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Mitochondrial DNA mutation m.5512A > G in the acceptor-stem of mitochondrial tRNA^{Trp} causing maternally inherited essential hypertension

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ABSTRACT

Essential hypertension (EH) is a common complex disorder with high heritability. Maternal inherited pattern was observed in some families with EH, which was known as maternally inherited essential hypertension (MIEH). Mitochondrial DNA (mtDNA) mutations were identified to account for some MIEH in previous studies. In the present study, we characterized clinical manifestations and the complete mitochondrial genome of a Chinese family with MIEH. Through analyzing the whole mtDNA genome of the proband, we identified a mutation m.5512A > G in the *MT-TW* gene that changed a highly conserved nucleotide and could potentially affect the function of tRNA^{Trp}. Furthermore, significantly exercise intolerance, left ventricular remodeling and increased arterial stiffness were observed in carriers with mutation m.5512A > G, which further supported the potentially pathogenic effect of m.5512A > G in MIEH.

1. Introduction

Essential hypertension (EH [MIM 145500]) is one of the most common complex disorders, and genetic factor is a well-known risk factor account for blood pressure (BP) variability [1]. Maternally inherited essential hypertension (MIEH) is an inherited mode of essential hypertension occasionally observed in clinic, and mitochondrial DNA (mtDNA) mutations, which are maternally inherited, were suggested to be involved in the genetic risk of MIEH [2,3]. Recently, several mtDNA point mutations have been identified to be associated with MIEH [4–8]. Among these mutations, mitochondrial transfer RNA (tRNA) is a hotspot for pathogenic mutations. These mutations included the m.4263A > G [6], m. 4291T > C [5] and m. 4295 A > G [7] in the *MT-TI* gene, m.4435 A > G in the *MT-TM* gene [8], etc. Due to their essential role in mitochondrial protein synthesis, mitochondrial tRNA mutations was considered as a major contribution to mitochondrial disorders [9]. However, mutations in all of the above mentioned genes account for only a small percentage of patients. The etiology of MIEH has not been completely determined and clinical manifestations are not well understood.

In this study, we analyzed the entire mtDNA in a Chinese family with MIEH that presented with an early onset, severe increased BP and exercise capacity limitation associated with stroke mainly in affected female. A homoplasmic mutation m.5512A > G in the mitochondrial tRNA^{Trp}(*MT-TW*) gene was identified and further analysis revealed the potential pathogenicity of this mutation to cause MIEH.

2. Materials and methods

2.1. Subjects

For molecular genetics studies, A Dai Chinese family (family EH28) with EH was recruited (Fig. S1). Members of this family were interviewed, and comprehensive health and life questionnaires were performed for each member. Physical measurements for each member were conducted following the WHO MONICA Project standards. BP of each participant was measured by using a mercury sphygmomanometer with a standardized fashion. Individuals with

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EH were diagnosed according to the Joint National Committee VI criteria [10]. Informed consent was obtained from each individual following the principles of the Declaration of Helsinki. The study protocol was approved by the ethical committee of the Kunming medical university and the institutional review board of the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS).

2.2. Special examinations and biochemical test

The ambulatory blood pressure monitoring (ABPM) was measured non-invasively for 24 h by the Spacelab 90207 or 90217 devices (Spacelab, CA, USA) to measure BP every 15 min during daytime (6:00–23:00) and every 30 min during nighttime (23:00–6:00). The arm cuff was positioned on the nondominant upperlimb. Records containing less than 80% of measures were excluded for evaluation. The 24 h BP, daytime BP and nighttime BP, 24 h heart rate (24hHR), ambulatory arterial stiffness index (AASI) and symmetric ambulatory arterial stiffness index (S-AASI) [11] were calculated in every subject.

All subjects underwent transthoracic echocardiography at rest in left lateral decubitus position, using Vivid 7 Dimension (GE, CT, USA) and standard echocardiographic evaluation techniques. All studies were performed and analyzed by the same experience echocardiographer. Two-dimensionally guided M-mode echocardiograms were obtained following the American Society of Echocardiography [12]. Measurements included Left atrium (LA), left ventricular septal thickness (IVS), posterior wall thickness at end-diastole (LVPW), left ventricular internal diameter at end-diastole (LVd) and end-systole (LVs), and left ventricular mass (LVM) was estimated by using the anatomically validated equation reported by Devereux et al. [13]. The left ventricular mass index (LVMI) was obtained by correcting for body surface area. For show of systolic and diastolic function, we measured ejection fraction (EF) and mitral pulse-wave Doppler E/A ratio.

Exercise testing (ET) were performed with the same device (CASE 8000, GE, CT, USA). The protocol for ET has been previously reported [14]. In brief, modified Bruce protocol was employed. Since our institution uses a heart rate of at least 90% of the adjusted age-predicted maximal heart rate (220 - age) to indicate adequate stress, the patients were encouraged to continue until 90% of maximal heart rate was achieved. During exercise and post-exercise, a 12-lead electrocardiography(ECG) and BP were recorded at the end of each 3-min stage and at peak exercise. The recovery period remained for 8 min or was prolonged in case the symptoms or electrocardiographic changes were persistent. The estimated workload in metabolic equivalents (METs) (1 MET = 3.5 mL/kg per minute of oxygen consumption, which corresponds to the resting state) were obtained. Heart rate reserve (percentage) was calculated by the following formula [15]: 100 × (Peak heart rate - Resting heart rate)/([220 - Age] - Resting heart rate).

1.5-T magnetic resonance imaging (MRI) with MR cine phase-contrast of head, thorax and abdomen (Siemens, Munich, Germany) was performed on each participant. We measured aortic pulse wave velocity (PWV) according to the methods of the distance between the ascending aorta and the abdominal aorta over the time required for the pulse wave to travel from the ascending aorta to the abdominal aorta.

Blood chemistry tests, including fasting blood sugar (FBS), total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), blood urea nitrogen (BUN), creatinine (Cr) and urea acid (UA), were performed using an automatic biochemistry analyzer (OLYMPUS AU-5400, Tokyo, Japan). Blood routine examination was determined by hematology analyzer (Sysmex XN-3000, Chuo-ku, Japan).

2.3. Mutational analysis of mitochondrial genome

The genomic DNA was extracted from whole blood by using the commercial DNA Isolation Kit (Tiangen Biotech Co., Beijing, China). The entire mtDNA genome of proband (II: 3) was amplified and sequenced as described in previous study [16]. Sequences were analyzed by using the DNASTAR software package (DNASTAR Inc., WI, USA). Sequence variations in the proband mtDNA sequences were scored relative to the revised Cambridge Reference Sequence (rCRS) [17]. We followed the PhyloTree Build 17 (http://www.phylotree.org; 18 Feb 2016) [18] and MitoTool (http://www.mitotool.org) [19] to classify haplogroup. The phylogenetic approach was used to define the (potentially) pathogenic mutation [20]. mtDNA sequence variation of the proband together with the rCRS [17] and two mtDNA sequences from GenBank (Accession numbers GU392069.1 and HM156677.1), were presented in a haplogroup tree to show the relationship among these mtDNAs. Evolutionary conservation analysis for m.5512A > G mutation was evaluated with a total of 20 different vertebrate species using the same approach as described in previous study [16], and the conservation index was calculated based on 43 primate sequences from GenBank by using the MitoTool project (http://www.mitotool.org) [19].

