

Mitochondrial polymorphisms in rat genetic models of hypertension

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Abstract Hypertension is a complex trait that has been studied extensively for genetic contributions of the nuclear genome. We examined mitochondrial genomes of the hypertensive strains: the Dahl Salt-Sensitive (S) rat, the Spontaneously Hypertensive Rat (SHR), and the Albino Surgery (AS) rat, and the relatively normotensive strains: the Dahl Salt-Resistant (R) rat, the Milan Normotensive Strain (MNS), and the Lewis rat (LEW). These strains were used previously for linkage analysis for blood pressure (BP) in our laboratory. The results provide evidence to suggest that variations in the mitochondrial genome do not account for observed differences in blood pressure between the S and R rats. However, variants were detected among the mitochondrial genomes of the various hypertensive strains, S, SHR, and AS, and also among the normotensive strains R, MNS, and LEW. A total of 115, 114, 106, 106, and 16 variations in mtDNA were observed between the comparisons S versus LEW, S versus MNS, S versus SHR, S versus AS, and SHR versus AS, respectively. Among the 13 genes coding for proteins of the electron transport chain, 8 genes had nonsynonymous variations between S, LEW, MNS, SHR, and AS. The lack of any sequence variants between the mitochondrial genomes of S and R rats

provides conclusive evidence that divergence in blood pressure between these two inbred strains is exclusively programmed through their nuclear genomes. The variations detected among the various hypertensive strains provides the basis to construct conplastic strains and further evaluate the effects of these variants on hypertension and associated phenotypes.

Introduction

Hypertension is a complex trait and an important risk factor for common cardiovascular diseases such as myocardial infarction, stroke, renal failure, and congestive heart failure. Changes in mitochondrial functionality are observed in parallel with progression of hypertension (Chan et al. 2009; Thomas et al. 2008; Zimmerman and Zucker 2009), which suggests that variations in the mitochondrial genome could account for differences in blood pressure. Studies on the genetic contribution to hypertension in both humans and animal models are, however, largely reported on the nuclear genome (Aneas et al. 2009; Cicila et al. 2009; Cowley 2006; Cowley et al. 2004; Deng 2007; Gilibert et al. 2009; Graham et al. 2007; Joe and Garrett 2005, 2006; Joe et al. 2009; Lee et al. 2007; Mattson et al. 2007, 2008; Moreno et al. 2003a, 2007; Saad et al. 2007a, b, 2008; Stoll et al. 2001; Toland et al. 2008a, b) with some exceptions such as recent reports of mitochondrial tRNA mutations observed in a Chinese population with essential hypertension (Li et al. 2009; Liu et al. 2009). Confirming these association studies in humans poses a significant problem that could be readily resolved using homozygous genomes of mammalian models of hypertension. Inbred rat and mouse strains with variations in their mitochondrial genomic sequences serve as good substrates for

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construction of conplastic strains as models for examining genetic contributions of the mitochondrial genome. Such limited experiments in mice and a single study in rats have reported that several complex traits are controlled by genetic elements of the mitochondrial genome (Chen et al. 2008b, c; Gregorova et al. 2008; Gusdon et al. 2007; Pravenec et al. 2007; Yu et al. 2009a, b). However, there are no reports of any studies on the mitochondrial genomic effects on blood pressure.

The goal of this report was to identify inbred rat models widely used in hypertension research as strains that can also be exploited to understand the contributions of the mitochondrial genome to the onset and progression of hypertension. Specifically, the entire mitochondrial genomes of the two selectively bred divergent models of blood pressure, the Dahl Salt-Sensitive (S) rats (also referred to as S/Jr) and the Dahl Salt-Resistant (R) rats (also referred to as R/Jr), the definitive colonies of which are maintained at the University of Toledo College of Medicine (formerly the Medical College of Ohio), two other genetically hypertensive strains, i.e., the Spontaneously Hypertensive Rat (SHR) and the Albino Surgery (AS) rat, and two normotensive strains Milan normotensive strain (MNS) and Lewis (LEW) which were previously used as parental strains for BP linkage analysis (Deng and Rapp 1995; Dukhanina et al. 1997; Garrett et al. 1998) were determined. The results obtained point to mitochondrial genomes of the S or R rat as not being contrasting, but point to comparisons between S and the hypertensive strains SHR or AS or to comparisons between S and the normotensive strains MNS and LEW as appropriate contrasting strains to study the genetic contributions of the mitochondrial genome.

