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Research Report

MitoH3: Mitochondrial Haplogroup and Homoplasmic/Heteroplasmic Variant Calling Pipeline for Alzheimer's Disease Sequencing Project

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Abstract.

Background: Mitochondrial DNA (mtDNA) is a double-stranded circular DNA and has multiple copies in each cell. Excess heteroplasmy, the coexistence of distinct variants in copies of mtDNA within a cell, may lead to mitochondrial impairments. Accurate determination of heteroplasmy in whole-genome sequencing (WGS) data has posed a significant challenge because mitochondria carrying heteroplasmic variants cannot be distinguished during library preparation. Moreover, sequencing errors, contamination, and nuclear mtDNA segments can reduce the accuracy of heteroplasmic variant calling.

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Objective: To efficiently and accurately call mtDNA homoplasmic and heteroplasmic variants from the large-scale WGS data generated from the Alzheimer's Disease Sequencing Project (ADSP), and test their association with Alzheimer's disease (AD).

Methods: In this study, we present MitoH3—a comprehensive computational pipeline for calling mtDNA homoplasmic and heteroplasmic variants and inferring haplogroups in the ADSP WGS data. We first applied MitoH3 to 45 technical replicates from 6 subjects to define a threshold for detecting heteroplasmic variants. Then using the threshold of 5% ≤ variant allele fraction ≤ 95%, we further applied MitoH3 to call heteroplasmic variants from a total of 16,113 DNA samples with 6,742 samples from cognitively normal controls and 6,183 from AD cases.

Results: This pipeline is available through the Singularity container engine. For 4,311 heteroplasmic variants identified from 16,113 samples, no significant variant count difference was observed between AD cases and controls.

Conclusions: Our streamlined pipeline, MitoH3, enables computationally efficient and accurate analysis of a large number of samples.

Keywords: Alzheimer's disease, haplogroup, homoplasmic and heteroplasmic variant calling, mitochondrial DNA, whole genome sequencing

INTRODUCTION

Mitochondria are intracellular organelles and have multiple functions beyond oxidative phosphorylation which generate energy for cells. They also play important roles in multiple cell metabolism, signaling, etc. Mitochondrial (MT) biology contributes to complex cellular and organismal processes [1]. Mitochondrial impairments increase with age and are correlated with several age-related diseases including Alzheimer's disease (AD) [2]. Mitochondrial DNA (mtDNA) is a double-stranded circular DNA with 16,569 bases. Mitochondrial genes primarily encode proteins required for oxidative phosphorylation. Hundreds of mtDNA mutations have been associated with clinical phenotypes, including multiple neurodegenerative diseases [3]. Unlike nuclear DNA which typically has two copies, mtDNA can have multiple copies in each cell. The number of copies of mtDNA within each cell ranges from a few hundred in blood cells to several thousand in neurons or muscle cells, thus the mtDNA variants have different status, homoplasmy or heteroplasmy. Besides the copy number difference, mtDNA's mutation rate is higher than nuclear DNA due to a relative lack of DNA-protective histones and efficient DNA repair mechanisms [4], it is also reported that AD brains had an average 63% increase in heteroplasmic/somatic mtDNA control-region mutations [5].

Furthermore, mtDNA's circular morphology has introduced an artificial breakpoint on the MT reference sequence. Reads that should align around the breakpoint may be discarded when aligned to the linear reference sequence, which may cause variant detection failure, especially in the control region. In addition, nuclear mtDNA segments (NuMTs) can introduce variant calling errors due to the sequence similarity to mtDNA. Accurate identification of NuMT reads and heteroplasmic variants is still challenging in mtDNA studies. Sample contamination is a major issue in both nuclear DNA and mtDNA studies, and external or cross-contamination may also introduce false heteroplasmic calls [6, 7]. VerifyBamID [8] is commonly used to detect contamination levels in nuclear DNA, while haplocheck [9] identifies contamination based on the coexistence of mitochondrial haplogroup incompatibility, using the mitochondrial phylogeny and the concept of haplogroups.

Accurate calling of mtDNA variants is essential for the analysis of their association with disease and a separate variant calling workflow from nuclear DNA is necessary. There are multiple tools designed for mtDNA variants calling. MToolBox [10] realigns reads that have been mapped to mtDNA onto the nuclear genome to discard NuMTs. mtDNA-Server is a web-based server using Mutserve for variant calling, and it performs a contamination check based on a phylogenic tree of mtDNA variation [11]. MitoHPC performs the second iteration of variant identification using a sample's unique mtDNA sequence as the reference [12] and it also claims that GATK Mutect2 outperforms Mutserve. We recently developed a pipeline for calling mtDNA homoplasmic single nucleotide variants (SNVs) and MT haplogroups in whole-exome sequencing (WES) data obtained from nearly 11,000 subjects in the Alzheimer's Disease Sequencing Project (ADSP) Discovery Phase cohort with a degree of accuracy comparable to genotypes called from whole genome sequence (WGS) data [13]. However, it is challenging to identify mtDNA heteroplasmic variants and to estimate MT copy numbers from WES data. Indeed, because WGS data are rapidly emerging from large studies including the ADSP, there is an urgent need to streamline the computational pipeline for the accurate and efficient calling of mtDNA SNVs.

