Neurobiology of Aging 36 (2015) 1604.e7–1604.e16

Contents lists available at ScienceDirect

Neurobiology of Aging

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

Mitochondrial DNA haplogroup B5 confers genetic susceptibility to Alzheimer's disease in Han Chinese

Rui Bi [a,b], Wen Zhang [a], Dandan Yu [a], Xiao Li [a,b], Hui-Zhen Wang [a,c], Qiu-Xiang Hu [a,b], Chen Zhang [d], Weihong Lu [d], Jianliang Ni [e], Yiru Fang [d], Tao Li [f], Yong-Gang Yao [a,*]

[a] Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences & Yunnan Province, Kunming Institute of Zoology, Kunming, Yunnan, China
[b] Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan, China
[c] School of Life Sciences, University of Science and Technology of China, Hefei, Anhui, China
[d] Division of Mood Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong, University School of Medicine, Shanghai, China
[e] First Geriatric Department, Tongde Hospital of Zhejiang Province, Hangzhou, Zhejiang, China
[f] The Mental Health Center & Psychiatric Laboratory, West China Hospital, Sichuan University, Chengdu, Sichuan, China

ARTICLE INFO

Article history:
Received 14 June 2014
Received in revised form 7 September 2014
Accepted 10 October 2014
Available online 22 October 2014

Keywords:
Alzheimer's disease
mtDNA haplogroup
Chinese
Genetic susceptibility

ABSTRACT

Mitochondrial dysfunction has been widely reported in psychiatric and neurodegenerative diseases. We aimed to investigate the association between matrilineal structures of Han Chinese populations and Alzheimer's disease (AD) by a 2-stage case-control study: A total of 341 AD patients and 435 normal individuals from Southwest China were analyzed for mitochondrial DNA sequence variations and were classified into respective haplogroups. A total of 371 AD patients and 470 normal individuals from East China, as validation samples, were genotyped for the variants defining the risk haplogroup. Haplogroup B5 had a significantly higher frequency in AD patients (7.33%) than in control subjects (3.68%) from Southwest China, and we found a similar pattern of higher frequency of B5 in patients in the case-control sample from East China. In the combined population, association of haplogroup B5 with AD risk was strengthened ($p = 0.02$; odds ratio = 1.74; 95% confidence interval = 1.10–2.76). In lymphoblastoid cell lines belonging to haplogroup B5a, we observed significantly increased reactive oxygen species and decreased mitochondrial mass. Hela cells with stable expression of the *MT-ATP6* gene with B5-defining variant m.8584G>A also showed a significantly decreased mitochondrial function. Taken together, our results indicated that haplogroup B5 conferred genetic susceptibility to AD in Han Chinese, and this effect was most likely mediated by ancient variant m.8584G>A. The predisposing effect of B5 to AD is consistent with the ancestral-susceptibility model of complex diseases.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease, which mainly leads to severe memory loss in elderly people older than 60 years (Huang and Mucke, 2012; Querfurth and LaFerla, 2010). Intracellular neurofibrillary tangles formed by hyperphosphorylated tau proteins and extracellular senile plaques because of deposition of amyloid-β (Aβ) peptide are 2 hallmarks observed in AD brains (Huang and Mucke, 2012; Querfurth and LaFerla, 2010). Only about 5% AD cases have a family history, and mutations in the amyloid precursor protein (*APP*) and presenilin (*PS*) genes have been identified to be involved in these familial AD cases (Querfurth and LaFerla, 2010; Tanzi, 2012). Recent large-scale genetic screening revealed several susceptible genes for AD (Cruchaga et al., 2014; Jonsson et al., 2013; Karch et al., 2014; Lambert et al., 2013). However, genetic susceptible factors for the remaining 90%–95% sporadic cases are still largely unknown.

Owing to the important roles of mitochondria in cellular metabolism and energy production, the nervous system, with high energy demands, is especially vulnerable to mitochondrial defects (Mattson et al., 2008). In fact, mitochondrial dysfunction is widely reported in neurodegenerative diseases (Mattson et al., 2008). Hitherto, large amounts of reports have prompted the significant role of mitochondrial dysfunction in the pathogenesis of AD. Mitochondrial abnormalities, including reduced adenosine triphosphate (ATP) production, increased reactive oxygen species (ROS) generation,

* Corresponding author at: Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences & Yunnan Province, Kunming Institute of Zoology, Kunming, Yunnan 650223, China. Tel./fax: +86 871 65180085.
E-mail address: ygyaozh@gmail.com (Y.-G. Yao).

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http://dx.doi.org/10.1016/j.neurobiolaging.2014.10.009

decreased activity of respiration chain complexes, impaired balance of mitochondrial fission and fusion, as well as mitochondrial DNA (mtDNA) damage were observed in AD patient brains, AD animal models, and Aβ-treated neurons (Cho et al., 2009; DuBoff et al., 2013; Krishnan et al., 2012; Liang et al., 2008; Rhein et al., 2009; Yao et al., 2009). The mitochondrial cascade hypothesis of AD further highlighted the important role of mtDNA variations and/or mutations in the development of AD (Swerdlow et al., 2014).

Human mtDNA encodes 2 ribosomal RNAs, 22 transfer RNAs, and 13 proteins which are components of mitochondrial oxidative phosphorylation (Falkenberg et al., 2007). During the origin and migration of modern human beings, mtDNA accumulated mutations in a chronological order to generate a group of related haplotypes that was termed as a haplogroup (namely, a group of mtDNAs that share certain ancient variants), which might mirror the ancient adaptation to environment and natural selection pressure (Ruiz-Pesini et al., 2004; Yao et al., 2002). However, with the change of environment and lifestyle, some of these ancient mtDNA variations and/or haplogroups may confer genetic susceptibility to disease according to the ancestral-susceptibility model (Di Rienzo and Hudson, 2005). For instance, mtDNA haplogroups were identified to affect susceptibility of many kinds of neurodegenerative diseases such as Leber hereditary optic neuropathy (Ji et al., 2008), Parkinson disease (Ghezzi et al., 2005), schizophrenia (Zhang et al., 2014), and AD (Mancuso et al., 2009). Haplogroups H and U and their subhaplogroups were consistently found to be risk factors for AD in several independent studies of European populations (Coto et al., 2011; Fesahat et al., 2007; Lakatos et al., 2010; Maruszak et al., 2009, 2011; Santoro et al., 2010; Tranah et al., 2012; van der Walt et al., 2004). However, some of these initial conclusions were controversial and lacked functional assessment (Hudson et al., 2012). Haplogroup L1 was found to be associated with increased dementia risk in African Americans (Tranah et al., 2014). Furthermore, haplogroups U5b1, U5b1b2, K1a1b, and K1a1b2a1 were identified to be associated with AD biomarkers (Ridge et al., 2013). However, there is still no related study in Chinese population. Because of different genetic structures between Chinese and European and/or American populations, it is essential to investigate the mtDNA haplogroup effect on AD in Chinese population and to test whether ancient mtDNA variants could serve as an example of the ancestral-susceptibility alleles for AD.

In this study, we dissected the matrilineal components in 2 well-matched AD case-control cohorts of Han Chinese. The frequency of mtDNA haplogroup B5 was found to be significantly over-represented in AD patients. Lymphoblastoid cell lines with haplogroup B5 background exhibited decreased mitochondrial function. Allotopic expression of B5 defining variant m.8584G>A (p.A20T in the MT-ATP6) in Hela cells could affect mitochondrial function, which might account for the association between haplogroup B5 and AD.

2. Methods

2.1. Subjects

Two independent case-control cohorts from Southwest China and East China were recruited in this study. The stage-I (discovery stage) sample set was composed of 341 AD patients and 435 normal individuals from Southwest China, which were collected at the Mental Health Center of West China Hospital. The stage-II (validation stage) sample set comprised 371 AD patients and 470 normal individuals from East China, which were collected at the Shanghai Mental Health Center and the Tongde Hospital of Zhejiang Province. All subjects were of Han Chinese origin, and around 66% of these AD patients and control subjects have been described in our previous study (Bi et al., 2014). The diagnosis of AD was performed following the DSM-IV and the NINCDS-ADRDA criteria, and all patients were identified as sporadic AD as none of their first-degree relatives developed dementia. The healthy control subjects were confirmed to have normal cognitive function. Informed consents according to the tenets of the Declaration of Helsinki were obtained from all participants or the supervisors of the patients before this study. The institutional review board of the Kunming Institute of Zoology, Chinese Academy of Sciences approved this study.

2.2. mtDNA sequence analyses and haplogroup classification

Total genomic DNA was extracted from peripheral blood by using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen, USA). The mtDNA control region sequences of the stage-I samples from Southwest China were amplified and sequenced using our previously reported method (Zhang et al., 2011, 2014). Sequence variants in each mtDNA were scored relative to the revised Cambridge Reference Sequence (Andrews et al., 1999). The haplogroup status of each mtDNA was determined according to the updated East Asian mtDNA tree and the latest version of Phylotree (http://phylotree.org/tree/main.htm; mtDNA tree Build 16, 19 Feb 2014) (van Oven and Kayser, 2009) and was double checked by MitoTool (www.mitotool.org) (Fan and Yao, 2013). The mtDNA-coding region motifs in certain samples were screened to confirm the haplogroup assignments.

We screened haplogroup-specific variants of mtDNA haplogroup B5 (m.709G>A and m.9950T>C) in stage-II samples by using the SNaPshot assay, to validate the positive association observed in stage-I samples. The SNaPshot assay was composed of a multiplex polymerase chain reaction (PCR) and subsequent single-base extension process, following the detailed step-by-step procedure in our previous study (Bi et al., 2014). Primers for multiplex PCR and single-base extension procedure were listed in Supplementary Table 1. The complete mtDNA genome of the B5a-2 cell line was sequenced and analyzed following our previous method (Ji et al., 2008; Zhang et al., 2014).

2.3. Statistical analysis

Power value was calculated by using the Quanto software 1.2.4. (Gauderman, 2002). Considering an average population minor allele frequency of 10%, the power to detect odds ratio range from 1.5 to 2.0 for a risk haplogroup was from 59.3% to 97.2%.

To avoid potential bias caused by small sample size and to ensure the statistical power, only those haplogroups shared by at least 3% of individuals were considered as variables in the statistical analysis (minor allele frequency >3% in at least 1 population). Fisher exact test (2 tailed) was performed by MitoTool (www.mitotool.org) (Fan and Yao, 2013) to investigate whether the frequency of certain haplogroup was different between case and control groups. A p-value < 0.05 was regarded as statistically significant. Assuming that each haplogroup is uniformly distributed in the entire samples, a permutation test was conducted with 1,000,000 replications to further validate the prevalence of certain mtDNA haplogroups in patient and control groups following the same procedure described in our recent study (Zhang et al., 2014).

Principal component analysis was performed based on the mtDNA haplogroup distribution frequency using the POPSTR software (Henry Harpending, 1997) (http://harpending.humanevo.utah.edu/popstr/) to show the overall clustering pattern of case and control populations with reported Han Chinese across China (Zhang et al., 2011, 2014 and references therein). Median-joining network for these mtDNAs belonging to the AD-associated

haplogroup was constructed by Network 4.5.1.6 (http://www.fluxus-engineering.com/sharenet.htm) (Bandelt et al., 1999). To illustrate regional distribution pattern of the AD-related mtDNA haplogroup, we (re)analyzed the previously reported mtDNA data from the general populations across China (Supplementary Table 2). A contour map of spatial frequency of the AD-related haplogroup was depicted by using Golden Software Surfer 8.0 (Golden Software Inc Golden, CO, USA). We compared the frequency distribution pattern of susceptible haplogroups with the pattern of AD prevalence based on the regional epidemiological data (Supplementary Table 3) to assess if there was a correlation.

2.4. Functional characterization of mtDNA haplogroup B5 and its defining variants

2.4.1. Cell culture

Blood samples from 5 subjects with different mtDNA haplogroup background were collected for establishing lymphoblastoid cell lines, including C-1 (haplogroup B4), C-2 (haplogroup A), C-3 (haplogroup D4), B5a-1 (haplogroup B5a, a subhaplogroup of B5), and B5a-2 (haplogroup B5a). Donors of the 2 B5a cell lines were maternally related.

Lymphoblastoid cell lines were immortalized by using the Epstein-Barr virus and were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) (Gibco, USA, 11,875). Hela cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA, 11,966) supplemented with 10% fetal bovine serum (Gibco, 10,099), 5 mM HEPES (Sigma-Aldrich, USA, H9136), and 1 mM sodium pyruvate (Gibco, 11,360). Considering the Warburg effect in cancer cells (Hsu and Sabatini, 2008; Warburg, 1956) (cancer cells prefer to producing ATP through glycolysis but not oxidative phosphorylation), the glucose in DMEM was replaced with 10 mM galactose (Sigma-Aldrich, G5388) to force cell to produce ATP via mitochondria. HEK293T cells were cultured in DMEM (Gibco, 11,965) containing 10% heat inactivated FBS. All cells were cultured at 37 °C in 5% CO_2.

2.4.2. Plasmid construction

Nuclear-encoded mtDNA coding gene *ATP6* in pCMV-Tag 4A plasmids (kind gifts from Dr Corral-Debrinski) (Kaltimbacher et al., 2006), together with upstream mitochondrial targeting sequence and downstream flag tag, were subcloned into FUGW lentivirus vector (Addgene, England, 14,883) with or without GFP to form constructs FUGW-ATP6 and FUGW-ATP6-GFP. Variant m.8584G>A was introduced into FUGW-ATP6 vector by using site-directed mutagenesis PCR method. All constructs were validated by sequencing.

2.4.3. Construction of Hela cells with stable expression of the MT-ATP6 gene

HEK293T cells that were cultured in 6-well plate were transfected with 10 μL Lipofectamine 2000 transfection reagent (Invitrogen, USA, 11,668) and 4 μg plasmid DNA mixtures per well, which was constituted by the FUGW-ATP6 construct, packaging plasmid psPAX2 (Addgene, England, 12,260) and envelope plasmid PMD2.G (Addgene, 12,259) with the ratio of 3:2:1, respectively. The cell supernatant containing lentivirus was harvested 48 hours after transfection. Hela cells cultured in a 12-well plate at approximately 10% confluence were incubated in a solution containing 500 μL lentivirus supernatant, 500 μL DMEM with 10% FBS, and 5 μg/mL hexadimethrine bromide (Sigma-Aldrich, H9268). After incubating for 24 hours at 37 °C in 5% CO_2, single Hela cell was seeded into 96-well plate by serial dilution to select single cell clones with stable expression of the *ATP6* gene. Genomic DNA of each clone was extracted by using the AxyPrep Genomic DNA Miniprep Kit (Axygen, AP-MN-BL-GDNA-250) and was amplified and sequenced using the FUGW specific primers hubc-f (5′-GCACCTTTTGAAATGTAATCATTTG-3′) and WPRE-R (5′-CAAAGGCATTAAAGCAGCGTATC-3′) to confirm successful lentivirus transduction.

2.4.4. Fluorescence microscopy and Western blot

To investigate whether the allotopically expressed ATP6-GFP protein could be translocated into mitochondria, Hela cells were seeded on coverslips and grown to 50% confluence in a 12-well plate, followed by a cotransfection of 0.9 μg of FUGW-GFP vector or FUGW-ATP6-GFP construct and 0.1 μg of pDsRed2-mito vector (which express mitochondrial targeting red fluorescent protein) (Clontech, USA, 632421) using FuGENE HP (Roche, Switzerland, 06366236001) as recommended by the manufacturer. After 48 hours of transfection, living cells were imaged using the Olympus FluoView 1000 confocal microscope (Olympus, Melville, NY, USA) at 488 nm and 563 nm, respectively. Acquired images and multichannel overlaying were analyzed by FV10-ASW 2.1 viewer software (Olympus).

Cells were lysed in cell lysis buffer (Beyotime, China, P0013) to obtain total cell protein. Crude mitochondria preparations were isolated by using Mitochondria Crude Isolation Kit (GENMED, China, GMS10006). Cell lysate concentration was determined by BCA Protein Assay Kit (Beyotime, P0012). To investigate whether ATP6 protein was translocated into mitochondria, 20 μg of crude mitochondria fraction were treated with 75 μg/mL proteinase K for 30 minutes on ice, and subsequently 1 mM phenylmethylsulfonyl fluoride (Sigma-Aldrich, P7626) were added to stop proteinase K reaction. Protein denaturation was performed at 70 °C for 10 minutes in sodium dodecyl sulfate loading buffer. A total of 20 μg protein was separated in 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Bio-Rad, USA, 162–0177). After blocking with 5% (wt/vol) nonfat dry milk in TBST for 2 hours at room temperature, the membrane was incubated with primary monoclonal mouse antibodies against flag tag (Abmart, China, M20008) (1:5000, overnight at 4 °C) or GAPDH (EnoGene, China, E12-042; as protein-loading control) (1:2000, overnight at 4 °C), followed by the anti-mouse IgG peroxidase-conjugated secondary antibody (KPL, Gaithersburg, MD, USA, 474–1806) (1:10,000, 1 hour at room temperature). The epitope was visualized by using Immobilon Western Chemiluminescent HRP Substrate (Millipore, USA, WBKLS0500).

2.4.5. Measurement of cellular ROS level and mitochondrial mass level

Lymphoblastoid cell lines were cultured in a 12-well plate for 48 hours before measurement of ROS and mitochondrial mass. Hela cells with stable expression of the *ATP6* gene were cultured in a 12-well plate for 48 hours to reach a confluence of 80%–90%. The cellular ROS level and mitochondrial mass level were assessed following our previously described methods (Guo et al., 2012). In brief, for ROS determination, Hela cells and lymphoblastoid cells were respectively incubated in phosphate buffer saline (PBS) containing 2 μM and 0.5 μM DCFH-DA probe (Sigma-Aldrich, D6883) at 37 °C for 20 minutes, then washed with PBS and analyzed by using flow cytometry (BD, Vantage SE, USA) at 535 nm. For mitochondrial mass measurement, cells were incubated in prewarm medium with 100 nM MitoTracker Red FM (Molecular Probe, USA, M22425) at 37 °C for 30 minutes, then washed with prewarm PBS and analyzed by using flow cytometry (BD) at 644 nm.