2.4. Statistical analysis

Continuous variables were expressed as mean ± SD, and discrete variables in groups were expressed as frequency. Data were tested for normality using the one-sample Kolmogorov-Smirnov test (2-tailed). The Levene's test was applied to evaluate the equality of variances. Non-parametric methods (Mann-Whitney *U* test and two-sample Kolmogorov-Smirnov test) and parametric method (Independent samples *t*-test) were performed between maternally-related members and non-maternal members. Discrete variables were analyzed by Fisher's exact test (two-tailed). The increasing rate of systolic blood pressure and heart rate were compared between different groups by using general linear model. All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA), and $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Clinical features of the family with MIEH

The proband (II:2) began suffering from hypertension at the age of 37 years. Her highest BP[systolic blood pressure (SBP)/diastolic blood pressure (DBP)] was 205/100 mmHg (Table 1), and cannot be controlled with calcium channel blocker, angiotensin-receptor blocker and hydrochlorothiazide. Routine ECG showed axis leaning left and atrial premature beats. The ET had to be truncated because of fatigue and dyspnea, while the results showed no evidence of cardiac ischaemia, although her BP and pulse rose above expected values. The exercise data showed a marked reduced oxygen uptake response and a markedly reduced metabolic equivalent suggestive of an underlying abnormality of muscle energy metabolism (Table 2). The echocardiogram showed LA enlargement, IVS thickening and E/A ratio abnormality (Supplementary Table S1), which were the most common changes of cardiac abnormalities in hypertension. Degeneration of aorta's compliance such as AASI (0.65), S-AASI (0.53) and PWV (13.5 m/s) were identified. Laboratory assessment showed high levels of TG and low levels of HDL cholesterol.

As shown in Supplementary Figure S1, this familial history is

Table 1
Detailed Clinical information for members in family EH28.

Subject	Gender	Maternal members of proband	Age of test (years)	Age of onset (years)	Age of death (years)	SBP (mmHg)	DBP (mmHg)	Diagnosis	Cause of death
I:1	M	No	–	–	59	–	–	GU	UGIH
I:2	F	Yes	–	No detail	59	–	–	EH	Stroke
II:1	F	Yes	–	–	25	–	–	RHVD	AHF
II:2	F	Yes	77	37	–	205	100	EH	–
II:3	M	No	–	–	73	–	–	2DM	DN
II:4	F	Yes	–	43	46	–	–	EH	Stroke
II:5	M	No	76	–	–	130	75	Normal	–
III:1	M	Yes	57	40	–	220	110	EH, 2DM	–
III:2	F	No	52	–	–	125	70	Normal	–
III:3	F	Yes	56	41	–	200	100	EH, CHS	–
III:4	M	No	61	–	–	100	60	Normal	–
III:5	M	Yes	55	–	–	135	70	Normal	–
III:6	F	No	53	–	–	110	70	Normal	–
III:7	F	Yes	46	43	–	200	110	EH	–
III:8	F	Yes	44	–	–	130	70	Normal	–
III:9	M	Yes	40	–	–	135	80	Normal	–
IV:1	M	No	30	–	–	100	60	Normal	–
IV:2	M	Yes	32	–	–	115	65	Normal	–
IV:3	F	No	29	–	–	95	60	Normal	–

Note: F: female; M: male; SBP: systolic blood pressure; DBP: diastolic blood pressure; GU: gastric ulcer; UGIH: upper gastrointestinal hemorrhage; RHVD: rheumatic valvular heart disease; AHF: acute heart failure; EH: essential hypertension; 2DM: 2-diabetes mellitus; DN: diabetic nephropathy; CHS: cerebral hemorrhagic stroke.

consistent with a maternal inheritance. Only the offspring of affected mothers had hypertension, none of the offspring of affected fathers had hypertension. Among the 19 subjects in this family, 5 individuals died (3 maternal members and 2 non-maternal members); living individuals included 8 maternal members (4 hypertensives and 4 normotensives) and 6 non-maternal members (6 normotensives). Comprehensively medical examination for all members in this family showed no sign for hearing problem, vision impairments, muscular diseases and neurological disorders. There is no evidence that any member of this family had any other known cause to account for hypertension. As shown in Supplementary Figure S1 and Table 1, the penetrance of this Chinese hypertension family is 54.55% (6/11), and the sex ratio is 1:5 (male: female). The onset age in this family is from thirty years old to forty years old. The subject III:1 experienced the hypertension (BP was 220/110 mm Hg) at the age of 40 years, whereas his sisters (III:3 and III:7) had hypertension (BP were 200/100 mm Hg and 200/110 mm Hg) at the age of 41 years and 43 years, respectively. The subject I:2 and her daughter II:4 died of sudden death caused by stroke, and the subject III:3 also experienced cerebral hemorrhagic stroke with paralysis on the right side of the body at the age of 46 years.

3.2. Whole mtDNA sequencing revealed one potentially pathogenic mutation m.5512A > G in the MT-TW gene

We analyzed the entire mtDNA sequence of the proband and presented sequence variation of the proband, together with related mtDNAs which belonged to same haplogroup or sharing the same private mutation in a tree (Fig. 1). The complete mtDNA sequence of the proband was deposited in GenBank under Accession No. KX762108. Analysis of the complete mtDNA sequence of the proband identified a total of 48 homoplasmic variants relative to the rCRS [17], in which 42 were haplogroup-characteristic variants, suggesting that the proband belongs to haplogroup M7b1a1e1 (Fig. 1). Among 6 private variants, two (m.523-524del and m.16320C > T) were located in the control region, two synonymous variants were located in the coding region (m.4832C > T in the *MT-ND2* gene and m.11914G > A in the *MT-ND4* gene), one was rRNA variant (m.1709G > A in the *MT-RNR2* gene) as well as haplogroup defining variant for some common

Table 2
Exercise responses data of 14 members in family EH28.