Here, we present a pipeline for calling mtDNA homoplasmic and heteroplasmic variants, and MT haplogroups from WGS data obtained from approximately 17,000 participants of the ADSP extension and FUS1.0 cohort (Release 3 dataset, https://adsp.niagads.org/data/data-summary/). This pipeline, named **MitoH3** (**Mito**chondria DNA **H**aplogroup, **H**omoplasmic and **H**eteroplasmic variants calling pipeline) can be applied to WGS data obtained from as many as several hundred thousand subjects. The pipeline integrates the GATK Best Practices Mitochondrial Analysis pipeline [14] and haplocheck [9] which are used for mtDNA variant calling, quality control, and contamination level estimation.

METHODS

Pipeline development

The MitoH3 pipeline has two main steps. First, it generates an individual-level VCF file that includes variants flagged with filters that they failed to pass. Next, it retrieves variants that pass all the filters and uses them to infer haplogroup and call heteroplasmic and homoplasmic variants. Details of each step are described below.

Variant calling

The pipeline code was written in WDL, a user-friendly scripting language maintained by the OpenWDL community and executed by Cromwell which is an open-source workflow execution engine that supports WDL and accepts as input a CRAM formatted file that contains information of WGS sequence alignment against the human reference genome. There are multiple processing steps including several implemented in the GATK mitochondria pipeline. First, mtDNA paired-end reads (chrM) were retrieved, and then reads with an unmapped mate or mapped to other contigs were excluded. The remaining chrM reads were mapped to two mtDNA reference sequences, namely the human-revised Cambridge Reference Sequence (rCRS) and a version of the rCRS that is shifted by 8,000 base pairs called shifted-rCRS. The use of the shifted-rCRS enhances the quality of variant calling in the control/D-loop region that contains the artificial break of the circular genome. Next, GATK Mutect2 was implemented to detect variants from both realigned mtDNA reads. Variants called from shifted-rCRS were assigned their original coordinates and merged with variants called from rCRS. Finally, multiple GATK [15] filtering protocols were used to flag problematic variants including FilterMutectCalls for strand bias and base quality, NuMTFilterTool for possible nuclear mtDNA segments variants based on median autosomal coverage, MTLowHeteroplasmyFilterTool for low heteroplasmy if the total low heteroplasmy exceeds the default threshold of 3, and VariantFiltration for excluding sites that likely contain unwanted artifacts. The resultant VCF file contained depths for the reference and alternative alleles which is essential information for homoplasmic and heteroplasmic variant calling. Variants that passed all filters were retained for subsequent analysis.

Haplogroup inference and heteroplasmic and homoplasmic variant calling

The haplocheck tool was employed to infer haplogroup and estimate the contamination status for each sample using all variants. Next, we separated the variants into homoplasmic and heteroplasmic groups. The variant allele fraction (VAF) was calculated as the proportion of alternate reads to total reads for one site [14] and was used to group the variants into homoplasmic or heteroplasmic. For example, we can define a site to be homoplasmic for the rCRS reference allele if its VAF < 3% and a site to be homoplasmic for alternative allele if its VAF > 97%. A site is defined as heteroplasmic if its VAF is between 3% and 97%, which is referred as "3_97%" for simplicity. We tested a series of thresholds from "1_99%" to "5_95%" to find an optimal VAF cutoff. To make the results comparable, under each VAF cutoff and within each set of technical replicates of same subject, we calculated the ratio of the number of replicates who has the variant to total number of replicates for each variant, if the ratio is larger than 0.5, it will be replaced by 1-ratio. This ratio is regarded as the inconsistent ratio for the variant of one subject. After calculating the sum of inconsistent ratio for each subject, we found that a VAF threshold of "5_95%" had the lowest overall inconsistent ratio (Fig. 1 and Supplementary Table 1) and therefore was used for calling homoplasmic and heteroplasmic variants across all

