14$, $T = -3.41$, $p = 0.001$). No other individual lifestyle variable showed significant effects on BrainAGE (Supplementary Table 1, $\beta_{\text{alcohol consumption}} = 0.05$, $p = 0.233$; $\beta_{\text{social integration}} = -0.05$, $p = 0.170$).

3.3.3 Sex differences in the association between lifestyle and BrainAGE

3.3.3.1 Combined lifestyle risk

The between-subjects ANCOVA did not show a significant interaction between sex and the combined lifestyle risk score on BrainAGE [$F(4, 617) = 0.18$, $p = 0.672$], even after outlier correction [$F(4, 617) = 0.37$, $p = 0.545$]. After stratifying the group by the two sexes to account for overall sex differences in BrainAGE, higher combined lifestyle risk was still associated with higher BrainAGE in both males ($\beta = 0.19$, $T = 3.66$, $p < 0.001$, [$F(2,346) = 6.46$, $p < 0.001$, $R^2 = 0.04$ for the whole regression model including age]) and females ($\beta = 0.16$, $T = 2.63$, $p = 0.009$, [$F(2, 269) = 5.55$, $p = 0.005$, $R^2 = 0.04$ for the whole model including age]). Neither introducing education as a second covariate changed these associations (Supplementary Table 1), nor outlier exclusion (Supplementary Table 1, Figure 4, outliers marked with diamond shapes).

3.3.3.2 Individual lifestyle variables

We did not find any significant interaction effects of sex and the individual lifestyle variables on BrainAGE in the between-subjects MANCOVA. After stratifying for the two sexes, smoking and physical activity showed significant effects (Supplementary Table 2). Here, the association between higher amount of smoking and higher BrainAGE was still significant for male [$\beta = 0.18$, $T = 3.46$, $p = 0.001$, $F(2, 340) = 6.92$, $p = 0.001$, $R^2 = 0.04$ for the whole model], as well as for female participants [$\beta = 0.134$ $T = 2.33$, $p = 0.021$, $F(2, 266) = 4.90$, $p < 0.01$, $R^2 = 0.04$ for the whole model], even after outlier correction (Supplementary Table 2, Fig. 4A, outliers marked with diamond shapes) and adding education as a second covariate (Supplementary Table 2). The association between higher physical activity and lower BrainAGE, though, remained significant for male participants [$\beta = -0.19$, $T = -3.50$, $p = 0.005$, $F(2, 346) = 7.05$, $p = 0.003$, $R^2 = 0.04$ for the whole model], but not for female participants [$\beta = -0.10$, $T = -1.60$, $p = 0.110$, $F(2, 265) = 3.53$, $p < 0.05$, $R^2 = 0.03$ for the whole model]. In males, this association was significant despite outlier correction and adding education and BMI as covariates (Supplementary Table 2).

3.3.4 Exploration of non-linear lifestyle effects

Finally, we tested for quadratic effects of the combined lifestyle risk score and individual lifestyle variables on BrainAGE. Lifestyle variables that were used as regressors in the curve fitting process were already corrected for age, sex, education, and, in case of physical activity, BMI. Therefore, the R^2 values within this estimation differ from those in the purely linear regressions. In the following section, the subscript "q" indicates that the regressors belong to the quadratic function.

3.3.4.1 Combined lifestyle risk

Adding a quadratic term to the linear regression of BrainAGE on combined lifestyle risk did not result in a better fit than the linear regressions, with both functions explaining 3.1% of the variance and the quadratic term itself not being significant, which was also true after outlier correction. After splitting the sample by sex, adding the quadratic terms did show a

slightly better fit to the data than the linear model in male participants ($R^2_{linear} = 0.040$, $R^2_{quadratic} = 0.046$; $\beta_{2q} = -0.077$, $T = -1.43$, $p = 0.155$), but not in female participants ($R^2_{linear} = 0.024$, $R^2_{quadratic} = 0.024$; $\beta_{2q} = 0.015$, $T = 0.25$, $p = 0.803$). All regression statistics can be found in Supplementary Table 3.

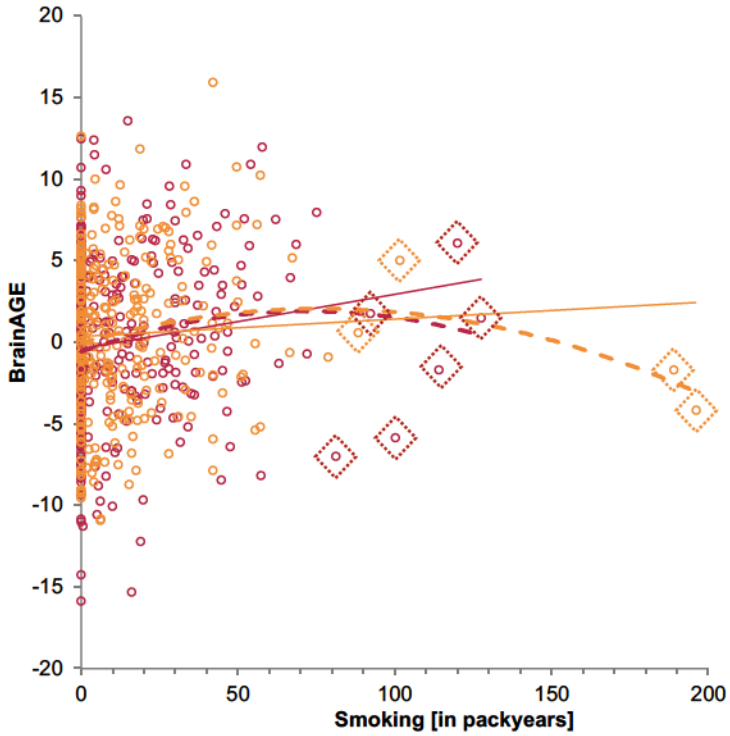

Figure 4. Association between smoking and BrainAGE. Higher BrainAGE was associated with higher amount of smoking. Orange dots represent men, magenta dots represent women. The interaction between sex and amount of smoking on BrainAGE was not significant. Dashed lines represent the fit of a quadratic function into the data.

