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Whole genome sequences of two octogenarians with sustained cognitive abilities

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Abstract

Although numerous genetic variants affecting aging and mortality have been identified, e.g. APOE ε4, the genetic component influencing cognitive aging has not been fully defined yet. A better knowledge of the genetics of aging will prove helpful in understanding the underlying biological processes. Here, we describe the whole genome sequences of two female octogenarians. We provide the repertoire of genomic variants that the two octogenarians have in common. We also describe the overlap with the previously reported genomes of two supercentenarians - individuals aged ≥ 110 years. We assessed the genetic disease propensities of the octogenarians and non-aged control genomes and could not find support for the hypothesis that long-lived healthy individuals might exhibit greater genetic fitness than the general population. Furthermore, there is no evidence for an accumulation of previously described variants promoting longevity in the two octogenarians. These findings suggest that genetic fitness, as currently defined, is not the sole factor enabling an increased lifespan. We identified a number of healthy-cognitive-aging candidate genetic loci awaiting confirmation in larger studies.

Keywords

aging; APOEε4; genetics; cognition; next generation sequencing; personalized medicine

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1. Introduction

Besides lifestyle and other environmental factors, genes determine up to 30% (Deelen, et al., 2013) and 50% (Harris and Deary, 2011) of human lifespan and cognitive aging, respectively. For instance, different genetic variants in the apolipoprotein E (APOE) gene have been shown to impact both the risk to develop Alzheimer's disease (AD), the major cause for dementia in the elderly (Liu, et al., 2013) and lifespan (Smith, 2002). Hence, studying the genetics of healthy (cognitive) aging is of utmost importance to better understand determinants of longevity and aging as well as to potentially identify mechanisms to ameliorate physical and mental decline.

While it has been challenging to unequivocally link genetic loci to cognitive aging (Payton, 2009), numerous variants and genes have been assigned roles in regulating lifespan (Budovsky, et al., 2013). These genetic associations implicate immune regulation and growth hormone/insulin signaling as well as pathways associated to lipoprotein metabolism, amongst others (Deelen, et al., 2013). Recently, the genomes of two centenarians have been published (Sebastiani, et al., 2011) that unexpectedly were neither characterized by an outstanding number of protective, putatively longevity-promoting variants nor an exceptionally low load in disease causing mutations (Sebastiani, et al., 2011).

In an effort to expand our knowledge of the genetic foundation of extraordinary healthy aging, we here describe the whole genomes of two female octogenarians with sustained cognitive abilities despite being heterozygous for APOE ε4, an AD and mortality risk variant.

2. Material and Methods

A detailed description of all methods can be found in the Supplementary Methods.

The genomes of two non-Hispanic white female octogenarians, participating in the UCSF Memory and Aging Center Hillblom Network Program on Aging and heterozygous for the APOE ε4 allele, were sequenced and aligned by Complete Genomics Inc. (CGI, Mountain View, CA). Genome sequences will be available at the European Genome-Phenome Archive (EGA) under the accession number EGAS00001000842. To define a genetic background of non-aged controls, we used 75 publicly available and four UCSF, ethnicity matched genomes that were also sequenced by CGI.

Using the Online Mendelian Inheritance in Man (OMIM) (http://www.omim.org/) and the ClinVar (Landrum, et al., 2014) databases, the number of non-synonymous variants in disease-associated genes and clinically relevant variants, respectively, were determined for each genome. A Chi-Squared Test was used to test whether the relative number of mutations differed significantly between octogenarians and non-aged controls. For each trait listed in the Genome-Wide Association Study (GWAS) catalog (Welter, et al., 2014), a relative genetic risk was calculated for every genome by summing up the relative disease risk conferred by each trait-associated variant with a reported odds ratio.

Variants were annotated using ANNOVAR and RegulomeDB (Boyle, et al., 2012). VAAST2.0.4 was run to identify genes harboring more SNPs in aged subjects (both the reported octogenarians' genomes and the previously published genomes of two centenarians (Sebastiani, et al., 2011)) than in non-aged controls (Hu, et al., 2013).

3. Results and discussion

3.1. Genetic inventory of two octogenarians with sustained cognitive function

We sequenced at > 40x coverage the genomes of two female octogenarians, referred to as 12664 and 12665, using high quality control thresholds for analysis (Supplementary Table S1). Subjects were selected because of their advanced age and maintained cognitive abilities (Supplementary Table S2), despite them being heterozygous for the AD and mortality genetic risk variant APOEε4. Their family history of longevity also suggested a strong genetic component for this trait.