Subject	Gender	Age of test (years)	Maternal members of proband	Resting heart rate (beats/min)	Exercise duration (min)	Maximal heart rate (beats/min) (% of predicted)	Maximal SBP (mm Hg)	METs	VO_{2max} (mL/kg/min)	Heart rate reserve (%)
II:2	F	77	Yes	72	6:45	123(86)	190	4.30	15.05	71.83
II:5	M	76	No	68	11:50	129(90)	160	7.00	24.50	80.26
III:1	M	57	Yes	75	7:35	146(90)	200	4.60	16.10	80.68
III:2	F	52	No	81	9:28	151(90)	150	5.70	19.95	80.46
III:3	F	56	Yes	72	8:16	147(90)	180	4.60	16.10	81.52
III:4	M	61	No	86	12:39	144(91)	130	9.00	31.50	79.45
III:5	M	55	Yes	81	11:50	148(90)	160	7.00	24.50	79.76
III:6	F	53	No	80	14:39	150(90)	130	10.10	35.35	80.46
III:7	F	46	Yes	75	12:10	141(81)	144	7.50	26.25	66.67
III:8	F	44	Yes	76	9:43	158(90)	150	6.30	22.05	82.00
III:9	M	40	Yes	80	11:45	165(92)	160	7.00	24.50	85.00
IV:1	M	30	No	68	18:04	171(90)	130	13.50	47.25	84.43
IV:2	M	32	Yes	94	9:33	175(93)	140	5.90	20.65	86.17
IV:3	F	29	No	84	16:50	172(90)	130	13.40	46.90	82.24

Note: F: female; M: male; SBP: systolic blood pressure; METs: metabolic equivalents; VO_{2max}: maximum oxygen consumption.

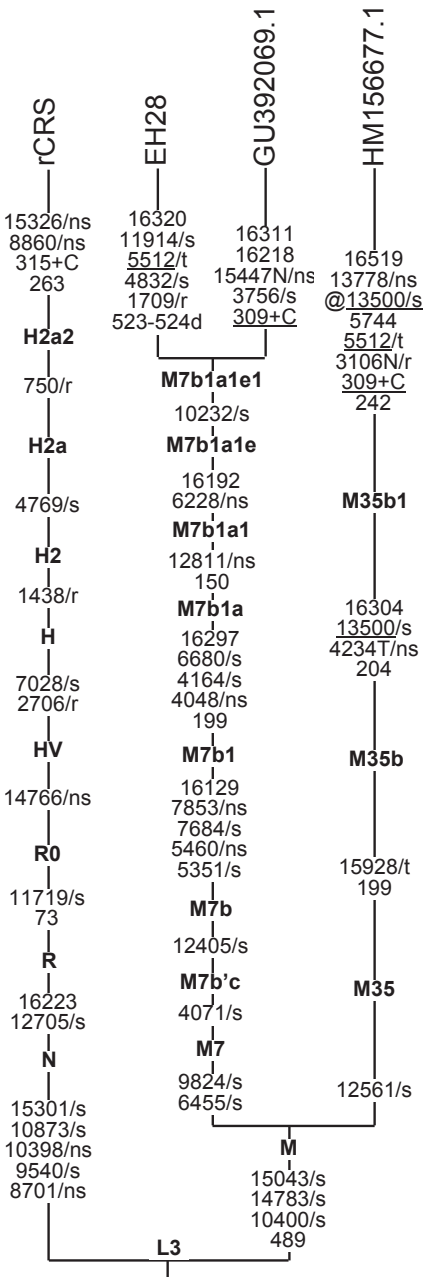

Fig. 1. Haplogroup tree of the proband mtDNA and two near-matched sequences from published sources. Sequences GU392069.1 and HM156677.1 were retrieved from GenBank. Sequence variation was scored relative to the revised Cambridge reference sequence (rCRS) [17]. The order of mutations on each uninterrupted branch section is arbitrary. Recurrent variations are underlined; back mutations are underlined and marked by "@". Suffix "G" refers to transversion and "+C" indicates an insertion of cytosine. The synonymous and non-synonymous coding-region variants in each mtDNA are further denoted by "/s" and "/ns", respectively. Variations in the ribosomal RNA genes are denoted by "/r", "/t" indicates the variation occurs in the tRNA gene.

mtDNA haplogroups, one was tRNA variant (m.5512A > G in the *MT-TW* gene) (Table 3). All these private variants were previously reported and were widely distributed in the general population except for m. 5512A > G, which showed low frequency in the general population (Fig. 2A and Table 3). Variant m. 5512A > G is located in the 1st position of the *MT-TW* gene, which changes a highly conserved position (CI = 88.0%) of the acceptor (AA) stem of $tRNA^{Trp}$ and may affect the steady-state level of $tRNA^{Trp}$ according to previous studies [21], indicating the potential pathogenicity of this mutation (Fig. 2B–C).

3.3. Carriers with mutation m.5512A > G showed left ventricular remodeling

In order to further verify the association between m.5512A > G and MIEH, we compared the clinical data between m.5512A > G carriers and non-carriers. No significant differences were observed in blood biochemical examination and blood routine examination. Other factors including age, gender, smoking, body mass index (BMI), waist hip ratio (WHR) were similar between the maternal members (m.5512A > G carriers) and non-maternal members (non-carriers) (Supplementary Table S2 and Table S3).

As compared with non-carriers, the m.5512A > G carriers were significantly more likely to have cardiac hypertrophy with the reduced diastolic function, as shown by significantly reduced levels of LA, IVS, LVPW and LVMI in mutation carriers (Fig. 3A–D and Supplementary Table S2). Considering left ventricular hypertrophy is one of the most important organ damage targets in hypertension, we also evaluated the association of echocardiographic parameters of left ventricular remodeling with normotensive carriers. Results showed that normotensive carriers also present significant thickness of LVPW than individuals without mutations (Fig. 3A–D and Supplementary Table S3), indicating that mutation m.5512A > G was significantly associated with the left ventricular remodeling, which was independent of disease status.

3.4. Carriers with mutation m.5512A > G showed increased arterial stiffness

The 24 h pulse pressure (24hPP), S-AASI and AASI derived from ABPM were considered as important index of arterial stiffness and PWV was the golden standard of arterial stiffness. We compared the ABPM parameters and PWV between the maternal and non-maternal members by using both non-parametric tests and parametric tests. The maternally-related members had a significantly higher value of 24hPP, day pulse pressure (dPP), night pulse pressure (nPP), S-AASI, AASI and PWV ($P < 0.05$) than the non-maternal members, which indicates arterial stiffness occurred significantly in the m.5512A > G carriers (Fig. 3E–H and Supplementary Table S2). This association persisted after excluding the mutation carriers with hypertension (Fig. 3E–H and Supplementary Table S3), suggesting that mutation m.5512A > G was directly related to arterial stiffness, irrespective of the hypertension status.

3.5. Carriers with mutation m.5512A > G showed exercise intolerance

None of the proband or her maternal relatives had previously been diagnosed with myopathy, but as shown in Table 2, Fig. 4A–B and Supplementary Table S2, submaximal ET (modified Bruce protocol) showed that the m.5512A > G-positive subjects had significantly lower treadmill exercise duration and oxygen consumption (Fig. 4A–B). Accordantly, the increasing rate of SBP and HR were significantly higher in m.5512A > G carriers than in non-carriers at lower stages of exercise (Fig. 4C–D). These results indicated that m.5512A > G carriers in the maternal lineages were more likely to have decrease exercise tolerance and oxygen consumption, which were probably attributed to the decreased ATP production and mitochondrial dysfunction caused by mtDNA mutation m.5512A > G.

Table 3
Private mt-tRNA and mt-rRNA variants in family EH28.