2.4.6. Determination of cellular ATP level and cellular oxygen consumption rate

Cells were seeded in 12-well plate and were cultured for 48 hours, then were lysed in 100 μL lysis buffer (GENMED, China,

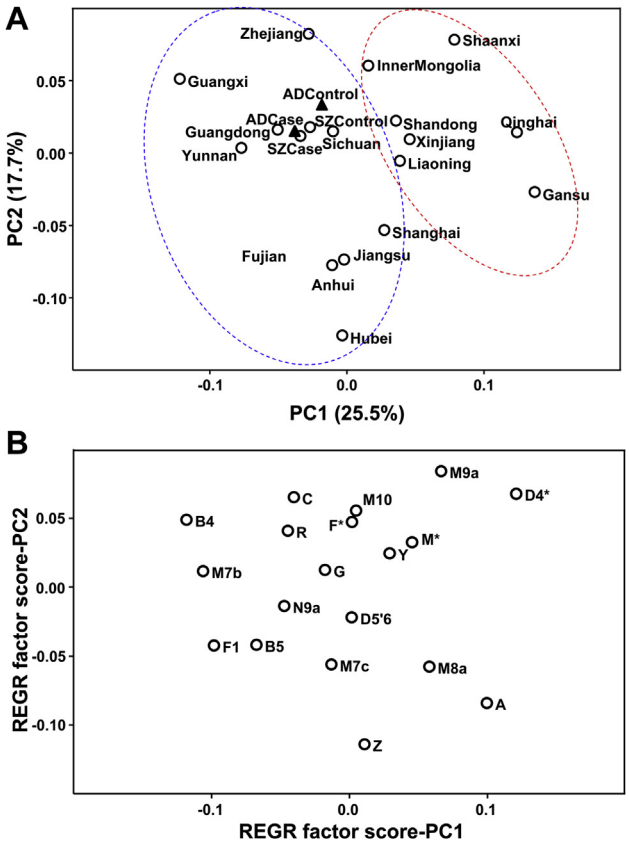

Fig. 1. Principal component analysis of AD patient and control samples from Southwest China and the previously reported Han Chinese populations across China. (A) PC map of Han regional populations based on mtDNA-haplogroup frequencies. Case and control populations were marked by solid triangles. The reported Han Chinese populations (Zhang et al., 2011 and references therein and those populations with a sample size less than 25 were discarded for avoiding potential bias) were marked by open circles. The schizophrenia (SZ) cases and control subjects from Hunan Province that were reported in our recent study (Zhang et al., 2014) were also included in this analysis. Han Chinese populations from northern and northwestern provinces were clustered in red ellipse and those from southern and eastern provinces were clustered together in blue ellipse. (B) Plot of mtDNA haplogroup contribution to the first and second PCs. We arbitrarily grouped mtDNAs belonging to R9b, R11, and R9* as R*; F, F2, F3, and F4 as F*; D* and D4 as D4*; M11, M12, M13, M20, M33, M71, M74, M75, and M76 as M*. Abbreviations: AD, Alzheimer's disease; mtDNA, mitochondrial DNA; PC, principal component.

Table 1
Haplogroup frequencies in 341 AD patients and 435 control subjects from Southwest China

Haplogroup	Number of case (N = 341)	Number of control (N = 435)	*p*-value[a]	OR (95% CI)	*PP*-I[b]
B	71	73	0.16	1.30 (0.91–1.87)	0.09
B4	46	55	0.75	1.08 (0.71–1.64)	0.41
B5	25	16	0.03	2.07 (1.09–3.95)	0.02
B5a	19	12	0.06	2.08 (1.00–4.35)	0.04
R9	71	97	0.66	0.92 (0.65–1.29)	0.34
F	58	87	0.31	0.82 (0.57–1.18)	0.17
F1	38	50	0.91	0.97 (0.62–1.51)	0.49
A	13	21	0.60	0.78 (0.39–1.58)	0.30
N9a	12	12	0.54	1.29 (0.57–2.90)	0.34
D	65	94	0.42	0.85 (0.60–1.22)	0.22
G	20	15	0.12	1.75 (0.88–3.46)	0.08
M7	31	39	1.00	1.02 (0.62–1.67)	0.53
M7b	21	29	0.88	0.92 (0.51–1.64)	0.45
M8	28	46	0.32	0.76 (0.46–1.24)	0.20
Others	30	38	—	—	—

Key: AD, Alzheimer's disease; CI, confidence interval; OR, odds ratio.
[a] Two tailed *p*-value of the Fisher exact test.
[b] Posterior probability of permutation test-I (Zhang et al., 2014), with smaller value meaning more robust test.

Data were presented as mean values with standard errors of multiple independent tests. Statistical analysis was performed with GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA) with Student *t* test.

3. Results

3.1. Association of mtDNA haplogroup B5 with AD

Based on the mtDNA sequence variations of the stage-I sample (sequences are available in GenBank under accession numbers KF306416-307191), all 776 Han Chinese with or without AD could be classified into respective haplogroups (Supplementary Table 4). The AD case-control samples from Southwest China showed a close affinity in the principal component map constructed on the basis of mtDNA haplogroup distribution frequency (Fig. 1), which indicated that there was no apparent potential population stratification between the case and control samples. Consistent with the clustering pattern described in our previous study (Yao et al., 2002), Han

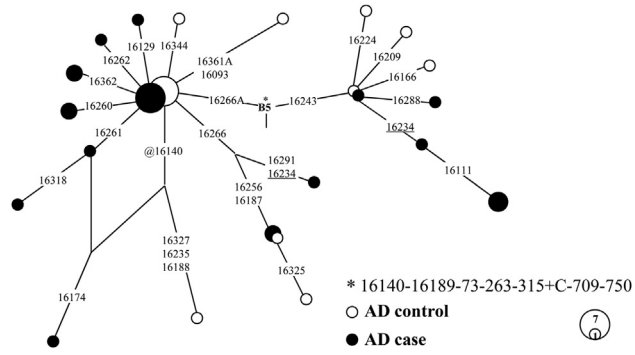

Fig. 2. Network of haplogroups B5 in Han Chinese with or without AD. The order of variants in region 16,090–16,365 of the mtDNA control region is arbitrary on the branch. Each circle represents an mtDNA haplotype, with the area of the circle being proportional to frequency of the haplotype. Length mutation of C-tract in region 16,184–16,193 was not considered except for the haplogroup-defining variants (e.g., 16,189). "@" means back mutation and recurrent variants were underlined in the network. The asterisks denote ancestral nodes of haplogroup B5. Abbreviations: AD, Alzheimer's disease; mtDNA, mitochondrial DNA.

GMS10050). 10 μL of cell lysate was used to measure cellular ATP level according to the manufacture's manual for ATP Determination Kit (Invitrogen) on GloMax 96 Luminometer (Promega). Final ATP value was normalized by protein concentration of each sample. For assessment of cellular oxygen consumption rate, cells were cultured in 6-well plate for 48 hours. Oxygen consumption rate was assessed on a Clark-type oxygen sensor (Hansatech instruments, England) at 25 °C as previously described (Guo et al., 2012). The cellular respiration rate was determined by recording the respiration of cells in culture medium for 10 minutes. Subsequently, the ATP synthase-coupled respiration rate and basal mitochondrial respiration rate were determined by sequentially adding 1 μM oligomycin (Sigma-Aldrich, 75351) and 1 μM rotenone (Sigma-Aldrich, R8875), each for 10 minutes, respectively. The final respiration rate was normalized by the number of cells.

For measurement of cellular ROS level, cellular ATP level, mitochondrial mass level, and oxygen consumption rate, each assay was independently performed at least 3 times to validate the consistency of the result. Results were normalized to the control cells.

Table 2
Distribution of mtDNA haplogroup B5 in 2 independent AD case-control cohorts from Southwest China and East China

Population	B5/others		*p*-value[a]	OR (95% CI)	*PP*-I[b]
	Case	Control			
Southwest China	25/316	16/419	0.02	2.07 (1.09–3.95)	0.02
East China	19/352	17/453	0.31	1.44 (0.74–2.81)	0.17
Combined	44/668	33/872	0.02	1.74 (1.10–2.76)	0.009

Key: AD, Alzheimer's disease; CI, confidence interval; mtDNA, mitochondrial DNA; OR, odds ratio.
[a] Two-tailed *p*-value of the Fisher exact test.
[b] Posterior probability of permutation test-I (Zhang et al., 2014), with smaller value meaning more robust test.

Chinese populations from South and Southwest China showed a more diverse pattern than those from East and Northeast China.

Among these haplogroups (each was shared by at least 12 individuals in the case or control population) listed in Table 1, haplogroup B5 had a significantly higher frequency in AD patients than control subjects (7.33% vs. 3.68%; $p = 0.03$; odds ratio [OR] = 2.07; 95% confidence interval [CI] = 1.09–3.95), and this result was also supported by the permutation test (Table 1 and Supplementary Fig. 1). The network of haplogroup B5 constructed based on the hypervariable segment 1 of the mtDNA control region sequences revealed a distinct distribution pattern between AD cases and control subjects: only 3 of 21 (14.3%) mtDNA haplotypes belonging to B5 were shared between the 2 populations (Fig. 2).

To further validate the association of mtDNA haplogroup B5 with AD, we screened B5 defining variants m.709G>A and m.9950T>C in stage-II sample from East China. Samples with alleles 709A and 9950C belonged to B5. The frequency of haplogroup B5 in AD patients was higher than control subjects (5.12% vs. 3.62%; $p = 0.31$; OR = 1.44; 95% CI = 0.74–2.81), and the direction of effect was same as that of the stage-I sample (Table 2), although the difference did not reach a statistically significant level. When we pooled the 2-stage samples together, the association was strengthened in the combined sample set (6.18% vs. 3.65%; $p = 0.02$; OR = 1.74; 95% CI = 1.10–2.76) (Table 2).

Intriguingly, when we compared the prevalence of AD and the spatial frequency of haplogroup B5 across China, we observed a seemingly overlapping pattern between the prevalence of AD and regional distribution of haplogroup B5 (Fig. 3). However, the

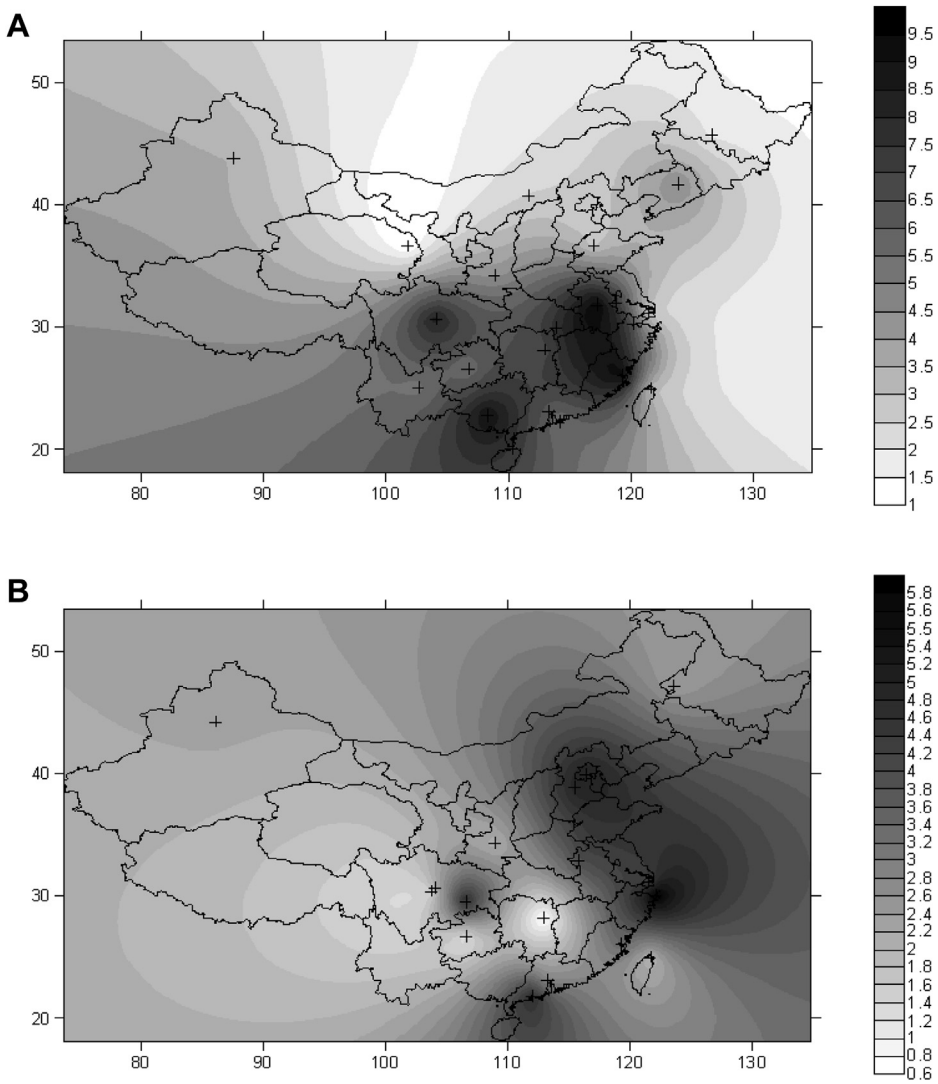

Fig. 3. Spatial frequency distribution pattern of haplogroup B5 (A) and the prevalence of Alzheimer's disease (B) across China. Detailed information regarding populations and data sources were listed in Supplementary Tables 2 and 3. The color scales in the right side are measured in percentage. Note that the pattern emerged from the contour map was influenced by the density of populations on the map. Regions with epidemiological data and/or mtDNA haplogroup data were marked by "+".

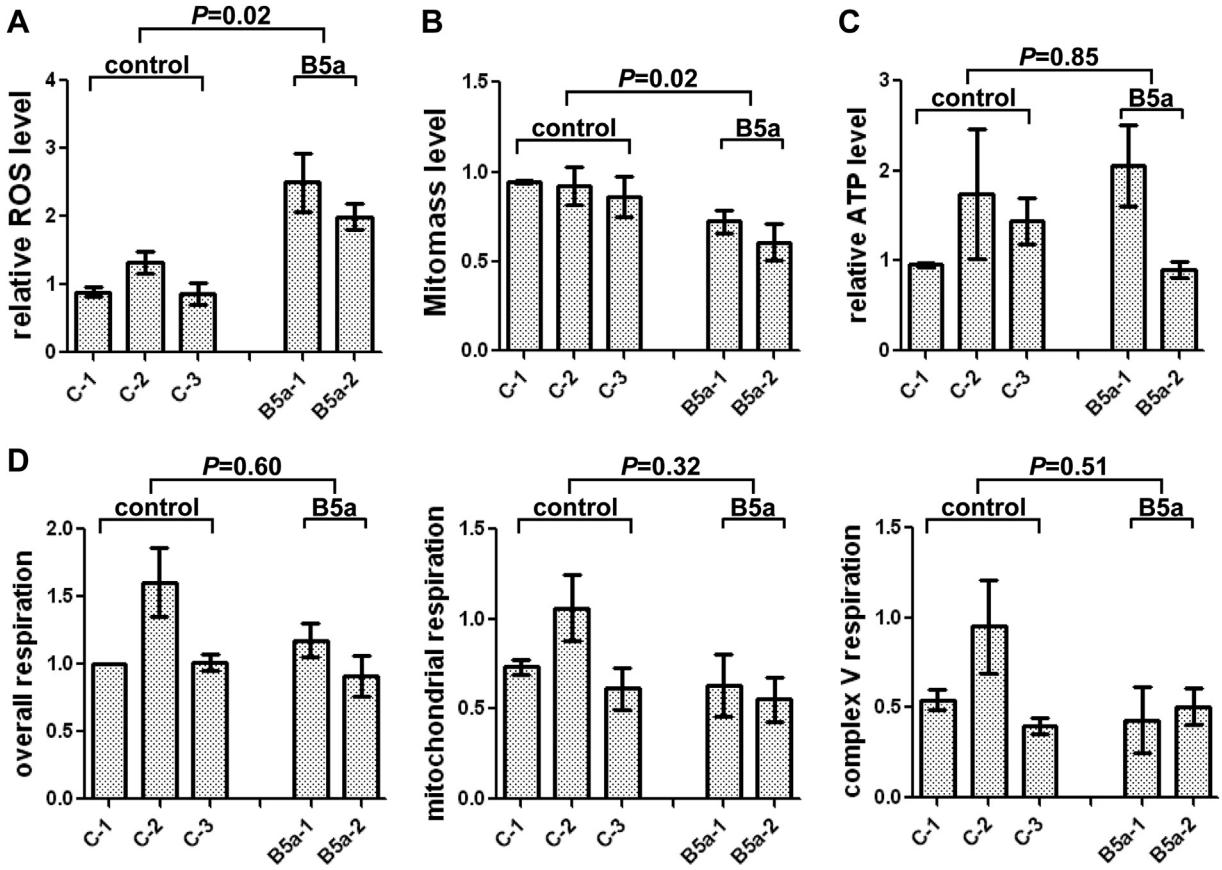

Fig. 4. Alteration of mitochondrial functions in lymphoblastoid cell lines belonging to haplogroup B5a (n = 2) and other haplogroups (n = 3). (A) Cellular ROS level in lymphoblastoid cell lines. Cells were incubated in phosphate buffer saline containing 0.5 μM DCFH-DA probe (Sigma-Aldrich) at 37 °C for 20 minutes, then were analyzed by flow cytometry at 535 nm. (B) Mitochondrial mass level in lymphoblastoid cell lines. Cells were incubated in 100 nM MitoTracker Red FM at 37 °C for 30 minutes, then were analyzed by using flow cytometry at 644 nm. (C) Cellular ATP level in lymphoblastoid cell lines. ATP level was assessed by ATP Determination Kit (Invitrogen); final ATP value was normalized by protein concentration of each cell strain. (D) Oxygen consumption rate in lymphoblastoid cell lines. About 3×10^6 cells were analyzed first in culture medium for 10 minutes for detection of the overall cellular respiration rate. Subsequently, the ATP synthase-coupled respiration rate and basal mitochondrial respiration rate were determined by sequentially adding 1 μM oligomycin and 1 μM rotenone, each for 10 minutes. The final oxygen consumption rate was normalized by the total number of cells. All results were normalized to C-1 cell line. Data was presented as mean ± standard errors of multiple independent tests. For each experiment, at least 3 independent tests were performed with consistent results. Abbreviation: ROS, reactive oxygen species.

correlation between the 2 frequency parameters did not reach a significant level ($r^2 = 0.044$, $p = 0.511$), most likely because of insufficient sampling locations with the availability of B5 frequency and the prevalence rate of AD.