In addition, during the course of our study, errors in public databases of the rat mitochondrial genome are identified and documented as a valuable reference to future investigators of the rat mitochondrial genome.

Materials and methods

Animals

All animal experiments were conducted as per preapproved protocols by the Institutional Animal Care and Use Committee (IACUC) of the University of Toledo College Of Medicine (UTCOCM). All rats were between 2 and 3 months of age and maintained on a low-salt (0.4% NaCl) diet. Dahl Salt-Sensitive (S) rats and Dahl Salt-Resistant (R) rats were from our colony maintained at UTCOCM. The Spontaneously Hypertensive Rat strain (SHR/Hsd/Mco), referred to as SHR, was originally obtained from Harlan Sprague-Dawley (Indianapolis, IN) and maintained in our

colony. Albino Surgery (AS) rats were obtained previously (Garrett et al. 2002) from the National Institute for Medical Research (Mill Hill, UK). The Lewis (LEW/NCrIBR) rats referred to as LEW, were originally obtained from Charles River Laboratories (Wilmington, MA). The Milan Normotensive Strain (MNS) was originally obtained from Veterinary Resources Branch, National Institutes of Health (Bethesda, MD). Both LEW and MNS were maintained in our animal facility.

Isolation of DNA and sequencing

DNA was extracted from tail biopsies using the Promega Wizard kit (Promega, Madison, WI). Polymerase chain reaction (PCR) was carried out using 23 pairs of primers to amplify the entire mitochondrial genome. Each set of primers was designed using the primer 3 program (<http://frodo.wi.mit.edu/primer3/>) and attached with M13 tags to amplify PCR products that were approximately 500–1000 bp. A list of primer sequences is provided in Supplementary Table 1. These custom primers were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). Amplified products were subsequently purified using the Qiagen PCR purification kit (Qiagen, Valencia, CA). The purified products were sequenced by MWG DNA Sequencing Services (High Point, NC) and analyzed using the software Sequencher v4.9 (Gene Codes Corp., Ann Arbor, MI). All mitochondrial DNA sequences of the S, R, SHR, and AS rats from the current study were deposited in GenBank (Accession Nos. GU997608—SS/Jr, GU997611—SR/Jr, GU997609—AS, GU997610—SHR, HM152027—LEW, HM152028—MNS).

Results

Comparisons of mitochondrial genome sizes

Mitochondrial DNA of the three hypertensive strains studied differed in their overall sizes (Table 1). The mitochondrial DNA of S and R rats, which were both derived from Sprague-Dawley rats, were the shortest (among the strains tested), with 16,309 bp. The strains derived from Wistar rats, i.e., SHR, MNS, LEW, and AS, had mitochondrial genomes longer than that of the strains derived from the Sprague-Dawley rats and ranged from 16,312 to 16,316 bp (Table 1).

Strainwise comparisons of mitochondrial variations

All sequence data obtained from the current study were compared with the reference BN sequence (AC_000022.1). Note that the sequence data obtained from the mtDNA of

Table 1 Comparisons of total mitochondrial genome sizes

Strain	mtDNA size (bp)
Brown Norway (BN)	16,313
Dahl Salt-Sensitive (SS/Jr)	16,309
Dahl Salt-Resistant (SR/Jr)	16,309
Spontaneously Hypertensive Rat (SHR)	16,312
Albino Surgery (AS)	16,314
Lewis (LEW)	16,316
Milan Normotensive Strain (MNS)	16,316

both the hypertensive Dahl S and the relatively normotensive R rats were identical. Both MNS and LEW mtDNA sequences were identical except for a synonymous variation at position 8844 (position number compared to the BN as reference sequence) where a guanine in LEW was substituted by an adenosine in MNS. Variations were observed among all the hypertensive strains compared. Specifically, 106 variations in mtDNA were observed between two of the most commonly used hypertensive rat models, S and SHR. Of these, 11.3% were nonsynonymous variants, 26.4% were within noncoding RNA gene sequences, and 62.3% were synonymous variants (Fig. 1). Likewise, the mtDNA sequence of the hypertensive AS rat was compared with that of the S rat. A total of 106 variants were detected between S and AS. Among these, 11.3% were in the gene-coding regions, 27.4% were within non-coding RNA gene sequences, and 61.3% were synonymous variants (Fig. 1). There were only 16 variants between SHR and AS mtDNA sequences. Of these, 37.5% were within gene-coding sequences, 18.8% were within non-coding RNA sequences, and 43.7% were synonymous variants (Fig. 1).

