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Whole-Exome Sequencing of an Exceptional Longevity Cohort

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Abstract

Centenarians represent a unique cohort to study the genetic basis for longevity and factors determining the risk of neurodegenerative disorders, including Alzheimer's disease (AD). The estimated genetic contribution to longevity is highest in centenarians and super-centenarians, but few genetic variants have been shown to clearly impact this phenotype. While the genetic risk for AD and other dementias is now well understood, the frequency of known dementia risk variants in centenarians is not fully characterized. To address these questions, we performed whole-exome sequencing on 100 individuals of 98–108 years age in search of genes with large effect sizes towards the exceptional aging phenotype. Overall, we were unable to identify a rare protein-altering variant or individual genes with an increased burden of rare variants associated with exceptional longevity. Gene burden analysis revealed three genes of nominal statistical significance associated with extreme aging, including *LYST*, *MDN1*, and *RBML1*. Several genes with variants conferring an increased risk for AD and other dementias were identified, including *TREM2*, *EPHA1*, *ABCA7*, *PLD3*, *MAPT*, and *NOTCH3*. Larger centenarian studies will be required to further elucidate the genetic basis for longevity, and factors conferring protection against age-dependent neurodegenerative syndromes.

Keywords: Centenarian, Whole-exome sequencing, SKAT, Alzheimer's disease, Dementia

The precise genetic mechanisms underlying human longevity remain elusive. The relative risk of living beyond the age of 90, if a sibling has reached this age, has been estimated to be only 1.7–1.8 (1). With increasing age however, the genetic contribution is likely more relevant, with a relative risk of up to 35 in sibling pairs where one has reached the age of 105 or higher (1). Genome-wide association (GWA) studies have identified loci in several nominated genes conferring an increased risk for extreme longevity, including *FOXO3*, *TOMM40/APOE*, *CDKN2B*, *SH2B3/ATXN2*, *ABO*, *USP42*, *TMTCC2*, and a longevity “signature” consisting of 281 single nucleotide polymorphisms (SNPs) (2–7). Few studies employing whole-exome or whole-genome sequencing in centenarian populations to identify risk variants relevant to extreme longevity have been

performed to date. A mutation in *CLEC3B*, and several variants in *HLA-DQB1* have been reported from whole-exome sequencing studies in East Asian nonagenarian/centenarian populations (8,9), and *COL25A1* in a whole-genome study in healthy Caucasians >80 years old (10). A small whole-genome sequencing study of 17 super-centenarians did not report significant findings (11).

By living beyond 100 years of age, centenarians have also escaped or delayed the onset of neurodegenerative diseases, including Alzheimer's disease (AD). In contrast to exceptional longevity, the major genetic risk factors for late onset AD are now well established, and recent widespread use of next-generation sequencing (NGS) has allowed the identification of additional rare risk variants in *TREM2*, *CLU*, *SORL1*, *ABCA7*, *APP*, *PLD3*, *EPHA1*, *CR1*, and *BIN1* (12). Other

rare genetic mutations have also been described in non-AD dementias (13). While these variants are increasingly being replicated in diverse clinical cohorts, their frequency in centenarians is not fully known. Characterizing this overlap better informs the role of many genetic variants reported to confer an increased risk for neurodegeneration, both with respect to dementia onset as well as extreme longevity.

Here, we performed whole-exome sequencing on 100 largely centenarian individuals from the Georgia Centenarian Study (GCS) (14). We sought to identify rare variants with large effects sizes enriched in centenarians, or an increased burden of rare variants in individual genes. We further explored the frequency of rare deleterious coding variants reported to increase the risk of AD as well as non-AD dementias.

Materials and Methods

Cohorts

The work was approved by the Yale Institutional Review Board. 100 DNA samples from the Georgia Centenarian Study (GCS) (14) were obtained from the Coriell Institute (catalogue #AGPLONG3). Individual consents were not obtained as all the samples were fully anonymized. The subjects' age range was from 98 to 108 (40 were 98–99 years old, 60 were >100 years old), with 17 males and 83 females. We defined this age range as extreme longevity/aging and exceptional longevity/aging, which is similar to previously used criteria for extreme longevity (15). For the SKAT non-centenarian reference population, 100 random and anonymous DNA samples from a Yale University cancer repository were sequenced on the same platform as the centenarians. Caucasian ethnicity was confirmed in 97/100 using the EthSEQ (16), and the remaining three were excluded from the analysis (Supplementary Figure 1). The majority of subjects in the SKAT non-centenarian reference population were males (96/97).