3.3.4.2 Individual lifestyle variables

Significant quadratic associations between the individual lifestyle variables and BrainAGE were found for smoking and physical activity.

Adding the quadratic term to the linear model describing the association between smoking and BrainAGE resulted in marginally higher explained variance ($R^2_{linear} = 0.021$, $R^2_{quadratic} = 0.023$), while the quadratic term itself was not significant ($\beta_{2q} = -0.056$, $T = -0.99$, p

= 0.323). This was also true in male participants only (R^2_{linear} = 0.021, $R^2_{quadratic}$ = 0.24; β_{2q} = -0.082, T = -1.05, p = 0.293, Suppl. Table 4). After adding the quadratic term to the linear model for female participants, the model no longer showed a significant effect on BrainAGE (Suppl. Table 4).

Regarding the association between physical activity and BrainAGE, the quadratic term showed an additional effect on BrainAGE (R^2_{linear} = 0.011; $R^2_{quadratic}$ = 0.019; β_{2q} = 0.125, T = 2.171, p = 0.030), which was no longer significant after outlier correction (Suppl. Table 4). In male participants, including the quadratic term did result in a slightly better model (R^2_{linear} = 0.024; $R^2_{quadratic}$ = 0.029), while the additional quadratic term was not significant (β_{2q} = 0.11, T = 1.36, p = 0.174) and the better model fit disappeared after outlier correction. In female participants, neither the quadratic nor the linear model showed a significant association between physical activity and BrainAGE (Suppl. Table 4).

3.3.5 Quantification of lifestyle effects

For one increase in the combined risk score (risk score = 1), BrainAGE was estimated at 3.84 months older in addition to the effect of sex and age (*BrainAGE = 15.40 -0.57 x sex - 0.23 x age + 0.32 x riskscore).* For each packyear of cigarettes, BrainAGE was estimated at 0.36 months older (*BrainAGE = 5.07 -0.01 x sex -0.07 x age + 0.03 x packyears).* Finally, we calculated an increase in BrainAGE of 0.36 months per packyear in males and 0.48 months per packyear in females ($BrainAGE_{male}$ *= 3.52 -0.05 x age – 0.03 x packyears;* $BrainAGE_{female}$ *= 4.89 -0.08 x age – 0.04 x packyears).* Comparing never, moderate and severe smokers revealed, that the brains of severe smokers (BrainAGE = 1.61) appeared significantly older than those of never (BrainAGE = -0.05) and moderate (BrainAGE = -0.03) smokers, but that there was no significant difference between never-smokers and moderate smokers in average BrainAGE.

For each metabolic equivalent that was expended per week, BrainAGE was estimated 0.48 month younger (*BrainAGE = 6.53 -0.49 x sex -0.07 x age + 0.04 x activity).* Finally, we calculated a decrease in BrainAGE of 0.6 months per metabolic equivalent in males and 0.48

months per metabolic equivalent in females ($BrainAGE_{male} = 4.94 - 0.06 \times age - 0.05 \times packyears$; $BrainAGE_{female} = 7.12 - 0.09 \times age - 0.03 \times activity$).

3.3.6 Power analysis. The post-hoc power analysis for the regression of BrainAGE onto combined lifestyle risk within the whole subsample using $\alpha = 0.05$, $n = 622$ and *number of predictors* = 3 revealed a *power* of 0.99 (power male = 0.93; $power_{female} = 0.84$). Similar values were obtained for the regression of BrainAGE onto packyears using $\alpha = 0.05$, $n = 622$ and *number of predictors* = 3 *with* a *power* of 0.99 (power male = 0.93; $power_{female} = 0.84$), and onto physical activity using $\alpha = 0.05$, $n = 622$ and *number of predictors* = 4 with a *power* of 0.95 (power male = 0.89; $power_{female} = 0.72$).

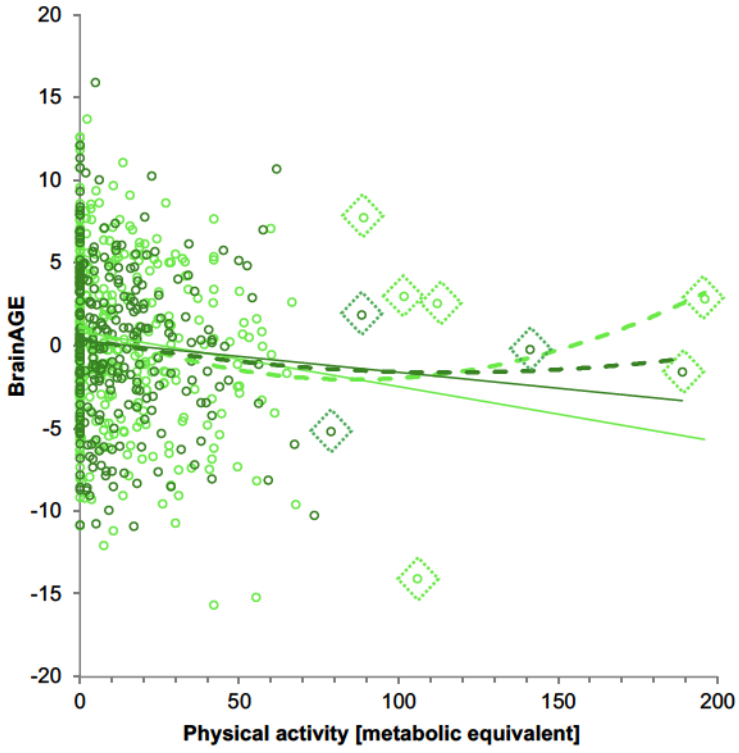

Figure 5. Association between physical activity and BrainAGE. Lower BrainAGE was associated with higher physical activity as measured with the metabolic equivalent. Dark green dots represent female, light green dots represent male participants. The interaction effect between sex and physical activity on BrainAGE was not significant. The dashed lines represent quadratic trends. Regression lines were significant for males, whereas they were not significant for females.