Nonsynonymous variants

Among the 13 protein-coding genes of the mtDNA, nonsynonymous variations were observed between S, SHR, and AS in eight genes (Table 2). The SHR and AS forms of NADH-coenzyme Q oxidoreductase gene subunit 2 (*mt-Nd2*) were conserved except at nucleotide position

3956, resulting in substitution of Ala/Val¹⁸ in SHR/AS. The *mt-Nd2* sequence of the S rat differs from both the SHR and AS rats resulting in five and four amino acid substitutions, respectively. Prominent among these substitutions is the deletion of a histidine residue (His³¹⁶) in the S rat compared to SHR and AS. The amino acid sequences of the other subunits, *mt-Nd3* and *mt-Nd5*, are identical between S and SHR, which differs from that of the AS rat. Isoleucine⁹ of *mt-Nd3* in S and SHR is replaced by Thr⁹ in AS rats. Threonine³⁷ of *mt-Nd5* in S and SHR is replaced by Ala³⁷ in AS rats.

Four nonsynonymous variants were detected within the *mt-Nd4* gene. Three of these variants resulted in Ile/Thr²³, Ala/Thr³⁵⁶, and Val/Ile⁴⁰¹ substitutions between S and SHR (Table 2). A significant substitution at amino acid 419 from leucine in both S and SHR to proline in the AS was noted (Table 2). Gene sequence for *mt-Nd6* was conserved between SHR and AS, with only one amino acid substitution from Val/Ile¹³⁹ observed in S rats. All the other genes were also conserved between SHR and AS. The S, however, differed from both the SHR and AS with three nonsynonymous substitutions in each of the genes coding for cytochrome c-oxidase subunit-2 (*mt-Cox-2*), F⁰-F¹-ATP synthase (*mt-Atp-6*), and the subunit of ubiquinol cytochrome-c-reductase (*mt-Cyt b*) (Table 2).

The protein-coding sequences of the two normotensive strains MNS and LEW are similar to that of the AS except for one substitution within the *mt-Nd3* gene (Table 2).

Variations in genes for ribosomal and transfer RNA

When compared with the other genetically hypertensive and normotensive strains, S and R rat mitochondrial DNA coding for 12s rRNA differs from that of SHR, AS, MNS, and LEW at two positions, 935 and 942 bp (Table 3). The S rat 16s rRNA sequence is also considerably different from that of the SHR (13 variants) and AS (15 variants) (Table 3). Both of the normotensive strains MNS and LEW were identical in their 16s rRNA sequences but were different from that of the S (17 variants) (Table 3).

Fig. 1 Overall strainwise comparisons of mitochondrial genomic variations. Sequences obtained from all strains were compared with S as the reference sequence

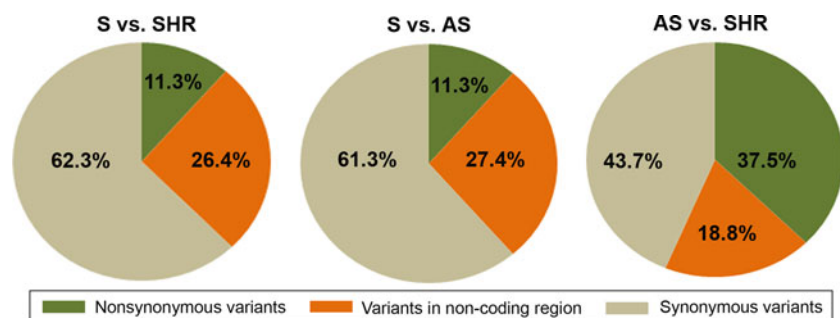


Table 2 Mitochondrial DNA variants predicted to cause amino acid substitutions in rat models used in genetic analysis of hypertension