3.2. Altered mitochondrial function in lymphoblastoid cell lines belonging to haplogroup B5a

To perform functional characterization of mtDNA haplogroup B5, 5 lymphoblastoid cell lines belonging to haplogroups B4 (C-1), A (C-2), D4 (C-3), and B5a (B5a-1, B5a-2) were established. As haplogroup B5a is a subset of B5 and shares the same ancient variants that define this background, cells of haplogroup B5a could represent the circumstance of haplogroup B5, whereas cells of other haplogroups could be considered as controls to investigate whether haplogroup B5 was associated with decreased mitochondrial function. We sequenced complete mtDNA genome of one of maternally related donors of the 2 B5a cell lines (B5a-2) to exclude the possibility of other potentially functional mtDNA variants in this lineage (sequence has been deposited in GenBank under accession number KM457636). None of the private variants in B5a-2 cell line seemed to be pathogenic according to an *in silico* prediction (Fan and Yao, 2013). All variants occurred in the general populations (Supplementary Fig. 2).

Mitochondrial functional parameters, including ROS level, mitochondrial mass level, ATP level, and oxygen consumption rate were determined in these lymphoblastoid cell lines. Compared with cells belonging to other haplogroups, cells with haplogroup B5a background showed a significantly increased cellular ROS level ($p = 0.02$) and significantly decreased mitochondrial mass level ($p = 0.02$) (Fig. 4A and B). There was no significant difference in mitochondrial mass level among the 3 control cell lines. Note that there were individual differences between cell lines, which might account for insignificant alteration in ATP and oxygen consumption rate levels between cells with and without B5 (Fig. 4C and D).

3.3. Alteration of mitochondrial function in cells overexpressing the ATP6 gene with ancient variant m.8584G>A

We speculated that haplogroup B5 defining variant m.8584G>A (p.A20T), which is a nonsynonymous variant in the *MT-ATP6* gene, would account for the association between haplogroup B5 and the altered mitochondrial function. To confirm this speculation, a previously reported efficient allotopic expression system of the nuclear-encoded *ATP6* gene ($SOD2^{MTS}ATP6\text{-}^{3'UTR}SV40$) (Kaltimbacher et al., 2006) was utilized

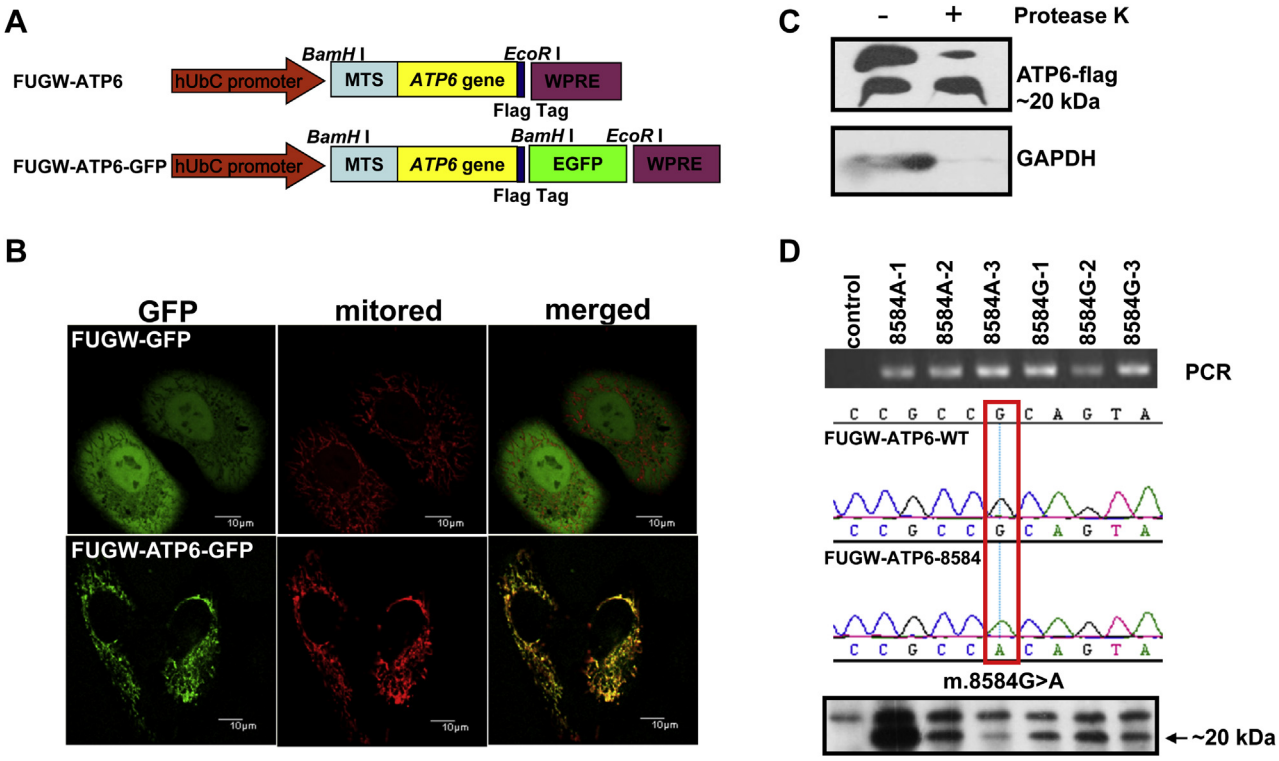

Fig. 5. Allotopic expression of the *ATP6* gene in Hela cells. (A) A schematic diagram of allotopic expression constructs for the *ATP6* gene. The nuclear-encoded *ATP6* gene was constructed into FUGW vector with or without GFP. (B) Fluorescence microscopy assay of Hela cells expressing GFP or ATP6-GFP. Hela cells were cultured to 50% confluence on glass slides in a 12-well plate and were cotransfected with 0.1 μg of pDsRed-mito vector and 0.9 μg of FUGW-ATP6-GFP construct or FUGW-GFP vector for 48 hours. Living cells were imaged using the Olympus FluoView 1000 confocal microscope at 488 nm and 563 nm, respectively. (C) Western blot analysis to validate the successfully expression and translocation of ATP6 protein to mitochondria. Hela cells were transfected with FUGW-ATP6 construct. Crude mitochondria were isolated 48 hours after transfection, and 20 μg of crude mitochondria fraction were treated with 75 μg/mL proteinase K for 30 minutes on ice, subsequently 1 mM phenylmethylsulfonyl fluoride were added to stop proteinase K reaction. A total of 20 μg protein was separated in 12% SDS-PAGE. Antibody against flag tag was used to detect the allotopically expressed ATP6 protein. (D) PCR, sequencing, and Western blot analysis to validate cell strains with stable expression of the *ATP6* gene with m.8584G (vector FUGW-ATP6-WT) or m.8584A (vector FUGW-ATP6-8584). Antibody against flag tag was used to identify successfully stable expression of ATP6 protein. Abbreviations: GFP, green fluorescent protein gene; PAGE, polyacrylamide gel electrophoresis; PCR, Polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

to discern potential function of variant m.8584G>A (Fig. 5A). The allotopic expression of ATP6 protein was successful in our system based on 2 lines of evidence, namely: (1) the ATP6-GFP product was colocalized with mitochondrial red fluorescent protein in Hela cells cotransfected with FUGW-ATP6-GFP construct and pDsRed2-mito vector (Fig. 5B); and (2) in the protease protection assay, crude mitochondria fraction was treated with proteinase K. The allotopically expressed ATP6 protein could be protected by the mitochondrial membrane from proteinase K digestion, indicating that it had been transported into mitochondria. The GAPDH protein, which is mainly located in the cytoplasm, was almost digested by proteinase K (Fig. 5C). For the purpose of achieving stable and efficient expression of the ATP6 protein, we constructed Hela cells with stable expression of the *ATP6* gene with alleles m.8584G and m.8584A and picked up 3 strains for each allele (Fig. 5D). The incorporation of exogenous *ATP6* gene into the genome of the cell strains could be confirmed by the amplification and sequencing using the FUGW vector specific primers. Furthermore, Western blot analysis with flag antibody exhibited that the ATP6 protein was expressed in all strains, albeit at different levels (Fig. 5D).

Compared with cell strains stably expressing the *ATP6* gene with m.8584G allele, cell strains with m.8584A allele presented a significantly increased cellular ROS level ($p = 0.01$) and decreased levels of mitochondrial mass ($p = 0.03$), cellular ATP ($p = 0.05$), and mitochondrial ATP synthase-coupled oxygen consumption rate ($p = 0.03$) (Fig. 6). These alterations of mitochondrial function in cell strains with m.8584A allele of the *ATP6* gene implied a potentially deleterious effect of this ancient variant on mitochondrial function.

4. Discussion

According to the ancestral-susceptibility model of modern human disease, those variants which reflect the ancient adaptation to environment and lifestyle of ancient human populations, may confer disease susceptibility because of the change of environment and lifestyle in modern time (Di Rienzo and Hudson, 2005). One typical example was the well known AD risk allele APOE ε4, which is the ancestral allele of the *APOE* gene and was proved to be associated with higher cholesterol level (Corbo and Scacchi, 1999). Carriers of the APOE ε4 allele would survive well in the ancient environment with limited food supply because of its active role in lipid transport and absorption. However, these carriers would have increased metabolic disease risk in modern environment with redundant food intakes (Corbo and Scacchi, 1999). Similarly, mtDNA haplogroups were formed during the evolution and migration of modern human and presented continental specific distributions (Mishmar et al., 2003; Ruiz-Pesini et al., 2004; Yao et al., 2002). In line with the ancestral-susceptibility model (Di Rienzo and Hudson, 2005), mtDNA haplogroup, as a product of ancient adaptation to environment, can confer susceptibility to disease in modern populations because of the change of environment. Indeed, accumulating evidence showed that mtDNA haplogroups were associated with a variety of diseases (Ghezzi et al., 2005; Ji et al., 2008;

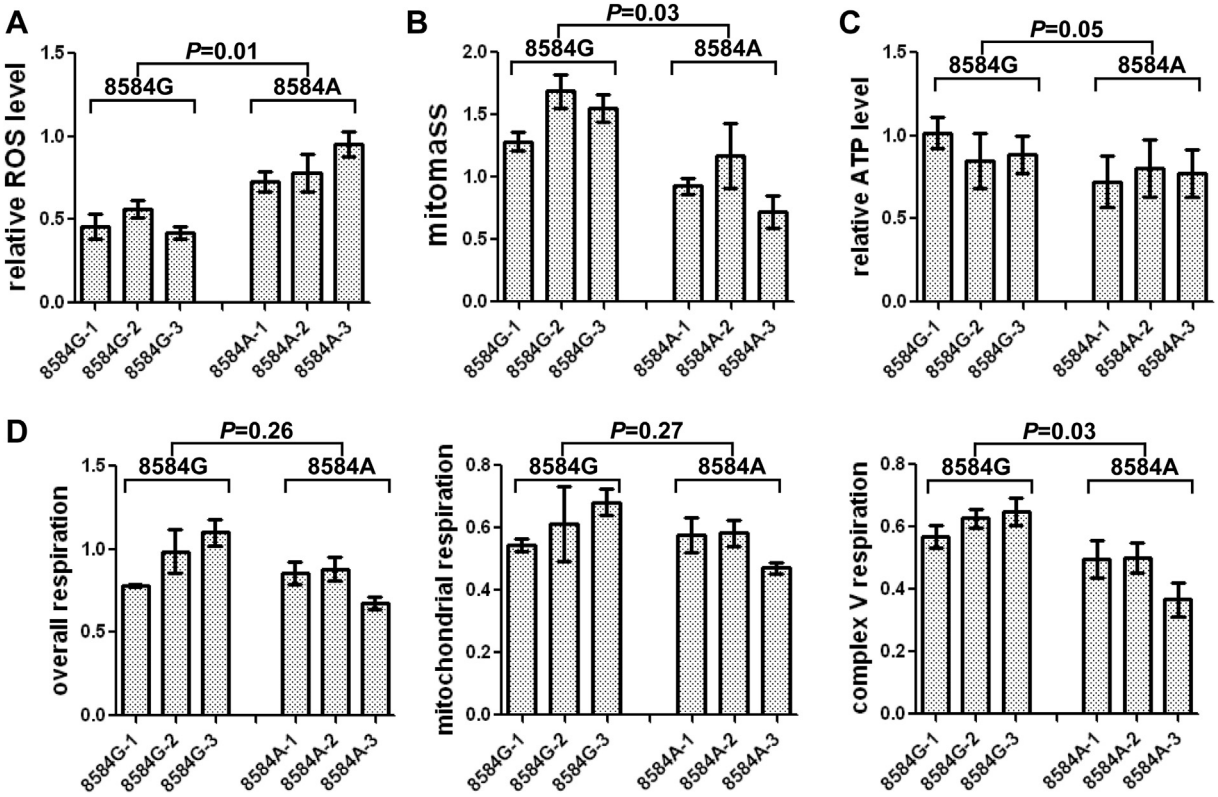

Fig. 6. Alteration of mitochondrial functions in Hela cells with stable expression of the *ATP6* gene with allele m.8584A or m.8584G. Three strains of Hela cells were picked for each allele. There is a significant increase of cellular ROS (A), decrease of mitochondrial mass (B), and cellular ATP (C) in Hela cells with stable expression of the *ATP6* gene with allele m.8584A compared with cells with allele m.8584G. In Hela cells with stable expression of the *ATP6* gene with allele m.8584A, a mild reduction of oxygen consumption rate was observed compared with cells overexpressing m.8584G. We followed the same condition for measurement as described in the legend of Fig. 4. Results were normalized to control Hela cells. Data was presented as mean ± standard errors of at least 3 independent tests. Abbreviation: ROS, reactive oxygen species.

Mancuso et al., 2009). In this study, we aimed to investigate whether mtDNA haplogroups and/or ancient variants confer susceptibility to AD in Han Chinese.

Our 2-stage genetic association analysis revealed that mtDNA haplogroup B5 conferred a susceptibility to AD (Tables 1 and 2). Moreover, a seemingly overlapping pattern between the prevalence of AD and regional distribution of haplogroup B5 was observed (Fig. 3), which supported the association of haplogroup B5 with AD. However, such an observation should be received with caution, as the epidemiological data of AD were incomplete for these regions. Interestingly, our recent study on schizophrenia identified haplogroup B5a, a subhaplogroup of B5, as the risk factor for schizophrenia in Han Chinese (Zhang et al., 2014). How did mtDNA haplogroups B5 and B5a enact their influence on disease? Are there any functional fundamentals for this "double-disease hit" association? These are very interesting yet important questions.

To discern the potential effect of haplogroup B5, we performed functional characterization of lymphoblastoid cells with and without B5 status and Hela cells overexpressing B5-defining variant m.8584G>A. The increased ROS level and decreased mitochondrial mass level in lymphoblastoid cell lines of haplogroup B5a (Fig. 4) suggested that haplogroup B5 (including its subhaplogroup B5a) indeed affected mitochondrial function. We then focused on the ancestral variant(s) which could lead to amino acid change in the root of the phylogenetic tree of haplogroup B5 (van Oven and Kayser, 2009). There are 2 nonsynonymous haplogroup-defining variants for B5 and its subhaplogroups, m.10398A>G and m.8584G>A. The 10398G allele was an ancient allele according to human mtDNA phylogenetic tree (PhyloTree), and m.10398A>G variant had arisen multiple times in macro-haplogroup N lineages (Supplementary Fig. 3A). Variant m.8584G>A, which leads to a change of alanine to threonine in the 20th amino acid site of the MT-ATP6 protein, is a nonsynonymous haplogroup-defining variant for several haplogroups including B5, M8, R30, R6a1, and V20 (Supplementary Fig. 3B). Considering a fact that the *MT-ATP6* gene played a very important role in ATP production and was previously reported to be evolutionarily sensitive to climate difference (Mishmar et al., 2003), we hypothesized that m.8584G>A might account for the association between AD risk and haplogroup B5. However, functional characterization of mtDNA variants was hardly performed, simply because the genetic code of mtDNA is different from the universal code, and the lack of robust system to fulfill the aim. One feasible way is to transform the mtDNA gene to nuclear-encoded version, express the protein in cytosol and then imported into mitochondria. This method was known as allotopic expression and has been successfully used to investigate the contribution of mtDNA mutation to mitochondrial dysfunction (Bonnet et al., 2008; Ellouze et al., 2008; Kaltimbacher et al., 2006; Manfredi et al., 2002). Allotopic expression assay of the *ATP6* gene with m.8584G>A revealed that this variant led to a series of decreased mitochondrial function including upregulation of ROS level, decrease of ATP productivity, loss of mitochondrial mass, and reduced oxygen consumption rate (Fig. 6). These functional changes caused by m.8584G>A alone might not be sufficient to account for disease onset but would be one of the susceptible factors to AD. Taken together, it is evident that ancestral m.8584A allele is functional, with a dependence on its haplogroup and environmental context, similar to m.3394T>C in LHON and hypoxia adaptation

(Ji et al., 2012). The APP/Aβ could interact with the α-subunit of ATP synthase and decrease its activity (Schmidt et al., 2008); further studies are necessary to investigate whether the ancient variant m.8584G>A (p.A20T) in the MT-ATP6 subunit of ATP synthase could influence the interaction between APP/Aβ and ATP synthase.

There are 2 limitations in this study. First, frequency difference of B5 between the case and control groups was not as significant as one would expect for a strong association. As sporadic AD is a very complex disease, it is understandable that haplogroup B5 may not account for the entire risk. This is the probable reason for a seemingly marginal association of B5 with AD as revealed by the *p*-values. Nonetheless, we remedied this limitation by permutation tests and validated the trend in an independent cohort; both analyses confirmed the association of haplogroup B5 with AD in Han Chinese (Table 1, Table 2, and Supplementary Fig. 1). Second, individual differences between lymphoblastoid cell lines were observed in our study, which might be attributed to different sex, age, and mtDNA genetic background of different cell donors. Furthermore, we failed to collect more lymphoblastoid cell lines of haplogroup B5a. However, we utilized an optimized allotopic expression assay to further discern the effect of ancestral B5 alleles, which could effectively avoid individual background effect among lymphoblastoid cell lines.

5. Conclusion

In summary, mtDNA haplogroup B5 was identified to be associated with AD risk in 2 independent AD case-control cohorts and the combined Han Chinese sample. Ancient variant m.8584G>A might account for the association between haplogroup B5 and AD in Han Chinese, which could serve as an example of the ancestral-susceptibility model. Future studies are necessary to further explore the pathway by which mtDNA haplogroup B5 affects the risk of AD.

Disclosure statement

There are no actual or potential conflicts of interest.

Acknowledgements

The authors thank the participants in this study. They are grateful to Dr Corral-Debrinski for sharing plasmid pCMV-Tag 4A with the *MT-ATP6* gene, and Dr Min-Sheng Peng, Dr A-Mei Zhang, Miss Ling Xu, Miss Yu Fan, and Mr. Jia-Qi Feng for technical assistance. This study was supported by the Strategic Priority Research Program of Chinese Academy of Sciences (XDB02020000), the MOST of China (2011CB910902), and the National Natural Science Foundation of China (31171225 and 30925021).