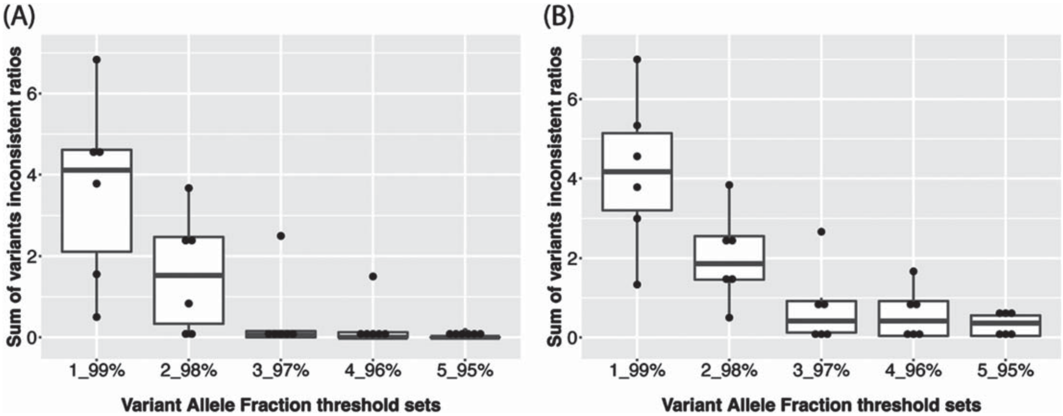


Fig. 1. Sum of inconsistent ratios of (A) Homoplasmic calls and (B) Heteroplasmic calls among technical replicates of each subject ($n = 6$) according to 5 different VAF thresholds.

subjects in the ADSP 17k WGS data. Therefore, variants with VAF > 95% will be homoplasmic for the alternative allele while 5% ≤ VAF ≤ 95% will be denoted as heteroplasmic. Of note, "5_95%" is the same VAF threshold applied in a recent analysis of the TOPMed WGS data [16]. Individual-level VCFs were merged to create a project-level VCF file for homoplasmic and heteroplasmic variants, respectively.

Datasets

WGS data were obtained from the ADSP which is an NIH-funded initiative to identify novel genes and rare genetic factors associated with AD risk in a large sample of AD cases and cognitively normal controls from multiple ancestry populations. Details of subject ascertainment and classification, library preparation, and sequencing protocols were described previously [17]. We applied the MitoH3 pipeline to WGS data included in the ADSP Release 3 dataset that was generated from 16,930 individuals. After excluding 239 samples that lacked phenotypic information, 168 samples showing apparent contamination based on haplocheck results, 88 samples having low average coverage or a high ratio of singletons (e.g., borderline samples), 277 duplicate samples detected by IBD analysis using the GENESIS [18], and 45 technical replicate samples included as part of the original ADSP design, 16,113 subjects remained for further analysis. This sample included 2,954 largely African American Descent (AA), 3,345 genetically admixed Caribbean Hispanics (CH), and 9,814 individuals of largely European ancestry (EA) whose ancestry group assignments were determined by principal component (PC) analysis using Gaussian Mixture Models from scikit-learn [19]. Among the 16,113 DNA samples, 12,226 were extracted from blood and 2,314 from brain, few from cell line, saliva or with unknown information. There are 6,742 samples from cognitively normal controls and 6,183 from AD cases, and the remaining 3,188 samples had an unknown disease status.

RESULTS

Determination of an optimal VAF threshold for calling homoplasmic and heteroplasmic variants

The determination of plasmy status for a particular MT variant requires a choice of VAF threshold. Because there is no established VAF threshold in the literature, we leveraged technical replicate WGS data from ADSP samples in the ADSP WGS data to discern an optimal VAF threshold. There are 45 technical replicates from six unique subjects among the ADSP samples. Three of these subjects (1–3) have nine technical replicates and the other three subjects (4–6) have six technical replicates (Table 1). The VAF threshold does not affect haplogroup inference since all the variants were evaluated by haplocheck. Haplogroup calling for the technical replicates was perfectly consistent (Table 2). However, the consistency of plasmy progressively improved with percentage increases in the VAF threshold (Fig. 1 and Supplementary Table 1). MitoH3 called the same number of homoplasmic variants in replicates for all subjects except for Subject 4 which had one technical replicate with 37 homoplasmic variants and five technical replicates with 38 homoplasmic variants (Table 3). As shown in

Table 1
Characteristics of the 6 sets of technical replicates of 45 samples

Subject		1	2	3	4	5	6
Sex		Female	Female	Female	Female	Female	Female
Age		89	63	70	83	53	70
Mother		NA	Subject1	Subject1	NA	NA	NA
Ethnicity		CH	CH	CH	CH	EA	AA
# of technical replicates		9	9	9	6	6	6
Sample source		Blood	Blood	Blood	Blood	Blood	Brain
Sequencing platform	Illumina HiSeq 2000	3	3	3	0	0	0
	Illumina HiSeqX	6	6	6	6	6	6
Sequencing Center	Baylor	3	3	3	2	2	2
	Broad	3	3	3	2	2	2
	WashU	3	3	3	2	2	2
Read length	101 bp	3	3	3	0	0	0
	150 bp	6	6	6	6	6	6
PCR	Free	6	6	6	6	6	6
	Amplified	3	3	3	0	0	0

CH, genetically admixed Caribbean Hispanic; EA, largely European ancestry; AA, largely African American Descent; WashU, University of Washing; PCR, polymerase chain reaction.