Gene	mtDNA location (bp) BN ^a	Triplet codon					Amino acid					Amino acid No.
		S/R	SHR	AS	MNS	LEW	S/R	SHR	AS	MNS	LEW	
<i>mt-Nd2</i>	3956	GTA (3956)	GCA (3956)	GTA (3958)	GTA (3960)	GTA (3960)	Val	Ala	Val	Val	Val	18
<i>mt-Nd2</i>	4352	AAC (4352)	AGC (4352)	AGC (4354)	AGC (4356)	AGC (4356)	Asn	Ser	Ser	Ser	Ser	150
<i>mt-Nd2</i>	4696	GCA (4696)	ACA (4696)	ACA (4698)	ACA (4700)	ACA (4700)	Ala	Thr	Thr	Thr	Thr	265
<i>mt-Nd2</i>	4814	ACA (4814)	ATA (4814)	ATA (4816)	ATA (4818)	ATA (4818)	Thr	Met	Met	Met	Met	304
<i>mt-Nd2</i>	4855	: (4854.1)	CAC (4855)	CAC (4857)	CAC (4859)	CAC (4859)	:	His	His	His	His	316
<i>mt-Cox2</i>	7498	ATC (7494)	GTC (7497)	GTC (7499)	GTC (7501)	GTC (7501)	Ile	Val	Val	Val	Val	165
<i>mt-Atp6</i>	8021	GAA (8017)	AAA (8020)	AAA (8022)	AAA (8024)	AAA (8024)	Glu	Lys	Lys	Lys	Lys	35
<i>mt-Nd3</i>	9476	ATT (9472)	ATT (9475)	ACT (9477)	ATT (9479)	ATT (9479)	Ile	Ile	Thr	Ile	Ile	9
<i>mt-Nd4</i>	10227	ATC (10223)	ACC (10226)	ACC (10228)	ACC (10230)	ACC (10230)	Ile	Thr	Thr	Thr	Thr	23
<i>mt-Nd4</i>	11225	GCA (11221)	ACA (11224)	GCA (11226)	GCA (11228)	GCA (11228)	Ala	Thr	Ala	Ala	Ala	356
<i>mt-Nd4</i>	11360	GTC (11356)	ATC (11359)	GTC (11361)	GTC (11363)	GTC (11363)	Val	Ile	Val	Val	Val	401
<i>mt-Nd4</i>	11415	CTA (11411)	CTA (11414)	CCA (11416)	CCA (11418)	CCA (11418)	Leu	Leu	Pro	Pro	Pro	419
<i>mt-Nd5</i>	11844	ACT (11840)	ACT (11843)	GCT (11845)	GCT (11847)	GCT (11847)	Thr	Thr	Ala	Ala	Ala	37
<i>mt-Nd6</i>	13647	ATT (13643)	GTT (13646)	GTT (13648)	GTT (13650)	GTT (13650)	Ile	Val	Val	Val	Val	139
<i>mt-Cytb</i>	14775	GAC (14771)	AAC (14774)	AAC (14776)	AAC (14778)	AAC (14778)	Asp	Asn	Asn	Asn	Asn	214

^a mtDNA sequence in NCBI (GenBank accession No. AC-000022.1 or AY172581; <http://www.ncbi.nlm.nih.gov/nuccore/26983975?report=genbank>). Data presented was collected by setting BN as the reference sequence and aligning all strain sequences in a single contig using the Sequencher software v4.9

Genes for tRNA are conserved among S, SHR, and AS, except for tRNA for four amino acids that were polymorphic in the S compared with SHR and AS. Three variations were detected within the tRNA for cysteine and one each for tRNA sequences of tyrosine, aspartic acid, and threonine (Table 3). Within the functionally important D-loop region of mtDNA, four variants were noted between S and SHR at positions 15,460, 15,549, 15,589 and 16,313 (Table 3). The AS sequence was identical to the S at 15,460 but identical to the SHR at 15,549, 15,589, and 16,313 (Table 3). MNS and LEW sequences for all tRNAs are identical to that of the SHR and AS.