Family	Haplogroup	Nucleotide variant	Gene	Conservation index[a]	Frequency in mitotool	Reported (population context)[b]	Reported (disorder context)[b]	Haplogroup specific variant[c]
EH28	M7b1a1e1	m.1709G > A	*MT-RNR2*	0.08	82/16420	Yes	No	Yes(M76a, D4j3a, A5b1 et al.)
EH28	M7b1a1e1	m.5512A > G	*MT-TW*	0.88	3/16420	Yes	No	No

[a] The conservation analysis was evaluated on 43 primate sequences from GenBank automatically by the MitoTool project (http://www.mitotool.org) [19].
[b] The search was performed on Aug 24, 2016 following the same strategy described in Bandelt et al. [26], e.g., both 'A5512G mtDNA' and '5512A > GmtDNA' were queried.
[c] The column "Haplogroup-specific variant" refers to the presence or absence of the corresponding variants in the world mtDNA phylogeny displayed at http://www.phylotree.org (mtDNA tree Build 17; 18 Feb 2016) [18]. In round brackets we indicate the haplogroup status as it defined in that tree.

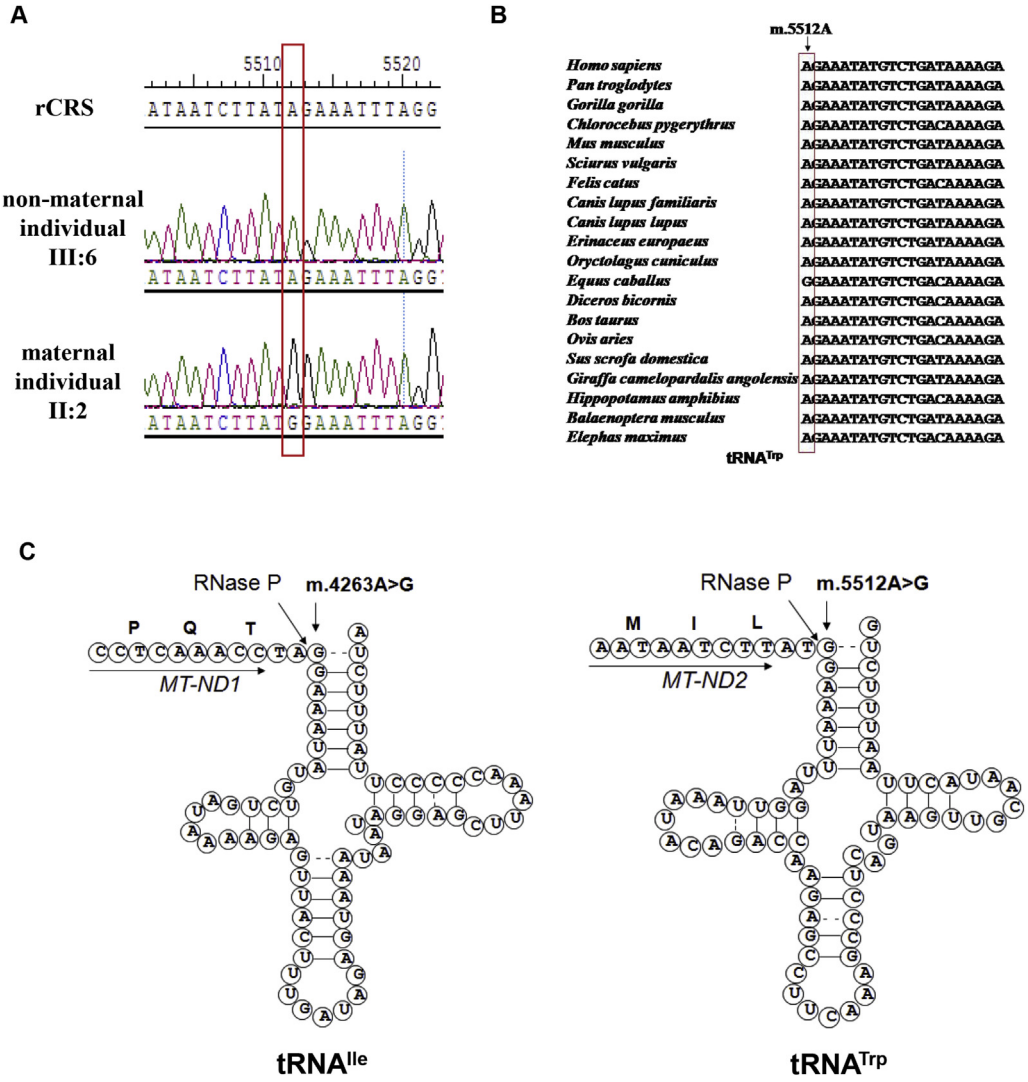

Fig. 2. Genetic analysis of the m.5512A > G mutation in the mitochondrial tRNA^{Trp}. (A) The sequence chromatograms of the tRNA^{Trp} gene from an affected individual (II-2) and a non-maternal individual (III-6). Arrow indicates the base change at position 5512. (B) Evolutionary conservation analysis of the private mtDNA variants m.5512A > G. mtDNAs of twenty different vertebrate species, including human (Homo sapiens, GenBank accession number NC_012920) chimpanzee (*Pan troglodytes*, NC_001643.1), gorilla (*Gorilla gorilla*, NC_001645.1), monkey (*Chlorocebus pygerythrus*, NC_009747.1), mouse (*Mus musculus*, NC_005089.1), squirrel (*Sciurus vulgaris*, NC_002369.1), cat (*Felis catus*, NC_001700.1), dog (*Canis lupus familiaris*, NC_002008.4), wolf (*Canis lupus lupus*, NC_009686.1), hedgehog (*Erinaceus europaeus*, NC_002080.2), rabbit (*Oryctolagus cuniculus*, NC_001913.1), horse (*Equus caballus*, NC_001640.1), rhinoceros (*Diceros bicornis*, NC_012682.1), cattle (*Bos taurus*, NC_006853.1), sheep (*Ovis aries*, NC_001941.1), pig (*Sus scrofa* domestica, NC_012095.1), giraffe (*Giraffa camelopardalis angolensis*, NC_012100.1), hippo (*Hippopotamus amphibius*, NC_000889.1), blue whale (*Balaenoptera musculus*, NC_001601.1), and Elephant (*Elephas maximus*, NC_005129.2) were aligned and compared by using the Cluster W method in MegAlign of DNASTAR Lasergene 7.1 (DNAS Inc, Madison, WI, USA). (C) The location of the m.5512A > G mutation in the mitochondrial tRNA^{Trp} and m.4263A > G mutation in the mitochondrial tRNA^{Ile}. Position 5512 and 4263 were marked by an arrow. Cloverleaf structure of human mitochondrial tRNA^{Ile} and tRNA^{Trp} is derived from Helm et al. [21]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

