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Brief communication

Whole genome sequences of 2 octogenarians with sustained cognitive abilities

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ABSTRACT

Although numerous genetic variants affecting aging and mortality have been identified, for example, apolipoprotein E 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 2 female octogenarians. We provide the repertoire of genomic variants that the 2 octogenarians have in common. We also describe the overlap with the previously reported genomes of 2 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 2 octogenarians. These findings suggest that genetic fitness, as currently defined, is not the sole factor enabling an increased life span. We identified a number of healthy-cognitive-aging candidate genetic loci awaiting confirmation in larger studies.

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 life span 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 life span (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.

Although it has been challenging to unequivocally link genetic loci to cognitive aging (Payton, 2009), numerous variants and genes have been assigned roles in regulating life span (Budovsky et al., 2013). These genetic associations implicate immune regulation, growth hormone and insulin signaling as well as pathways associated to lipoprotein metabolism, among others (Deelen et al., 2013). Recently, the genomes of 2 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 2 female octogenarians with sustained cognitive abilities despite being heterozygous for APOE ε4, an AD and mortality risk variant.

2. Methods

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

The genomes of 2 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 (Mountain View, CA). Genome sequences will be available at the European

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Genome-Phenome Archive under the accession number EGAS00001000842. To define a genetic background of non-aged control subjects, we used 75 publicly available and 4 UCSF, ethnicity-matched genomes that were also sequenced by Complete Genomics Inc.

Using the Online Mendelian Inheritance in Man (http://www.omim.org/) and the ClinVar (Landrum et al., 2014) databases, the number of nonsynonymous variants in disease-associated genes and clinically relevant variants, respectively, were determined for each genome. A χ^2 test was used to test whether the relative number of mutations differed significantly between octogenarians and non-aged control subjects. 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 annotate variation and RegulomeDB (Boyle et al., 2012). VAAST2.0.4 was run to identify genes harboring more single-nucleotide polymorphisms (SNPs) in aged subjects (both the reported octogenarians' genomes and the previously published genomes of 2 centenarians; Sebastiani et al., 2011) than in non-aged control subjects (Hu et al., 2013).

3. Results and discussion

3.1. Genetic inventory of 2 octogenarians with sustained cognitive function

We sequenced at >40× coverage the genomes of 2 female octogenarians, referred to as 12664 and 12665, using high quality control thresholds for analysis (Supplementary Table 1). Subjects were selected because of their advanced age and maintained cognitive abilities (Supplementary Table 2), 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.

To describe the 2 octogenarians' genetic makeup—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 control subjects (Table 1). We found 289 autosomal common (in ≥95% of control subjects) variants absent from the 2 studied octogenarians, 3 of which were nonsynonymous and 21 (13.2 % of SNPs with available information) likely to have gene-regulatory function (RegulomeDB score <4; Supplementary Table 3). One of these variants, an 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 individuals, and could hence be involved in healthy general aging rather than in cognitive aging. Seven thousand four hundred and eighty autosomal rare (allele frequency <0.05% in control subjects) variants were exclusive to the 2 elders, with 28 of them being nonsynonymous and 206 likely to affect gene expression (5.5 % of SNPs with available information; Supplementary Table 4). 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 nonsynonymous 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-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 5).

