Complete mtDNA genomes reveal similar penetrances of maternally inherited type 2 diabetes in two Chinese families

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Abstract

Previous work suggests that mitochondrial DNA (mtDNA) derived from the maternal genome has a close affinity with type 2 diabetes. This would support a familial pattern for type 2 diabetes. Thereby, we analyzed complete mtDNA genomes from two families, A and B, from Southwest China that demonstrated maternally inherited type 2 diabetes. Our data support that mtDNA lineages from families A and B belong to haplogroups A4 and D4h1, respectively. This suggests that maternally inherited type 2 diabetes with similar penetrances can arise in Chinese individuals with strikingly different maternal genetic backgrounds. Two private coding region mutations (G13759A in MT-ND5 and G15930A in tRNA-Thr) were identified in family B. Further evolutionary and phylogenetic analyses suggest that both these mutations have multiple origins and are unlikely to be disease causing.

Keywords

diabetes, haplogroup, mitochondrial DNA, multiple origins, private mutations

Introduction

Diabetes mellitus is a complex disease with its major form, type 2 diabetes, characterized by hyperglycemia in the context of insulin resistance and a relative lack of insulin action (Association, 2008; Lin & Sun, 2010). Type 2 diabetes has become a global public health concern in the past two decades with prevalence predicted to be approximately 300 million by 2025 (King et al., 1998). It is estimated there are 113.9 million Chinese adults with diabetes and 493.4 million that are pre-diabetics in 2013 (Xu et al., 2013).

In view of the crucial role of mitochondria in energy production and the required glucose utilization for insulin release, systemic mitochondrial dysfunction would be expected to lead to reduced insulin release and persistent hyperglycemia (Lowell et al., 2005; Rolo & Palmeira, 2006). Mitochondria have a significant role in cellular energy metabolism, and decreases in the capacity of the mitochondrial oxidative phosphorylation system are associated with insulin resistance in older people and in offspring of individuals with type 2 diabetes (Goto et al., 1990; Shoffner et al., 1990). Patients with mutations in their mitochondrial DNA (mtDNA) that cause diabetes, what is increasingly referred to as mitochondrial diabetes mellitus (mtDM), account for more than approximately 1.5% of reported world-wide diabetes cases (Holt et al., 1990). Specific mtDNA variants and/or mtDNA haplogroups are associated with type 2 diabetes (Poulton et al., 2002). For example, the mutation 3243A>G was suggested as a diabetogenic factor that shows no racial exclusivity (Suzuki et al., 1997; Zhong et al., 2000). However, mtDNA haplogroups N9a and D4 have been observed in Asian populations, and haplogroup J1 in west Eurasian groups, serve as protective factors providing resistance to the development of diabetes (Bodmer & Bonilla, 2008; O’Rahilly et al., 2005; Tanaka et al., 2013). In contrast, haplogroup B4 in Asians and haplogroup J/T in Europeans are considered to be risk factors for diabetes (Crispim et al., 2006; Liou et al., 2012).

Although large-scale case–control studies are a popular research approach, family-based studies based on individuals with the same genetic background are a promising method for identifying rare disease variants (Liou et al., 2012; Suzuki et al., 1997). To date, many genetic variants in mitochondria have been identified from family studies (e.g., 3243A>G, 3271T>C (Goto et al., 1990); 3460G>A, 8344A>G, and 8356T>C (Shoffner et al., 1990); 8993T>G (Holt et al., 1990); 3243A>G (Van den Ouweland et al., 1992); 11778G>A (Wallace et al., 1988); 14484T>C (Johns et al., 1992); 14709T>C (Hao et al., 1995); and 1555A>G (Prezant et al., 1993)) that have proved to be causative in various energy metabolism disorders. Thereby, we examined two Chinese families that show maternal inheritance of type 2 diabetes with similar levels of penetrance (61.5% and 58.3%). We hypothesized that mutations in mtDNA to be similar in these two Chinese families with varying maternal genetic backgrounds.