Table 2
Summary of mitochondrial haplogroup for 6 subjects with 45 technical replicates

	Mitochondrial Haplogroup			
	I1a1e	L0a1a2	L3b1a	L3b2
Subject 1	0	0	9	0
Subject 2	0	0	9	0
Subject 3	0	0	9	0
Subject 4	0	0	0	6
Subject 5	6	0	0	0
Subject 6	0	6	0	0

Subject 1 is the mother of subject 2 and subject 3 who share the same haplogroup

Table 3
Summary of mitochondrial homoplasmic variants for 6 subjects with 45 technical replicates at the non-reference variant allele fraction (VAF) threshold > 95%

	Number of homoplasmic variants			
	37	38	42	80
Subject 1	**9**	0	0	0
Subject 2	**9**	0	0	0
Subject 3	**9**	0	0	0
Subject 4	**1**	**5**	0	0
Subject 5	0	0	**6**	0
Subject 6	0	0	0	**6**

Table 4, the same number of heteroplasmic variants were called for all technical replicates for subjects #3 and #6 (i.e., concordance rate = 100%). Heteroplasmic variant concordance rates were lower for the other subjects ranging from 44.4% to 83.3%. Based on the results from the 45 technical replicates, we chose a VAF threshold of 5_95% for classifying variant genotypes as homoplasmic or heteroplasmic in the remaining sample of 16,113 subjects.

Application of the pipeline using a 5_95% VAF threshold

Haplogroups

Twenty-two known human mitochondrial haplogroups were assigned to each of the 16,113 subjects who were grouped by ancestry (AA, EA, and CH) as determined by PC analysis of nuclear genome variants. As shown in Fig. 2, each group is uniquely characterized by the haplogroup distribution, which is consistent with findings from a previous study [20]. For example, haplogroups A, B, C, and D are much more abundant in the CH group. Haplogroups H, J, K, T and U are more frequent in the EA group, whereas Haplogroup L was observed primarily in the AA (91.9%) and CH (41.3%) groups.

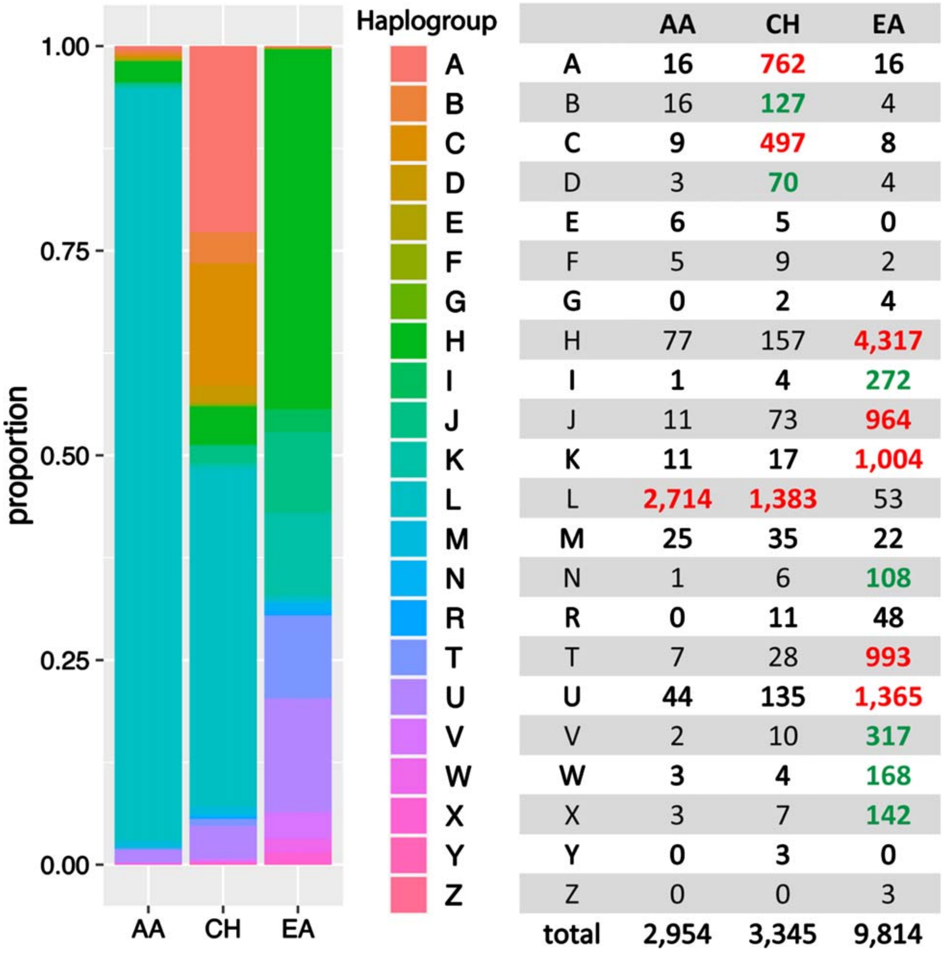
Distribution of homoplasmic and heteroplasmic calls and variants

