Experimental Gerontology 82 (2016) 166–174

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Employing biomarkers of healthy ageing for leveraging genetic studies into human longevity

Joris Deelen [a,b], Erik B. van den Akker [a,c], Stella Trompet [d,e], Diana van Heemst [d], Simon P. Mooijaart [d], P. Eline Slagboom [a,1], Marian Beekman [a,*,1]

[a] Department of Molecular Epidemiology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands
[b] Max Planck Institute for Biology of Ageing, P.O. Box 41 06 23, 50866 Cologne, Germany
[c] Delft Bioinformatics Lab, Delft University of Technology, P.O. Box 5031, 2600 GA Delft, The Netherlands
[d] Department of Gerontology and Geriatrics, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands
[e] Department of Cardiology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands

ARTICLE INFO

Article history:
Received 10 March 2016
Received in revised form 27 May 2016
Accepted 27 June 2016
Available online 29 June 2016

Editor: Chennai Guest Editor

Keywords:
Biomarkers
Healthy ageing
Human longevity
Genetics

ABSTRACT

Genetic studies have thus far identified a limited number of loci associated with human longevity by applying age at death or survival up to advanced ages as phenotype. As an alternative approach, one could first try to identify biomarkers of healthy ageing and the genetic variants associated with these traits and subsequently determine the association of these variants with human longevity. In the present study, we used this approach by testing whether the 35 baseline serum parameters measured in the Leiden Longevity Study (LLS) meet the proposed criteria for a biomarker of healthy ageing.

The LLS consists of 421 families with long-lived siblings of European descent, who were recruited together with their offspring and the spouses of the offspring (controls). To test the four criteria for a biomarker of healthy ageing in the LLS, we determined the association of the serum parameters with chronological age, familial longevity, general practitioner-reported general health, and mortality.

Out of the 35 serum parameters, we identified glucose, insulin, and triglycerides as biomarkers of healthy ageing, meeting all four criteria in the LLS. We subsequently showed that the genetic variants previously associated with these parameters are significantly enriched in the largest genome-wide association study for human longevity.

In conclusion, we showed that biomarkers of healthy ageing can be used to leverage genetic studies into human longevity. We identified several genetic variants influencing the variation in glucose, insulin and triglycerides that contribute to human longevity.

1. Introduction

Chronological age is the main risk factor for many diseases. However, the rate of ageing is different between persons and some individuals become very old and seem to delay or even escape age-related disability (Christensen et al., 2008; Terry et al., 2008). Hence, by investigating longevity as a trait, we may be able to identify mechanisms that promote healthy ageing and protect against age-related disease. Since human longevity is heritable and clusters in families (Perls et al., 2000; Westendorp et al., 2009), genetic determinants of healthy ageing have been investigated using genome-wide association studies (GWAS). Thus far, only a limited number of longevity loci have been identified, i.e. *TOMM40/APOE/APOC1*, *FOXO3A* and chromosome 5q33.3 (Broer et al., 2015; Deelen et al., 2014a). Together, these loci only explain a small part of the heritability of human longevity, which has been estimated to be around 25% (Herskind et al., 1996; Hjelmborg et al., 2006) and is assumed to be explained by many genes with relatively small effects (Finch and Tanzi, 1997; Kirkwood et al., 2011). The lack of identified loci is most likely caused by the small sample size of the GWAS, which is attributable to limited availability of the studied outcomes, i.e. prospective survival or survival up to advanced ages, i.e. above 90 or 100 years (exceptional longevity).