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Materials and methods

Study design

Two families, 96 unrelated individuals with T2DM and 90 unrelated healthy individuals, were collected from the Department of Endocrinology, the Second People's Hospital of Yunnan Province. Informed consents conforming to the tenets of the Declaration of Helsinki and following the guidance of sample collection of Human Genetic Disease (863 programs) by the Ministry of Public Health of China were obtained from each participant prior to the study. The institutional review boards of the Second People's Hospital of Yunnan Province and Yunnan University approved this study. Diagnosis and classification of diabetes were based on clinical features, laboratory data, and the guidelines of the Expert Committee Report of the American Diabetes Association.

DNA extraction, mtDNA genome sequencing, and data quality control

Genomic DNA was extracted from whole blood using the standard phenol/chloroform method. Complete mtDNA genome sequences were amplified and then sequenced according to the reported protocol (Wang et al., 2008). The entire mtDNA sequences of two probands (family A: III:7 and family B: III:4) were sequenced. For family A, the maternal incidence rate of type 2 diabetes for females was significantly higher than that of males with a proportion of 8:0, which was significantly higher than that of family B (ratio = 5:2) (Chi-square test, p < 0.05). Therefore, we also sequenced complete mtDNA sequences from all other sampled maternal members in family A (six females [II:1, II:7, II:9, II:13, II:14, and III:7]) and two males [II:3 and III:6]) to determine whether the differences in type 2 diabetes incidence were associated with a gender preference. In total, nine complete mtDNA sequences from samples of family A (n = 8) and B (n = 1) were sequenced and deposited into GenBank under accession numbers KF898135, KF898137–KF898140, and KF898142–KF898145.

Population screening

The mtDNA susceptible locus G13759A was screened in 96 unrelated individuals with type 2 diabetes and 90 unrelated healthy individuals from Southwest China by sequencing. Sequences were obtained after amplification with the primers L13612 (5'-AAGCGCCTATAGCCTCGAA-3') and H14591 (5'-AAGCCTTCTTCATT TATGG-3') (Wang et al., 2008).

Data analysis

Variation in the complete mtDNA sequences was scored relative to the revised Cambridge reference sequence (rCRS) (Andrews et al., 1999). The hypervariable loci 309insC, 315insC, and T16519C were disregarded. The status of the mtDNA haplogroup of the two pedigrees was determined using the observed variations with reference to the most recently updated PhyloTree [http://www.phylotree.org/; version: Build 16; retrieve date: 2 January 2014]. We searched for the presence of private mutations, which occur at the tips of the mtDNA tree, from greater than 22,514 available (near) complete mtDNAs from across the world (including MITOMAP [http://www.mitomap.org/; version: r98; retrieve date: 2 January 2014], mtDB [http://www.mtdb.igp.uu.se/; version: Mar 1, 2007; retrieve date: 2 January 2014], or mtSNP database [http://mtSNP.tmig.or.jp/mtSNP/index_e.shtml; version: Jan 1, 2006; retrieve date: 2 January 2014]) to discern the novelty or recurrent status of the private mutations as previously described (Bandelt et al., 2009).

The following evolutionary analyses were performed on the new sequences. Conservation of particular human mtDNA variations, from the mtSNP and NCBI database, were estimated by comparisons with sequences from multiple vertebrate species (Table 1). The predicted impact of the amino acid substitutions on the structure and function of the proteins was assessed using the PolyPhen tool (http://genetics.bwh.harvard.edu/pph/) (Adzhubei et al., 2013).

Results

Clinical features

Both the families have relatively high penetrance levels of type 2 diabetes, more than 50%. Family A, including 26 subjects (13 maternal and 13 non-maternal members), and family B, containing 17 subjects (12 maternal and 5 non-maternal members), were recruited (Figure 1). Type 2 diabetes cases were diagnosed as having a history of hyperglycemia (plasma glucose > 7.0 mmol/L, fasting; Appendix A) at the Second People’s Hospital of Yunnan Province, Kunming, China. The average ages at onset of diabetes were 40 ± 12 and 33 ± 24 years in families A and B, respectively. The maternal penetrances of type 2 diabetes within these two families, 61.5% (8/13) and 58.3% (7/12) for families A and B, respectively, being similar. For family A, 88.89% (8/9) of the maternal type 2 diabetes patients were female. In family B, all members of generation II had type 2 diabetes as well as the two sons of member II:5 (who died of diabetes at a young age (36 years old)), where both sons of II:5 had early onset type 2 diabetes (both diagnosed at 9 years of age). We also examined 96 unrelated individuals with type 2 diabetes and 90 unrelated healthy individuals from Southwest China. These subjects were sampled randomly without age and gender preference.