The VAF distribution of all the calls in the total sample (Fig. 3A) as well as in each ancestry group (Supplementary Figure 1A) is bimodal with a higher proportion of homoplasmic calls in the tails of the distribution. In the total sample, 96.8% of the calls were homoplasmic (Fig. 3B), but the proportion is slightly higher in AAs than in the other ancestry groups (Supplementary Figure 1B). Although in the total sample the proportions of unique variants that were observed only as heteroplasmic or both homoplasmic and heteroplasmic were roughly equal (24.2% vs. 27.8%) and about one-half of the 8,286 unique variants were only called as homoplastic (48.0%) (Fig. 4A), the proportion of variants that were homo-

Table 4
Comparison of mitochondrial heteroplasmic variants for 6 subjects with 45 technical replicates at the 5% ≤ VAF ≤ 95%

	Number of heteroplasmic variants						Concordant
	0	1	2	3	4	5	(Percent)
Subject 1	**1**	**4**	**4**	0	0	0	44.4% (4/9)
Subject 2	0	**6**	**3**	0	0	0	66.7% (6/9)
Subject 3	**9**	0	0	0	0	0	100.0% (9/9)
Subject 4	0	**5**	**1**	0	0	0	83.3% (5/6)
Subject 5	0	0	0	**3**	**2**	**1**	50.0% (3/6)
Subject 6	0	**6**	0	0	0	0	100.0% (6/6)

VAF, variant allele fraction.



	AA	CH	EA
A	16	762	16
B	16	127	4
C	9	497	8
D	3	70	4
E	6	5	0
F	5	9	2
G	0	2	4
H	77	157	4,317
I	1	4	272
J	11	73	964
K	11	17	1,004
L	2,714	1,383	53
M	25	35	22
N	1	6	108
R	0	11	48
T	7	28	993
U	44	135	1,365
V	2	10	317
W	3	4	168
X	3	7	142
Y	0	3	0
Z	0	0	3
total	2,954	3,345	9,814

Fig. 2. MT haplogroup distribution in African American (AA), Caribbean Hispanic (CH), and European ancestry (EA) cohorts. Bolded numbers indicate that the haplogroup is more predominant in the particular ancestry group compared to other groups. Red-highlighted numbers signify that the haplogroup constitutes more than 5% within the ancestry, while green-highlighted numbers indicate that the haplogroup comprises less than 5%, yet the frequency is 5-fold greater than in other ancestral groups at least.

plasmic only was highest in the AA group (68.6% each) and lowest in the EA group (53.0%), noting that the number of unique variants observed in each ancestry group is proportional to the sample size (Supplementary Figure 2A). Most unique variants are rare (MAF < 1%, Fig. 4B). Not surprisingly, the proportion of uniquely homoplasmic or heteroplasmic variants decreases as a function of the VAF in the total sample (Fig. 4C) and within the EA and CH groups (Supplementary Figure 2C). The mean number of

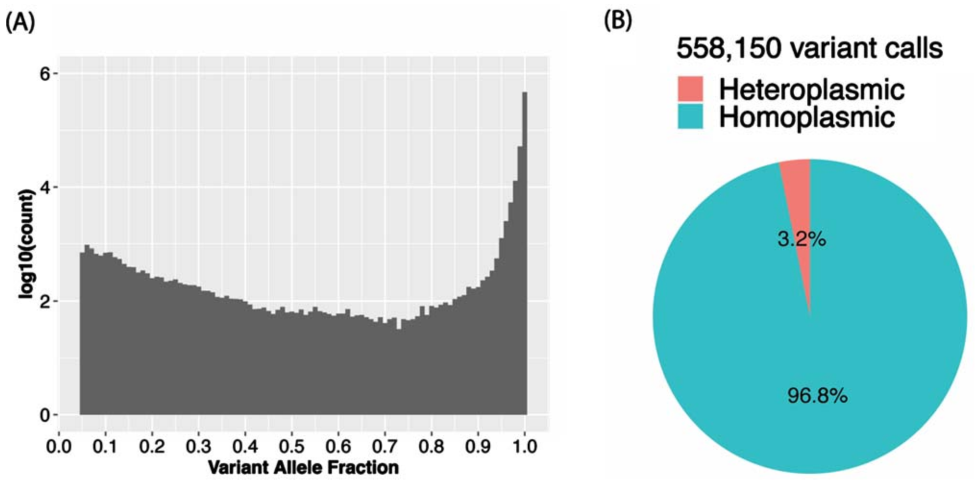


Fig. 3. Among individuals in the total sample, (A) variant allele fraction (VAF) distribution of mtDNA variant calls and (B) proportion of heteroplasmic calls and homoplasmic calls.