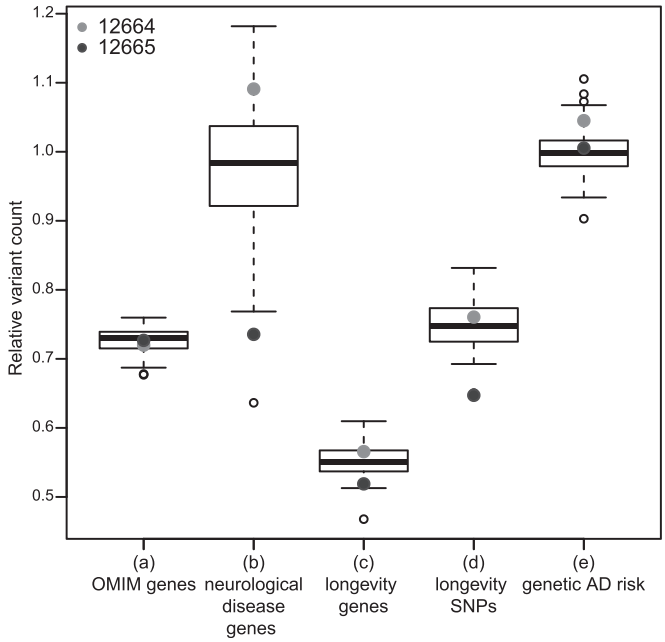

Fig. 1. Genetic burden of the 2 studied cognitive healthy octogenarians as compared with 79 non-aged control subjects. The number of nonsynonymous variants within (A) all disease-causing genes, as reported in OMIM, with information in our data set (n = 3463), (B) genes reported in OMIM to be causally linked to neurologic 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 and/or 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 and/or risk score for non-aged controls is visualized as boxplots, data of the 2 octogenarians are superimposed as gray (12664) and black (12665) dots. Abbreviations: OMIM, Online Mendelian Inheritance in Man; SNPs, single-nucleotide polymorphisms.

In addition to small sequence variants, we identified 5 chromosomal junctions and 11 copy number variants (CNVs) that were exclusive to both elders, but showed a frequency <0.05 in the control genomes (Supplementary Table 6). The identified CNVs do not overlap with previously reported age-associated CNVs (Kuningas et al., 2011). 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 2 octogenarians

It has also been reported that long-lived individuals avoid and/or 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, life span extending variants. We therefore tested whether the presence of disease genetic risk factors differed between our 2 octogenarians and the non-aged control subjects at 4 different levels: nonsynonymous variants either (1) affecting genes associated with inherited diseases; or (2) affecting genes associated with diseases impacting brain function; (3) clinically relevant variants; (4) variants associated with

common (disease) traits. At neither of these levels was there a (consistent) difference between the 2 octogenarians and the non-aged control subjects (Fig. 1; Supplementary Table 7). Interestingly, there were a number of traits for which at least one of the 2 octogenarians showed slightly more extreme risk scores than observed in non-aged control subjects. For instance, individual 12665 had an increased genetic risk for basal cell carcinoma; this subject indeed had 12 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 2 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 8). We also tested the mutation load in genes that had been previously described to be linked to longevity and/or aging (Supplementary Table 9). No difference between the 2 octogenarians and the control genomes could be observed (Fig. 1). For instance, although one of the 2 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 also be found in 25 of the non-aged control subjects. Our results are in line with Sebastiani et al. (2011) who reported that the genomes of 2 centenarians did neither harbor most of previously described longevity-associated variants, nor did they have a different genetic susceptibility profile compared with non-centenarians. Together, these results challenge the hypothesis 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, that is, more genetic influences on longevity remain to be established.

3.3. The 2 octogenarians share genetic variants with 2 centenarians

To suggest novel longevity variants, we provide a list of the 26 exclusive variants that the 2 octogenarians shared with the recently published genomes of 2 centenarians (Sebastiani et al., 2011) in Supplementary Table 10. One of them is close to (approximately 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 14 genes with an excessive number of potentially damaging SNPs in the elderly genomes as compared with non-aged control subjects (Supplementary Table 11). 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.

Table 1
General characterization of variants that were either absent in or exclusive to the 2 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) (nonsyn.), %	1.0 (100)	0.8 (47.5)
Nonexonic with gene regulatory function[a], %	7.1	2.9

Key: non-syn, nonsynomymous; SNP, single-nucleotide polymorphism.
[a] RegulomeDB score <2.

Disclosure statement

The authors state no conflicts of interest. Joel H. Kramer was supported by the National Institutes of Health (NIH) grants P50 AG023501 and R01AG032289. Jorge R. Oksenberg was supported by the NIH grant R01NS76492.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2014.11.003.

References

Andersen, S.L., Sebastiani, P., Dworkis, D.A., Feldman, L., Perls, T.T., 2012. Health span approximates life span among many supercentenarians: compression of morbidity at the approximate limit of life span. J. Gerontol. A Biol. Sci. Med. Sci. 67, 395–405.