In order to describe the two octogenarians' genetic make-up – presumably permissive for healthy aging – we first contrasted small sequence variants found in their genomes (SNPs, small substitutions, deletions and insertions) with variants present in the genomes of 79 non-aged controls. We found 289 autosomal common (in >= 95% of controls) variants absent from the two studied octogenarians, 3 of which were non-synonymous and 21 (13.2 % of SNPs with available information) likely to have gene-regulatory function (RegulomeDB score < 4; Supplementary Table S3). One of these variants, a SNP in the intron of C9orf114, also shows very low frequency (0.004) in the Wellderly Study, a genomic study by the Scripps Institute enrolling healthy elderly, and could hence be involved in healthy general aging rather than in cognitive aging. Seven thousand four hundred eighty autosomal rare (allele frequency < 0.05% in controls) variants were exclusive to the two elders, with 28 of them being non-synonymous and 206 likely to affect gene expression (5.5 % of SNPs with available information; Supplementary Table S4). Three exclusive intergenic variants also showed high frequencies (> 0.98) in the Wellderly Study. Interestingly, genes harboring exclusive variants were significantly enriched in genes reported to play a role in longevity (data not shown). Another noteworthy observation was that multiple genes with exclusive non-synonymous variants were involved in protein processing and transport along the endoplasmic reticulum (ER)-Golgi apparatus-vesicle axis: the ER-resident aminopeptidase ERAP2; PIGN, involved in glycosylphosphatidylinositol (GPI)-anchor biosynthesis in the ER; the Golgi protein GOLGA2 and SEC24C, both likely regulators of vesicle trafficking. It is conceivable that transport is of crucial importance for cognition, as neuronal axons and dendrites depend on organelle and protein deliveries from the cell body. Variants in C9orf114, GOLGA2 and SEC24C as well as a random selection of 15 other exclusive or absent variant positions, were validated in both elderly genomes by Sanger sequencing, underscoring the high quality and confidence of the whole genome sequences (Supplementary Table S5).

In addition to small sequence variants, we identified five chromosomal junctions and eleven CNVs that were exclusive to both elders, but showed a frequency < 0.05 in the control genomes (Supplementary Table S6). The identified CNVs do not overlap with previously reported age-associated CNVs (Kuningas, et al.). No protein-coding gene was affected by

either exclusive junctions or CNVs, so the functional consequences of these structural variants remain unclear.

3.2. No evidence for decreased genetic (disease) burden in the two octogenarians

It has also been reported that long-lived individuals avoid/delay morbidity (Andersen, et al., 2012, Terry, et al., 2008), raising the possibility that their genomes might harbor less disease-causing mutations and more beneficial, lifespan extending variants. We therefore tested whether the presence of disease genetic risk factors differed between our two octogenarians and the nonaged controls at four different levels: non-synonymous variants either (i) affecting genes associated with inherited diseases or (ii) affecting genes associated with diseases impacting brain function, (iii) clinically relevant variants, (iv) variants associated with common (disease) traits. At neither of these levels was there a (consistent) difference between the two octogenarians and the non-aged controls (Fig. 1; Supplementary Table S7). Interestingly, there were a number of traits for which at least one of the two octogenarians showed slightly more extreme risk scores than observed in non-aged controls. For instance, individual 12665 had an increased genetic risk for basal cell carcinoma; this subject indeed had twelve carcinomas removed. Of note, despite heterozygosity for the AD risk variant APOE risk variants ε4, there was no indication for an increased genetic susceptibility for AD in the two healthy females (Fig. 1). In summary, these findings suggest that reduced genetic disease propensity cannot explain extreme healthspan and maintained cognitive functions in our study participants.

Complementing our analysis of missing detrimental variants, we next assessed the occurrence of SNPs that were reported to be associated with longevity, hence might be protective (Supplementary Table S8). We also tested the mutation load in genes that had been previously described to be linked to longevity/aging (Supplementary Table S9). No difference between the two octogenarians and the control genomes could be observed (Fig. 1). For instance, while one of the two octogenarians was heterozygous for SNP rs9536314, tagging an allele in the longevity gene KLOTHO that has recently been described to boost cognition (Dubal, et al., 2014), this variant could was also found in 25 of the non-aged controls. Our results are in line with Sebastiani and colleagues who reported that the genomes of two centenarians did neither harbor the majority of previously described longevity-associated variants, nor did they have a different genetic susceptibility profile compared to non-centenarians (Sebastiani, et al., 2011). Together, these results challenge the hypothesis that that increased life span is significantly facilitated by greater genetic fitness (Beekman, et al., 2010). They also imply that currently known longevity variants are not universal indicators of life span, i.e. more genetic influences on longevity remain to be established.

3.3. The two octogenarians share genetic variants with two centenarians

To suggest novel longevity variants, we provide a list of the 26 exclusive variants that the two octogenarians shared with the recently published genomes of two centenarians (Sebastiani, et al., 2011) in Supplementary Table S10. One of them is close to (~ 195 kb) the longevity gene GATA4, a transcription factor of the zinc finger family. Four further variants are also supported by high frequencies (> 0.75) in the Wellderly Study. In addition, VAAST

analysis (Hu, et al., 2013) identified fourteen genes with an excessive number of potentially damaging SNPs in the elderly genomes as compared to non-aged controls (Supplementary Table S11). Although none of these genes have been previously described in the context of longevity, they include potentially interesting candidates, such as SETX and PCDHAC1 that have been reported to fulfill functions in the central nervous system. However, larger sequencing confirmatory endeavors are needed to provide unequivocal evidence of relevance for any of the here suggested genetic loci. Specifically, integrating genome sequencing data with large-scale transcriptional profiling and epigenetic studies, thus putting genetic variation into context, holds great promise to disclose more of the secret of a healthy long life.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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- The genetics of cognitive aging have not been fully defined yet.
- Whole genomes of two octogenarians were sequenced and shared variants determined.
- Genetic fitness of the octogenarians was not enhanced compared to controls.
- The octogenarians' genomes were not enriched in longevity-promoting variants.
- We suggest a number of cognitive-aging candidate genetic loci.