Table 4

Descriptive statistics of lifestyle variables

		Min	Max	*Mean*	*SD*
Packyears	total sample	0.00	204.00	13.61	21.73
	(Male, Female)	(0.00, 0.00)	(204.00, 123.00)	(16.27, 1.19)	(24.37, 17.21)
Alcohol consumption	total subsample	0.00	198.50	11.25	19.87
	(Male, Female)	(0.00, 0.00)	(198.50, 79.40)	(15.85, 5.33)	(23.86, 10.46)
Physical activity	total subsample	0.00	196.00	14.32	20.94
	(Male, Female)	(0.00, 0.00)	(196.00, 189.00)	(14.69, 13.86)	(21.07, 20.81)
Social integration	total subsample	3.00	53.00	12.99	6.40
	(Male, Female)	(3.00, 4.00)	(53.00, 44.00)	(13.19, 12.72)	(6.53, 6.24)
Combined lifestyle risk	total subsample	-9.60	10.36	-1.02	2.06
	(Male, Female)	(-9.60, -9.08)	(10.36, 4.96)	(0.72, 1.40)	(2.19, 1.81)

Note. Descriptive statistics for all four individual lifestlye variables, as well as the combined lifestlye risk score for the total older subsample (n = 622), as well as for older males (n = 350) and older females (n = 272).

Table 5

Correlations between level of eduction and lifestyle variables

General level of education	Packyears	Alcohol consumption	Physical activity	Social integration	Combined lifestyle risk score
Whole subsample (n = 622)	ρ = -0.21, p < 0.001	ρ = -0.02, p = 0.066	ρ = 0.10, p = 0.017	ρ = 0.13, p = 0.001	ρ = -0.16, p < 0.001
Male (n = 350)	ρ = -0.22, p < 0.001	ρ = 0.01, p = 0.841	ρ = 0.10, p = 0.064	ρ = 0.17, p = 0.001	ρ = -0.19, p < 0.001
Female (n = 272)	ρ = -0.12, p = 0.046	ρ = 0.06, p = 0.311	ρ = 0.14, p = 0.014	ρ = 0.12, p = 0.059	ρ = -0.14, p = 0.026

Note. ρ=Spearman correlation coefficient.

4. Discussion

The present study showed that lifestyle habits contribute to differences in brain aging in a population-based cohort of older adults and gives promising insights into why people age so differently. We used two approaches: We used a novel lifestyle risk score (Bittner et al., 2019) combining different lifestyle variables into one value, while additionally investigating each lifestyle habit alone. In addition, we examined differences in brain structure using BrainAGE as a meaningful imaging biomarker (Franke & Gaser, 2012; Gaser et al., 2013; Loewe et al., 2016; Franke et al., 2014), which showed a very good performance in our sample (Cole & Franke, 2017). Further, our results hint at sex differences in the association between lifestyle and BrainAGE that are often not examined in studies on lifestyle associated differences in the brain, e.g. in terms of smoking (Karama et al., 2015), alcohol consumption (Vergara et al., 2017) and physical activity (Kramer & Colcombe, 2018).

4.1 Associations between combined lifestyle risk and BrainAGE

Prior studies focused mostly on the effect of single lifestyle variables on the brain, considering co-occurrences of various lifestyle behaviors as nuisance factors rather than as effects of interest. In contrast, the current study considered four different lifestyle behaviors as a combined concept (Bittner et al., 2019) to examine if lifestyle explains variability in BrainAGE and which lifestyle behaviors contribute the most to this association. Regarding a phenotype as complex and multi-dimensional as lifestyle, it is reasonable to assume that one specific behavior can only account for parts of the variance in brain aging, providing necessity for investigation of different variables together (Bittner et al., 2019; Flöel et al., 2008; Vergara et al., 2017). Considering several behaviors as well as composite scores seem to provide a better prediction of differences between older adults, e.g. in verbal memory, than the individual measures alone (Flöel et al., 2008). Comparable approaches have also been

used in imaging genetics, where polygenic risk scores (several genetic markers aggregated into one score) can explain more variability in neurological diseases and brain phenotypes than individual genetic markers alone (Dudbridge et al, 2013; Harrison et al., 2016; Torkamani et al., 2018; Ursini et al., 2018). Therefore, we hypothesized that our combined lifestyle risk score would explain more variance than each single behavior alone.

As hypothesized, we found a lifestyle-dependent acceleration of structural brain aging, where higher lifestyle risk was associated with higher BrainAGE scores, thus older looking brains. This observation is particularly important, since it helps explain a significant proportion of the large interindividual variability in structural brain aging of older adults (Dickie et al., 2013), which cannot be accounted for by age, sex, education or clinical markers, such as BMI or uric acid (Arenaza-Urquijo et al., 2015; Christie et al., 2017, Eavani et al., 2018; Fjell et al., 2012; Franke et al., 2014; Jagust 2013; Lockhardt & DeCarli, 2014). In the present sample, the model consisting of age, sex, and all individual lifestyle variables explained a comparable amount of the variance in BrainAGE (5%) as the model consisting of age, sex, and the combined lifestyle risk score. This is comparable to the amount of explained variance reported for lifestyle behaviors or health markers in other large epidemiological studies (Franke et al., 2014; Jockwitz et al., 2017; Miller et al., 2016). In a former study, individual lifestyle variables did not show a significant effect on cortical surface measures, whereas the combined lifestyle risk score did (Bittner et al., 2019). However, it is important to note, that the former study was a vertex-wise whole brain approach, sensitive to lifestyle-related regional differences, whereas the present study examined the association between lifestyle and BrainAGE, reflecting the multidimensional pattern of aging aggregated into one marker. Importantly, we quantified the effect of combined lifestyle risk on structural brain aging in terms of years. This was inspired by Franke et al. (2014), who estimated mean BrainAGE in a