mtDNA tree and evolutionary analysis

We scored out 40 variants in the complete mtDNA genome of the proband (III:7) of family A compared with rCRS (Andrews et al., 1999), which allowed it to be assigned o haplogroup D4h1c based on the 37 characteristic variants (Figure 2). However, the complete mtDNA genomes of eight members of family A were identical, which complied with maternal inheritance. For family B, a total of 32 variants in the complete mtDNA sequence of the proband (III:4) were identified, allowing it to be assigned to haplogroup A4 (Figure 2).

Table 1. The private mutations in the mitochondrial protein-coding regions detected for family B: III:4.

| Locus      | Nucleotide position | Nucleotide change | Amino acid change | Conservation among mammalian species | Grantham value | Asian type 2 diabetes | Asian | mtDB (%) | MITOMAP (%) |
|------------|---------------------|-------------------|-------------------|--------------------------------------|----------------|------------------------|-------|----------|-------------|
| MT-ND5     | 13759               | G>A               | Ala 475 Thr       | 31 (31/61, 51%)                      | 58             | 4                      | 24    | 1.40     | 3.38        |
| tRNA-thr   | 15930               | G>A               | No                | 2 (2/17, 12.5%)                      | –              | 0                      | 13    | 1.37     | 2.88        |

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Figure 1. Pedigree information for two Chinese families with maternally inherited type 2 diabetes. Affected individuals were marked by filled symbols. The sequenced families members were marked by an arrow. Penetrances of two families showed by the ratio of maternally inherited affected individuals and maternally inherited individuals.

To further investigate the potential roles of mutation in the mtDNA on type 2 diabetes in these families, we constructed a phylogenetic tree incorporating 20 additional complete mtDNA genomes retrieved from PhyloTree (three from type 2 diabetes individuals and the others from healthy individuals) to identify private mutations in these two pedigrees. A single private variation (G16213A), located in the control region, was detected in the proband (III:7) in family A. For family B, eight private variants were detected in the proband (III:4), including three in the control region (C16214T, G16274A, and C16527T) and two coding region variants (G13759A in MT-ND5 and G15930A in tRNA-Thr). Searches for these variants among more than 24,187 complete mtDNA genomes (MITOMAP [http://www.mitomap.org/]) found that all these private mutations had been previously detected.

We also screened 96 type 2 diabetes patients and 90 unrelated healthy individuals for the variation G13759A to indentify its distribution frequency. The G13759A mutation was found at a slightly lower frequency (4.1%; 4/96) in the sampled type 2 diabetes population than that of observed in the control group (4.4%, 4/90) (Table 2). The haplogroup status of the G13759A mutation was then confirmed by sequencing the entire control region sequences. The four type 2 diabetes patients with G13759A carriers was then confirmed by sequencing the entire control region sequences. The four type 2 diabetes patients with G13759A could be assigned to haplogroups F1b, F1a, and M10a, while the four healthy individuals with G13759A were from haplogroups F1a, N9a, and A4c (Table 2).

**Discussion**

Based on scored variants, the mtDNA lineages of families A and B can be assigned into haplogroups D4h1c and A4, respectively (Figure 2). The sequences of the complete mtDNA genomes from different members of family A were identical, which suggests that the female preference type 2 diabetes pathogenic feature observed in this family was not related to variation in the mtDNA. These results also indicate that type 2 diabetes with significant maternal inheritance, and similar levels of penetrance, can originate from different maternal backgrounds.