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.2014.10.009.

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Supplementary Data

Table S1. Primers for genotyping variants m.709G>A and m.9950T>C by using SNaPshot assay.

variant	Primer (5'-3') [a]
m.709G>A	F: AAAATGTTTAGACGGGCTCAC
	R: TTGTTCCTTTTGATCGTGGT
	E: GACTTCTTAGTAAGATTACACATGCAAGCATCCCC
m.9950T>C	F: AAACATCACTTTGGCTTCGA
	R: ATACTAAAAGAGTAAGACCCTCATCAATA
	E: CTGACTGACTCCGCCTGATACTGGCATTTTGTAGATGTGGT

[a] F: forward primer; R: reverse primer; E: extension primer.

Table S2. Published mtDNA data sets reanalyzed in this study.

Location [a]	Population	Sample size	No. of B5 (%) [b]	References
Hefei, Anhui	Han Chinese	42	4 (9.52)	(Wen et al., 2004a)
Changting and Longyan, Fujian	Han Chinese	54	5 (9.26)	(Wen et al., 2004a)
Gansu	Han Chinese	45	0 (0.00)	(Wen et al., 2004a)
Guangdong	Han Chinese, Teochew, Pou, Mien, Hakka	545	34 (6.24)	(Chen et al., 2008b; Kivisild et al., 2002; Li et al., 2007b; Wang et al., 2010; Wen et al., 2005; Yao et al., 2002)
Guangxi	Han Chinese, Zhuang, Hmong, Mien, Caolan, Maonan, Mulam, Palyu, Blue-Gelao, Yerong, Sui, E	561	51 (9.09)	(Li et al., 2007b; Wen et al., 2005; Wen et al., 2004a)
Guizhou	Tujia, Dong, Yi, Gelao, Qau, Ai-Cham, Mak, Mollao, Then	253	14 (5.53)	(Li et al., 2007a; Li et al., 2007b)
Hainan	Jiamao, Cun, Hlai-Qi, Lingao, Danga	162	12 (7.41)	(Li et al., 2007b)
Heilongjiang	Han Chinese, Daur, Hezhen	107	2 (1.87)	(Powell et al., 2007)
Hubei	Han Chinese, Dong	52	4 (7.69)	(Li et al., 2007b; Yao et al., 2002)
Hunan	Han Chinese, Hmong, Mien, Tujia	322	22 (6.83)	(Oota et al., 2002; Wen et al., 2005; Wen et al., 2004a; Wen et al., 2004b)
Nanjing, Jiangsu	Han Chinese	67	5 (7.46)	(Wen et al., 2004a)
Liaoning	Han Chinese, Manchu	133	6 (4.51)	(Powell et al., 2007; Wen et al., 2004a; Yao et al., 2002)
Inner Mongolia	Han Chinese, Korean, Daur, Mongolia, Ewenki	356	9 (2.53)	(Kong et al., 2003; Powell et al., 2007; Wen et al., 2004a)
Qinghai	Han Chinese, Tu, Tibetan	208	2 (0.96)	(Chen et al., 2008a; Wen et al., 2004a)
Shandong	Han Chinese	174	5 (2.87)	(Wang et al., 2000; Yao et al., 2002; Yao et al., 2003)
Shaanxi	Han Chinese	137	6 (4.38)	(Oota et al., 2002; Wen et al., 2004a)
Shanghai	Han Chinese, DornQdayc	73	2 (2.74)	(Li et al., 2007b; Wen et al., 2004a)
Sichuang	Han Chinese, Tibetan	132	11 (8.33)	(Wen et al., 2004a; Zhao et al., 2009)
Taiwan	Han Chinese	155	5 (3.23)	(Tsai et al., 2001)
Xinjiang	Han Chinese, Xibe, Uygur	173	6 (3.47)	(Powell et al., 2007; Yao et al., 2002)
Yunnan	Han Chinese, Bai, Naxi, Yi, Aini, Hani, Jino, Lahu, Buyang, Dai-lu, Lachi, Mien, Gelao, Pubiao, Hmong, Bugan, Va, Tibetan	844	45 (5.33)	(Li et al., 2007b; Qian et al., 2001; Wen et al., 2005; Wen et al., 2004a; Yao et al., 2002; Yao and Zhang, 2002)
Hangzhou, Zhejiang	Han Chinese,	61	4 (6.56)	(Wen et al., 2004a)
Hong Kong	Residents of urban Hong Kong with diverse Chinese ancestry	377	21 (5.57)	(Irwin et al., 2009)

[a] We used the latitude and longitude of the capital of each province in contour map construction.

[b] Haplogroup B5 was defined according to the presence of haplogroup motif “16140-16189”.

Table S3. Prevalence of Alzheimer's disease across China.

Location [a]	Population	Sample size	Prevalence (‰)	References
Kaokaoping, Taiwan	General Chinese	2915	1.99	(Lin et al., 1998)
Yingzhou, Anhui	General Chinese	2749	3.96	(Wang et al., 1999)
Baoding, Heibei	General Chinese	2308	4.90	(Wei et al., 2008)
Beijing	General Chinese	2788	5.02	(Tang et al., 2002)
Chengdu, Sichuan	General Chinese	5353	2.05	(Tang et al., 2001)
Xinjin, Sichuan	General Chinese	5987	1.44	(Tang et al., 1999)
Fuzhou, Fujian	General Chinese	2373	3.41	(Chen et al., 2009)
Guangdong [b]	General Chinese	1018	4.72	(Yu et al., 1998)
Guangzhou, Guangdong	General Chinese	9056	2.83	(Ma et al., 2005; Tang et al., 2007)
Guiyang, Guizhou	General Chinese	3229	1.27	(Huang et al., 2007)
Changsha, Hunan	General Chinese	5125	0.64	(Gao et al., 1994)
Shanghai	General Chinese	15910	4.32	(Zhou et al., 2001)
Xian, Shaanxi	General Chinese	4807	2.08	(Qu et al., 2001)
Yuzhong, Chongqing	General Chinese	1519	4.80	(Zou et al., 2002)
Dinghai, Zhejiang	General Chinese	1689	5.98	(Lv et al., 1998)
Qiqihaer, Heilongjiang	General Chinese	3698	2.4	(Sun et al., 2011)
Shihezi, Xinjiang	General Chinese	1230	2.3	(Yang et al., 2003)

[a] We used the latitude and longitude of the capital of each province in contour map construction unless the location was well defined to city or county level in the original reference.

[b] Data from three counties (Enping, Yangdong and Yangxi) of Guangdong Province.

Table S4. mtDNA sequence variations of 776 Han Chinese analyzed in this study

Sample	Haplogroup	Region 1 (16000+) [a]	Region 2 [a]	5176*AluI* [b]	5108 8718 8281-8289d [b]	Additional polymorphisms [c]
control samples						
Z019	A	223 290 319 362	073 146 152 235 263 315+C 523-524d 654 663 750			
Z064	A	192 223 274 290 319 362 519 527	073 152 235 263 315+C 523-524d 663 750			
Z066	A	223 256 290 319 362 381	073 093 151 152 235 263 309+C 315+C 523-524d 663			
Z076	A	223 260 290 319	064 073 146 195 235 263 309+C 315+C 523-524d 663 750			
Z082	A	223 290 319 362	073 152 235 263 309+C 315+C 523-524d 663			
Z095	A	223 290 319 362	073 152 263 309+C 315+C 523-524d 663			
Z101	A	179 223 290 311 319 362	073 152 235 263 309+C 315+C 522 523-524d 663 750			
Z151	A	223 290 319 362 519	073 152 235 263 309+C 315+C 523-524d 663 750			
Z211	A	223 267 290 304 319 362	073 152 235 315+C 316 513 523-524d 663			
Z247	A	223 290 304 311 319 362	073 152 235 263 315+C 523-524d 663 750			
Z250	A	126 223 235 290 319 362 519	073 235 263 309+C 315+C 523-524d 663 750			
Z277	A	179 192 223 290 319 362 519	073 146 152 207 235 309+2C 315+C 523-524d			
Z291	A	209 223 234 290 293C 319 519 527	073 152 189 235 263 309+C 315+C 523-524d 663			
Z293	A	223 290 319 362	073 152 207 235 309+C 315+C 523-524d 663			
Z306	A	223 260 290 319	073 146 152 195 235 263 315+C 523-524d 663 750			
Z347	A	223 290 311 319 362 365	073 152 234 235 263 309+C 315+C 523-524d 663 750			
Z350	A	223 274 290 319 362 519 527	073 152 235 263 315+C 523-524d 663 750			
Z355	A	223 290 319 362 519	073 151 152 200 235 263 315+C 523-524d 663 735 750			
Z411	A	126 223 235 290 319 519	073 235 263 309+C 315+C 523-524d 663			
Z416	A	093 179A 223 290 319	073 152 235 263 309+C 315+C 523-524d 663			
Z420	A	223 290 293C 304 319 519	073 152 235 263 309+2C 315+C 523-524d 663 750			
Z406	B	051 182C 183C 189 519	073 152 214 263 315+C 356+C 750		+	
Z038	B4	183C 189 217 519	073 263 309+C 315+C 316			
Z062	B4	093 178 182C 183C 189 217	073 214 263 309+2C 315+C 368 709 750			
Z072	B4	182C 183C 189 217 304	073 150 195 263 279 309d 315+C 750			
Z346	B4	183C 189 217 519	073 263 309+C 315+C 316			
Z408	B4	182C 183C 189 217 519	073 200 263 315+C 316			
Z056	B4a	182C 183C 189 217 261 299 519	073 152 193 263 309+2C 315+C 523-524d 709 750			
Z073	B4a	182C 183C 189 217 261 327 519	073 146 263 309+2C 315+C 523-524d 709 750			
Z083	B4a	182C 183C 189 217 261 299 519	073 152 193 263 309+2C 315+C 523-524d 709 750			
Z088	B4a	182C 183C 189 217 261 299 519	073 152 193 263 309+2C 315+C 523-524d 709 750			
Z103	B4a	092 182C 183C 189 217 261 399 519	073 263 309+C 315+C 523-524d 750			
Z148	B4a	182C 183C 189 217 261 299 311 519	073 189 193 195 263 309+C 315+C 523-524d			
Z167	B4a	182C 183C 188 189 214 217 243 261 519	073 146 263 309+C 315+C 523-524d 709 750			
Z207	B4a	182C 183C 189 217 261 299 355 390 519	073 263 309+2C 315+C 750			
Z290	B4a	182C 183C 189 217 261 519	073 146 263 309+2C 315+C 523-524d 750			
Z331	B4a	093 182C 183C 188 189 214 217 261 519	073 146 263 309+C 315+C			
Z353	B4a	129 182C 183C 189 217 261	073 146 263 315+C 523-524d 750			
Z386	B4a	167 182C 183C 189 217 261 519	073 146 152 263 308-309d 315+C 350C 523-524d 709 750			
Z430	B4a	182C 183C 189 194C 195 217 261 519	073 146 150 152 263 309+2C 315+C 523-524d 709 750			
Z457	B4a	182C 183C 189 217 261 519	073 146 263 307-310d 315+C 523-524d 573+3C			
Z157	B4a'g'h'i	093 179 182C 183C 189 217 261 303 519	073 263 315+C 499 523-524d 750			
Z253	B4a'g'h'i	182C 183C 189 217 219 261 286 519	073 263 309+2C 315+C 523-524d 750			
Z278	B4a'g'h'i	182C 183C 189 217 261 360 519	073 263 309+2C 315+C 523-524d 750			
Z295	B4a'g'h'i	093 182C 183C 189 217 261	073 263 315+C 750			
Z336	B4a'g'h'i	093 182C 183C 189 217 261	073 263 315+C 750			
Z403	B4a'g'h'i	182C 183C 189 217 261 519	073 263 307-310d 315+C 523-524d 709 750			
Z425	B4a'g'h'i	093 182C 183C 189 217 261	073 263 315+C 750			
Z449	B4a'g'h'i	182C 183C 189 217 240 261	073 263 309+2C 315+C 523-524d 750			
Z012	B4b	136 183C 189 217 218 355 519	073 263 309+2C 315+C 499 750 827			
Z055	B4b	136 182C 183C 189 217 519	073 207 263 309+2C 315+C 499 750 827			
Z080	B4b	136 182C 183C 189 217 270 298 519	073 150 152 263 308-310d 315+C 499 750 827			
Z106	B4b	136 183C 189 217 218 519	073 263 309+2C 315+C 456 499 750 827			
Z114	B4b	136 183C 189 217 298 519	073 152 200 263 315+C 499 750			
Z118	B4b	136 183C 189 217 298 519	073 152 200 263 315+C 499			
Z119	B4b	136 183C 189 217 298 519	073 152 200 263 315+C 499			

Z280	B4b	136 183C 189 217 519	073 152 207 263 309+2C 315+C 499 750 827	
Z303	B4b	136 183C 189 217 218 292 519	073 263 309+2C 315+C 499 636	
Z311	B4b	136 183C 189 217 218 292 519	073 263 309+2C 315+C 499 636	
Z312	B4b	136 183C 189 217 218 292 519	073 263 309+2C 315+C 499 636 750 827	
Z318	B4b	136 183C 189 217 309 354 519	073 207 263 309+C 315+C 499	
Z319	B4b	136 183C 189 217 257 519	073 207 263 315+C 499 750 827	
Z324	B4b	136 183C 189 217 519	073 199 263 315+C 499 750 827	
Z009	B4c	051 140 182C 183C 189 217 274 335 519	073 146h 150 189 195 263 309+C 315+C 709 750	
Z316	B4c	129 140 166 183C 188+T 189 217 274	073 150 152 263 309+2C 315+C 709 750	
Z469	B4c	126 129 140 166 182C 183C 189 217 274 335 519	073 150 263 306-309d 315+C	
Z216	B4g	181C 182C 183C 189 213 217 261 292 519	073 152 263 315+C 523-524d 750	
Z333	B4g	181C 182C 183C 189 213 217 242 261 292 301 519	061A 062 073 183 263 309+2C 315+C 523-524d 750	
Z454	B4g	181C 182C 183C 189 213 217 261 292 519	073 263 309+2C 315+C 523-524d 750	
Z455	B4g	181C 182C 183C 189 213 217 261 292 519	073 263 309+2C 315+C 523-524d 750	
Z459	B4g	181C 182C 183C 189 213 217 261 292 519	073 263 309+2C 315+C 523-524d 750	
Z027	B4h	129 182C 183C 189 217 261 362 519	073 263 307-310d 315+C 458 477 523-524d 709 750	
Z150	B4h	129 182C 183C 189 217 261	073 093 263 315+C 523-524d	
Z172	B4h	093 129 182C 183C 189 217 261	073 093 263 315+C 469A 523-524d 750	
Z180	B4h	129 182C 183C 189 217 261 311	073 185 263 309+2C 315+C 459A 523-524d 750	
Z285	B4h	129 182C 183C 189 217 261	073 185 263 315+C 523-524d 750	
Z385	B4h	129 182C 183C 189 217 261 488	073 263 315+C 523-524d	
Z016	B5a	140 187 189 256 266G 519	073 093 210 263 315+C 523-524d 709	
Z026	B5a	140 182C 183C 189 266A 344 519	073 210 263 315+C 523-524d 709 750	
Z070	B5a	140 187 189 256 266G 325 519	073 093 210 263 315+C 523-524d 709 750	
Z112	B5a	140 182C 183C 189 266A 519	073 210 263 315+C 523-524d 593 709 750	
Z141	B5a	140 183C 189 266A 519	073 210 263 309+C 315+C 523-524d 709 750	
Z158	B5a	140 183C 189 266A 519	073 210 263 309+C 315+C 523-524d 709 750	
Z226	B5a	140 183C 189 266A 482 519	073 210 263 309+2C 315+C 523-524d 709 750	
Z229	B5a	093 140 183C 189 266A 361 399 519	073 152 153 204 210 263 309+C 315+C 523-524d 709 750	
Z288	B5a	188 189 235 266A 327 519	073 210 263 309+C 315+C 523-524d 709 750	
Z332	B5a	140 183C 189 266A 519	073 210 263 309+C 315+C 523-524d 709 750	
Z464	B5a	140 183C 189 266A 519	073 210 263 309d 315+C 523-524d 709 750	
Z465	B5a	140 183C 189 266A 519	073 210 263 315+C 523-524d 709 750	
Z206	B5b	140 182C 183C 189 243 519	073 103 195 263 309+2C 315+C 523-524d 709 750	
Z256	B5b	140 166 183C 189 243 519	073 103 152 195 204 263 309+C 315+C 523-524d 573+3C	
Z377	B5b	140 182C 183C 189 224 243 519	073 103 195 204 263 309+C 315+C 523-524d 573+2C 709 750	
Z471	B5b	140 183C 189 209 243	073 103 204 263 309+2C 315+C 523-524d 709 750	
Z400	B6	093 179 182C 183C 189	073 150 263 309+2C 315+C 750	
Z030	C	223 298 327 399 519	073 249d 263 309+C 315+C 489	
Z044	C	223 266 298 327 519	073 249d 263 309+C 315+C 489 750	
Z081	C	189 223 298 327 357 519	073 249d 263 309+2C 315+C 489	
Z086	C	223 298 327 390 519	073 249d 263 309+2C 315+C 489 524+AC 750	
Z104	C	223 288 298 327 390 519	073 249d 263 309+C 315+C 489 595+C 750	
Z105	C	223 298 327 390 519	073 249d 263 309+2C 315+C 489 524+AC 750	
Z108	C	175 223 298 327 519	073 146 249d 263 309+C 315+C 489 750	
Z135	C	093 129 223 298 327 519	073 194 249d 263 315+C 489	
Z214	C	051 144A 189 223 298 311 327 519	073 195 200 249d 263 309+C 315+C 489 750	
Z237	C	189 223 298 327 519	073 207 249d 263 309+2C 315+C 489 750	
Z268	C	037 189 223 298 327 519	073 249d 263 309+C 315+C 489	
Z289	C	129 223 298 327 519	073 151 195 249d 263 309+C 315+C 489 750	
Z357	C	145 183C 186 189 223 298 311 327 519	073 249d 263 309+C 315+C 489	
Z462	C	129 150 223 298 327 519	073 195 249d 263 315+C 489 549 750	
Z463	C	223 298 327 519	044+C 073 249d 263 315+C 489 750	
Z466	C	129 223 298 327	073 249d 263 315+C 489 523-524d 750	
Z139	CZ	189 223 298 357 519	073 200 249d 263 315+C 489 750	
Z008	D4	174 192 223 362	073 152 195 263 309+C 315+C 489 523-524d 750	-
Z011	D4	223 362	073 146 263 309+C 315+C 485 489	-
Z034	D4	185 189 223 232A 319 362	073 263 315+C 489 523-524d 750	-
Z053	D4	174 223 311 362	073 152 263 309+C 315+C 489 750	
Z068	D4	092 172 223 362	073 094 146 263 309+C 315+C 489	
Z069	D4	185 223 311 362 365 526	073 263 298 315+C 489 523-524d	-
Z078	D4	092 223 362	073 094 152 263 309+C 315+C 489 750	
Z085	D4	172 223 362	073 263 309+C 315+C 489 629 750	-
Z090	D4	189 223 362	073 263 309+C 315+C 488 489 750	-
Z115	D4	223 362 519	073 204 263 315+C 489 750	-