"risky" and a "healthy" group in terms of clinical markers, such as BMI or uric acid. Instead of quantifying group differences in terms of years, as done by Franke et al. (2014), we estimated the linear increase in BrainAGE for each increase in lifestyle risk of the specific variable. In consequence, with each increase in combined lifestyle risk, brains appear 3.8 months older than the "normal" age-related difference in brain structure, which the statistical model corrected for. In comparison, brains appear 0.24 months older with each packyear and 0.48 months younger with each increase in metabolic equivalent (MET) per week (with, e.g., 4 MET reflecting one hour of 10mph bicycling). Hence, the combined lifestyle risk score explained more than 3 months in Brain AGE "in addition" to smoking in packyears, as hypothesized. Therefore, the combined lifestyle risk score seems to have higher explanatory power, presumably via consideration of over-additive and interacting effects between the individual factors when quantifying the harmful and protective effects of lifestyle. Considering different behaviors of interest simultaneously may thus be a fruitful way to explain additional variance in brain aging by investigating their cumulative effects.

4.2 Associations between individual lifestyle variables and BrainAGE

Investigating the four lifestyle behaviors individually revealed more smoking and lower physical activity to be the strongest contributors to the positive association between lifestyle risk and BrainAGE.

4.2.1 Smoking

One of the compelling results of the current study were the negative effects of smoking on the aged brain quantified in months. Prior studies already hinted at an association between smoking and changes in GM. Regionally lower GM volume and density for smokers compared to non-smoking control participants have been reported in the prefrontal cortex and the cerebellum (Brody et al., 2004), the posterior cingulum, precuneus,

right thalamus, and bilateral frontal cortex (Almeida et al., 2008), as well as the substantia nigra (Gallinat et al., 2006). Importantly, all of these studies had a fairly small sample size that either included younger adults only (n = 45, age range: 22.4 – 38.3 years; Gallinat et al., 2006), older adults only (n = 78, age range 71.6 – 78.9 years; Almeida et al., 2008), or a large age range (n = 36, 21 – 65 years; Brody et al., 2004). Additionally, results on the association of smoking with other brain metrics are quite heterogenous. Higher numbers of white matter hyperintensities (Longstreth et al., 2005), lower microstructural integrity (Gons et al., 2011), or infarcts (Howard et al., 1998) in smokers compared to non-smokers were reported. With population-based cohort imaging available, the sample sizes have substantially increased (e.g. Bamberg et al. 2015; Caspers et al., 2014; Miller et al., 2016; Van Essen et al., 2012), thus increasing generalizability of results to the general population. For example, Karama et al. (2015) showed that smoking was associated with widespread cortical thinning in a sample of 504 older adults particularly in prefrontal cortex, mostly omitting primary sensory areas. Still, none of the studies provided a quantification of the effect of smoking on the brain. For the first time, we were able to associate each packyear with an increase of 0.36 months of BrainAGE. Translating this result to our older adult study sample taking into account the average smoking behavior of 13.61 packyears, an overall increase of 4.9 years of BrainAGE (13.61 x 0.36 month of BrainAGE) only by smoking can be stated.

There was a high variance in BrainAGE in individuals, who never smoked (139 female, 114 male, Figure 4). Within this group, BrainAGE scores were very high, as well as very low, aggregating to a mean BrainAGE of almost zero. This finding is comparable to the considerable variance in cortical thickness in the rarely smoking participants observed by Karama et al. (2015). In the current study, the more the participants smoked, the stronger was the relationship between higher packyears and higher BrainAGE, suggesting that this

effect was mostly driven by high lifetime smoking (Figure 4). This was also revealed when comparing never, moderate and severe smokers, where the brains of severe smokers (BrainAGE = 1.61) appeared significantly older than those of never (BrainAGE = -0.05) and moderate (BrainAGE = -0.03) smokers. It is particularly important to note that this observation cannot be translated simply to the assumption that rare smoking has no effect on the brain. Rather, rare smoking may manifest in other metrics for healthy brain aging, even if alterations in brain structure would not be present: For example, in our previous study we found no association between smoking and cortical surface measures in older adults. Instead, more smoking was associated with higher resting-state functional connectivity (RSFC), which may be a compensation mechanism for accelerated brain aging (Bittner et al., 2019). Additionally, activity differences in task-based fMRI (Lawrence et al., 2002; Tanabe et al., 2011), as well as receptor differences between smokers and non-smokers (Feduccia et al., 2012; Mukhin et al., 2008) were described. Studies on RSFC in relation to longterm effects of smoking and not acute effects of nicotine are rather rare. It may thus be of particular interest to investigate general differences in brain function, e.g. in RSFC, associated with light smoking (Janes et al., 2012; Pariyadath et al., 2014; Zhou et al., 2017), even though light smoking seems not to be heavily associated to differences in brain structure.

The underlying mechanisms driving the association between smoking and changes in brain structure are still unclear. Smoking could potentially act via atherosclerotic processes, which may impact the aging brain and thus accelerate brain aging (Prescott et al., 1998, Freund et al., 1993; Mucha et al., 2006; Pujades-Rodriguez et al., 2014). Possibly, the measured increase in BrainAGE might also be attributable to the direct toxic effects of tobacco smoke onto the cerebro-vascular system, which includes oxidative stress within the cells and results in apoptosis (Swan & Lessov-Schlaggar, 2010). However, since BrainAGE

takes the whole GM volume of an individual into account, drawing inferences about any molecular mechanisms or disentangle regional differences that drive the association between stronger smoking and accelerated brain aging as reflected by higher BrainAGE remains for future studies.