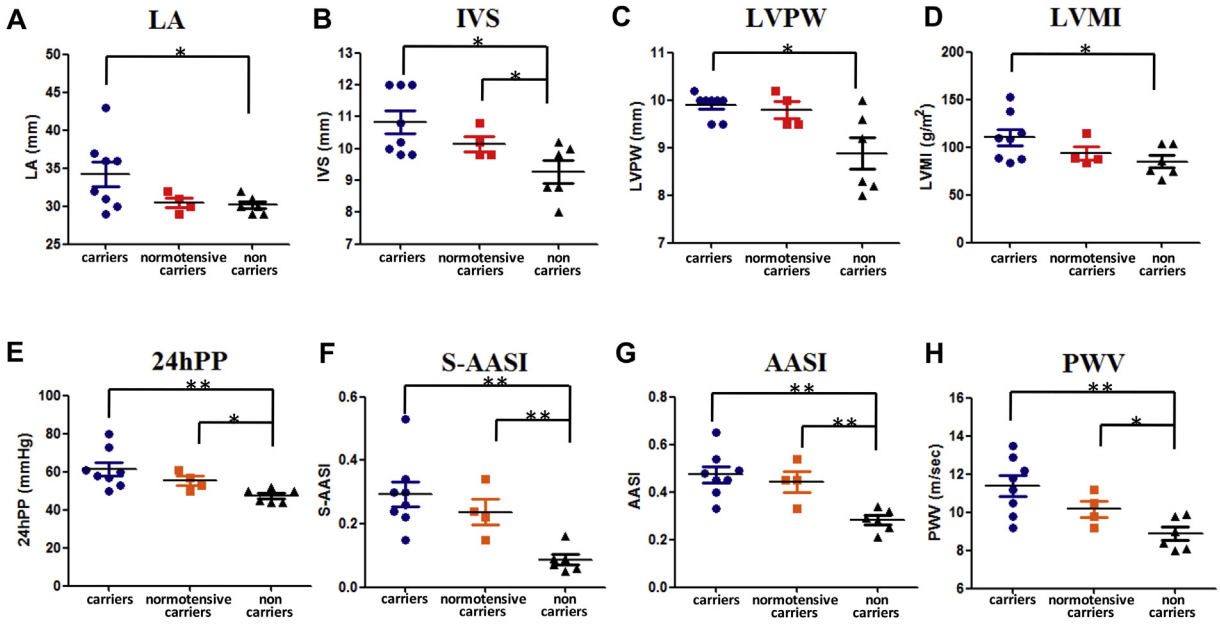

Fig. 3. Change of echocardiographic parameters and arterial stiffness index between m.5512A > G carriers and non-carriers. LA: left atrium diameter; IVS: interventricular septum thickness; LVPW: left ventricular posterior wall thickness; LVMI: left ventricular mass index. 24hPP: 24 h pulse pressure; S-AASI: symmetric ambulatory arterial stiffness index; AASI: ambulatory arterial stiffness index; PWV: pulse wave velocity. The difference between two groups was calculated by using Independent samples *t*-test with SPSS 16.0. A *P* value < 0.05 was marked by a "*", and a *P* value < 0.01 was marked by a "**".

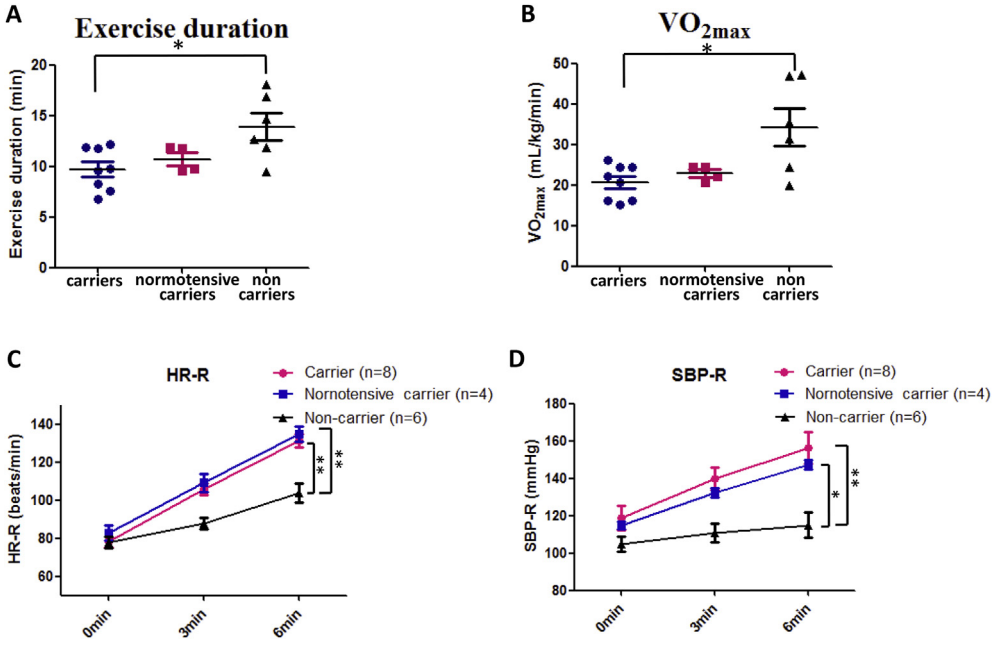

Fig. 4. Comparison of Exercise responses data between m.5512A > G carriers and non-carriers. VO_{2max}:maximum oxygen consumption; HR-R: the increasing rate of heart rate; SBP-R: the increasing rate of systolic blood pressure. (A–B) The difference between two groups was calculated by using Independent samples *t*-test with SPSS 16.0. (C–D) The difference between two groups was calculated by using general linear model with SPSS 16.0. A *P* value < 0.05 was marked by a "*", a *P* value < 0.01 was marked by a "**".

4. Discussion

In the present study, we characterized clinical manifestations and the complete mitochondrial genome of a Chinese family with MIEH. The mitochondrial tRNA (mt-tRNA) genes comprise only a small fraction of the mitochondrial genome, but are responsible for more than half of pathogenic mtDNA point mutations [22]. The pathogenic mutations can affect tRNAs at various levels, including their structure, 3'-end maturation, posttranscriptional modifications, aminoacylation, complex formation with translation factors, association with ribosomes, and the decoding process [23].