Z130	D4	223 362 526	073 146 263 309+C 315+C 489 750	-	
Z134	D4	092 223 362	073 094 152 263 309+C 315+C 489 750		
Z160	D4	172 223 362	073 146 263 309+C 315+C 489 709		4529-5098=4595 4769 4883
Z169	D4	168 184 223 311 362	073 263 309+C 315+C 489	-	
Z175	D4	140 223 362 519	073 152 263 298 315+C 489	-	
Z178	D4	126 166h 172 174 223 343 362	073 263 315+C 489	-	
Z179	D4	126 172 174 223 343 362	073 263 315+C 489 750	-	
Z186	D4	223 286 362 519	073 150 194 263 315+C 489 523-524d		
Z188	D4	192 223 316 362	073 152h 263 309+C 315+C 489 750	-	
Z203	D4	126 166h 172 174 223 343 362	073 263 315+C 489	-	
Z204	D4	140 223 362 519	073 152 263 298 315+C 489 750	-	
Z210	D4	223 362 519 525	073 263 309+C 315+C 489	-	
Z231	D4	223 362 497 519	073 094 151 204 263 315+C 489 750	-	
Z233	D4	223 311 319 362	073 263 315+C 489 523-524d 750	-	
Z236	D4	145 223 362 519	073 263 309+C 315+C 489	-	
Z239	D4	192 223	073 195 263 309+C 315+C 489 750	-	
Z251	D4	223 294 311 362	065A 073 195 237 263 315+C 489 501 750	-	
Z262	D4	129 162 223 362 519	073 152 263 309+C 315+C 489 709 750	-	
Z272	D4	184 223 256 311 362	073 152 263 315+C 489 750		
Z308	D4	223 304 319 362	073 150 263 309+C 315+C 489	-	
Z314	D4	223 362	073 200 263 309+C 315+C 489	-	
Z315	D4	223 362	073 200 263 309+C 315+C 489	-	
Z326	D4	092 223 362	073 094 263 315+C 489 750		
Z339	D4	093 223 249 362	073 263 315+C 489	-	
Z361	D4	223 259A 274 362 519	073 263 298 309+C 315+C 489 573+2C	-	
Z362	D4	223 259A 274 362 519	073 263 298 309+C 315+C 489 573+2C 750	-	
Z368	D4	126 172 189 223 319 362	073 185 195 263 315+C 489 523-524d 573+C	-	
Z372	D4	150 223 362	073 263 309+C 315+C 489 750	-	
Z374	D4	172 362 519	073 194 263 309+C 315+C 489 523-524d		
Z387	D4	223 274 362	073 263 298 309+C 315+C 489 750	-	
Z391	D4	223 274 362	073 263 298 309+C 315+C 489	-	
Z412	D4	189 190 193+C 223 362	073 184 199 204 263 309+C 315+C 489 495 523-524d	-	
Z413	D4	174 223 355 362	073 263 309+C 315+C 489 523-524d	-	
Z427	D4	094 223 274 362	073 263 298 309+2C 315+C 489 523-524d 750	-	
Z445	D4	223 290 362 519	150 263 309+2C 315+C 489 523-524d 750	-	
Z467	D4	174 192 223	073 195 263 309+2C 315+C 489 750	-	
Z470	D4	223 293T 362	073 263 309+C 315+C 489	-	
Z472	D4	092 223 362	073 094 200 263 309+C 315+C 489 750	-	
Z063	D4a	129 223 263 362	073 152 263 279 315+C 489 750		
Z074	D4a	093 129 223 249 362 399	063 064 073 152 263 309+2C 315+C 489		
Z096	D4a	129 223h 362 519	073 152 263 315+C 489 750		
Z283	D4a	129 162 223 362 519	073 152 263 309+C 315+C 489 750		
Z381	D4a	129 223 263 311 362 519	073 152 263 309+C 315+C 489 507 523-524d 750		
Z404	D4a	129 172 223 270 362 519	073 152 263 309+C 315+C 489 750		
Z040	D4b	223 362 519	073 194 263 315+C 489 523-524d 750		
Z098	D4b	093 223 362 519	073 194 263 315+C 489 523-524d 750		
Z102	D4b	093 223 362 519	073 194 263 315+C 489 523-524d 750		
Z165	D4b	223 362 519	073 194 199 263 309+C 315+C 489		
Z194	D4b	223 286 362 519	073 150 194 263 315+C 489 523-524d 750		
Z195	D4b	223 286 362 519	073 150 194 263 309+C 315+C 489 523-524d 750		
Z217	D4b	223 362 519	073 194 199 263 309+C 315+C 489		
Z235	D4b	093 223 287 319 362 380	073 263 309+C 315+C 431 489 523-524d 750		
Z241	D4b	223 362 519	073 194 263 315+C 489 523-524d 750		
Z242	D4b	223 362 519	073 194 263 315+C 489 523-524d 750		
Z244	D4b	093 223 287 319 362 380	073 263 309+C 315+C 431 489 523-524d 750		
Z365	D4b	223 362 519	073 194 263 315+C 489 523-524d 750		
Z369	D4b	223 362 519	073 194 263 309+C 315+C 489 750		
Z426	D4b	184A 189 223 356 362 519	073 194 263 309+C 315+C 489 523-524d 750		
Z468	D4e	093 129 176 223 362	073 094 194 263 309+C 315+C 489 750		
Z156	D5	092 182C 183C 189 223 360 362	073 150 263 309+C 315+C 489		
Z021	D5a	092 164 182C 183C 189 223 266 362	073 150 195 263 315+C 489 523-524d 750 752		
Z036	D5a	164 172 182C 183C 189 223 266 362	073 150 263 309+C 315+C 489 523-524d 750 752		
Z132	D5a	164 172 182C 183C 189 223 266 362	073 150 152 263 309+C 315+C 489 523-524d		
Z187	D5a	172 182C 183C 189 223 266 362 519	073 150 263 309+C 315+C 489 523-524d		
Z243	D5a	164 182C 183C 189 223 266 362	073 150 204 207 263 309+2C 315+C 489 523-524d 750 752		

Z271	D5a	092A 164 182C 183C 189 223 266 362	073 150 152 263 309+C 315+C 489 523-524d	
Z348	D5a	172 182C 183C 189 223 266 324 362	073 150 263 309+2C 315+C 489 523-524d 750 752	
Z359	D5a	092 182C 183C 189 223 360 362	073 150 263 309+C 315+C 489 752 752	
Z363	D5a	164 172 182C 183C 189 223 266 362	073 150 263 309+C 315+C 489 523-524d	
Z398	D5a	164 172 182C 183C 189 223 266 362 519	073 150 263 309+C 315+C 489 523-524d	
Z415	D5a	164 182C 183C 185 189 193d 223 266 362 519	073 150 194 207 263 309+C 315+C 489 523-524d 750 752	
Z437	D5a	092 182C 183C 189 223 360 362	073 150 263 309+C 315+C 489 750 752	-
Z004	D5b	183C 189 223 357 362 519	073 150 263 309+2C 315+C 456 489 499 681 750	
Z060	D5b	183C 189 223 326 357 362 519	073 146 150 207 263 309+C 315+C 456 489 681 750	
Z089	D5b	189 216 223 362 519	073 150 263 309+C 315+C 456 489	
Z176	D5b	189 223 362 519	073 150 185 309+2C 315+C 456 489 524+2AC	
Z307	D5b	182C 183C 189 223 362	073 150 263 309+C 315+C 456 489	
Z405	D5b	178 183C 189 223 311 362	073 150 263 315+C 456 489 681	
Z443	D5b	182C 183C 189 223 311 362	073 146 150 263 309+C 315+C 456 489 681 750	
Z029	D5c	189 190 193+2C 223 311 316 362 519	073 150 151 152 263 309+C 315+C 489	
Z447	D5c	086 126 189 190 193+2C 223 295 297 299 311 362	073 150 151 152 263 309+2C 315+C 489 750	
Z448	D5c	086 126 189 190 193+2C 223 295 297 299 311 362	073 150 151 152 263 309+2C 315+C 489 750	
Z450	D5c	086 126 189 190 193+2C 223 295 297 299 311 362	073 150 151 152 263 309+2C 315+C 489 750	
Z460	D5c	086 126 189 190 193+2C 223 295 297 299 311 362	073 150 151 152 263 309+2C 315+C 489 750	
Z122	F	221 304 519	073 152 195 249d 263 275 309+C 315+C 471 750	
Z137	F	221 304 519	073 152 195 249d 263 275 309+C 315+C 471	
Z192	F	224 304	073 249d 263 309+2C 315+C	
Z273	F	176 304 527	073 249d 263 315+C 750	
Z421	F	304	073 195 249d 263 279 315+C 750	
Z452	F	192 304 527	073 249d 263 315+C 709	
Z045	F1	183C 189 304 519	073 249d 263 309+2C 315+C 523-524d 750	
Z218	F1	183C 189 304 519	073 249d 263 309+C 315+C 523-524d 750	
Z224	F1	183C 189 300 304 357 519	073 131 150 195 204 249d 263 309+C 315+C 523-524d 750	
Z267	F1	129 183C 189 304 519	073 249d 263 309+2C 315+C 523-524d 750	
Z304	F1	172 189 304 465 519	073 194 249d 263 309+C 315+C 521-524d 750	
Z313	F1	172 304 465 519	073 249d 263 309+C 315+C 521-524d	
Z028	F1a	129 162 172 258 304 399 519	073 249d 263 309+C 315+C 523-524d	
Z116	F1a	129 172 304 519	073 249d 263 315+C 523-524d 750	
Z149	F1a	086 129 172 189 304 519	073 249d 263 315+C 523-524d 750	
Z163	F1a	162 172 304 519	073 249d 263 309+2C 315+C 523-524d	
Z164	F1a	162 172 304 519	073 249d 263 309+2C 315+C 523-524d	
Z170	F1a	129 162 172 304 519	073 249d 263 315+C 523-524d 750	
Z208	F1a	129 172 304 320 519	073 249d 263 315+C 523-524d 750	
Z220	F1a	172 304 465 519	073 249d 263 315+C 521-524d 750	
Z222	F1a	108 129 162 172 304 519	073 249d 263 315+C 523-524d 750	
Z223	F1a	129 162 172 304 519	073 249d 263 315+C 523-524d 750	
Z230	F1a	129 162 172 304 311 519	073 249d 263 315+C 523-524d 750	
Z252	F1a	129 162 172 304 519	073 249d 263 315+C 523-524d 548 750	
Z255	F1a	129 172 304 311 497 519	073 152 249d 263 309+C 315+C 523-524d 709 750	
Z257	F1a	108 129 162 172 179 183 293 304 519	073 150 249d 263 315+C 523-524d	
Z264	F1a	129 162 172 304 519	073 094 146 249d 263 309+2C 315+C 523-524d	
Z292	F1a	129 162 172 304 519	073 094 146 249d 263 309+2C 315+C 523-524d	
Z301	F1a	129 162 172 278 304 399 519	073 152 249d 263 309+C 315+C 523-524d 750	
Z305	F1a	129 162 172 278 304 399 519	073 152 249d 263 309+C 315+C 523-524d	
Z309	F1a	108 129 162 172 214 304 519	073 249d 263 315+C 513 523-524d	
Z320	F1a	129 172 304 519	073 249d 263 309+C 315+C 523-524d 750	
Z322	F1a	129 172 179 274 304 519	073 093 146 249d 263 309+C 315+C 523-524d 750	
Z325	F1a	129 172 304 354 519	073 195 249d 263 315+C 523-524d 750	
Z328	F1a	129 162 172 304 497 519	073 234 249d 263 315+C 523-524d 750	
Z343	F1a	129 162 172 304 497 519	073 234 249d 263 315+C 523-524d 750	
Z344	F1a	129 162 172 304 497 519	073 234 249d 263 315+C 523-524d 750	
Z345	F1a	108 129 162 172 304	073 150 195 249d 263 315+C 523-524d 750	
Z389	F1a	129 162 172 304	073 249d 263 315+C 523-524d 750	
Z393	F1a	129 162 172 304 519	073 249d 263 315+C 523-524d 750	
Z399	F1a	129 172 304 519	052 053 054T 071d 073 249d 263 309+C 315+C 318 523-524d	
Z419	F1a	129 172 304 519	073 249d 263 315+C 523-524d 750	
Z431	F1a	129 162 172 304 519	073 249d 263 315+C 523-524d 548 750	
Z439	F1a	093 129 162 168 172 304 399 519	073 249d 263 309+C 315+C 750	
Z461	F1a	037 108 111 129 162 172 304 519	073 150 195 249d 263 315+C 523-524d	
Z473	F1a	172 274 304 519	073 249d 263 309+C 315+C 523-524d 750	

Z032	F1b	129 183C 189 232A 249 304 519	073 152 234 249d 263 309+C 315+C 523-524d 750			
Z128	F1b	182C 183C 189 232A 249 304 311 519	073 204 249d 263 309+2C 315+C 523-524d 750			
Z051	F1c	111 129 268 304 519	073 152 234 249d 263 315+C 523-524d 750			
Z077	F1c	111 129 268 304 519	073 152 234 249d 263 315+C 523-524d 750			
Z286	F1c	111 129 266 304 519	073 152 249d 263 315+C 523-524d 750			
Z317	F1c	111 129 266 304 519	073 152 249d 263 309+C 315+C 523-524d			
Z417	F1c	111 129 266 304 519	073 152 249d 263 309+C 315+C 523-524d 709			
Z456	F1c	111 129 266 304 519	073 152 249d 263 315+C 523-524d 750			
Z061	F1d	183C 189 304 519	073 146 249d 263 309+C 315+C 523-524d 750			
Z281	F1d	183C 189 304 519	073 146 249d 263 309+C 315+C 523-524d			
Z025	F2	203 304 311 519	073 199 249d 263 309+C 315+C 750			
Z199	F2	086 203 304 519	073 249d 263 315+C 750			
Z245	F2	066 167 203 304 318 519	073 182 249d 263 315+C 750			
Z401	F2	203 304 519	073 150 249d 263 309+C 315+C			
Z168	F2a	203 291 304 519	073 249d 263 309+2C 315+C			
Z212	F2a	203 259 291 304 519	073 249d 263 315+C 750			
Z228	F2a	203 291 304 311 335 519	073 249d 263 309+2C 315+C			
Z424	F2a	126 203 259 291 295 304 352 519	073 146 249d 263 315+C 750			
Z358	F2b	092A 291 304	073 249d 263 309+C 315+C 523-524d 533			
Z378	F2b	092A 166 249 291 304	073 249d 263 315+C 523-524d 750			
Z396	F2b	092A 291 297 304	073 249d 263 309+C 315+C 523-524d 750			
Z166	F2d	rCRS	073 235 249d 263 315+C 750			
Z215	F2d	rCRS	073 235 249d 263 315+C			
Z260	F2d	286 295	073 235 249d 263 315+C 750			
Z458	F2d	rCRS	073 235 249d 263 315+C 750			
Z107	F3	260 298 355 362	073 204 207 249d 263 309+C 315+C 709 750			
Z120	F3	260 298 355 362	073 204 207 249d 263 309+C 315+C 709 750			
Z124	F3	260 298 355 362	073 207 249d 263 309+C 315+C			
Z140	F3	260 298 355 362	073 204 207 249d 263 309+C 315+C 709 750			
Z143	F3	260 298 355 362	073 204 207 249d 263 309+C 315+C 709 750			
Z227	F3	260 298 355 362	073 204 207 249d 263 309+C 315+C 709 750			
Z323	F3	187 298 355 362	073 207 249d 263 295 309+C 315+C			
Z341	F3	093h 260 298 355 362	073 204 207 249d 263 309+C 315+C 709 750			
Z351	F3	111 192 249 263 298 355 362 390	073 249d 263 309+C 315+C 709 750			
Z438	F3	093 260 278 298 355 362	073 182 207 249d 263 309+C 315+C 709 750			
Z451	F3	093h 111 192 249 263 298 355 362 390 519	073 150 207 249d 263 309+C 315+C			
Z133	F3b	220C 227 298 362 519	073 152 249d 263 309+2C 315+C			
Z091	F4a	129 185 207 399 519	073 146 249d 263 281 315+C 573+5C			
Z209	F4a	126 207 304 362 399	073 146 249d 263 309+2C 315+C 317A			
Z299	F4a	093h 126 140 207 304 362 399	073 146 150 249d 263 309+C 315+C 317A 750			
Z300	F4a	304 399	073 146 249d 263 309+2C 315+C 524+2AC 750			
Z006	G	114A 145 223 243 261 362 519	073 191+A 194 203h 204 263 309+2C 315+C 489			
Z010	G	223 278 362 519	073 152 263 315+C 489 709 750	+		
Z024	G	219 223 278 362	073 150 152 263 315+C 489	+		
Z035	G	223 362 519	073 263 315+C 489 593 709 750	+		
Z043	G	189 223 274 319 362	073 143 152 226 263 309+C 315+C 489 709 750	+		
Z067	G	223 274 311 362	073 143 195 263 315+C 489 709 750	+		
Z092	G	129 223 278 362	073 263 315+2C 489			
Z110	G	223 278 362	073 152 263 309+C 315+C 489 573+3C	+		
Z184	G	223 362 519	073 263 315+C 489 593 709 750	+		
Z193	G	223 234 362	073 263 309+C 315+C 489 709 750	+	C	4529-5098=4769 4833 4853 5108
Z202	G	192 209 223 274 362	073 152 189 263 309+C 315+C 489 524+ACh	+		
Z371	G	223 274 311 362	073 263 309+C 315+C 489 709	+	C	4529-5098=4769 4833 5108
Z410	G	129 223 274 311 362	009 073 143 263 309+2C 315+C 489 709	+		
Z414	G	129 223 278 362 519	073 263 315+C 489 709 750			
Z418	G	223 278 362	073 146 152 263 309+2C 315+C 489 709 750			
Z125	M*	223 234 390 519	073 152 249d 263 279 309+C 315+C 489			
Z127	M*	223 234 390 519	073 152 249d 263 279 309+C 315+C 489 750			
Z129	M*	223 234 390 519	073 152 249d 263 279 309+C 315+C 489 750			
Z131	M*	223 234 390 519	073 152 249d 263 279 309+C 315+C 489 750			
Z153	M*	223 234 390 519	073 146h 152 185 249d 263 279 309+C 315+C 489			
Z265	M*	042 183C 189 209 223 269 291 390 519	073 150 194 207 263 315+C 489 523-524d 750			
Z254	M10	093 193 223 311 357 497	073 146 263 315+C 489 523-524d 573+3C			
Z294	M10a	093 193 223 311 357 497	073 146 263 315+C 489 523-524d 573+4C			
Z360	M10a	093 129 223 311 394	073 263 309+C 315+C 489 573+3C			