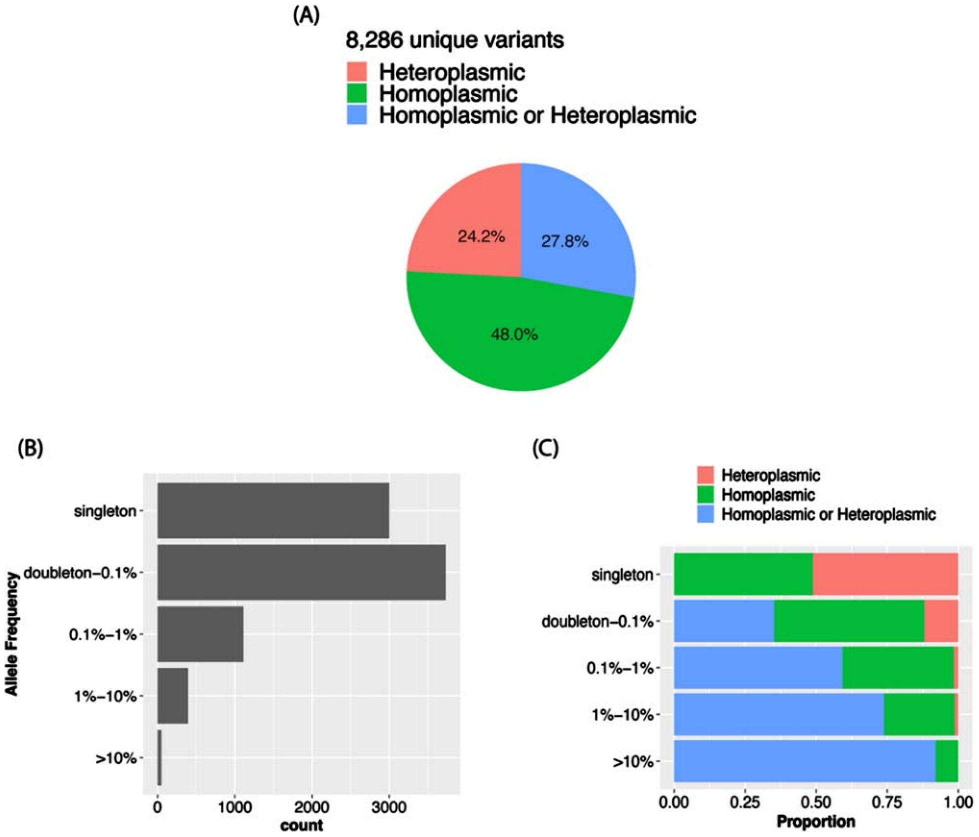


Fig. 4. MT variant distributions in the total sample. A) Proportion of variants by plasmy status. B) Number of variants detected according to allele frequency strata. C) Proportion of variants by plasmy status in each frequency strata.

homoplasmic variants among individuals significantly differed by ancestry group (AA = 54.9 ± 21.0, CH = 44.1 ± 18.8, EA = 23.5 ± 11.2; $p < 2 \times 10^{-16}$) (Fig. 5A) and was slightly greater in EA samples derived from blood (23.7 ± 11.3) compared to brain (23.0 ± 11.0) (Fig. 5B). The mean of num-