4.2.1.1 Sex differences in the associations between smoking and BrainAGE

Most prior studies assessing the effect of smoking on brain structure did not examine sex differences or the interaction of sex and smoking (Almeida et al., 2008; Brody et al., 2004; Gallinat et al., 2006, Karama et al., 2015; Longstreth et al., 2005). To our knowledge, there are only two studies addressing this issue, showing that structural differences associated to smoking may regionally differ depending on sex (Duriez et al., 2014; Franklin et al., 2014). In the present study, we addressed this issue and found no interaction between sex and smoking, hinting at a comparable direction and strength of association in both sexes. Nevertheless, future studies should carefully address sex-differences in lifestyle effects. With imaging research focusing more on sex differences (Franke et al., 2014; Gur & Gur, 2017; Richie et al., 2018; Ruigrok et al., 2014; Wierenga et al., 2018) it may be of particular interest to identify lifestyle behaviors that differentially affect male and female brains, such that interventions that slow or delay manifestations of aging can be tailored for sex.

4.2.2 Physical activity and BrainAGE

The protective effect of physical activity on GM volume has been discussed to be regionally specific (see review by Erickson et al., 2014). Our results support an association between higher physical activity and lower BrainAGE, thus younger looking brains. Physical activity therefore does not only seem to affect specific brain regions, but also the multi-dimensional pattern of brain aging itself. Most previous studies comprised intervention trainings, where training was systematic, regular, and highly controlled (Erickson et al.,

2014). The present study adds to this by demonstrating that higher physical activity is associated with decelerated brain aging (lower BrainAGE scores) in a population-based sample of older adults using a comprehensive, epidemiologically motivated measurement of physical activity, i.e. the metabolic equivalent (Ainsworth et al., 1992; Bus et al., 2011; Flöel et al, 2010; Milanović et al., 2013; Pierce et al., 2007; Ruscheweyh et al., 2011; Wagner et al., 2012). This measurement is drawn from self-reports that summarize all sorts of sports older adults engage in and is likely to reflect the average daily physical activity, in contrast to highly controlled intervention settings. The present association between self-reported physical activity and BrainAGE is therefore not as strong as reported effects of fitness training on e.g. the hippocampus (Erickson et al., 2011), but is likely reflecting a natural and therefore more generalizable relationship.

Several mechanisms how higher physical activity or fitness levels may act protectively on the aging brain have been discussed, such as the upregulation of neurotrophic factors, including brain-derived-neurotrophic factor (BDNF, de Melo Coelho et al., 2013; Neeper et al., 1996, Piepmeier & Etnier, 2015) and granulocyte-colony stimulating factor (G-CSF; Flöel et al., 2010), which significantly impact synaptic efficacy, neuronal connectivity, and use-dependent plasticity. Here, use-dependent plasticity may play a crucial role in the sense of the "lose-it-or-use-it"-hypothesis (Swaab et al., 2002). This hypothesis states that those neurons needed and therefore stimulated in daily life are better preserved during the lifespan. As a consequence, physical activity as one kind of training would lead to better preservation of those brain structures needed to perform the activity engaged in (Bittner et al., 2019; Colcombe et al., 2003; Vaynman et al., 2004; Vaynman & Gomez-Pinilla, 2005). Several studies have shown that training-induced preservation or even adaptation of

brain regions is possible in adults (Churchill et al., 2002; Draganski et al., 2004; Erickson et al., 2011, Kramer & Erickson, 2007), older adults in particular (Boyke et al., 2008).

4.2.2.1 Sex differences in the associations between physical activity and BrainAGE

Interestingly, the strength of this association seems to differ between the two sexes. Stratifying the sample for the two sexes revealed the relationship to be significant in male ($p = 0.005$), but not in female participants ($p = 0.169$). However, the power to find an effect of physical activity was lower in female participants (0.72) than in male participants (0.89), with a power of 0.72 translating to 28% probability of assuming no effect of physical activity, even though the effect is truly present. Hence it is possible, that even larger sample sizes are needed to detect the small effect of physical activity in females as well. A recent review concluded that the sex proportion in physical activity intervention studies may impact the effect sizes (Kramer & Colcombe, 2018). A potential reason may be expression of BDNF and its effect on physical activity, which has been shown to differ between the sexes in mice with lower expression in females (Venezia et al. 2016). Further, estrogens or hormone replacement therapy seem to be related to levels of neurotrophins (Garcia-Segura et al., 2000). One study showed that longer periods of hormone therapy corroborated the positive effect of high physical activity on cognitive performance in women (Erickson et al., 2007). If the enhanced release of neurotrophic factors like BDNF drive the relationship between physical activity (Flöel et al., 2010, Ruscheweyh et al., 2011) and brain structure and the release of neurotrophic factors differs between the two sexes, different levels of neurotrophins may be another reason for the sex differences reported in the current study. There may also be further differences between the two sexes that co-occur with physical activity, such as dietary habits (Kramer & Colcombe, 2018), the specific kind of activity (Churchill et al., 2002; Colcombe et al., 2003; Flöel et al., 2010; Hayes et al., 2013; Kramer & Colcombe, 2018), as well

as differences in metabolism (Burd et al., 2009; Wu & O´Sullivan, 2011) to be considered in future studies. Taken together, higher physical activity seems to be one lifestyle behavior that contributes to decelerated brain aging, in line with previous studies. Still, there seems to be a lack of studies addressing the possibility of sex-differences within this association such that underlying mechanisms could be identified. Most previous studies statistically controlled for sex as a covariate, but did not examine interaction effects, as done in classic psychological research or specifically conducted sex-stratified analyses, as done in epidemiological research (Erickson et al., 2014; Flöel et al., 2008; Flöel et al, 2010, Ho et al., 2011). As Kramer & Colcombe (2018) state in their recent review, it can be of great help to disentangle the association between physical activity and BrainAGE to facilitate e.g. large exercise programs within the communities. Here, programs tailored for the specific sex may have higher acceptance and long-term maintenance rates.