Whole-Exome Capture and Sequencing

Genomic DNA was captured on a NimbleGen 2.1M human exome array with modifications to the manufacturer's protocol, followed by single-read cluster generation (Illumina, San Diego, CA). Exome library sequencing was performed using the HiSeq2000, paired end analysis, with six samples per lane. Image analysis and base calling were performed using the Illumina pipeline (version 1.8). Sequencing reads were aligned to the human genome (NCBI37/hg19) by Maq and BWA software. Coverage and error rates were quantified using perl scripts, available on request. Average read depth was 90x. Single nucleotide variations were identified and annotated using Enlis Genome Research software v1.9 (Enlis, Berkeley, CA), which incorporates data from 1000 Genomes, the Exome Aggregation Consortium (ExAC) (17), the Exome Variant Server (EVS), and the dbSNP databases (build 142). All variant call files were added to the genome server Neuroseq (<https://neuroseq.ca>) for additional annotation from the genome Aggregation Database (gnomAD) (17).

Sanger Sequencing

Select variants underwent validation by Sanger sequencing, using standard protocols. Primer pairs were used to sequence amplicons containing exome variants of interest by polymerase chain reaction (PCR). PCR products were purified using Agencourt bead technology (Beverly, MA) with Biomek FX automation (Beckman Coulter, Fullerton, CA). Electropherograms were analyzed with 3730xl DNA Analyzer using SeqScapev2.1.1 (Life Technologies, Carlsbad, CA).

Statistical Analysis

SNPs predicted to be deleterious were first filtered based on a population minor allele frequency of <1% in publically available databases (Enlis Research). For statistical analysis, minor allele frequencies (MAFs) of individual SNPs in centenarians were then compared to the MAFs in ExAC using a Fisher exact test (JMP v13.0, SAS Institute, Cary, NC). Gene burden analysis was performed by sequence kernel association test (SKAT) using R statistical software (18).

Results

Genomes of Extreme-Aged Individuals Are Not Enriched for a Rare Protein-Altering SNP or Genes with an Increased Variant Burden

We sought to identify novel and rare variants enriched in our cohort of 100 subjects of age 98–108 years compared to large population control databases containing data from more than 30,000 individuals (ExAC). In total, we found 26,011 rare, high quality, non-synonymous coding variants predicted to be deleterious, with an average read depth of >91x. Using Fisher Exact test, a novel variant would have to be present in at least three centenarians to reach genome-wide statistical significance after Bonferroni correction ($p = .05/26,011 = 1.92 \times 10^{-6}$). For known, rare variants (<1% population MAF) a similar threshold was used. With stringent quality filters, we did not find a statistically significant enrichment of a rare deleterious SNP in centenarians compared to population controls.

While centenarians may not share a rare SNP affecting longevity, a single gene may be affected by different deleterious variants impacting protein function. To address this possibility, we assessed genomes from individuals achieving extreme age for enrichment of rare protein-altering variants within each gene compared to 97 control individuals from our cancer repository. Using SKAT, none of the genes enriched for rare coding variants were statistically significant after correction for multiple comparisons. However, using a less stringent p -value of <.001, without correcting for multiple comparisons, we identified increased variant burden in *LYST*, *MDN1*, and *RBMXL1* in extreme aging individuals compared to control subjects (Table 1, Supplementary Table 1).

AD Risk Variants are Present in Subjects with Exceptional Longevity

To assess risk variants in AD-associated genes, we compiled a list of AD risk genes derived from recent NGS publications which included *ABCA7*, *TOMM40/APOE*, *APP*, *BIN1*, *CASS4*, *CD2AP*, *CD33*, *CLU*, *EPHA1*, *FERMT2*, *HLA-DRB1*, *HLA-DRB5*, *INPP5D*, *MEF2C*, *MS4A6A*, *NME8*, *PICALM*, *PLD3*, *PSEN1*, *PSEN2*, *PTK2B*, *RIN3*, *SORL1*, *TREM2*, *ZCWPW1*, and *CELF1* (reviewed in (12)). Rare (<1% population MAF) coding variants in these genes predicted to be deleterious (DANN criteria) were found in *TREM2*, *EPHA1*, *ABCA7*, and *PLD3* (Table 2). The *TREM2* p.D87N variant