In this study, we detected two coding region private variants for family B, G15930A in tRNA-Thr and G13759A in MT-ND5. We were highly interested in their impacts. For the variant G15930A, it was located at an extra loop in the secondary structure of the tRNA (Appendix B) and did not alter a classic Watson–Crick base pair (Pei et al., 2014). A low conservation index (12.5%) was identified by a comparison of mtDNA genomes from 24 diverse vertebrate species for this site (Table 1 and Appendix B). With the database of 24,187 mtDNA genomes (MITOMAP), the variant was also found that distributed among many haplogroups including: B, C, D, E, F, H, J, K, L, M1, M13, M18, M5, N, P, Q, R, T, U, V, W, and X (Appendix C). These haplogroups are among Eurasian, Asian, and Africa populations (Van Oven & Kayser, 2009), which suggest that the G15930A variant may be a polymorphism which distributes all over the world populations and has multiple origins. In addition, the G15930A variant was not found in any Asians with type 2 diabetes, when the mtSNP database was searched (Table 1), but was identified in normal Chinese subjects (5/512, 0.98%; Pei et al., 2014). Although variants in mt-tRNA genes may be associated with clinical diseases (Pei et al., 2014), our result support a recent study showing that the G15930A variant might not be associated with diabetes (Qin et al., 2014).

However, the G13759A variant in MT-ND5 gene occurs at the first base of a codon and causes an amino acid change from alanine to threonine (Ala475Thr). A comparison of the mtDNA genomes from 61 diverse vertebrate species showed that this site G13759A (Ala475Thr) has low conservation (<60%). The Grantham value for this change is also relatively low (<60, Table 1) suggesting that there is only a small physicochemical difference between the original and altered amino acid residues. Low conservation (<60%) and Grantham (<60) values are in agreement with the PolyPhen prediction that this change is unlikely to cause damage or impair protein function. The presence of the G13759A variant is not a germ line mutation leading to the proband, as the presence of this mutation in family B was confirmed by screening all family members maternally related to the proband (III:4) (Appendix D). These results, thus, suggest that the G13759A change is most likely a polymorphism rather than a disease causing mutation.

The G13759A variation was also found in 717 different complete mtDNA genomes within the database of 24,187 mtDNA genomes (MITOMAP) and distributed among many haplogroups including A, B, C, D, E, F, H, J, K, L, M1, M7, M49, M71, N9, N13, R*, R0, R12, T, U, V, and W (Appendix E). These haplogroups are among West Eurasian, East Eurasian, South
Asian, and Southeast Asian populations (Van Oven & Kayser, 2009), suggesting that the G13759A mutation occurred multiple times. Searches of the mtSNP database identified this polymorphism in four Asians with type 2 diabetes and 28 individuals that are not reported to have diabetes. The G13759A mutation was also found at a slightly higher frequency (4.1%; 4/96) in the sampled type 2 diabetes Southwest China population than at the worldwide level (3%, 717/24,187); however, this frequency was not significantly different (T-test, \( p > 0.05 \)) from the frequency observed in the control group (4.4%, 4/90). Although the haplogroup status of four type 2 diabetes patients with G13759A was different from that of the four healthy individuals with G13759A, A4c, they were in line with the pattern of health individuals based on their complete mtDNA genomes. Thus, our results confirm that the G13759A polymorphism present in the maternally inherited diabetes family B is unlikely a type 2 diabetes disease causing mutation in Southwest China.

**Conclusions**

In summary, by analyzing complete mtDNA genomes from two Chinese families with maternally inherited type 2 diabetes, with similar penetrance, we found that they did not share haplogroup status or share private mtDNA mutations indicating that they have different genetic causes. Two coding region private mutations (G13759A in the *MT-ND5* gene and G15930A in *tRNA-Thr*) were identified in family B. The mutation G13759A occurs at the first base pair of the codon and causes an amino acid change from alanine to threonine. However, evolutionary and phylogenetic analyses showed that both mutations have multiple origins and are most likely polymorphisms, rather than disease causing mutations. Further genetic studies on nuclear genes and the cross talk....