4.2.3 Alcohol consumption and social integration

We did not find associations between BrainAGE and social integration or alcohol consumption. Regional differences in brain structure associated to social integration, as well as alcohol consumption could be present, but might not have been identifiable with the specific approach of the current study. Several explanations might hold for these observations. To date, the number of studies investigating social integration in relation to structural brain decline in older adults is relatively small (for a recent review, see Anatürk et al., 2018). Additionally, most studies report effects for composite measurements of cognitive and social activities, which do not clearly differentiate between social and cognitive components (Gow et al., 2012; Hafsteinsdottir et al., 2012; Vaughan et al., 2014). Further, composite measures for social activities when investigated in combination with additional lifestyle behaviors (Bittner et al., 2019) have been assessed. Therefore, future studies could

shed light on effects of social activities with low cognitive versus high cognitive demands to answer the question whether the cognitive or the social component of social integration contributes to brain reserve, the amount to which age-related GM loss can be tolerated without showing deficiencies (Stern, 2012). Additionally, we used a quantitative measurement of social integration. Studies have shown that older adults engage in relationships with a focus on quality rather than quantity (Carstensen et al., 1999). Hence, future studies would be needed to address the association between quality of relationships and brain aging. Further, differences in brain structure related to social integration may be regional or subtle (James et al., 2012), which also seems to be the case for alcohol consumption (Topiwala et al., 2017). Even though accelerated brain aging has been shown in patients with alcoholism (Pfefferbaum et al., 1992), differences related to alcohol consumption in the normal population may not be as strong or only identifiable if several risk behaviors co-occur (Bittner et al., 2019). Further, alcohol consumption may affect other brain parameters earlier such as WM lesions (den Heijer et al., 2004) or RSFC (Vergara et al., 2017). Additionally, effects of alcohol consumption may also be non-linear (Mukamal et al., 2001), which we could not identify in the present study, but might be interesting for future studies to further investigate into.

4.3 Strengths and limitations

Strengths of the present study include the large sample size, the older age range of the sample, and the use of BrainAGE as a state-of-the art imaging biomarker to quantify the effects of lifestyle behavior in years. The present study has a cross-sectional design which does not allow conclusions about directionality of effects. Even without a longitudinal design, though, our approach hints at individuals with higher risk for accelerated brain aging: each participant's image-based brain aging pattern is compared to his or her own

chronologically expected brain aging and not only to the average brain aging pattern of the sample. BrainAGE thus approximates intra-individual trajectories of brain aging rather than displaying average inter-individual brain aging patterns of groups. BrainAGE therefore provides a useful framework to capture relevant aspects of variability in structural brain aging beyond average values and hence provides a meaningful framework to examine the high variability in brain reserve (Stern, 2012).

Further, it is important to mention that, based on established approaches in epidemiological research (Schmermund et al., 2002), the lifestyle variables included in our combined lifestyle risk score were measured using different time windows (e.g., physical activity was assessed for the last four weeks, smoking as the number of cigarettes smoked over the whole lifetime). Assessments that refer to specifically defined short time frames (e.g., a month, a week) seem to be more reliable indicators of long-term behavior than self-reports referring to longer time frames (e.g., a whole year, Del Boca & Darkes, 2003).

Additionally, all lifestyle habits were assessed using self-reports, which makes it impossible to rule out memory effects or social desirability bias. Self-report measurements have nevertheless been shown to be valid and reliable (Del Boca & Darkes, 2003) and thus suitable in such an epidemiological population-based cohort setting.

4.4 Conclusion

Higher lifestyle risk, represented by a combined lifestyle risk score, contributes to accelerated brain aging as revealed by BrainAGE, a meaningful imaging biomarker. Higher lifetime smoking, as well as lower physical activity contributed most to this association. Especially the differential relation between physical activity and BrainAGE between the sexes, i.e. older men showing a stronger relationship between higher physical activity and decelerated brain aging, underlines the need for sex tailored lifestyle interventions. Our

results therefore highlight the importance of considering sex differences in the relationship between lifestyle and alterations in brain structure and future studies are warranted to examine the underlying mechanisms. More research is needed to elucidate the relation between alcohol consumption and brain structure, as well as social integration and brain health, e.g. by disentangling the cognitive and social components. In summary, lifestyle seems to be a fruitful target for identifying behaviors that may slow neuronal changes and related or resulting cognitive impairment. Considering co-occurrences between several lifestyle behaviors as effects of interests, rather than as a nuisance may enable us to better understand individual trajectories of brain aging in the older population and why people age differently.

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Supplementary Tables

Supplementary Table 1

Regression statistics, multiple linear regression examining the combined lifestyle risk score

Sample	Model including	Combined lifestyle risk score	*F* (whole model)	R^2
Whole subsample (n = 622)	Age, sex, combined lifestyle risk score	$\beta = 0.18$, $T = 4.54$, $p < 0.001$	8.77****	0.04
Outlier corrected (n = 615)	Age, sex, combined lifestyle risk score	$\beta = 0.17$, $T = 4.28$, $p < 0.001$	7.92****	0.04
	Age, sex, education, combined lifestyle risk score	$\beta = 0.17$, $T = 4.18$, $p < 0.001$	5.95***	0.04
Male (n = 350)	Age, combined lifestyle risk score	$\beta = 0.19$, $T = 3.66$, $p < 0.001$	6.46****	0.04
Outlier corrected, (n = 345)	Age, combined lifestyle risk score	$\beta = 0.18$, $T = 3.32$, $p = 0.001$	6.19**	0.04
	Age, education, combined lifestyle risk score	$\beta = 0.18$, $T = 3.23$, $p = 0.001$	4.13**	0.04
Female (n = 272)	Age, combined lifestyle risk score	$\beta = 0.16$, $T = 2.63$, $p = 0.009$	5.55***	0.04
Outlier corrected (n = 270)	Age, combined lifestyle risk score	$\beta = 0.16$, $T = 2.64$, $p = 0.009$	5.56***	0.04
	Age, education, combined lifestyle risk score*	$\beta = 0.16$, $T = 2.58$, $p = 0.010$	3.72*	0.04

Note. **** $p < 0.0001$, *** $p <= 0.001$, ** $p < 0.01$, * $p < 0.05$

Supplementary Table 2

Regression statistics, multiple linear regression examining individual lifestyle variables