Comparisons with published hypertensive rat mitochondrial genomic sequences

There are two other published mtDNA sequences of different stocks of S rats used in hypertensive research, i.e., that of SS_{MCW} (Schlick et al. 2006) and that of DS (Chen et al. 2008a). A single variant was detected between S and the reported mtDNA sequence of SS_{MCW} (C to A, respectively, DQ673914) at 11,334 bp of the S mitochondrial genome [the location corresponds to 11,338 bp in the reference BN mtDNA (GenBank accession No. AC_000022.1)]. However, there were seven variants between the mtDNA sequences of S and the published DS (Table 4). Besides the S rat, the sequence of the SHR obtained was compared with the available mtDNA sequence of SHR/OlaIpcv (Pravenec et al. 2007). There are no variants between SHR sequenced

in our laboratory and the published SHR mtDNA (Pravenec et al. 2007).

Finally, a short note on an inconsistency that we have resolved for future reference: The GenBank accession number of the BN (BN/SsNHsdMCW) strain is cited in previous references (Chen et al. 2008a; Pravenec et al. 2007; Schlick et al. 2006) as AC_000022. However, the current record (November 2009) of AC_000022 in GenBank is that of the Wistar rat, which when compared to the BN rat mtDNA (BN/SsNHsdMCW) harbors 330 variants (data not shown). The correct accession numbers and their historical derivatives of these accession numbers is presented clearly in Table 5 to serve as a future reference for investigators.

Discussion

Inbred rat strains previously have been used extensively in our laboratory to study the contributions of their nuclear genomes to hypertension (Joe and Garrett 2005, 2006; Joe et al. 2009; Saad et al. 2007a, b, 2008; Toland et al. 2008b). The purpose of this study was to define combinations of these rats that can be exploited to study the genetic contributions of the mitochondrial genome to hypertension. The lack of any sequence variants between the mitochondrial genomes of S and R rats provides conclusive evidence that divergence in blood pressure between these two inbred strains is programmed exclusively through their nuclear

Table 3 Variations in mitochondrial genes for ribosomal and transfer RNA in inbred rat strains

Gene	Nucleotide location BN ^a	Single nucleotide polymorphism				
		S/R ^b	SHR	AS	MNS	LEW
rRNA (12-s)	935	A (935)	G (935)	G (935)	G (935)	G (935)
rRNA (12-s)	942	C (942)	T (942)	T (942)	T 942)	T (942)
rRNA (16-s)	1130	A (1130)	C (1130)	C (1130)	C (1130)	C (1130)
rRNA (16-s)	1136	: (1135.1)	C (1136)	C (1136)	C (1136)	C (1136)
rRNA (16-s)	1136.1	: (1135.2)	C (1137)	C (1137)	C (1137)	C (1137)
rRNA (16-s)	1136.2	: (1135.3)	: (1137.1)	C (1138)	C (1138)	C (1138)
rRNA (16-s)	1136.3	: (1135.4)	: (1137.2)	C (1139)	C (1139)	C (1139)
rRNA (16-s)	1136.4	: (1135.5)	: (1137.3)	: (1139.1)	C (1140)	C (1140)
rRNA (16-s)	1136.5	: (1135.6)	: (1137.4)	: (1139.2)	C (1141)	C (1141)
rRNA (16-s)	1137	A (1136)	C (1138)	C (1140)	C (1142)	C (1142)
rRNA (16-s)	1223	A (1222)	G (1224)	G (1226)	G (1228)	G (1228)
rRNA (16-s)	1248	C (1247)	T (1249)	T (1251)	T (1253)	T (1253)
rRNA (16-s)	1521	G (1520)	A (1522)	A (1524)	A (1526)	A (1526)
rRNA (16-s)	1585	T (1584)	C (1586)	C (1588)	C (1590)	C (1590)
rRNA (16-s)	1653.1	A (1653)	: (1654.1)	: (1656.1)	: (1658.1)	: (1658.1)
rRNA (16-s)	1653.2	C (1654)	: (1654.2)	: (1656.2)	: (1658.2)	: (1658.2)
rRNA (16-s)	1716	T (1717)	C (1717)	C (1719)	C (1721)	C (1721)
rRNA (16-s)	1832	G (1833)	A (1833)	A (1835)	A (1837)	A (1837)
rRNA (16-s)	2170	T (2171)	C (2171)	C (2173)	C (2175)	C (2175)
tRNA (Cys)	5200	A (5196)	G (5199)	G (5201)	G (5203)	G (5203)
tRNA (Cys)	5202	A (5198)	G (5201)	G (5203)	G (5205)	G (5205)
tRNA (Cys)	5237	T (5233)	A (5236)	A (5238)	A (5240)	A (5240)
tRNA (Tyr)	5269	G (5265)	C (5268)	C (5270)	C (5272)	C (5272)
tRNA (Asp)	6978	G (6974)	A (6977)	A (6979)	A (6981)	A (6981)
tRNA (Thr)	15333	G (15329)	A (15332)	A (15334)	A (15336)	A (15336)
D-Loop region	15460	C (15456)	T (15459)	C (15461)	C (15463)	C (15463)
D-Loop region	15549	C (15545)	T (15548)	T (15550)	T (15552)	T (15552)
D-Loop region	15589	A (15585)	G (15588)	G (15590)	G (15592)	G (15592)
D-Loop region	16313	A (16309)	G (16312)	G (16314)	G (16316)	G (16316)