This study showed for the first time that mtDNA mutation m.5512A > G may associate with the pathogenesis of hypertension in the Chinese Dai population. We have found several lines of evidence supporting the causal role of the m.5512A > G variant in MIEH. First, it was localized in a hot-spot region of tRNAs. After

reviewing the published tRNA mutations with pathogenicity, we found that the majority of pathogenic mutations in tRNAs are located in stem structures (68%–73%), with mutation hotspots in the anticodon and the aminoacyl acceptor stems [21]. Second, evolutionary conservation analysis indicated that adenine at the 1st nucleotide in human *MT-TW* gene is a highly conserved nucleotide throughout evolution in 43 primate species and with high conservation index (CI = 88.0%). Third, this variant disrupts a highly conserved Watson-Crick base pairing in all mammalian mt-tRNAs [21], which might decrease stabilization of tRNA (Fig. 2C). Fourth, according to Mitotool database, the frequency of m.5512A > G in reported worldwide mtDNAs was very low (3/16420), and we could not find this variant in specific motifs of known haplogroup (Table 3). Fifth, the arterial stiffness and left ventricular remodeling of the members carrying mutation m.5512A > G was significantly increased than that of non-maternal members without this mutation (Fig. 3 and Supplementary Table S2), even after excepting hypertensive patients of maternal member. Coincidently, vascular remodeling caused by mutation m.14484T > C also were observed in maternal member of MIEH [4]. Finally, the mutation m.5512A > G may cause mitochondrial defects in that exercise intolerance (the short increases in heart rate from rest to maximum, significantly lower treadmill exercise duration and oxygen consumption) was observed in mutation carriers (Fig. 4). All these findings strongly indicated the potential pathogenic effect of mutation m.5512A > G in the development of MIEH.

Imitation of the present study was that the molecular mechanisms mediating m.5512A > G mutation to cause EH was not determined. One plausible explanation for the pathogenicity of these tRNA mutations may be based on the disruption of structure and steady state level of tRNA [9]. It is possible that mutations located in the same position in the cloverleaf structure of different tRNA may resulted in similar clinical phenotypes, as reviewed in Ref. [9]. Interestingly, one previous study reported that the homoplasmic m.4263A > G mutation, which located in the 1st nucleotide at the acceptor (AA) stem of the $tRNA^{Ile}$ cloverleaf structure, may be implicated in the pathogenesis of MIEH in a Chinese family [6]. Functional assessment of m.4263A > G revealed that this mutation may affect the $tRNA^{Ile}$ precursor cleavage and result in reduced steady-state level of $tRNA^{Ile}$ as well as the rate of mitochondrial translation [6]. Furthermore, m.4263A > G mutation was found to affect the mitochondrial Ca^{2+} cycling [24,25]. Similarly, mutation m.5512A > G identified in MIEH patients in this study also located in the 1st nucleotide at the acceptor (AA) stem of the $tRNA^{Trp}$, we thus infer that this mutation may also affect the steady state level of $tRNA^{Trp}$ and lead to mitochondrial dysfunction. Consistent with this inference, carriers with m.5512A > G showed significantly decreased oxygen consumption and exercise duration, indicating that mitochondrial dysfunction due to m.5512A > G mutation would be responsible for MIEH in this family.

In conclusion, mtDNA mutation m.5512A > G was identified as a potentially pathogenic mutation that may cause mitochondrial dysfunction, which might further exert a deleterious effect on oxygen consumption, exercise duration and arterial stiffening, and finally result in hypertension. Future studies with functional assessment of this mutation at cellular and animal level will be essential to uncover the role of m.5512A > G in the development of MIEH.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2016.09.129.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2016.09.129.

Conflict of interest

None declared.

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Supplementary material

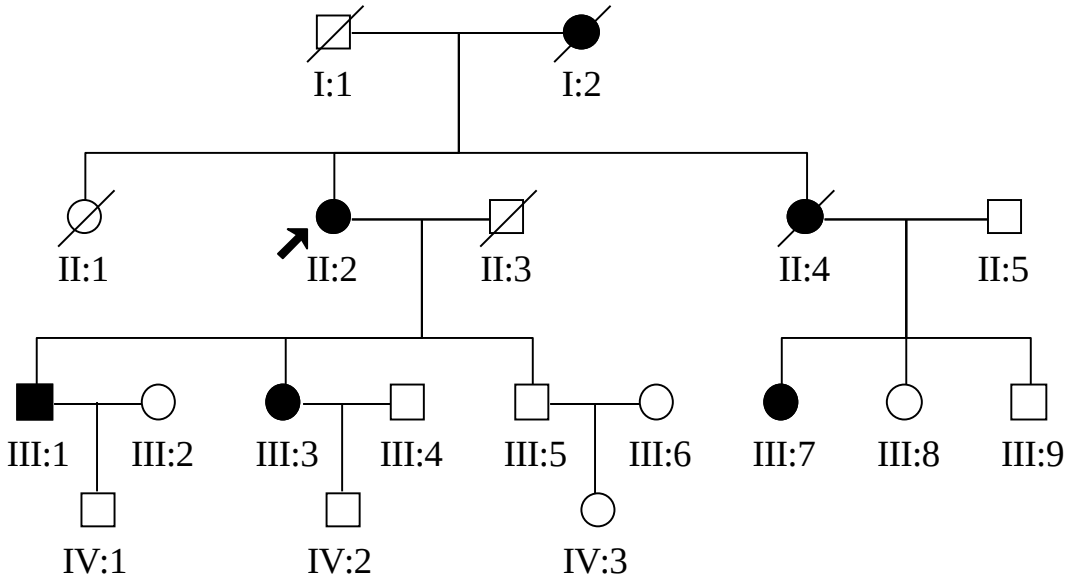

Figure S1. Pedigree information for a Dai Chinese family (family EH28) with maternally inherited EH. Affected individuals were marked by filled symbols. The proband that was analyzed for the complete mtDNA sequence was marked by an arrow.

Table S1. Echocardiographic data of 14 members in family EH28

Subject	Gender	Age at test (year)	LVd (mm)	LA (mm)	IVS (mm)	LVPW (mm)	LVMI (g/m^2)	EF (%)	E (m/s)	A (m/s)	E/A
Maternal members of proband											
II:2	F	77	47.00	37.00	12.00	10.00	152.26	68.80	0.58	1.04	0.56
III:1	M	57	44.00	36.00	12.00	10.00	108.22	69.60	0.75	0.91	0.82
III:3	F	56	48.00	43.00	12.00	10.00	137.72	73.30	0.95	1.10	0.86
III:5	M	55	42.00	30.00	9.80	9.50	83.65	63.70	0.95	0.66	1.44
III:7	F	46	43.00	36.00	10.00	10.00	109.42	75.60	0.74	0.91	0.81
III:8	F	44	40.00	29.00	9.80	9.50	88.39	70.30	0.89	0.66	1.35
III:9	M	40	47.00	32.00	10.80	10.00	114.89	66.90	0.76	0.52	1.46
IV:2	M	32	41.00	31.00	10.20	10.20	87.36	61.20	0.82	0.65	1.26
Non-maternal members of proband											
II:5	M	76	45.00	32.00	10.00	10.00	103.21	63.60	0.85	0.65	1.31
III:2	F	52	41.00	29.00	8.80	8.20	74.25	60.50	0.98	0.56	1.75
III:4	M	61	46.00	31.00	10.20	9.60	104.08	65.40	1.00	0.75	1.33
III:6	F	53	38.00	30.00	8.00	8.00	65.51	65.30	1.20	0.78	1.54
IV:1	M	30	44.00	30.00	9.80	9.20	85.60	68.20	0.96	0.65	1.48
IV:3	F	29	39.00	29.00	8.80	8.30	75.91	66.70	0.91	0.70	1.30

LVd: left ventricular diastolic diameter; LA: left atrium diameter; IVS: interventricular septum thickness; LVPW: left ventricular posterior wall thickness; LVMI: left ventricular mass index; EF: ejection fraction; E: early mitral inflow velocity; A: late mitral inflow velocity.