Beekman, M., Nederstigt, C., Suchiman, H.E., Kremer, D., van der Breggen, R., Lakenberg, N., Alemayehu, W.G., de Craen, A.J., Westendorp, R.G., Boomsma, D.I., de Geus, E.J., Houwing-Duistermaat, J.J., Heijmans, B.T., Slagboom, P.E., 2010. Genome-wide association study (GWAS)-identified disease risk alleles do not compromise human longevity. Proc. Natl. Acad. Sci. U. S. A. 107, 18046–18049.

Boyle, A.P., Hong, E.L., Hariharan, M., Cheng, Y., Schaub, M.A., Kasowski, M., Karczewski, K.J., Park, J., Hitz, B.C., Weng, S., Cherry, J.M., Snyder, M., 2012. Annotation of functional variation in personal genomes using RegulomeDB. Genome Res. 22, 1790–1797.

Budovsky, A., Craig, T., Wang, J., Tacutu, R., Csordas, A., Lourenco, J., Fraifeld, V.E., de Magalhaes, J.P., 2013. LongevityMap: a database of human genetic variants associated with longevity. Trends Genet. 29, 559–560.

Deelen, J., Beekman, M., Capri, M., Franceschi, C., Slagboom, P.E., 2013. Identifying the genomic determinants of aging and longevity in human population studies: progress and challenges. Bioessays 35, 386–396.

Dubal, D.B., Yokoyama, J.S., Zhu, L., Broestl, L., Worden, K., Wang, D., Sturm, V.E., Kim, D., Klein, E., Yu, G.Q., Ho, K., Eilertson, K.E., Yu, L., Kuro-o, M., De Jager, P.L., Coppola, G., Small, G.W., Bennett, D.A., Kramer, J.H., Abraham, C.R., Miller, B.L., Mucke, L., 2014. Life extension factor klotho enhances cognition. Cell Rep. 7, 1065–1076.

Harris, S.E., Deary, I.J., 2011. The genetics of cognitive ability and cognitive ageing in healthy older people. Trends Cogn. Sci. 15, 388–394.

Hu, H., Huff, C.D., Moore, B., Flygare, S., Reese, M.G., Yandell, M., 2013. VAAST 2.0: improved variant classification and disease-gene identification using a conservation-controlled amino acid substitution matrix. Genet. Epidemiol. 37, 622–634.

Kuningas, M., Estrada, K., Hsu, Y.H., Nandakumar, K., Uitterlinden, A.G., Lunetta, K.L., van Duijn, C.M., Karasik, D., Hofman, A., Murabito, J., Rivadeneira, F., Kiel, D.P., Tiemeier, H., 2011. Large common deletions associate with mortality at old age. Hum. Mol. Genet. 20, 4290–4296.

Landrum, M.J., Lee, J.M., Riley, G.R., Jang, W., Rubinstein, W.S., Church, D.M., Maglott, D.R., 2014. ClinVar: public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res. 42, D980–D985.

Liu, C.C., Kanekiyo, T., Xu, H., Bu, G., 2013. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat. Rev. Neurol. 9, 106–118.

Payton, A., 2009. The impact of genetic research on our understanding of normal cognitive ageing: 1995 to 2009. Neuropsychol. Rev. 19, 451–477.

Sebastiani, P., Riva, A., Montano, M., Pham, P., Torkamani, A., Scherba, E., Benson, G., Milton, J.N., Baldwin, C.T., Andersen, S., Schork, N.J., Steinberg, M.H., Perls, T.T., 2011. Whole genome sequences of a male and female supercentenarian, ages greater than 114 years. Front. Genet. 2, 90.

Smith, J.D., 2002. Apolipoproteins and aging: emerging mechanisms. Ageing Res. Rev. 1, 345–365.

Terry, D.F., Sebastiani, P., Andersen, S.L., Perls, T.T., 2008. Disentangling the roles of disability and morbidity in survival to exceptional old age. Arch. Intern. Med. 168, 277–283.

Welter, D., Macarthur, J., Morales, J., Burdett, T., Hall, P., Junkins, H., Klemm, A., Flicek, P., Manolio, T., Hindorff, L., Parkinson, H., 2014. The NHGRI GWAS catalog, a curated resource of SNP-trait associations. Nucleic Acids Res. 42, D1001–D1006.