Z379	M10a	066 223 311 519	073 152 263 315+C 489 573+3C		
Z297	M10a1	129 223 311	073 263 309+2C 315+C 489 573+3C		
Z269	M10a2	066 223 311 519	073 263 309+2C 315+C 489 523-524d 573+3C		
Z340	M11a	114 189 223	073 188 198 200 215 263 309+C 315+C 318 326 489 750		
Z338	M12a	093 223 234 266 290 311	073 125 127 128 146 152 263 315+C 318 326 489 750		
Z375	M12a	223 290 362	073 125 127 128 263 315+C 318 489 513 523-524d 750		
Z409	M12a	223 290 362	073 125 127 128 263 315+C 318 489 513 523-524d 750		
Z020	M61	193 215 223 270 311 362 381	003 073 152 263 315+C 489		
Z017	M71	223 271 293T	073 152 263 315+C 489 750	G	8660-9300=8701 8718 8860
Z013	M76	183C 189 293C 325 362	073 146 234 263 315+C 489 513 750		
Z005	M7b	129 192 223 297	073 150 199 263 309+C 315+C 489 750		
Z007	M7b	129 189 223 297	073 150 199 263 309+C 315+C 489 573+4C		
Z022	M7b	129 189 223 297	073 150 199 263 309+2C 315+C 456 489 750		
Z037	M7b	129 192 223 297	073 150 199 263 315+C 489 750		
Z047	M7b	129 189 223 248 293 297	073 150 199 204 207 263 315+C 489 750		
Z052	M7b	129 223 297	073 150 199 263 309+2C 315+C 489 538 750		
Z054	M7b	129 189 192 223 297	073 150 199 263 315+C 489		
Z058	M7b	038 129 297 324 343 362	073 199 263 309+2C 315+C 489		
Z059	M7b	129 223 297	073 150 159 199 263 315+C 489 750		
Z065	M7b	129 192 223 297	073 150 199 263 309+C 315+C 489		
Z094	M7b	223 297 327	073 150 199 204 263 315+C 489		
Z097	M7b	223 297 327	073 150 199 204 263 315+C 489		
Z100	M7b	129 189 223 248 297	073 150 199 204 207 263 315+C 489 750		
Z121	M7b	129 192 223 297	073 150 199 263 315+C 489 750		
Z142	M7b	092 129 223 297	072 073 150 159 199 263 315+C 489		
Z177	M7b	223 297	073 150 199 204 263 315+C 489 750		
Z200	M7b	129 189 223 248 297	073 150 199 204 207 263 315+C 489 750		
Z201	M7b	223 297	073 150 199 204 263 315+C 489 750		
Z205	M7b	223 297	073 150 199 204 263 315+C 489		
Z213	M7b	129 189 214 223 297 357 399 519	073 150 199 263 309+C 315+C 489 750		
Z232	M7b	129 223 297 527	073 150 199 263 309+C 315+C 489 750		
Z279	M7b	129 189 223 297	073 150 199 204 263 309+C 315+C 456 489 524+AC 750		
Z334	M7b	086 297 324 399	073 199 263 315+C 489		
Z367	M7b	129 189 223 248 297	073 150 199 204 207 263 315+C 489 750		
Z376	M7b	094 223 297	073 199 204 263 315+C 489		
Z432	M7b	092 129 223 297	072 073 150 159 199 263 315+C 489 750		
Z434	M7b	129 223 297 354	073 150 159 199 263 309+C 315+C 489		
Z446	M7b	129 192 223 297 362 400	073 150 199 263 309+C 315+C 489 750		
Z453	M7b	129 171 189 214 223 297 399 519	073 150 199 263 309+C 315+C 489		
Z159	M7c	223 295 519	073 146 199 263 309+C 315+C 489 523-524d		
Z174	M7c	223 295 519	073 146 152 199 263 309+C 315+C 489 523-524d 750		
Z196	M7c	223 278 295 519	073 146 199 263 309+C 315+C 489 523-524d		
Z238	M7c	086 172 187 223 519	073 146 199 263 309+C 315+C 489 523-524d 750		
Z259	M7c	223 519	073 146 152 199 263 315+C 489 523-524d 750		
Z274	M7c	223 519	073 146 199 263 315+C 489 523-524d 750		
Z302	M7c	223 293T 295	072 073 146 199 263 315+C 489 523-524d 750		
Z352	M7c	093 223 295 311 519	073 146 199 263 315+C 489 523-524d 750		
Z384	M7c	223 248 295 519	073 146 150 199 263 309+C 315+C 456 489 523-524d		
Z397	M7c	108 223 295 319 519	073 146 199 263 309+C 315+C 489 523-524d 750		
Z003	M8a	134 184 223 298 319	073 263 315+C 489		
Z049	M8a	093 184 223 293 298 319 519	073 152 263 309+C 315+C 489		
Z071	M8a	134 184 223 298 319	073 263 309+C 315+C 489		
Z075	M8a	184 223 293 298 319 519	073 146 152 263 309+C 315+C 489 750		
Z087	M8a	184 209 223 298 311 319	073 207 263 309+C 315+C 489 750		
Z138	M8a	184 185 189 223 298 311 319 390 468 470 471 473	073 146 263 309+2C 315+C 489		
Z171	M8a	184 223 294h 298 319	073 146 263 309+C 315+C 489 750		
Z197	M8a	184 189 223 298 311 319 327 390 468 470 471 473	073 263 266 309+C 315+C 489 750		
Z219	M8a	184 223 298 319	073 263 309+C 315+C 489 750		
Z225	M8a	184 185 189 223 298 319 470 471 473	073 152 263 309+2C 315+C 489 523-524d 750		
Z284	M8a	172 184 223 298 319 519	073 152 263 309+C 315+C 489 750		
Z364	M8a	184 223 298 319 362	073 263 315+C 489 750		
Z373	M8a	184 189 223 298 319 470 471 473	073 263 309+2C 315+C 489		
Z383	M8a	134 184 223 298 319	073 263 309+C 315+C 489 750		
Z435	M8a	134 184 223 298 319	073 263 309+C 315+C 489 750		
Z015	M9a	223 234 240 362	073 153 263 309+C 315+C 489		

Z033	M9a	223 234 240 362	073 153 263 309+C 315+C 489 750		
Z039	M9a	223 234 240 362	073 153 263 309+C 315+C 489		
Z042	M9a	223 234 240 362	073 153 263 309+C 315+C 489 750		
Z057	M9a	223 234 240 362	073 153 263 309+C 315+C 489		
Z079	M9a	223 234 316 362	073 263 309+C 315+C 489 593 750		
Z109	M9a	234 291 316 362	073 153 263 309+C 315+C 489 750		
Z126	M9a	093 223 234 316 362	073 146 153 263 309+C 315+C 489 750		
Z221	M9a	176 223 234 316 356 362	073 153 246 263 315+C 489 750		
Z282	M9a	114A 223 234 240 362	073 263 309+C 315+C 489 750	+	
Z249	M9b	051 209 223 362 519	073 153 263 315+C 489 573+5C		
Z161	N10	172 223 234 258C 291A 298	073 150 199 263 309+C 315+C 523-524d		5300-6060=5581
Z162	N10	172 223 234 258C 291A 298	073 150 199 263 309+C 315+C 523-524d		5300-6060=5581
Z031	N9a	129 189 223 257A 261 287	073 150 263 309+C 315+C 750		
Z147	N9a	111 129 223 257A 261 274	073 150 263 309+2C 315+C		
Z173	N9a	111 129 223 257A 261	073 150 263 309+2C 315+C 750		
Z181	N9a	111 129 223 257A 261	073 150 263 309+2C 315+C 750		
Z183	N9a	111 129 223 257A 261	073 150 263 309+2C 315+C 750		
Z342	N9a	086 223 257A 261 311	073 150 263 309+C 315+C		
Z354	N9a	129 223 257A 261	073 150 263 309+C 315+C		
Z388	N9a	223 257A 261 311	073 150 263 315+C		
Z390	N9a	223 257A 261 311	073 150 263 315+C 750		
Z428	N9a	086 223 257A 261 311	073 150 263 309+C 315+C 750		
Z429	N9a	111 129 153 223 257A 260 261	073 150 263 309+C 315+C 750		
Z433	N9a	111 129 182 223 257A 261	073 150 263 315+C 750		
Z048	R11	129 182C 183C 189 311 519	073 185 189 207 263 309+2C 315+C 523-524d 709 750		
Z145	R11	111 172 183C 189 223 362 519	073 185 189 195 234 263 315+C 523-524d		
Z310	R11	182C 183C 189 311 390 399 519	073 185 189 215 263 307-310d 315+C 709 750		
Z276	R9	157 304 324	073 151 263 309+C 315+C 479		
Z014	R9b1	124 148 304 309 390 519	073 263 309+C 315+C 750		
Z018	R9b1	189 192 304 309 390 519	073 263 309+C 315+C 523-524d 750		
Z023	R9b1	145 192 243 284 304 309 390 519	073 183 263 309+C 315+C 523-524d		
Z084	R9b1	192 304 309 390 519	073 152 263 309+C 315+C 523-524d 750		
Z240	R9b1	192 288 304 309 390 519	073 143 146 183 263 309+C 315+C 523-524d 573+4C		
Z246	R9b1	189 288 304 309 390 519	073 143 183 263 309+2C 315+C 479 523-524d 573+4C		
Z258	R9b1	189 192 304 309 390 519	073 152 263 309+C 315+C 523-524d		
Z380	R9b1	304 309 390 519	073 151 263 309+2C 315+C		
Z382	R9b1	124 148 304 309 390 519	073 263 309+2C 315+C		
Z366	U2e1	051 129C 183C 189 190 193+2C 362 519	073 217 228 263 309+C 315+C 340 508 750		
Z266	Y	126 209 213 231 266 519	073 146 263 315+C 385		
Z440	Y	126 231 311	073 199 263 309+C 315+C 482 750		
Z041	Z	185 223 260 298	073 152 210 249d 263 309+C 315+C 489 523-524d 750		
Z136	Z	185 223 260 298 302	073 151 152 249d 263 309+2C 315+C 489 750		
Z152	Z	185 209 223 260 298 519	073 143 152 249d 263 309+C 315+C 489 750		
Z154	Z	185 209 223 260 298 519	073 143 152 249d 263 309+C 315+C 489		
Z155	Z	185 223 248 260 298	073 146 152 249d 263 309+C 315+C 489		
Z321	Z	185 223 260 298 302	073 151 152 249d 263 309+C 315+C 489 750		
Z335	Z	185 209 223 260 298	073 143 152 249d 263 309+C 315+C 489 750		
Z370	Z	185 260 298	073 197 249d 263 315+C 489 750		
Z392	Z	185 223 260 298 317T	073 151 152 249d 263 309+C 315+C 489 750		
Z395	Z	185 189 223 260 298 317T	073 151 152 249d 263 309+C 315+C 489 750		
Z402	Z	185 223 248 260 298	073 146 152 249d 263 309+C 315+C 489 750		
Z441	Z	129 185 223 224 260 298 519	073 151 152 249d 263 309+C 315+C 489		
Z442	Z	185 223 260 298	073 152 195 249d 263 315+C 489 709 750		
Z444	Z	136 223 260 298	073 152 249d 263 309+C 315+C 489		

AD patients

A095	A	223 290 319 362	073 152 200 235 263 315+C 523-524d 663 750		
A106	A	223 274 290 319 362 519 527	073 152 235 263 315+C 523-524d 663 750		
A137	A	153 223 290 319 362	073 152 207 235 315+C 523-524d 663 750		
A138	A	125 158 223 234 290 311 319 362	073 152 235 263 309+C 315+C 523-524d 576 663		
A141	A	086 223 290 294 319 362	073 152 235 263 315+C 523-524d 663 750		
A162	A	086 092 223 290 362 519	073 152 235 263 309+C 315+C 663 750		
A283	A	092 223 290 319 362	073 152 235 263 309+C 315+C 663 750		
A290	A	223 290 311 319 362	073 152 235 263 309-310d 315+C 523-524d 663 750		
A299	A	223 290 311 319 362	073 152 235 263 309-310d 315+C 523-524d 663 750		

A304	A	223 290 319 362 519	073 152 235 263 309+C 315+C 523-524d 663 750
A327	A	223 290 319 362 365	073 151 152 200 235 263 309+C 315+C 523-524d 663
A337	A	126 223 234 235 290 319 519	073 152 235 263 309+C 315+C 523-524d 663 750
GZ19	A	126 223 234 290 319 445	073 235 263 315+C 523-524d 663 750
A013	B4	037 114h 172 183C 189 217 519	073 195 263 315+C 316 619 750
A082	B4	182C 183C 189 217	073 214 263 309+C 315+C 368 709 750
A203	B4	129 182C 183C 189 217 362	073 150 263 309+2C 315+C 523-524d 750
A307	B4	129 182C 183C 189 217 296	073 263 309+2C 315+C 523-524d 750
GZ30	B4	129 182C 183C 189 217	073 150 263 309+2C 315+C 523-524d 750
GZ33	B4	129 182C 183C 189 217	073 150 263 309+2C 315+C 523-524d 750
A023	B4a	150 182C 183C 189 217 261 519	073 146 263 309+2C 315+C 523-524d 709 750
A045	B4a	182C 183C 189 217 261 299 355 390 519	073 263 309+2C 315+C 750
A081	B4a	182C 183C 188 189 214 217 243 261 519	073 146 263 309+C 315+C 523-524d 709
A091	B4a	182C 183C 189 217 261 299 519	073 193 263 308-310d 315+C 523-524d 709 750
A136	B4a	182C 183C 188 189 214 217 243 261 519	073 146 263 309+C 315+C 523-524d 709 750
A183	B4a	182C 183C 189 217 261 299 519	073 189 193 263 309+C 315+C 489 523-524d
GZ12	B4a	182C 183C 189 217 261 519	073 146 263 309+2C 315+C 523-524d 750
GZ23	B4a	182C 183C 189 217 261 299 519	073 193 263 309d 315+C 523-524d
GZ38	B4a	168 182C 183C 189 217 261 311 519	073 263 309+C 315+C 523-524d 750
GZ73	B4a	182C 183C 189 217 261 299 519	073 193 263 309+2C 315+C 523-524d 709 750
A067	B4a'g'h'i	093 182C 183C 189 217 261 357 519	073 263 309+C 315+C 523-524d 750
A132	B4a'g'h'i	182C 183C 189 217 261	073 263 309+2C 315+C 523-524d 750
A144	B4a'g'h'i	182C 183C 189 217 219 261 289 519	073 263 309+C 315+C 523-524d 750
A161	B4a'g'h'i	182C 183C 189 217 261	073 263 309+C 315+C 466 523-524d 750
A179	B4a'g'h'i	093 182C 183C 189 217 261 519	073 263 309+C 315+C 523-524d 750
A201	B4a'g'h'i	182C 183C 189 217 261 519	073 263 307-310d 315+C 523-524d 709 750
A204	B4a'g'h'i	093 182C 183C 189 217 261 357 519	073 263 315+C 523-524d 750
GZ24	B4a'g'h'i	093 182C 183C 189 217 261 519	073 263 309+2C 315+C 523-524d 750
GZ45	B4a'g'h'i	094 150 182C 183C 189 217 261	073 263 309+2C 315+C 466 523-524d 750
A313	B4b	136 182C 183C 189 217 218 519	073 263 309+2C 315+C 499 750 827
GZ63	B4b	136 183C 189 217h 298 519	073 152 200 263 315+C 451d 499 750
GZ76	B4b	136 183C 189 217 309 354 519	073 152 207 263 315+C 499 750 827
A033	B4c	140 183C 189 217 311 335 519	073 150 263 315+C 319
A096	B4c	147 183C 184A 189 217 235 249 519	073 263 309+C 315+C 523-524d 750
A142	B4c	129 140 183C 193+C 217 274 335 519	073 150 185 263 309+2C 315+C 513 709 750
A198	B4c	140 182C 183C 189 217 274 519	073 150 263 309+2C 315+C 709 750
A291	B4c	147 183C 184A 189 217 235 519	073 263 309+C 315+C 523-524d
A300	B4c	147 183C 184A 189 217 235 519	073 263 309+C 315+C 523-524d
A316	B4c	136 140 183C 189 217 249 274 291 335 519	073 150 263 315+C 709
A324	B4c	129 140 145 166 183C 189 217 274 519	073 146 150 263 309+2C 315+C 573+C 709 750
GZ32	B4c	093 183C 189 214 217 218	073 150 195 214 263 315+C
GZ40	B4c	140 182C 183C 189 217 274 305T 335 519	073 150 195 263 309+2C 315+C 709 750
GZ48	B4c	140 182C 183C 189 217 242A 274 335 519	073 146 150 263 315+C 523-524d 709
GZ60	B4c	092 140 182C 183C 189 217 274 311 335 519	073 146 150 263 309+C 315+C 709 750
A312	B4g	181C 182C 183C 189 213 217 249 261 270G 292 51	073 263 309+2C 315+C 523-524d 750
A338	B4g	181C 182C 183C 189 213 217 260 261 292 301 519	061A 062 073 263 309d 315+C 523-524d 750
GZ05	B4g	181C 182C 183C 189 213 217 261 292 519	073 263 309+2C 315+C 523-524d 750
GZ20	B4g	181C 182C 183C 189 201 213 217 261 270 292 519	073 195 263 315+C 523-524d 750
A009	B4h	129 182C 183C 189 217 261	073 194 263 309+C 315+C
A176	B4h	086 129 182C 183C 189 217	073 150 263 309+2C 315+C 523-524d 750
A046	B5a	140 183C 189 261 266A 318	073 210 263 309+C 315+C 523-524d 709 750
A052	B5a	140 182C 183C 189 266A 519	073 210 263 309+C 315+C 523-524d 709 750
A054	B5a	140 183C 189 266A 519	073 210 263 315+C 523-524d 709 750
A056	B5a	140 183C 189 262 266A 519	064 073 210 263 315+C 523-524d 593 709 750
A075	B5a	140 183C 189 266A	073 210 263 309+C 315+C 523-524d 709 750
A155	B5a	140 187 189 256 266G 519	073 093 210 263 315+C 523-524d 709 750
A206	B5a	140 189 234 266G 291 519	073 210 263 315+C 523-524d 709 750
A282	B5a	140 182C 183C 189 266A 362 519	073 210 263 309+2C 315+C 523-524d 709 750
A285	B5a	140 183C 189 266A 519	073 210 263 309+2C 315+C 523-524d 573+C 709 750
A289	B5a	140 183C 189 260 266A 399 519	073 189 200 205 263 294 309+2C 315+C 709 750
A293	B5a	140 183C 189 266A 519	073 210 263 309+2C 315+C 523-524d 573+C 709 750
A298	B5a	140 183C 189 260 266A 399 519	073 189 200 205 263 294 309+2C 315+C 709 750
A311	B5a	140 183C 189 261 266A 519	073 210 263 309+2C 315+C 523-524d 709 750
A328	B5a	140 187 189 256 266G 519	073 093 210 263 315+C 523-524d
GZ15	B5a	174 182C 183C 189 261 266A 519	073 152 210 263 309d 315+C 523-524d 709 750