Sample	Variables included	β Pack-years	β Alcohol consumption	β Physical activity	β Social integration	*F* (whole model)	R^2
Whole subsample (n = 622)	Age, sex all individual lifestyle variables	β = 0.14 *T* = 3.41 *p* = 0.009	β = 0.05 *T* = 1.19 *p* = 0.233	β = -0.11 *T* = -2.75 *p* = 0.006	β = -0.05 *T* = -1.14 *p* = 0.170	5.14****	0.05
Male (n = 350)	Age, all individual lifestyle variables	β = 0.14 *T* = 2.60 *p* = 0.010	β = 0.06 *T* = 1.08 *p* = 0.279	β = -0.13 *T* = -2.45 p = 0.015	β = -0.07 *T* = -1.26 *p* = 0.208	3.68**	0.05
Female (n = 272)	Age, all individual lifestyle variables	β = 0.134 *T* = 2.16 *p* = 0.032	β = 0.04 *T* = 0.41 *p* = 0.680	β = -0.08 *T* = -1.34 *p* = 0.182	β = -0.03 *T* = 0-.54 *p* = 0.587	2.47**	0.04

Note. **** $p < 0.0001$, *** $p <= 0.001$, ** $p < 0.01$, * $p < 0.05$

Supplementary Table 3

Regression statistics, multiple linear regression examining pack-years

Sample	Covariates included	Statistics	*F* (whole model)	R^2
Whole subsample	Age, sex	$\beta = 0.17$, $T = 4.24$, $p < 0.0001$	8.15***	0.04
$n = 612$	Age, sex, education	$\beta = 0.17$, $T = 4.17$, $p < 0.0001$	6.13****	0.04
Male,	Age	$\beta = 0.18$, $T = 3.46$, $p = 0.001$	6.92***	0.04
$n = 342$	Age, education	$\beta = 0.18$, $T = 3.40$, $p = 0.001$	4.60**	0.04
Female,	Age	$\beta = 0.14$, $T = 2.33$, $p = 0.021$	4.90**	0.04
$n = 270$	Age, education	$\beta = 0.14$, $T = 2.31$, $p = 0.022$	3.29, $p = 0.021$	0.04

Note. **** $p < 0.0001$, *** $p <= 0.001$, ** $p < 0.01$, * $p < 0.05$, all statistics refer to linear regressions examined in the outlier corrected samples.

Supplementary Table 4

Regression statistics, multiple linear regression examining physical activity

Sample	Covariates included	Statistics	*F* (whole model)	R^2
Whole subsample,	Age, sex	$\beta = -0.15$, $T = -3.72$, $p < 0.001$	6.72***	0.03
($n = 613$)	Age, sex, education	$\beta = -0.15$, $T = -3.65$, $p = 0.001$	5.12***	0.04
	Age, sex, education, BMI	$\beta = -0.14$, $T = -3.41$, $p = 0.001$	4.41***	0.03
Male,	Age	$\beta = -0.19$, $T = -3.50$, $p = 0.005$	7.05***	0.04
($n = 345$)	Age, education	$\beta = -0.18$, $T = -3.47$, $p = 0.001$	4.75**	0.04
	Age, education, BMI	$\beta = -0.18$, $T = -3.32$, $p = 0.001$	3.61**	0.04
Female,	Age	$\beta = -0.10$, $T = -1.60$, $p = 0.11$	3.53*	0.03
($n = 268$)	Age, education	$\beta = -0.09$, $T = -1.52$, $p = 0.131$	2.42, $p = 0.067$	0.03
	Age, education, BMI	$\beta = -0.08$, $T = -1.36$, $p = 0.18$	2.23, $p = 0.066$	0.03

Note. **** $p < 0.0001$, *** $p <= 0.001$, ** $p < 0.01$, * $p < 0.05$, all statistics refer to linear regressions examined in the outlier corrected samples.

Supplementary Table 5

Regression statistics, quadratic effects of the combined lifestyle risk score

Sample	Combined lifestyle risk score	*F* (quadratic whole model)	R^2
Whole subsample	$\beta 1q = 0.177$, $T = 4.46$, $p < 0.001$	$F_{Linear} = 19.85^{****}$	$R^2_{linear} = 0.031$
($n = 622$)	$\beta 2q = -0.019$, $T = -0.47$, $p = 0.640$	$F_{Quadratic} = 10.02^{****}$	$R^2_{quadratic} = 0.031$
Outlier corrected	$\beta 1q = 0.172$, $T = 4.31$, $p < 0.001$	$F_{Linear} = 17.64^{****}$	$R^2_{linear} = 0.028$
($n = 615$)	$\beta 2q = -0.049$, $T = -1.234$, $p = 0.218$	$F_{Quadratic} = 9.59^{*}$	$R^2_{quadratic} = 0.030$
Male	$\beta 1q = 0.217$, $T = 4.01$, $p < 0.001$	$F_{Linear} = 14.38^{****}$	$R^2_{linear} = 0.040$
($n = 350$)	$\beta 2q = -0.077$, $T = -1.43$, $p = 0.155$	$F_{Quadratic} = 8.23^{****}$	$R^2_{quadratic} = 0.046$
Outlier corrected	$\beta 1q = 0.181$, $T = 3.38$, $p = 0.001$	$F_{Linear} = 10.56^{***}$	$R^2_{linear} = 0.030$
($n = 345$)	$\beta 2q = -0.070$, $T = -1.317$, $p = 0.189$	$F_{Quadratic} = 6.16^{**}$	$R^2_{quadratic} = 0.035$
Female	$\beta 1q = 0.157$, $T = 2.57$, $p = 0.011$	$F_{Linear} = 6.61^{*}$	$R^2_{linear} = 0.024$
($n = 272$)	$\beta 2q = 0.015$, $T = 0.25$, $p = 0.803$	$F_{Quadratic} = 3.33^{*}$	$R^2_{quadratic} = 0.024$
Outlier corrected	$\beta 1q = 0.162$, $T = 2.68$, $p = 0.008$	$F_{Linear} = 7.09^{**}$	$R^2_{linear} = 0.026$
($n = 270$)	$\beta 2q = -0.019$, $T = -0.320$, $p = 0.749$	$F_{Quadratic} = 3.58^{*}$	$R^2_{quadratic} = 0.026$

Note. **** $p < 0.0001$, *** $p <= 0.001$, ** $p < 0.01$, * $p < 0.05$, all models have been carried out using the residuals of the combined lifestyle risk score corrected for the covariates age and education and sex (when examining the non-sex-split sample).