As an alternative approach, one could first try to identify biomarkers of healthy ageing (Sprott, 2010) and the genetic variants associated with these traits and subsequently determine the association of these variants with longevity. We have previously proposed four criteria for a quantitative parameter that, we think, need to be fulfilled before

* Corresponding author at: Leiden University Medical Center, Molecular Epidemiology Section, Zone S5-P, P.O. Box 9600, 2300 RC Leiden, Einthovenweg 20, 2333 ZC Leiden, The Netherlands
E-mail addresses: Joris.Deelen@age.mpg.de (J. Deelen), E.B.van_den_Akker@lumc.nl (E.B. van den Akker), S.Trompet@lumc.nl (S. Trompet), D.van_Heemst@lumc.nl (D. van Heemst), S.P.Mooijaart@lumc.nl (S.P. Mooijaart), P.Slagboom@lumc.nl (P.E. Slagboom), M.Beekman@lumc.nl (M. Beekman).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.exger.2016.06.013

being considered a biomarker of healthy ageing (Deelen et al., 2013). In short, a biomarker of healthy ageing should show an association with (1) chronological age, (2) familial propensity for longevity, (3) known health parameters, and (4) morbidity and/or mortality (Box 1). Thus far, biomarker research has identified several potential biomarkers of healthy ageing, such as glucose and free triiodothyronine (fT3) serum levels, *CDKN2A* (p16) gene expression, leukocyte telomere length (LTL), and gait speed (Deelen et al., 2013; Njajou et al., 2009; Shiels, 2010). Some of these potential biomarkers of healthy ageing have been reported to associate with mortality, while others show a cross-sectional difference between young and old individuals, and therefore seem useful. However, previous studies for biomarkers of healthy ageing did not yet consistently investigate all four criteria or the studied biomarkers did not meet all criteria (e.g. LTL met three of the criteria, but was unable to discriminate individuals based on their familial propensity for longevity (Deelen et al., 2014b)). Identified biomarkers may be studied in GWAS with a larger sample size than GWAS for longevity. A similar approach has previously been applied in studies of complex diseases, such as type 2 diabetes and Alzheimer's disease, in which GWAS on endophenotypes successfully identified loci that play a role in progresses underlying these diseases (Cruchaga et al., 2013; Ingelsson et al., 2010). Hence, we will apply the same approach to identify loci contributing to human longevity.

In this study, we first test whether all available serum parameters in the Leiden Longevity Study (LLS) (Schoenmaker et al., 2006) meet the four criteria for a biomarker of healthy ageing. The LLS consists of 421 long-lived families (two generations), which have been followed for over 10 years. This enables us to test potential biomarkers for their association with mortality in two generations, i.e. the nonagenarians and the combined group of their offspring and controls. In addition, we will compare the levels of potential biomarkers between middle-aged members of long-lived families and controls. We previously showed that long-lived family members display a decreased prevalence of age-related diseases in comparison to controls (Westendorp et al., 2009; Bos et al., 2013). Hence, they are considered "healthy agers". Subsequently, we tested whether there is a significant enrichment of genetic loci contributing to the identified biomarkers of healthy ageing in a GWAS for human longevity in individuals of European descent, which is currently the largest GWAS for human longevity that has been performed (Deelen et al., 2014a).

2. Materials and methods

2.1. Study population

The LLS consists of 421 long-lived families of European descent. Families were included if at least two long-lived siblings were alive and fulfilled the age criterion of 89 years or older for males and 91 years or older for females, representing <0.5% of the Dutch population in 2001 (Schoenmaker et al., 2006). In total, 944 long-lived proband siblings (mean age = 94 years, range = 89–104), 1671 offspring (mean age = 61 years, range = 39–81) and 744 spouses thereof (mean age = 60 years, range = 36–79) were included. In the LLS, the spouses are considered controls. DNA from the LLS was extracted from white blood cells at baseline using conventional methods (Beekman et al., 2006) and genotyping was performed with Illumina Human660W-Quad and OmniExpress BeadChips (Illumina, San Diego, CA, USA) (Deelen et al., 2014a). In this study, we only used the individuals for which the identity was confirmed after genotyping.