^a mtDNA sequence in NCBI (GenBank accession No. AC-000022.1 or AY172581; <http://www.ncbi.nlm.nih.gov/nuccore/26983975?report=genbank>)

^b S and R rat mtDNA sequences are identical

genomes. The S and R rats originally were bred selectively from an outbred stock of Sprague-Dawley rats for differences in blood pressure in response to a salt diet. Because the mitochondrial genomes are maternally inherited, the identical mitochondrial genomic sequence between S and R indicates that they may have been derived from a single female rat. This inference is consistent with the documentation in the rat genome database (<http://rgd.mcw.edu>) that the outbred stock of Sprague-Dawley rats was indeed originally initiated in 1925 by R. Dawley at the Sprague-Dawley Company in Madison, WI, by breeding a hybrid hooded male of unknown origin to a white female and subsequently to his white female offspring for seven generations. The data also suggest that either during the selection process or during inbreeding and thereafter, no

polymorphisms/mutations have accumulated to date in the mitochondrial genomes of either S or R rats.

Rat mitochondrial genomic polymorphisms have been reported for two other S rat stocks that are used extensively in research on the genetics of hypertension (Chen et al. 2008a; Schlick et al. 2006). These are the Dahl S rats maintained at the Medical College of Wisconsin (SSM_{mcw}) and in Japan (Dahl-Iwai S or DS). Unfortunately, the inbred S rats commercially available from Harlan Sprague-Dawley were genetically contaminated in 1992-1993 (Lewis et al. 1994; St Lezin et al. 1994). Although a test of commercial stocks indicated that this problem was apparently corrected (Walder et al. 1996), recent genome-wide single nucleotide polymorphism data indicate that the nuclear genome of at least one of the S rat subsets, the

Table 4 Mitochondrial DNA variations between different stocks of Dahl salt-sensitive rats

S (S/Jr)	SS _{MCW}	DS(Iwai)	BN ^a mtDNA location (bp)
A	A	DEL	1,130
DEL	DEL	A	1,131
A	A	T	1,209
C	C	DEL	4,848
C	C	DEL	4,849
A	A	DEL	4,850
T	T	C	11,799
C	A	Unknown	11,334

S = sequence data presented in the current report from the original stock of Dahl salt-sensitive (S) rat at the University of Toledo (formerly Medical College of Ohio); SSMCW = sequence data from the Dahl salt-sensitive rat at the Medical College of Wisconsin (Schlick et al. 2006); DS(Iwai) = Dahl salt-sensitive rat data from Chen et al. (2008a); DEL = deletion

^a Positions obtained from the mtDNA sequence in NCBI (GenBank accession No. AC-000022.1 or AY172581, <http://www.ncbi.nlm.nih.gov/nuccore/26983975?report=genbank>)

SSMCw, is 2.52% polymorphic compared to the original Dahl S rat inbred at our institution (S compared to SSMCW at <http://rgd.mcw.edu>). Contrary to these polymorphisms reported on their nuclear genomes, data from the current study suggests that mitochondrial genomes of S rats inbred and maintained in our laboratory and SSMCW are nearly identical with only one variation, which may perhaps be attributed to an error in sequencing. The mitochondrial genome of the Dahl-Iwai S rat having more variations compared to either S or SSMCW remains puzzling. Unlike the S or SSMCW, which represent the lineage of inbred rats by Rapp and Dene (1985), the Dahl-Iwai S was inbred by Iwai and Heine (1986). Because all of the S and R rats originated from Sprague-Dawley rats, which in turn originated from a single female rat, it is likely that any variants observed between the mtDNA of stocks of S rats either represent errors in sequencing or mutations that have accumulated since their inbreeding.