Table S2. Clinical features of all 14 members in family EH28

Parameter	Maternal members (*n*=8)	Non-maternal members (*n*=6)	*P*-value [a]	*P*-value [b]	*P*-value [c]
General information					
Age, years	50.88±13.67	50.17±18.17	0.935	0.796	0.841
Female (%)	4 (50)	3 (50)	1.000	1.000	1.000
Smoking (%)	4 (50)	3 (50)	1.000	1.000	1.000
BMI, kg/m^2	24.26±2.22	23.92±1.78	0.759	0.699	0.983
WHR	0.85±0.05	0.84±0.05	0.710	0.650	0.932
Biochemical values					
FBS, mmol/L	4.43±0.38	5.01±0.67	0.064	0.071	0.267
TC, mmol/L	4.26±0.47	4.56±0.33	0.212	0.245	0.591
TG, mmol/L	1.97±1.07	1.90±1.05	0.904	0.897	0.841
HDL, mmol/L	1.05±0.26	1.21±0.25	0.250	0.219	0.841
LDL, mmol/L	2.31±0.54	2.47±0.44	0.553	0.796	0.983
UA, μmol/L	354.13±77.10	311.50±52.44	0.268	0.606	0.358
BUN, mmol/L	4.58±0.81	3.88±0.54	0.098	0.121	0.194
Cr, μmol/L	88.63±12.86	85.67±11.18	0.661	0.846	0.932
K	3.94±0.23	3.92±0.26	0.932	0.605	0.841
Na	140.70±1.57	138.92±1.68	0.064	0.120	0.267
Cl	105.59±1.51	105.83±1.66	0.777	0.651	0.983
Routine blood tests					
RBC	5.15±0.45	5.02±0.54	0.646	0.796	0.983
HGB	160.75±20.42	153.67±17.47	0.499	0.605	0.721
PLT	230.50±100.57	187.67±50.46	0.361	0.519	0.983
WBC	7.15±1.97	6.40±2.01	0.502	0.519	0.841
NEUT	60.01±6.99	64.12±14.13	0.486	0.439	0.467
LYM	31.45±6.04	28.30±11.71	0.523	0.606	0.932
MONO	6.28±1.68	6.05±2.07	0.826	0.698	0.998
BASO	0.29±0.24	0.18±0.12	0.343	0.454	0.983
EO	1.98±0.97	1.35±1.41	0.342	0.155	0.194
ABPM parameters					
24hSBP, mmHg	129.25±15.73	111.67±7.31	0.027*	0.014*	0.042*
24hDBP, mmHg	67.75±7.63	64.17±4.22	0.323	0.192	0.467
24hPP, mmHg	61.50±10.10	47.50±3.56	0.007*	0.004*	0.010*
dSBP, mmHg	130.50±14.97	114.17±7.22	0.031*	0.019*	0.194
dDBP, mmHg	69.38±6.52	66.33±3.44	0.322	0.516	0358
dPP, mmHg	61.13±10.38	47.83±4.40	0.013*	0.008*	0.042*
nSBP, mmHg	120.75±17.65	105.33±8.04	0.072	0.038*	0.267
nDBP, mmHg	63.75±5.50	61.33±3.39	0.363	0.395	0.721
nPP, mmHg	57.00±13.35	44.00±5.48	0.046*	0.020*	0.042*
S-AASI	0.29±0.11	0.09±0.39	0.001*	0.003*	0.010*
AASI	0.47±0.09	0.28±0.47	0.001*	0.003*	0.010*

24hHR, bpm	74.25±5.34	74.67±5.89	0.894	0.948	1.000
Aorta's compliance					
PWV, m/sec	11.39±1.50	8.87±0.84	0.003*	0.008*	0.042*
Echocardiographic parameters					
LV_d, mm	44.00±3.02	42.17±3.31	0.302	0.300	0.721
LA, mm	34.25±4.65	30.17±1.17	0.044*	0.067	0.358
IVS, mm	10.83±1.02	9.27±0.86	0.011*	0.026*	0.358
LVPW, mm	9.90±0.26	8.88±0.83	0.029*	0.019*	0.095
LVMI, g/m^2	110.24±24.66	84.76±15.96	0.048*	0.028*	0.137
EF, %	68.68±4.73	64.95±2.67	0.110	0.071	0.137
E, m/s	0.81±0.13	0.98±0.12	0.020*	0.014*	0.095
A, m/s	0.81±0.21	0.68±0.80	0.198	0.298	0.358
E/A	1.07±0.35	1.45±0.18	0.030*	0.053	0.137
Exercise responses data					
Resting heart rate,beats/min	78.13±7.20	77.83±7.91	0.944	0.795	0.841
Exercise duration,min	9.70±2.07	13.92±3.23	0.011*	0.020*	0.095
Maximal heart rate ,beats/min	150.38±15.82	152.83±16.46	0.782	0.699	0.932
Maximal systolic blood pressure, mmHg	165.50±22.08	138.33±13.29	0.021*	0.022*	0.095
SBP*HR	24702.00±2656.84	21017.00±1652.96	0.012*	0.010*	0.010*
Metabolic equivalents	5.90±1.26	9.78±3.23	0.031*	0.027*	0.095
VO_{2max} ,mL/kg/min	20.65±4.40	34.24±11.29	0.031*	0.027*	0.095
Heart rate reserve	79.20±6.65	81.22±1.82	0.437	0.897	0.932

BMI: body mass index; WHR: waist hip ratio; FBS: fasting blood sugar; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; UA: urea acid; BUN: blood urea nitrogen; Cr: creatine; ABPM: ambulatory blood pressure monitoring; 24hSBP: 24 hour systolic blood pressure; 24hDBP: 24 hour diastolic blood pressure; 24hPP: 24 hour pulse pressure; dSBP: day systolic blood pressure; dDBP: day diastolic blood pressure; dPP: day pulse pressure; nSBP: night systolic blood pressure; nDBP: night diastolic blood pressure; nPP: night pulse pressure; S-AASI: symmetric ambulatory arterial stiffness index; AASI: ambulatory arterial stiffness index; 24hHR: 24 hour heart rate; PWV: pulse wave velocity; LV_d: left ventricular diastolic diameter; LA: left atrium diameter; IVS: interventricular septum thickness; LVPW: left ventricular posterior wall thickness; LVMI: left ventricular mass index; EF: ejection fraction; E: early mitral inflow velocity; A: late mitral inflow velocity; SBP*HR:the rate-pressure product ; VO_{2max}:maximum oxygen consumption.

* A *P* value <0.05 was marked by a star.

[a] *P*-value obtained from Independent samples *t*-test or Fisher's exact test (two-tailed).