GZ50	B5a	140 183C 189 262 266A 519	073 210 263 315+C 523-524d 593 709 750	
GZ54	B5a	140 183C 189 266A	073 200 210 263 309+2C 315+C 523-524d 709 750	
GZ78	B5a	129 140 182C 183C 189 266A 519	073 210 263 315+C 523-524d 593 709 750	
GZ86	B5a	140 183C 189 266A	073 200 210 263 309+2C 315+C 523-524d 709 750	
A001	B5b	140 183C 189 243 519	073 103 204 263 315+C 357 523-524d 709 750	
A100	B5b	111 140 183C 189 234 243 463 519	073 103 131 263 309+C 315+C 523-524d 709 750	
A131	B5b	017 140 182C 183C 189 234 243 519	073 152 189 203 222 263 309+2C 315+C 523-524d 709 750	
A163	B5b	140 183C 189 243 288	073 103 204 207 263 315+C 523-524d 709 750	
A309	B5b	111 140 183C 189 234 243 463 519	073 131 204 263 315+C 523-524d 709 750	
A323	B5b	051 111 140 183C 189 234 243 463 519	073 103 263 309+C 315+C 481 523-524d 709 750	
A014	C	183C 189 223 261 298 327 519	073 249d 263 309+2C 315+C 489 750	
A071	C	223 298 311 327 357 519	073 249d 263 309-310d 315+C 489 750	
A092	C	189 223 298 327 519	073 249d 263 309+C 315+C 489 750	
A094	C	093 129 223 298 327 519	073 195 228T 249d 263 309+C 315+C 489 750	
A135	C	223 298 327 519	073 146 249d 263 309+C 315+C 489 750	
A154	C	223 298 327 519	073 146 249d 263 309+C 315+C 489 709 750	
A286	C	067 093 145 223 294 298 327 519	073 249d 263 309+C 315+C 489 750	
A295	C	067 093 145 223 294 298 327 519	073 249d 263 309+C 315+C 489 750	
A314	C	223 298 327 519	073 249d 263 309+C 315+C 489 750	
A340	C	092 189 223 298 327 355 519	073 249d 263 309+C 315+C 415 489 750	
GZ17	C	223 298 327 519	073 146 249d 263 309+C 315+C 489 750	
A002	D4	218 223 362	073 146 263 309+C 315+C 489 750	-
A008	D4	189 223 294 362	065A 073 195 237 263 315+C 489 501 750	-
A015	D4	223 362	073 263 309+C 315+C 489	-
A020	D4	223 294 319 362	073 234 263 309+C 315+C 489 523-524d	-
A031	D4	223 316 362	073 183 263 315+C 489 750	-
A034	D4	185 189 223 232A 319 362	073 263 315+C 489 523-524d 750	-
A050	D4	184 223 311 362	073 151 263 309+C 315+C 489 750	-
A062	D4	174 223 362	073 263 309+C 315+C 489 523-524d 750	-
A068	D4	223 242 256 311 362 519	073 200 263 489 750	-
A080	D4	223 287 310 319 352 362	073 263 315+C 431 489 523-524d 750	
A084	D4	183 223 274 290 319 327A 362 519	073 195 263 309+C 315+C 489 750	
A088	D4	129 192 223 249 362	073 152 263 309+C 315+C 489	
A098	D4	223 274 362	073 263 298 309+C 315+C 489 546 750	-
A108	D4	092 223 362	073 094 263 309+C 315+C 489 750	
A109	D4	223 362 519	073 194 263 315+C 489 523-524d 750	
A118	D4	223 249 362	073 263 309+2C 315+C 489	-
A139	D4	223 362 519	073 194 263 315+C 489 523-524d 750	
A140	D4	129 223 263 362 519	073 152 263 309+C 315+C 385 489 750	
A145	D4	172 189 223 240C 311 362	016T 068 073 152 263 315+C 489 709 750	-
A150	D4	172 223 319 327 362	073 263 315+C 489 750	-
A152	D4	223 362	073 152 263 309+C 315+C 489 750	-
A156	D4	223 362 519	041 073 194 263 309+C 315+C 489 523-524d 750	
A164	D4	223 362	073 152 263 315+C 489 750	-
A165	D4	092 223 311 362	073 094 195 214 263 279 315+C 417 489 750	
A168	D4	126 172 189 223 319 362	073 185 195 263 315+C 489 523-524d	-
A173	D4	223 362	073 263 315+C 489 750	-
A177	D4	150 184 223 311 362 399 519	073 263 309+C 315+C 489 750	-
A182	D4	092 223 362	073 094 263 309+C 315+C 489 750	
A185	D4	223 362 519	073 150 194 263 315+C 489 523-524d	
A199	D4	223 249 362	073 146 150 263 309+C 315+C 489 750	-
A200	D4	129 223 362	073 263 309+C 315+C 489 750	
A284	D4	223 356 362	073 195 263 309+C 315+C 489 750	-
A292	D4	223 356 362	073 195 263 309+C 315+C 489 750	-
A302	D4	129 223 362	073 263 309+C 315+C 489 750	-
A315	D4	223 320 362 519	073 194 251 263 315+C 489 523-524d	
A318	D4	223 362 519	073 150 194 263 315+C 489 523-524d 750	
A325	D4	093 223 362 519	073 199 263 309+C 315+C 489 750	-
A339	D4	223 294 362	065A 073 195 237 263 315+C 489 501	
GZ53	D4	126 223 362 519	073 194 263 279 315+C 489 523-524d 750	
GZ36	D4a	093 129 223 249 362	073 152 237 263 309+C 315+C 456 460 463+2C 489	
GZ43	D4a	129 223 263 362 519	073 152 263 309+2C 315+C 489 750	
GZ51	D4a	129 223 263 362	073 152h 263 309+C 315+C 489	
GZ69	D4b	223 271 287 319 356 362	073 263 309+C 315+C 431 489 523-524d 750	
A070	D5a	092 164 172 182C 183C 189 223 266 362	073 150 263 309+C 315+C 489 523-524d	

A148	D5a	092 164 172 182C 183C 189 223 266 362 482	073 150 263 315+C 489 523-524d 750 752	
A153	D5a	164 172 173 182C 183C 189 223 266	073 146 150 263 309+C 315+C 489 523-524d 750 752	
A287	D5a	158 164h 172 182C 183C 189 223 261h 266 362	073 150 263 309+2C 315+C 489 523-524d 750 752	
A296	D5a	158 164h 172 182C 183C 189 223 261h 266 362	073 150 263 309+2C 315+C 489 523-524d 750 752	
A310	D5a	092 164 182C 183C 189 223 266 362	073 150 228 263 309+C 315+C 489 523-524d 750 752	
A319	D5a	086 092 182C 183C 185h 189 223 266 362	073 150 263 309+C 315+C 489 523-524d 750 752	-
A320	D5a	086 092 182C 183C 185h 189 223 266 362	073 150 263 309+C 315+C 489 523-524d 750 752	
A334	D5a	086 092h 182C 183C 185h 189 223 266 362	073 150 263 309+C 315+C 489 523-524d 750 752	
A346	D5a	148 164 172 182C 183C 189 223 266 362	073 150 152 263 315+C 489 523-524d 750 752	
GZ22	D5a	164 172 182C 183C 189 223 266 311 362	073 150 217 263 315+C 489 523-524d 709 752	
A030	D5b	189 223 362 519	073 150 152 263 309+C 315+C 456 489 681	
A049	D5b	182C 183C 189 223 362	073 150 194 263 309+2C 315+C 456 489 681 750	
A083	D5b	189 223 362 519	073 150 185 309+C 315+C 456 489 524+AC 681 750	
A160	D5b	111 189 223 362 519	073 150 263 315+C 456 489 681 750	
A205	D5b	183C 189 223 357 362 519	073 150 263 309+C 315+C 456 489 681 750	
GZ31	D5b	182C 183C 189 223 362	073 150 263 309+2C 315+C 456 489 681 750	
GZ37	D5b	182C 183C 189 223 362	073 150 263 309+C 315+C 456 489 681 750	
A101	D5c	051 189 223 320 362 390	073 094 146 150 151 152 263 309+C 315+C 489 750	
GZ25	D5c	169 189 190 193+2C 223 311 316 362	073 150 151 152 263 309+2C 315+C 489 750	
A345	D6	183C 189 223 311 362	073 152 204 263 315+C 489 523-524d 709 750	
GZ34	D6	183Ch 189 223 311 362	073 146 152 263 309+C 315+C 489 709 750	
A151	F	221 304	073 152 195 249d 263 275 315+C 750	
A022	F1	183C 189 221 304 519	073 249d 263 309+C 315+C 523-524d	
A086	F1	129 168 266 304 519	073 152 249d 263 309+C 315+C 523-524d 750	
A332	F1	182C 183C 189 260h 304 371 519	073 093 249d 263 309+C 315+C 523-524d 750	
GZ89	F1	042 051 189 239 269 292 300 304 519	073 150 195 249d 263 309+C 315+C 523-524d 750	
A004	F1a	129 162 172 304 519	073 249d 263 309+C 315+C 523-524d 548	
A006	F1a	129 172 221 304 438 519	073 249d 263 309+C 315+C 523-524d	
A021	F1a	129 172 304 519	073 189 207 249d 263 309+2C 315+C 523-524d	
A024	F1a	108 129 162 172 304	073 150 195 249d 263 309+C 315+C 523-524d	
A037	F1a	129 172 304 519	073 249d 263 315+C 523-524d 750	
A064	F1a	172 304 465 519	073 249d 263 309+C 315+C 521-524d	
A077	F1a	108 129 162 172 304 519	073 249d 263 309+C 315+C 523-524d	
A087	F1a	129 162 172 189 304 519	073 094 249d 263 315+C 523-524d 750	
A103	F1a	129 172 304 519	073 249d 263 309+2C 315+C 521-524d	
A157	F1a	108 129 162 172 304 519	073 150 195 249d 263 315+C 523-524d 750	
A175	F1a	129 162 172 189 304 519	073 249d 263 315+C 523-524d 750	
A190	F1a	108 129 162 172 201 304 311 519	073 150 249d 263 315+C 523-524d 750	
A306	F1a	129 172 189 221 304 438 519	073 249d 263 309+C 315+C 523-524d 750	
A317	F1a	108 129 162 172 201 304 311 519	073 150 249d 263 315+C 523-524d 750	
A336	F1a	108 129 162 172 304	073 150 195 249d 263 315+C 523-524d 750	
GZ11	F1a	129 172 304 519	073 249d 263 309+C 315+C 521-524d 750	
GZ13	F1a	129 162 172 304 399 519	073 249d 263 309+C 315+C 523-524d 750	
GZ18	F1a	108 129 162 172 214 263+A 304 362h 519	073 249d 263 315+C 523-524d 750	
GZ26	F1a	129 172 304 519	073 249d 263 309+C 315+C 523-524d 750	
GZ27	F1a	129 172 304 519	073 249d 263 309+C 315+C 523-524d 750	
GZ29	F1a	172 274 304 519	073 249d 263 309+C 315+C 523-524d 750	
GZ35	F1a	108 129 162 172 304 362 519	073 150 195 249d 263 315+C 523-524d 750	
GZ41	F1a	108 129 162 172 304 519	073 195 249d 263 309+C 315+C 523-524d 750	
GZ66	F1a	129 172 304 519	073 249d 263 309+C 315+C 521-524d	
GZ74	F1a	129 172 295 304 519	073 249d 263 315+C 523-524d 750	
GZ80	F1a	129 172 209 239 304 519	073 249d 263 309+C 315+C 521-524d	
A093	F1b	172 179 183C 189 232A 249 304 311	073 152 204 249d 263 315+C 523-524d 750	
A116	F1b	172 179 183C 189 232A 249 304 311	073 152 204 249d 263 315+C 523-524d 750	
GZ42	F1b	183C 189 232A 249 304 519	073 152 249d 263 309+2C 315+C 523-524d 750	
A057	F1b'd'e'g	183C 189 304 519	073 249d 263 309+2C 315+C 523-524d 750	
A066	F1c	111 129 266 304 311 519	073 143 152 215 249d 263 309+C 315+C 523-524d	
A114	F1c	111 129 304 519	073 152 234 249d 263 315+C 513 523-524d 750	
A012	F1d	189 284 304	073 146 249d 263 315+C 523-524d 750	
A308	F1d	189 304 311 519	073 146 249d 263 309+C 315+C 523-524d	
A120	F2	092 291 294 304	073 146 249d 263 315+C 523-524d 709 750	
A330	F2	203 304 519	073 249d 263 309+C 315+C 750	
GZ52	F2	092A 291 304	073 249d 263 315+C 750	
GZ61	F2	167 203 304 318 519	073 182 249d 263 315+C 750	
GZ88	F2	092A 291 304	073 249d 263 315+C 750	

A053	F2b	092A 291 304	073 249d 263 309+C 315+C 523-524d			
A125	F2b	092A 291 304	073 249d 263 309+2C 315+C 523-524d 750			
A058	F2d	rCRS	073 204 228 235 249d 263 309+2C 315+C 574			
A191	F3	260 298 355 362	073 207 249d 263 309+C 315+C			
GZ28	F3	239 260 298 355 362 526	073 207 249d 263 309+C 315+C 709 750			
GZ49	F3	185 189d 223 224 260 261 298	073 152 185h 249d 263 315+C 489 750			
GZ59	F3	260 298 355 362	073 207 249d 263 309+2C 315+C 709 750			
GZ67	F3	260 298 355 362	073 207 249d 263 309+2C 315+C 709			
GZ83	F3	260 298 355 362	073 207 249d 263 309+C 315+C 709 750			
A016	F3a	260 266 298 355 362	073 204 207 249d 263 309+C 315+C			
A063	F3b	220C 227 298 362	073 152 249d 263 309+C 315+C 750			
A181	F4	093 207 304 362 399 497	073 146 152 207 249d 263 315+C 750			
A305	F4a	207 304 399	073 146 152 204 207 249d 263 281 309+C 315+C			
GZ70	F4a	207 304 362 399	073 146 152 182 207 249d 263 315+C 750			
A027	G	129 223 278 362	073 263 315+2C 489 709 750			
A029	G	051 189 223 362 519	073 263 309+2C 315+C 489 593 709 750			
A038	G	223 311 362	073 263 309+C 315+C 489 709 750	+	C	4529-5098=4769 4833 4853 5108
A047	G	223 319 362 519	073 263 309+C 315+C 489 593 709 750			
A085	G	093 223 227 278 304 362	073 309+C 315+C 489 709 750			
A089	G	093 223 227 278 304 362	073 309+C 315+C 489 709 750			
A126	G	156 209 223 274 362 390	073 263 315+C 390 489 573+3C	+		
A130	G	156 209 223 274 362 390	073 263 315+C 390 489 573+3C	+		
A134	G	126 223 362 519	073 263 315+C 489 593 709 750	+		
A170	G	207 223 278 362	073 152 263 309+C 315+C 489 573+3C	+		
A178	G	051 189 223 362 519	073 263 309+C 315+C 489 593 709 750			
A184	G	223 234 278 311 362	073 152 263 315+C 489 709 750	+		
A186	G	093 189 223 227 234 278 294 309 362	073 263 315+C 489 573+5C			
A188	G	223 278 362	073 260 263 309+C 315+C 489 709 750			
A189	G	051 223 227 278 362	073 263 309+2C 315+C 489 709 750			
A192	G	223 278 362	073 260 263 309+C 315+C 489			
A326	G	051 223 227 278 362	073 263 309+2C 315+C 489 709 750			
A331	G	093 189 223 227 234 278 309 362	073 263 315+C 489 573+4C			
A342	G	223 362 519	073 263 309+C 315+C 489 709 750	+		4529-5098=4769 4833
GZ57	H6	278 342 362 482 519	239 263 309+C 315+C 750			
GZ84	H6	278 342 362 482 519	239 263 309+C 315+C 750			
A099	M*/G	213 223 325 355 362 519	073 200 263 315+C 489 523-524d 709 750	+		
A133	M10	129 189 223 311	073 263 315+C 489 573+3C			
GZ62	M10	066 223 311	073 263 291d 315+C 489 573+CCTCCC 709			
A072	M10a1	093 129 223 311 394	073 263 309+C 315+C 489 573+C			
A149	M11	223	073 195 198 200 263 309+C 315+C 318 326 489 750			
GZ85	M11	189 223 294	073 198 215 263 315+C 318 326 489 750			
A073	M12a	093 223 234 266 290 311	073 125 127 128 146 152 263 315+C 318 326 489 750			
A107	M12a	086 172 223 234 290 311	073 125 127 263 309+C 315+C 489 523-524d 633 750			
A146	M12a	223 234 290 304 362	073 125 127 128 263 315+C 318 489 513 523-524d 750			
A174	M12a	093 223 234 266 290 309 311	073 125 127 128 146 152 263 309+C 315+C 318 326 489 750			
A281	M12a	129 172 189 223 234 290 295 519	073 125 126 127 128 146 195 263 309+2C 315+C 489 573+3C			
GZ79	M12a	051 223 234 288 290 362	073 125 127 128 228 263 309+C 315+C 318 489 513 523-524d 750			
GZ02	M12b	129 172 223 234 290 519	073 150 195 263 309+2C 315+C 489 523-524d 750			
GZ44	M12b	129 172 223 234 290 519	073 150 195 263 309+2C 315+C 489 523-524d			
A159	M20	129 172 209 223 272 311 362 519	073 152 225 249d 263 309+C 315+C 316 489 523-524d 750			
A048	M33c	093 104 111 223 235 362 519	073 263 309+2C 315+C 489 750			
A124	M76a	183C 189 293C 325 362	073 146 234 263 315+C 489 513 750			
A018	M7b	086 297 324 399	073 199 263 315+C 489			
A028	M7b	129 192 223 297	073 150 199 263 309+2C 315+C 489 538 750			
A035	M7b	129 140 183C 189 223 248 297	073 105-110d 150 199 204 207 263 309+C 315+C 489 750			
A060	M7b	129 223 297	073 150 159 199 263 309+C 315+C 489			
A061	M7b	129 223 297	073 150 159 199 263 309+C 315+C 489			
A104	M7b	129 189 192 223 297	073 150 199 263 309+C 315+C 489 750			
A122	M7b	129 192 223 297	073 150 199 263 315+C 489 750			
A143	M7b	129 183C 189 192 223 297	073 150 199 204 207 263 315+C 489 750			
A166	M7b	129 189 223 297 519	073 150 199 204 263 309+C 315+C 456 489 750			
A169	M7b	129 192 223 297	073 150 199 204 207 263 309+C 315+C 489 750			
A172	M7b	129 192 223 297	073 150 152 199 263 309+C 315+C 489 538 750			
A180	M7b	129 189 223 248 297	073 150 199 204 207 263 315+C 489 750			
A194	M7b	129 192 223 297	073 150 152 199 263 309+C 315+C 489 538			