For the purpose of assessing the contributions of the mitochondrial genomes to hypertension, the variations observed between the genetically hypertensive strains are more relevant than the variations observed between hypertensive and relatively normotensive strains. Some of these variations were nonsynonymous and within genes coding

proteins of the electron transport chain. These variants may or may not contribute to the status of hypertension but are nevertheless useful for dissecting key cellular mechanisms. For instance, molecules of the TCA cycle, including fumarate and succinate, are reported as being linked to the genetics of hypertension (Papadopoulos and Papademetriou 2009; Robben et al. 2009; Sadagopan et al. 2007; Tian et al. 2009; Toma et al. 2008), whereas mutations in *mt-Nd2* are reported to play a critical role in the production of mitochondrial reactive oxygen species (Gusdon et al. 2007), and a mutation in *mt-Atp6* is reported in the rat to cause age-related impaired glucose tolerance, a hallmark of type 2 diabetes mellitus (Mathews et al. 1995, 1999). The identification of four to five different nonsynonymous variants between S and SHR or AS provides the opportunity to examine the effects of these variants of *mt-Nd2* on levels of mitochondrial reactive oxygen species. Likewise, a distinct variation in *mt-Atp6* of Glu in S to Lys in either SHR or AS could be further investigated in the context of glucose tolerance.

Significant changes are observed also within tRNA genes, providing unique opportunities to assess and validate these variants as quantitative trait nucleotides for hypertension, especially because of recent associations reported between mitochondrial tRNA genes and a number of traits including hypertension, hypercholesterolemia, hypomagnesemia, and hypertrophic cardiomyopathy (Li et al. 2009; Liu et al. 2009).

Phylogenetically, the S and SHR are a large distance from each other (Thomas et al. 2003). The S is derived from non-Wistar Sprague-Dawley rats, whereas both the SHR and the AS rat are derived from Wistar rats. Interestingly, all of the variants between S and SHR/AS detected in our study are also reported as highly conserved variants between other non-Wistar and Wistar-derived strains (Abhyankar et al. 2009). One of the variable regions among the Wistar-derived strains is at position 1137 of 16s rRNA, wherein the number of C residues ranges between 2 to 5 (Abhyankar et al. 2009). The SHR strain reported in our study has two C residues, which is different from the SHR/Mol rat which is reported to have five C residues from 1137 (Abhyankar et al. 2009). The significance of these stretches of variable numbers of C residues in Wistar-derived strains remains to be determined.

The AS rat is believed to be a subline of the genetically hypertensive (GH) rat (<http://rgd.mcw.edu>). This is

Table 5 Clarification of GenBank accession numbers of rat mtDNA

Strain	GenBank accession no.			mtDNA length (bp)
	Current	Alternative	Origin of sequence	
BN (BN/SsNHsdMCW)	AC_000022.2	AY172581	GI:110189662	16,313
Wistar	AC_000022.1	AC_000022	X14848	16,300

reflected in the sequence of the mitochondrial genomes of AS and GH rats, which are identical. The stretch of four C residues described above is also identical in both AS and GH rats.

Construction of conplastic strains, wherein mitochondrial DNA from one strain is substituted with that of another strain, offers a good design by which to study a complex trait's genetic contribution of the mitochondrial DNA independent of the nuclear DNA. From the data presented it is clear that constructing conplastic strains between S and R is a moot point. This does not mean that the mitochondrial genomes of these strains do not harbor any elements that control the trait of BP. The data collected also suggest that such elements, if any, can be traced even among hypertensive strains by constructing and testing conplastic strains between S or R as one of the parental strains and SHR or AS as the other parental strain. These designs can be expected to be valuable tools to dissect the contributions of the mitochondrial genome to mechanisms of hypertension and other complex polygenic traits.

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