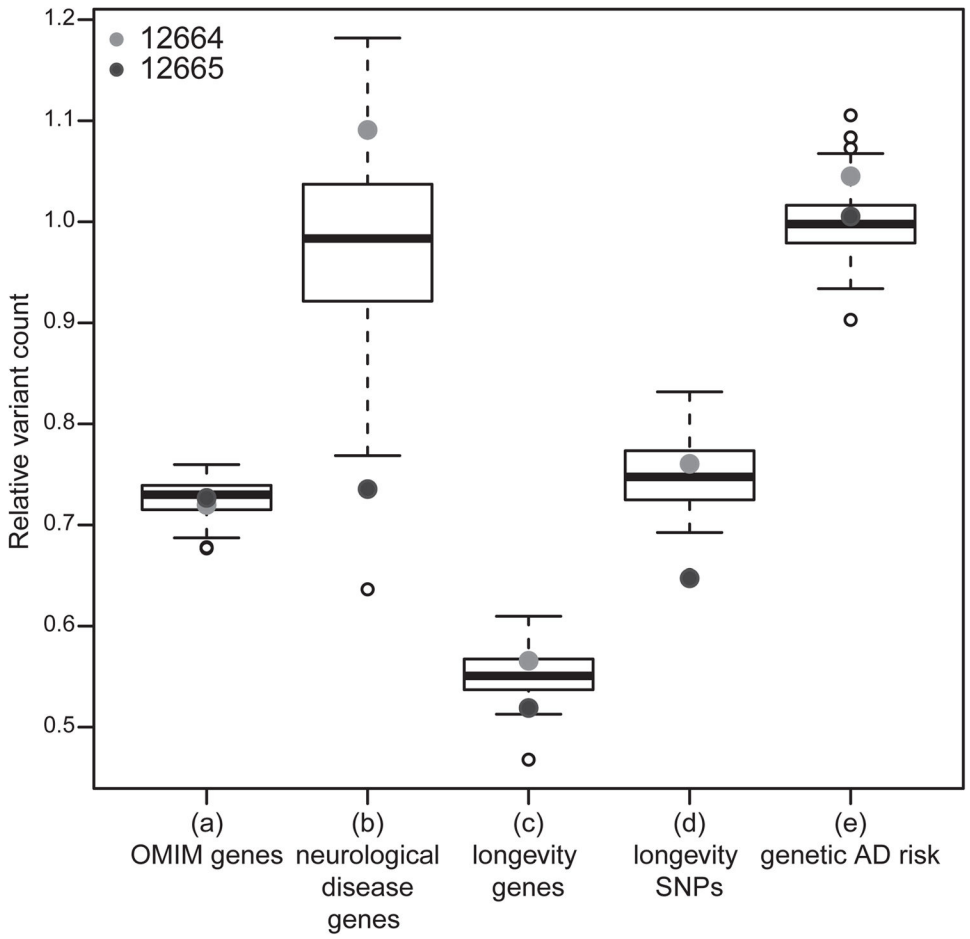

Figure 1. Genetic burden of the two studied cognitive healthy octogenarians as compared to 79 non-aged controls

The number of non-synonymous variants within (a) all disease-causing genes, as reported in OMIM, with information in our data set (n=3463), (b) genes reported in OMIM to be causally linked to neurological diseases (n=121), (c) genes reported to be involved in longevity (n=1135) and (d) SNPs previously associated with longevity (n=309) was assessed. (e) Further, the genetic risk for Alzheimer's disease (AD) was calculated (133 SNPs). Variant counts/risk scores were normalized by the number of assessed entities, which are genes in (a), (b) and (c) and SNPs in (d) and (e). The distribution of counts/risk score for non-aged controls is visualized as boxplots, data of the two octogenarians are superimposed as grey (12664) and black (12665) dots.

Table 1

General characterization of variants that were either absent in or exclusive to the two octogenarians' genomes.

category	absent	exclusive
Total	289	7480
% SNP	93.8	93.2
% deletion	2.4	3.7
% insertion	2.1	1.6
% substitution	1.7	1.4
% coding (exonic) [% non-syn.]	1.0 [100]	0.8 [47.5]
% non-exonic with gene regulatory function[a]	7.1	2.9

non-syn: non-synomymous,

[a] RegulomeDB score < 2