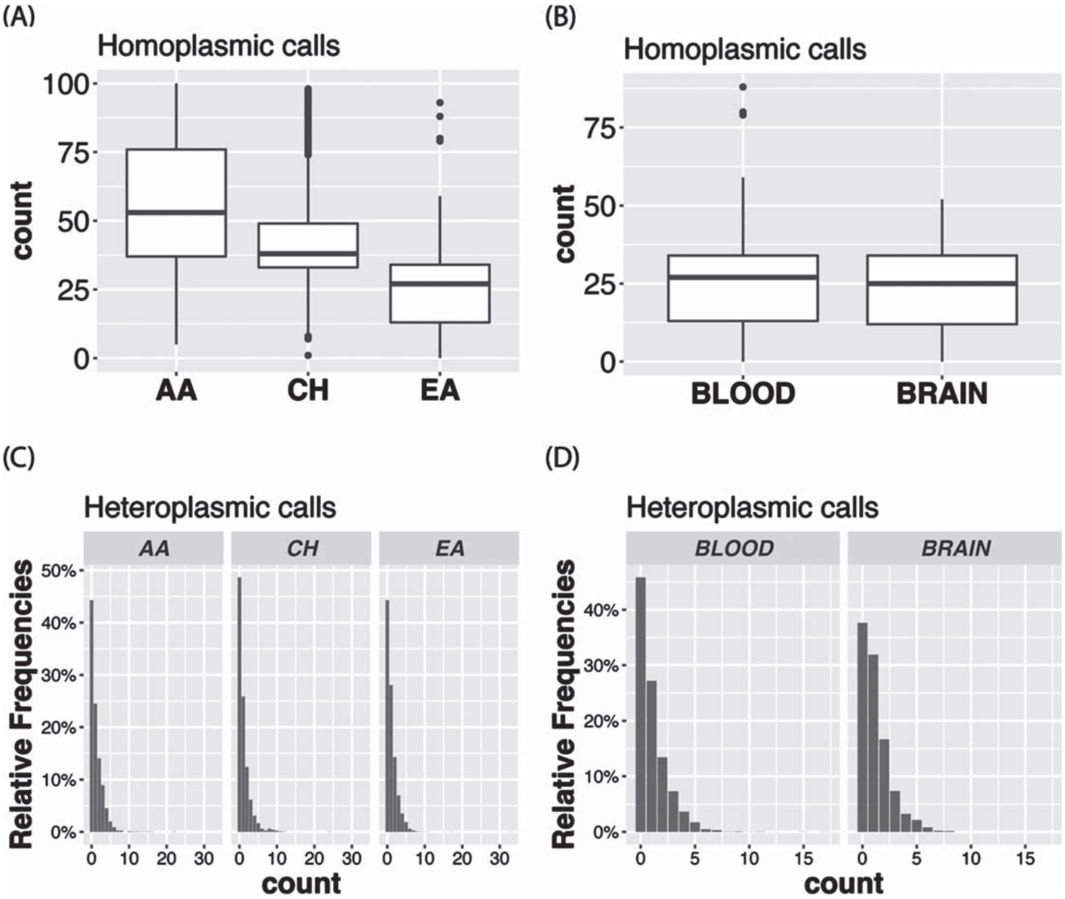


Fig. 5. Boxplots showing the mean number of homoplasmic calls according to (A) ancestry group and (B) tissue type among European ancestry individuals. Bar plots show the proportion of heteroplasmic calls according to (C) ancestry group and (D) tissue type among European ancestry individuals.

ber of heteroplasmic calls in AA individuals (1.21) was significantly greater than in CH individuals (1.08) and EA (1.08) individuals (ANOVA test $p = 5.61 \times 10^{-5}$, Fig. 5C). The difference between blood and brain in EA samples is also significant (*t* test *p*-value = 0.0003, mean of blood sample = 1.06, mean of brain sample = 1.18, Fig. 5D). However, no significant difference of homoplasmic or heteroplasmic variant count was found in AD cases and controls within each ethnic group or within the sample source.

DISCUSSION

We integrated multiple tools into a comprehensive and flexible pipeline for calling mtDNA haplogroup and homoplasmic/heteroplasmic variants in whole genome sequence data. The pipeline can be implemented easily, has multiple quality control features and the ability to estimate contamination levels, and enables computationally efficient and accurate analysis of a large number of samples. The software and scripts for using this pipeline are publicly available and packaged as one singularity image (https://github.com/MarchOnion/MitoH3).

Some of our findings are consistent with other studies. For example, we found that haplogroup L is primarily found in AAs and haplogroups H, I, J, and K are more frequent in individuals of European ancestry, patterns which are similar to those observed in data from the 1000 Genomes Project [20]. In addition, we leveraged ADSP WGS technical replicates to determine the optimal VAF threshold for separating homoplasmic and heteroplasmic call sets. Applying this information to the entire WGS sample of 16,113 individuals revealed that 96.8% of calls were homoplasmic, an estimate that is consistent with a recent study using the information in the Genome Aggregation Database [14]. We also found that EAs have a slightly smaller proportion of homoplasmic calls compared to the other ancestry groups included in our study, a difference which could be due to the relatively small sample size for non-EAs. The number of homoplasmic sites observed in blood and brain samples was similar in the EA group although a large difference in sample size for blood versus brain, which might be due to the complexity of brain tissues or the high heterogeneity of brain regions.

Furthermore, we found there is no significant difference in homoplasmic or heteroplasmic variant counts between AD cases and controls within ethnic groups and sample sources (i.e., blood and brain). This finding is supported by a recent study indicating that mtDNA heteroplasmy levels do not show an association with AD pathologies or cognitive function; rather, they are more strongly correlated with age. Klein et al. also suggests that single nucleotide polymorphisms (SNPs) or small insertions and deletions (indels) may not play a significant role in late-onset neurodegenerative diseases [21]. There are also multiple studies aimed at exploring the mechanism underlying the association between mitochondrial impairments and the pathogenesis of AD [22]. While no primary MT mutations were found to be associated with AD, many studies indicate that MT haplogroups play a role in AD [23]. However, the same AD-associated MT haplogroups cannot be replicated in an independent Caucasian population study, which could be explained by the geographic difference in the sub-haplogroup structure of mtDNA [24]. The technical differences including sampling, sequencing methods, and library preparation may also contribute to the inconsistent results across studies. Integrating mtDNA copy number, structure variants, and haplogroups along with homoplasmic and heteroplasmic levels to investigate the mechanism between mtDNA with AD would be a potential enhancement to the MitoH3 pipeline.