Supplementary Table 6

Regression statistics, quadratic effects of pack-years.

Sample	Pack-years	*F* (whole model)	R^2
Whole subsample	β_{1q} = 0.186, T = 3.25, p = 0.001	F_{Linear} = 13.34****	R^2_{linear} = 0.021
(n = 622)	β_{2q} = -0.056, T = -0.99, p = 0.323	$F_{Quadratic}$ = 7.16***	$R^2_{quadratic}$ = 0.023
Outlier corrected	β_{1q} = 0.182, T = 3.13, p < 0.001	F_{Linear} = 16.99****	R^2_{linear} = 0.027
(n = 612)	β_{1q} = -0.024, T = -0.407, p = 0.684	$F_{Quadratic}$ = 8.57****	$R^2_{quadratic}$ = 0.027,
Male	β_{1q} = 0.177, T = 3.31, p = 0.001	F_{Linear} =10.97***	R^2_{linear} = 0.031
(n = 350)	β_{1q} = -0.082, T = -1.05, p = 0.293	$F_{Quadratic}$ = 6.05**	$R^2_{quadratic}$ = 0.034
Outlier corrected	β_{1q} = 0.230, T = 314, p = 0.002	F_{Linear} = 6.27*	R^2_{linear} = 0.031
(n = 342)	β_{2q} = -0.078, T = -2106, p = 0.290	$F_{Quadratic}$ = 3.39*	$R^2_{quadratic}$ = 0.34
Female	β_{1q} = 0.125, T = 1.39, p = 0.166	F_{Linear} = 5.93*	R^2_{linear} = 0.021
(n = 272)	β_{2q} = 0.028, T = 0.315, p = 0.753	$F_{Quadratic}$ = 3.00, p = 0.051	$R^2_{quadratic}$ = 0.22
Outlier corrected	β_{1q} = 0.085, T = 0.775, p = 0.439	FL_{inear} = 6.27*	R^2_{linear} = 0.023
(n = 270)	β_{2q} = 0.079, T = 0.723, p = 0.470	$F_{Quadratic}$ = 3.39*	$R^2_{quadratic}$ = 0.25

Note. **** p < 0.0001, *** p <= 0.001, ** p < 0.01, * p < 0.05, all models have been carried out using the residuals of the metabolic equivalent per week corrected for the covariates age and education and sex (when examining the non-sex-split sample).

Supplementary Table 7

Regression statistics, quadratic effects of physical activity.

Sample	Pack-years	*F* (whole model)	R^2
Whole subsample	β_{1q} = -0.197, *T* = -3.41, *p* = 0.001	F_{Linear} = 7.03**	R^2_{linear} = 0.011
(*n* = 622)	β_{2q} = 0.125, *T* = 2.171, *p* = 0.030	$F_{Quadratic}$ = 5.89**	$R^2_{quadratic}$ = 0.019
Outlier corrected	β_{1q} = -0.145, *T* = -2.62, *p* = 0.009	F_{Linear} = 11.94***	R^2_{linear} = 0.019
(*n* = 613)	β_{2q} = 0.010, *T* = 0.175, *p* = 0.861	$F_{Quadratic}$ = 5.98**	$R^2_{quadratic}$ = 0.019
Male	β_{1q} = -0.233, *T* = -2.97, *p* = 0.003	F_{Linear} = 8.45**	R^2_{linear} = 0.024
(*n* = 350)	β_{2q} = 0.110, *T* = 1.36, *p* = 0.174	$F_{Quadratic}$ = 5.16**	$R^2_{quadratic}$ = 0.029
Outlier corrected	β_{1q} = -0.197, *T* = -2.64, *p* = 0.009	F_{Linear} =11.86***	R^2_{linear} = 0.033
(*n* = 345)	β_{2q} = 0.020, *T* = 0.268, *p* = 0.789	$F_{Quadratic}$ = 5.95**	$R^2_{quadratic}$ = 0.034
Female	β_{1q} = -0.082, *T* = -1.35, *p* = 0.178	F_{Linear} = 1.82, *p* = 0.178	R^2_{linear} = 0.007
(*n* = 270)	β_{2q} = -0.014, *T* = -0.16, *p* = 0.876	$F_{Quadratic}$ = 0.92, *p* = 0.438	$R^2_{quadratic}$ = 0.007
Female	β_{1q} = -0.065, *T* = -0.78, *p* = 0.436	F_{Linear} = 1.49, *p* = 0.224	R^2_{linear} = 0.006
(*n* = 268)	β_{2q} = -0.014, *T* = -0.16, *p* = 0.871	$F_{Quadratic}$ = 0.75, *p* = 0.472	$R^2_{quadratic}$ = 0.006

Note. **** $p < 0.0001$, *** $p <= 0.001$, ** $p < 0.01$, * $p < 0.05$, all models have been carried out using the residuals of the metabolic equivalent per week corrected for the covariates age, education, BMI and sex (when examining the non-sex-split sample).

Supplementary Table 8

Results of the power analysis

Effect of interest	Sample	*Effect size* F^2	*Number of predictors*	*Resulting Power*
Combined lifestyle risk	$n = 622$	0.04	3	0.99
	$n = 350$	0.04	2	0.93
	$n = 272$	0.04	2	0.84
Packyears	$n = 622$	0.04	3	0.99
	$n = 350$	0.04	2	0.93
	$n = 272$	0.04	2	0.84
Physical activity	$n = 622$	0.03	4	0.95
	$n = 350$	0.04	3	0.89
	$n = 272$	0.03	3	0.72

Note. For all calculations a error probability of $\alpha = 0.05$ was used.

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