[b] *P*-value obtained from Mann-Whitney U test or Fisher's exact test (two-tailed).

[c] *P*-value obtained from two-sample Kolmogorov-Smirnov test or Fisher's exact test (two-tailed).

Table S3. Clinical features of normotensive members in family EH28

Parameter	Maternal members (*n*=4)	Non-maternal members (*n*=6)	*P*-value [a]	*P*-value [b]	*P*-value [c]
General information					
Age, years	42.75±9.57	50.17±18.17	0.480	0.670	0.799
Female (%)	1(25)	3(50)	0.571	0.571	0.571
Smoking (%)	3(75)	3(50)	0.571	0.571	0.571
BMI, kg/m^2	24.30±1.99	23.92±1.78	0.759	0.670	0.952
WHR	0.83±0.06	0.84±0.05	0.766	0.748	0.998
Biochemical values					
FBS, mmol/L	4.37±0.47	5.01±0.67	0.140	0.088	0.388
TC, mmol/L	4.23±0.13	4.56±0.33	0.067	0.136	0.236
TG, mmol/L	1.69±1.45	1.90±1.05	0.803	0.286	0.388
HDL, mmol/L	1.13±0.23	1.21±0.25	0.589	0.593	0.952
LDL, mmol/L	2.33±0.47	2.47±0.44	0.630	0.831	0.952
UA, μmol/L	354.50±83.48	311.50±52.44	0.405	0.670	0.586
BUN, mmol/L	4.63±1.00	3.88±0.54	0.164	0.286	0.388
Cr, μmol/L	87.50±7.85	85.67±11.18	0.785	0.593	0.799
K	4.02±0.15	3.92±0.26	0.528	0.394	0.799
Na	140.00±1.48	138.92±1.68	0.328	0.388	0.799
Cl	106.23±1.31	105.83±1.66	0.703	0.915	0.952
Routine blood tests					
RBC	5.15±0.62	5.02±0.54	0.739	0.831	0.998
HGB	161.75±29.17	153.67±17.47	0.594	1.000	0.998
PLT	270.75±129.26	187.67±50.46	0.294	0.394	0.586
WBC	7.38±1.39	6.40±2.01	0.427	0.394	0.586
NEUT	57.93±2.57	64.12±14.14	0.420	0.201	0.388
LYM	33.38±2.35	28.30±11.71	0.426	0.394	0.586
MONO	5.70±1.57	6.05±2.07	0.782	0.915	1.000
BASO	0.43±0.28	0.18±0.12	0.178	0.151	0.388
EO	2.58±0.88	1.35±1.41	0.163	0.088	0.071
ABPM parameters					
24hSBP, mmHg	117.50±3.32	111.67±7.31	0.129	0.133	0.586
24hDBP, mmHg	62.25±5.19	64.17±4.22	0.537	0.829	0.799
24hPP, mmHg	55.25±4.79	47.50±3.56	0.018*	0.030*	0.134
dSBP, mmHg	119.75±2.87	114.17±7.22	0.132	0.194	0.586
dDBP, mmHg	65.50±4.36	66.33±3.44	0.744	0.519	0.388
dPP, mmHg	54.25±3.77	47.83±4.40	0.045*	0.066	0.388
nSBP, mmHg	108.00±4.69	105.33±8.04	0.570	0.388	0.799
nDBP, mmHg	59.50±4.04	61.33±3.39	0.458	0.330	0.799
nPP, mmHg	48.50±4.80	44.00±5.48	0.219	0.199	0.586
S-AASI	0.24±0.08	0.09±0.04	0.004*	0.019	0.071
AASI	0.44±0.09	0.28±0.05	0.005*	0.019	0.071

24hHR, bpm	71.50±4.93	74.67±5.89	0.402	0.333	0.799
Aorta's compliance					
PWV, m/sec	10.18±0.87	8.87±0.84	0.044*	0.069	0.236
Echocardiographic parameters					
LV_d, mm	42.50±3.11	42.17±3.31	0.877	0.748	0.952
LA, mm	30.50±1.29	30.17±1.17	0.682	0.660	1.000
IVS, mm	10.15±0.47	9.27±0.86	0.072	0.158	0.586
LVPW, mm	9.80±0.36	8.88±0.83	0.047*	0.108	0.236
LVMI, g/m^2	93.57±14.36	84.76±15.96	0.401	0.286	0.586
EF, %	65.53±3.95	64.95±2.67	0.788	0.670	0.952
E, m/s	0.86±0.08	0.98±0.12	0.100	0.055	0.236
A, m/s	0.62±0.07	0.68±0.08	0.259	0.386	0.586
E/A	1.38±0.09	1.45±0.18	0.467	0.522	0.586
Exercise responses data					
Resting heart rate,beats/min	82.75±7.80	77.83±7.90	0.480	0.667	0.952
Exercise duration,min	10.71±1.25	13.92±3.23	0.064	0.109	0.236
Maximal heart rate ,beats/min	161.50±11.39	152.83±16.46	0.061	0.394	0.799
Maximal systolic blood pressure, mmHg	152.50±9.57	138.33±13.29	0.390	0.094	0.236
SBP*HR	24570.00±1278.38	21017.00±1652.96	0.007*	0.011*	0.016*
Metabolic equivalents	6.55±0.54	9.78±3.23	0.106	0.131	0.236
VO_{2max} , mL/kg/min	22.93±1.91	34.24±11.29	0.057	0.131	0.236
Heart rate reserve	83.23±2.91	81.21±1.82	0.209	0.285	0.586

BMI: body mass index; WHR: waist hip ratio; FBS: fasting blood sugar; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; UA: urea acid; BUN: blood urea nitrogen; Cr: creatine; ABPM: ambulatory blood pressure monitoring; 24hSBP: 24 hour systolic blood pressure; 24hDBP: 24 hour diastolic blood pressure; 24hPP: 24 hour pulse pressure; dSBP: day systolic blood pressure; dDBP: day diastolic blood pressure; dPP: day pulse pressure; nSBP: night systolic blood pressure; nDBP: night diastolic blood pressure; nPP: night pulse pressure; S-AASI: symmetric ambulatory arterial stiffness index; AASI: ambulatory arterial stiffness index; 24hHR: 24 hour heart rate; PWV: pulse wave velocity; LV_d: left ventricular diastolic diameter; LA: left atrium diameter; IVS: interventricular septum thickness; LVPW: left ventricular posterior wall thickness; LVMI: left ventricular mass index; EF: ejection fraction; E: early mitral inflow velocity; A: late mitral inflow velocity; SBP*HR:the rate-pressure product ; VO_{2max}:maximum oxygen consumption.

* A *P* value <0.05 was marked by a star.

[a] *P*-value obtained from Independent samples *t*-test or Fisher's exact test (two-tailed).

[b] *P*-value obtained from Mann-Whitney U test or Fisher's exact test (two-tailed).

[c] *P*-value obtained from two-sample Kolmogorov-Smirnov test or Fisher's exact test (two-tailed).