Table 1. SKAT BurdenTest of Rare Variants in Subjects Aged 98–108 Years

Gene	# Centenarian Variants	# Control Variants	Nominal p -value
<i>LYST</i>	22	9	2.50×10^{-4}
<i>MDN1</i>	23	5	5.79×10^{-4}
<i>RBMXL1</i>	9	2	9.93×10^{-4}

Table 2. Frequency of Rare AD Risk Variants in Subjects Aged 98–108 Years

Gene	AA Change	dbSNP	Centenarian MAF	ExAC MAF	gnomAD MAF	AD MAF	Sex/Age	Ref.
<i>TREM2</i>	D87N	rs142232675	0.01 (2/200)	0.0018	0.00115	0.0028	F/99; F/100	(19)
<i>TREM2</i>	R47H	rs75932628	0.005 (1/200)	0.0026	0.00248	0.01	F/101	(19)
<i>EPHA1</i>	P460L	rs202178565	0.01 (2/200)	0.0004	0.00022	0.004	M/102;F/100	(20)
<i>ABCA7</i>	V1599M	rs117187003	0.005 (1/200)	0.0041	0.00339	0.018	F/100	(20)
<i>PLD3</i>	V232M	rs145999145	0.01 (2/200)	0.0045	0.00325	0.008	F/103; F/102	(21)

Note: All variants were validated by Sanger sequencing. AA = amino acid; AD = Alzheimer's disease; ExAC = Exome Aggregation Consortium; MAF = minor allele frequency; gnomAD = genome Aggregation Database.

which has been shown to be significantly associated with AD was found in 2/100 individuals aged 98–108, as were *EPHA1* (p.P460L) and *PLD3* (p.V232M) variants (19–21). Rare variants were also found in AD-associated genes where the particular SNP has not previously been associated with AD, including *EPHA1*, *SORL1*, *ABCA7*, *PLD3*, *BIN1*, *INPP5D*, *CD2AP*, *NME8*, *ZCWPW1*, *PTK2B*, *FERMT2*, and *RIN3* (Supplementary Table 2).

Risk Variants Associated with Age-Dependent Non-AD Dementias in Subjects Aged 98–108 Years

To characterize the risk variants in genes associated with non-AD dementias, we derived a panel of non-AD dementia risk genes from a recent NGS study (13), including frontotemporal lobar degeneration (FTLD) spectrum: *MAPT*, *GRN*, *VCP*, *TREM2*, *SQSTM1*, *FUS*, *TARDBP*, *CHMP2B*; Parkinson's dementia (PDD) or dementia with Lewy bodies: *LRRK2*, *SNCA*, *GBA*, and *SCARB2*; vascular dementia: *NOTCH3*, *HTRA1*, *COL4A1*; and other neurodegenerative disease: *PRNP*, *DNMT1*, *ITM2B*, *SERPINI1*, *CSF1R*, and *TYROBP*. Within these genes, one centenarian carried a rare variant (<1% population MAF) in *MAPT* (p.A152T; rs143624519), previously associated with an increased risk of both AD (OR 2.3) and FTD (OR 3.0) (22). In addition, two centenarians harbored the p.E365K (also referred to as p.E326K) (rs2230288) variant in the gene encoding for glucosylceramidase beta (*GBA*), known to increase the risk of Parkinson's disease (23). A novel variant in exon 13 of *NOTCH3*, the gene associated with the syndrome cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), p.C654F, was found in a woman 98 years of age (Supplementary Table 3). This variant leads to a cysteine–phenylalanine change, and is thus predicted to cause disease (24). Twenty-five variants of benign or unknown disease-related significance in Caucasians were found in non-AD associated risk genes in our extreme aging cohort (Supplementary Table 3).