Box 1
Biomarkers of healthy ageing

A biomarker of healthy ageing essentially (1) shows a change with chronological age, (2) discriminates individuals based on their familial propensity for longevity, and associates with (3) known health parameters and (4) morbidity and/or mortality in prospective studies (Deelen et al., 2013). Ideally, the measurement of a biomarker (of healthy ageing) should be inexpensive, fast, and show minimal methodological variation within and between laboratories (Martin-Ruiz and von, 2014).
There are three major classes of biomarkers of healthy ageing; (1) the *pro-longevity class*, for which a high level is considered healthy, (2) the *anti-longevity class*, for which a low level is considered healthy, and (3) the *role-switching class*, for which a level is considered anti-longevity at middle age and pro-longevity at advanced age, or vice versa (Moeller et al., 2014).

2.2. Biochemical measurements

All standard serum measurements were performed in non-fasting venous blood samples using fully automated equipment.

Glucose, fructosamine, high-sensitivity C-reactive protein (CRP), free fatty acids (FFA), triglyceride, and total and high-density lipoprotein (HDL) cholesterol levels were measured on the Hitachi Modular P 800. fT3, free thyroxine (fT4), and thyroid stimulating hormone (TSH) levels were measured on the Modular E170, while parathyroid hormone (PTH) and 25(OH) vitamin D3 were measured on the Cobas e 411 analyzer (all from Roche, Almere, the Netherlands) (Noordam et al., 2012; Rozing et al., 2009; Rozing et al., 2010; Rozing et al., 2011). LDL cholesterol levels were calculated using the Friedewald formula (Friedewald et al., 1972) and set to missing if plasma triglyceride levels exceeded 4.52 mmol/L.

Insulin, insulin-like growth factor 1 (IGF-1), and insulin-like growth factor-binding protein 3 (IGFBP3) levels were measured on the Immulite 2500 (DPC, Los Angeles, CA, USA) (Rozing et al., 2009). For calculation of the IGF-1/IGFBP3 molar ratio we used the following formula:

$$\text{IGF}-1/\text{IGFBP3 molar ratio} = \text{IGF}-1\ (\text{ng/ml}) * 0.130/\text{IGFBP3}\ (\text{ng/ml}) * 0.036$$

Triglyceride levels and low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particle sizes and concentrations were measured using proton nuclear magnetic resonance (NMR) spectroscopy (LipoScience Inc., Raleigh, NY, USA) (Vaarhorst et al., 2011).

Apolipoprotein E (ApoE), adiponectin (R&D Systems Europe, Ltd, Abingdon, United Kingdom), leptin (Diagnostics Biochem Canada Inc., Dorchester, Canada), and interleukin 6 (IL-6) levels (Sanquin Reagents, Amsterdam, The Netherlands) were determined using specific sandwich enzyme-linked immunosorbent assay (ELISA) (van Vlijmen et al., 1994; Mooijaart et al., 2006; Stijntjes et al., 2013).

Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) levels were measured using the NADH (with P-5′-P) methodology, albumin levels using the Bromcresol Purple methodology, and gamma-glutamyl transferase (γ-GT) levels using the L-gamma-glutamyl-3-carboxy-4-nitroanilide substrate methodology (Sala et al., 2014).

Creatine, folate and vitamin B12 levels were measured on the Abbott ci8200 (Architect, Abbott Laboratories, Illinois, USA), homocysteine levels were measured using a competitive immunoassay (Architect), while dehydroepiandrosterone sulfate (DHEAS) levels were measured with a delayed one-step immunoassay (Architect) (Wijsman et al., 2011).

For glucose, insulin, TSH, CRP, IL-6, FFA, triglyceride, ApoE, adiponectin, leptin, PTH, 25(OH) vitamin D, ALAT, albumin, ASAT, γ-GT, folate, vitamin B12, and DHEAS levels natural log transformed values were used for analyses due to non-normal distribution of the data, while triglyceride levels and LDL and HDL particle concentrations were batch corrected before analysis (Vaarhorst et al., 2011).