There are several notable limitations to our study. First, it is challenging to estimate the tissue-specific difference of homoplasmic and heteroplasmic calls across different ancestry groups due to the smaller sample sizes for the AA and CH groups, as well as the small number of samples from brain compared to blood. Brain samples were unavailable for the non-EA groups. Second, we suggested mitochondrial heteroplasmic variants be defined at the $5\% \leq \text{VAF} \leq 95\%$ based on 45 technical replicates from 6 subjects. This threshold could be adjusted for different ancestry groups to achieve ancestry-specific optimal results.

In summary, the pipeline described here can efficiently provide comprehensive and accurate information about mtDNA variants, plasmy and haplogroups derived from whole genome sequence data. Further studies in larger and more diverse samples are needed to validate our findings and suggest future modifications to the mitoH3 pipeline.

AUTHOR CONTRIBUTIONS

Congcong Zhu (Data curation; Formal analysis; Methodology; Project administration; Software; Visualization; Writing – original draft; Writing – review & editing); Tong Tong (Methodology; Writing – review & editing); John J. Farrell (Supervision); Eden R. Martin (Funding acquisition); William S. Bush (Funding acquisition); Margaret A. Pericak-Vance (Funding acquisition); Li-San Wang (Funding acquisition); Gerard D. Schellenberg (Funding acquisition); Jonathan L. Haines (Funding acquisition); Kathryn L. Lunetta (Methodology; Supervision); Lindsay A. Farrer (Funding acquisition; Supervision; Writing – review & editing); Xiaoling Zhang (Conceptualization; Funding acquisition; Investigation; Methodology; Supervision; Writing – original draft; Writing – review & editing).

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The ADGC cohorts include: Adult Changes in Thought (ACT) (U01 AG006781, U19 AG066567), the Alzheimer's Disease Research Centers (ADRC) (P30 AG062429, P30 AG066468, P30 AG062421, P30 AG066509, P30 AG066514, P30 AG066530, P30 AG066507, P30 AG066444, P30 AG066518, P30 AG066512, P30 AG066462, P30 AG072979, P30 AG072972, P30 AG072976, P30 AG072975, P30 AG072978, P30 AG072977, P30 AG066519, P30 AG062677, P30 AG079280, P30 AG062422, P30 AG066511, P30 AG072946, P30 AG062715, P30 AG072973, P30 AG066506, P30 AG066508, P30 AG066515, P30 AG072947, P30 AG072931, P30 AG066546, P20 AG068024, P20 AG068053, P20 AG068077, P20 AG068082, P30 AG072958, P30 AG072959), the Chicago Health and Aging Project (CHAP) (R01 AG11101, RC4 AG039085, K23 AG030944), Indiana Memory and Aging Study (IMAS) (R01 AG019771), Indianapolis Ibadan (R01 AG009956, P30 AG010133), the Memory and Aging Project (MAP) (R01 AG17917), Mayo Clinic (MAYO) (R01 AG032990, U01 AG046139, R01 NS080820, RF1 AG051504, P50 AG016574), Mayo Parkinson's Disease controls (NS039764, NS071674, 5RC2HG005605), University of Miami (R01 AG027944, R01 AG028786, R01 AG019085, IIRG09133827, A2011048), the Multi-Institutional Research in Alzheimer's Genetic Epidemiology Study (MIRAGE) (R01 AG09029, R01 AG025259), the National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD) (U24 AG021886), the National Institute on Aging Late Onset Alzheimer's Disease Family Study (NIA-LOAD) (U24 AG056270), the Religious Orders Study (ROS) (P30 AG10161, R01 AG15819), the Texas Alzheimer's Research and Care Consortium (TARCC) (funded by the Darrell K Royal Texas Alzheimer's Initiative), Vanderbilt University/Case Western Reserve University (VAN/CWRU) (R01 AG019757, R01 AG021547, R01 AG027944, R01 AG028786, P01 NS026630, and Alzheimer's Association), the Washington Heights-Inwood Columbia Aging Project (WHICAP) (RF1 AG054023), the University of Washington Families (VA Research Merit Grant, NIA: P50AG005136, R01AG041797, NINDS: R01NS069719), the Columbia University Hispanic Estudio Familiar de Influencia Genetica de Alzheimer (EFIGA) (RF1 AG015473), the University of Toronto (UT) (funded by Wellcome Trust, Medical Research Council, Canadian Institutes of Health Research), and Genetic Differences (GD) (R01 AG007584). The CHARGE cohorts are supported in part by National Heart, Lung, and Blood Institute (NHLBI) infrastructure grant HL105756 (Psaty), RC2HL102419 (Boerwinkle) and the neurology working group is supported by the National Institute on Aging (NIA) R01 grant AG033193.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

DATA AVAILABILITY

NIAGAD: Data for this study were prepared, archived, and distributed by the National Institute on Aging Alzheimer's Disease Data Storage Site (NIAGADS) at the University of Pennsylvania (U24-AG041689), funded by the National Institute on Aging (Accession number: NG00067).

GitHub repository/Code availability: https://github.com/MarchOnion/MitoH3

SUPPLEMENTARY MATERIAL

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