Discussion

This study aimed to identify novel genetic variants contributing to extreme longevity, and the frequency of variants conferring an increased risk of AD and other dementias in a cohort aged 98–108 years. Through whole-exome sequencing of these 100 extreme longevity subjects, we did not find a statistically significant SNP likely to contribute to longevity, nor genes harboring an increased burden of rare variants in this cohort compared to control subjects. Genes of nominal statistical significance from the latter analysis may serve to guide future studies in centenarians, including *LYST*, *MDN1*, and *RBMXL1*. Lysosomal trafficking regulator

(*LYST*) is a widely expressed gene encoding a protein thought to regulate intracellular protein trafficking from secretory lysosomes (25). Recessive mutations in *LYST* cause Chediak-Higashi syndrome (CHS), a fatal childhood immunodeficiency syndrome. Adult forms of CHS are rare, but reported cases exclusively present with neurological dysfunction, including cerebellar, extrapyramidal, and cognitive symptoms (25). A non-coding variant in *LYST* has previously been reported as part of a genetic signature panel for exceptional longevity in humans (2). While neuronal lysosomal dysfunction is an important mechanism involved in several neurodegenerative syndromes, including AD and FTLD, it remains speculative whether an increased variant burden in *LYST* may promote longevity. Midasin AAA ATPase 1 (*MDN1*) encodes the protein midasin which functions as a nuclear chaperone required for the maturation and export of pre-60s ribosomal subunits (26). While midasin has not previously been implicated in human longevity, depleting the 60s ribosomal subunit in yeast extends lifespan by up to 40% (27). In humans, the *MDN1* transcript is upregulated in the aged brain which may be related to an age-dependent requirement for repair and replacement of compromised macromolecules (28). Taken together, *MDN1* may drive the molecular aging process, and its partial inhibition could serve a protective role. Future experiments are needed to test this hypothesis. RNA-binding motif protein, X-linked like 1 (*RBMXL1*) is a 43-kDa RNA binding protein with an unclear function. RNA binding proteins have been implicated in cellular senescence and neurodegeneration, but additional studies will be required to determine whether *RBMXL1* also plays a role in these conditions.

Several known genetic variants conferring AD risk are present in our extreme aging population. While it is well established that these genetic variants are also present at a lower rate in healthy non-demented subjects, our data add that the presence of such variants does not preclude extreme longevity. A rare variant in *PLD3* (p.V232M) with a MAF of 0.0045 in ExAC was suggested to increase the risk of sporadic AD, with a MAF of 0.008 and a carrier frequency of 1.36% in patients (21). The same variant is present in 2/100 centenarians, with a carrier frequency of 1.98% (MAF 0.01), suggesting further studies may be necessary to define the role of this variant in AD risk. While variants in both *TREM2* and *ABCA7* have received significant attention as genetic risk factors for AD, their presence in our centenarian cohort indicate that these variants do not preclude extreme aging. Finally, the *MAPT* p.A152T variant has been shown to weakly associate with both AD and FTD (22). This variant is present in non-Finnish Europeans with a MAF of 0.002 in the ExAC database (17), and 1 of 100 centenarians in our cohort is a carrier (MAF 0.005). Of known variants conferring an increased risk of non-AD dementias, the novel *NOTCH3* p.C554F variant was found in a 98-year-old woman, suggesting that not all mutations

leading to a gain or loss of cysteine produce the CADASIL phenotype. This latter finding highlights the utility of a centenarian cohort to help interpret biological significance of reported pathologic variants across neurodegenerative syndromes and beyond.

There are several important limitations to our analysis, most important of which is the small cohort size. Moreover, our analysis includes individuals <105 years of age, which may limit discovery of variants relevant for extreme longevity (1). We also recognize that while the majority of subjects in the SKAT control group were males, 83/100 centenarians were females. While the overall gene burden of rare variants may be different in men and women independent of age, our reported variant burden in *LYST* and *RBMXL1* was well balanced by gender in centenarians. For *MDN1*, 1/23 variants in centenarians was in a male subject, and thus future studies are required to fully assess whether gender independently impacts the variant burden in this gene.

In conclusion, NGS is a valuable tool to further elucidate not only the genetic basis of extreme longevity, but also factors relevant to age-dependent neurodegeneration such as AD. Our report adds to a small number of published whole-exome sequencing studies in centenarians, and forms the basis for future work in this unique cohort.

Aggregate sequencing data will be fully available to the academic community through the annotated genome server Neuroseq (<https://neuroseq.ca>).

Supplementary Material

Supplementary data is available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None declared.

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