A195	M7b	129 192 223 297	073 150 152 199 263 309+C 315+C 489 538 750
A321	M7b	129 192 223 297	073 150 182 199 263 315+C 459d 489 750
A335	M7b	129 297 324 343 362	073 199 263 309+C 315+C 489
A344	M7b	129 189 223 297 298	073 150 199 204 263 315+C 489 750
GZ14	M7b	223 297	073 150 152 199 263 271 309+2C 315+C 489
GZ71	M7b	092 129 192 223 254 297	073 150 159 182 199 263 315+C 489 750
GZ77	M7b	038 129 220C 223 297	073 150 199 263 309+2C 315+C 489 750
GZ96	M7b	129 192 223 297	073 150 199 263 309+C 315+C 489 750
A097	M7c	519	073 146A 199 263 309+2C 315+C 489 523-524d 750
A119	M7c	095 223 295 519	073 146 199 262 263 315+C 489 523-524d 709 750
A127	M7c	172 223 295 519	073 146 199 263 309+2C 315+C 489 523-524d
A303	M7c	173 223 295 362 519	073 146 199 263 315+C 489 523-524d 750
A333	M7c	095 223 256 519	073 146 199 262 263 309+C 315+C 489 523-524d 709 750
GZ08	M7c	095 223 295 519	073 146 199 262 263 315+C 489 523-524d 709
GZ16	M7c	519	073 146A 199 204 263 315+C 489 523-524d 750
GZ95	M7c	519	073 146A 152 199 204 263 309+C 315+C 489 523-524d 750
A171	M7e	172 223 311 519	073 146 263 315+C 489 523-524d 750
GZ10	M7g	086 184 223 235 519	073 263 315+C 489 523-524d 709 723 750
A003	M8a	129 184 223 293 298 311 319	073 152 263 309+2C 315+C 489 508
A005	M8a	129 184 223 293 298 311 319	073 152 263 309+C 315+C 489 508 523-524d
A026	M8a	129 184 223 293 298 311 319	073 152 263 309+C 315+C 489 508 523-524d 750
A039	M8a	184 223 298 311 319	073 263 315+C 489 574 750
A059	M8a	134 184 223 298 319	073 263 309+2C 315+C 450 489
A129	M8a	184 189 223 298 319	073 263 309+2C 315+C 489 750
A329	M8a	184 223 298 319	073 152 263 309+2C 315+C 489 750
GZ46	M8a	184 189 223 298 311 319 390 391 468 470 471 473	073 263 309+C 315+C 489 750
GZ58	M8a	184 189 223 298 319	073 150 263 309+2C 315+C 444 489 523-524d 750
GZ68	M8a	134 184 223 298 319	073 263 309+2C 315+C 489
A128	M9a	145 169 223 234 316	073 153 263 309+C 315+C 489 513 750
A288	M9a	092 223 234 291 316 362	073 153 263 309+C 315+C 489 750
A297	M9a	092 223 234 291 316 362	073 153 263 309+C 315+C 489 750
A065	N11a	145 189 223 355 519	073 195 240 263 315+C 523-524d 750
A051	N9a	093 189 223 257A 261	073 150 263 309+C 315+C 709 750
A076	N9a	129 223 257A 261	073 150 263 309+C 315+C 750
A079	N9a	223 257A 261	073 150 228T 263 309+C 315+C 750
A147	N9a	092 145 172 223 245 257A 261	073 150 263 309+C 315+C 524+2AC
A193	N9a	172 223 257A 261	073 150 263 309+C 315+C 750
A196	N9a	111 129 223 257A 261 327	073 150 263 309+C 315+C 750
A202	N9a	172 189 223 257A 261 293C	073 150 195 204 263 315+C 750
A301	N9a	223 257A 261	073 150 263 309+C 315+C 750
A341	N9a	184 223 257A 261 274 519	073 150 152 263 309+C 315+C 573+3C
GZ21	N9a	111 129 171 223 257A 261 298 519	073 150 263 315+C 750
GZ39	N9a	129 223 257A 261	073 150 195 263 309+C 315+C 750
GZ47	N9a	086 182C 183C 189 223 257A 261	073 150 263 315+C
A322	R11	182C 183C 189 311 519	073 185 189 263 309+2C 315+C 709 750
GZ90	R11	182C 183C 189 311 468 519	073 185 189 263 315+C 709 750
GZ91	R11	111 172 183C 189 223 362 519	073 185 189 195 234 263 315+C 523-524d
A078	R22	051 249 288 304 519	073 146 263 315+C 329 750
A036	R9	093 157 304	073 146 151 263 309+C 315+C 479 750
A158	R9	234 304	073 151 263 309+C 315+C 479 750
GZ06	R9	093 157 201 219 304	073 151 263 309+C 315+C 374 479 750
GZ87	R9	304 362 519	073 263 315+C 750
A011	R9b1	124 148 304 309 390 519	073 263 309+C 315+C 573+C
A025	R9b1	124 148 304 309 390 519	073 263 309+C 315+C
A055	R9b1	304 309 390 519	073 183 263 309+C 315+C 523-524d 750
A123	R9b1	145 192 243 304 390 519	073 183 184 263 315+C 523-524d 750
A343	R9b1	124 148 304 309 390 519	073 263 309+C 315+C 750
GZ09	R9b1	192 239 304 309 390 519	073 152 263 309+C 315+C 523-524d 750
GZ55	R9b1	093 189 288 304 309 390 519	073 143 183 263 309+C 315+C 523-524d 573+3C
GZ97	R9b1	192 304 309 390 519	073 152 263 309+C 315+C 523-524d 750
GZ64	R9c1	157 256 266 304 311 335	073 249d 263 309+C 315+C 750
A069	Y	126 231 266 319 399 519	073 146 263 315+C 504 709 750
A167	Y	126 231 266 519	073 146 263 309+C 315+C 750
GZ65	Y	126 231 266 399 519	073 146 263 309+2C 315+C 750
GZ81	Y	126 176 231 266 399 519	073 146 263 309+C 315+C

A032	Z	185 223 260 298	073 152 210 249d 263 309+C 315+C 489 523-524d 750
A102	Z	185 223 260 298 380	073 152 249d 263 315+C 489 723 750
A187	Z	185 223 260 298 299	073 150 152 249d 263 315+C 489 750
A197	Z	146 185 223 260 298	073 146 152 249d 263 309+C 315+C 489 523-524d 750
GZ01	Z	136 185 223 260 298 519	073 152 249d 263 315+C 489 750
GZ03	Z	185 223 260 298 519	073 152 214 249d 263 309+C 315+C 489 709
GZ56	Z	185 223 260 287 298 302	073 151 152 249d 263 315+C 489 750

Note: Positions are numbered according to the revised Cambridge reference sequence (rCRS); mtDNAs that show no sequence variation in a sequenced region compared with the revised Cambridge reference sequence are labeled as "rCRS". "A", “G”, "C", "T" in column "F" to column "H" represent the nucleotide at certain position by sequencing certain coding regions.

[a] Suffixes “A”, “G”, “C”, and “T” indicate transversions, suffix “h” denotes heteroplasmic mutation; ‘‘d’’ indicates deletions, and ‘‘+’’ means insertion; indels are recorded at the last possible site.

[b] ‘‘-’’ and ‘‘+’’ denote the absence and presence of the restriction site at position 5176 for *AluI* or deletion at position 8281-8289 in certain mtDNA. All mtDNAs belonging to haplogroup D (including its subhaplogroups) were characteristic of -5176 *Alu* I. When sequence information was not available, items have been left blank.

[c] Sequence variants in coding regions that were sequenced for further confirmation of haplogroup status of certain mtDNA. When sequence information was not available, items have been left blank.

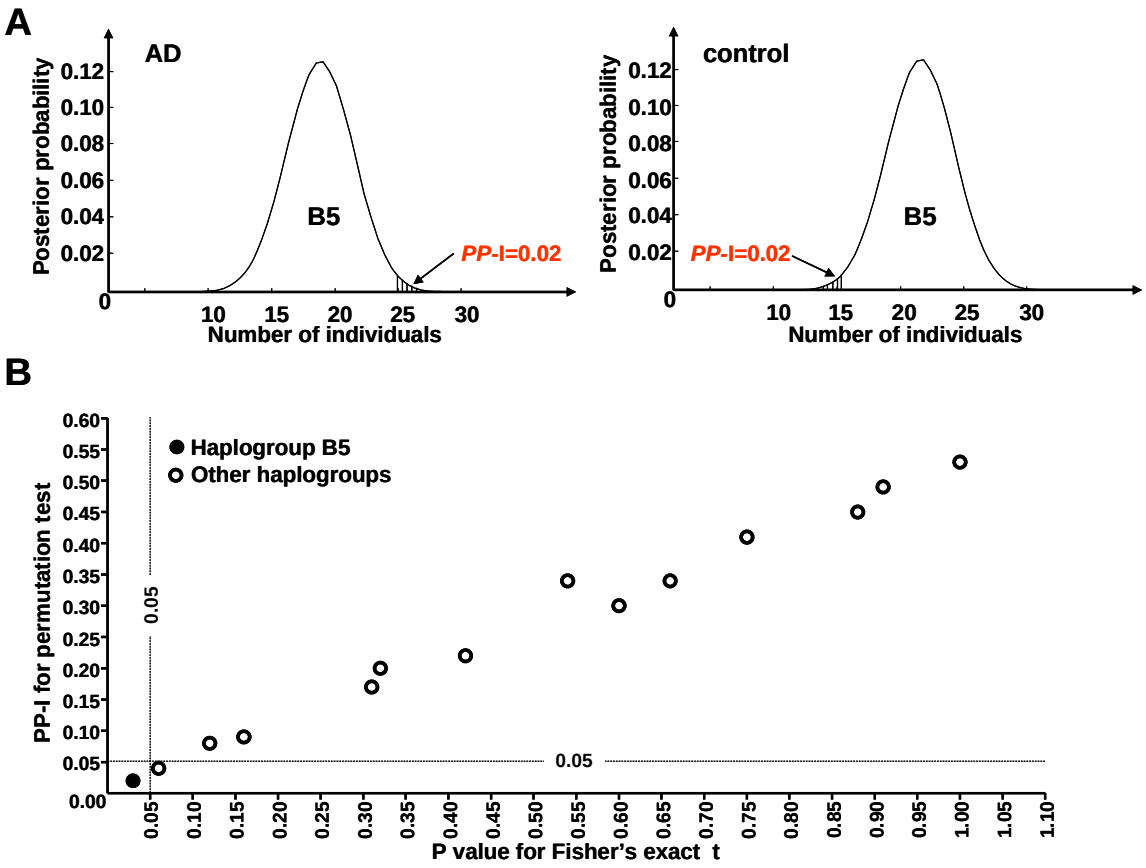

Figure S1. Permutation test for further validation of the prevalence of haplogroup B5 in patient and control groups. (A) Estimation of posterior distribution for haplogroup B5 in patients and healthy controls by using permutation test. The *P* values referred to posterior probabilities of the observed number of individuals belonging to B5 in AD samples during the simulation that was larger than 25 (for cases) or fewer than 16 (for controls), respectively. (B) Correlation of the *P* values of Fisher's exact test and posterior probabilities of permutation test for each haplogroup.

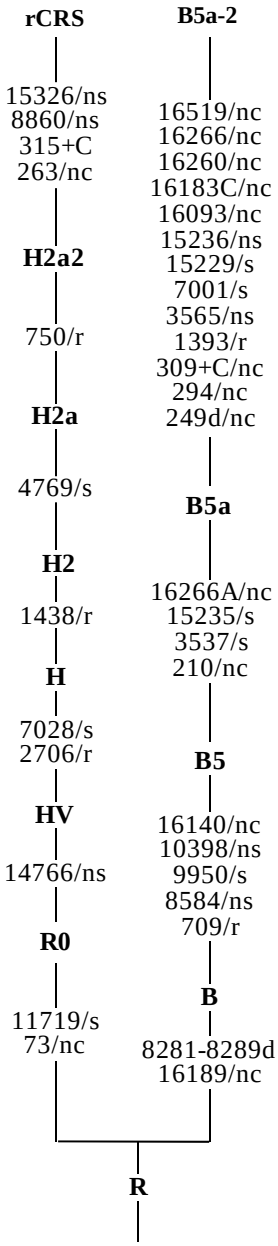

Figure S2. Haplogroup tree of complete mtDNA sequence of B5a-2 cell line and the rCRS (Andrews et al., 1999). Deletions and insertions are denoted by a “d” and “+”, respectively; “r” indicates the variant occurs in the rRNA genes; “t” indicates the variant occurs in the tRNA genes; “nc” indicates the variant occurs in the non-coding region; synonymous and non-synonymous variants are labeled as “s” and “ns”, respectively; Suffixes A means transversions.

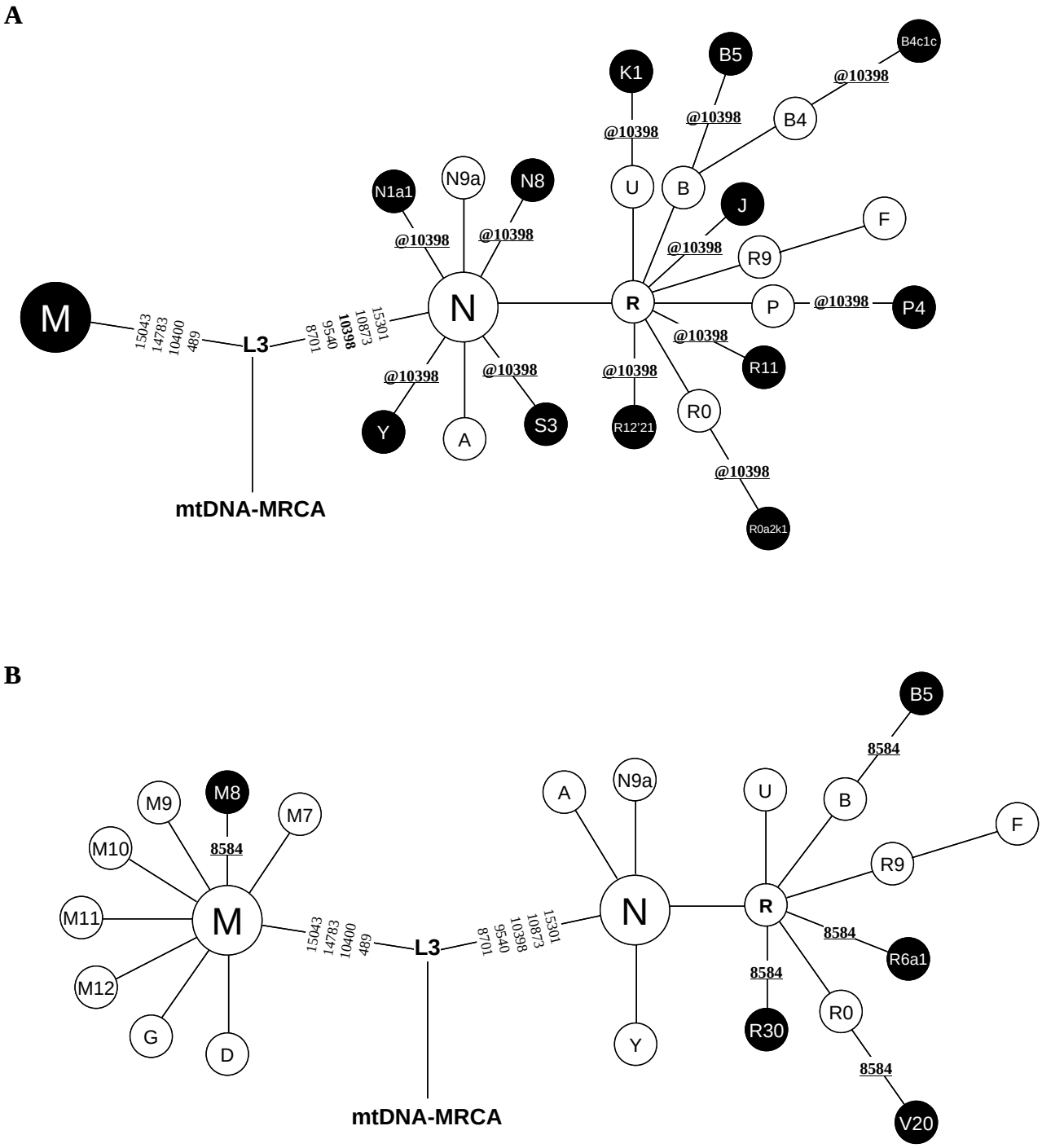

Figure S3. Distribution pattern of variants m.10398A>G (A) and m.8584G>A (B) in human mtDNA phylogenetic tree. mtDNA-MRCA: most recent common ancestor for modern human mtDNA. mtDNA-MRCA possessed m.10398G and m.8584G allele. Solid circles mean haplgroups with m.10398G allele in (A) and m.8584A allele in (B), whereas hollow circles mean haplgroups with m.10398A allele in (A) and m.8584G allele in (B).

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