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**Table 2. Distribution of m.13759G>A among two population.**

| T2DM (96/4, 4.1%) | Control (90/4, 4.4%) |
|-------------------|----------------------|
| Heplogroup | Number | Heplogroup | Number |
| F1a | 2 | F1a | 2 |
| F1b | 1 | N9a | 1 |
| M10a | 1 | A4c | 1 |
| Sum | 4 | Sum | 4 |

*aT2DM case (96) and control (90) for Southwest China.*
between mtDNA and nuclear genes should shed additional light on understanding the genetic basis of type 2 diabetes in Chinese populations.

**Declaration of interest**

The authors declare that they have no conflicts of interest. This work was supported by grants from the National Natural Science Foundation of China (No. 81260135), the training fund of Young and Middle-Aged Academic Technology leaders in Yunnan Province (No. 2011CL045) and the fund of medical leader in Yunnan Province (No. D-201217). The Youth Innovation Promotion Association, Chinese Academy of Sciences provided support to M.-S. P.

**References**

Adzhubei I, Jordan DM, Sunyaev SR. (2013). Predicting functional impact of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet. Chapter 7: Unit 20.

Andrews RM, Kubacka I, Chinnery PF, Lightowler RN, Turnbull DM, Howell N. (1999). Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23:147.

Association AD. (2008). Diagnosis and classification of diabetes mellitus. Diabetes Care 31:S55–60.

Bandelt HJ, Salas A, Taylor RW, Yao YG. (2009). Exaggerated status of ‘null’ and ‘pathogenic’ mtDNA sequence variants due to inadequate database searches. Hum Mutat 30:191–6.

Bodmer W, Bonilla C. (2008). Common and rare variants in multifactorial susceptibility to common diseases. Nat Genet 40:695–701.

Crispim D, Canani L, Gross J, Tschiedel B, Souto K, Roisenberg I. (2006). The European-specific mitochondrial cluster J/T could confer an increased risk of insulin-resistance and type 2 diabetes: An analysis of the m. 4216T>C and m. 4917A>G variants. Ann Hum Genet 70: 488–95.

Goto Y-i, Nonaka I, Horai S. (1990). A mutation in the tRNALeu (UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature 348:651–3.

Hao H, Bonilla E, Manfredi G, DiMauro S, Moraes CT. (2015). Segregation patterns of a novel mutation in the mitochondrial tRNA glutamic acid gene associated with myopathy and diabetes mellitus. Am J Hum Genet 56:1017–25.

Holt I, Harding A, Petty R, Morgan-Hughes J. (1990). A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. Am J Hum Genet 46:428–33.

Johns DR, Neufeld MJ, Park RD. (1992). An ND-6 mitochondrial DNA mutation associated with Leber hereditary optic neuropathy. Biochem Biophys Res Co 187:1551–7.

King H, Aubert RE, Herman WH. (1998). Global burden of diabetes, 1995–2025: Prevalence, numerical estimates, and projections. Diabetes Care 21:1414–31.

Lin Y, Sun Z. (2010). Current views on type 2 diabetes. J Endocrinol 204: 1–11.

Liou CW, Chen JB, Tiao MM, Weng SW, Huang TL, Chuang JH, Chen SD, et al. (2012). Mitochondrial DNA coding and control region variants as genetic risk factors for Type 2 diabetes. Diabetes 61: 2642–51.

Lowell BB, Shulman GI. (2005). Mitochondrial dysfunction and type 2 diabetes. Science 307:384–7.

O’Rahilly S, Barroso I, Wareham NJ. (2005). Genetic factors in type 2 diabetes: The end of the beginning? Science 307:370–3.

Pei H, Peng Q, Lan C, Chi Liu B. (2014). Variations in mitochondrial tRNAThr gene may not be associated with coronary heart disease. Mitochondrial DNA [Epub ahead of print]. doi: 10.3109/19401736.2014.905862.

Poulton J, Luan Ja, Macaulay V, Hennings S, Mitchell J, Wareham NJ. (2002). Type 2 diabetes is associated with a common mitochondrial variant: Evidence from a population-based case–control study. Hum Mol Genet 11:1581–3.

Prezant TR, Agapian JV, Bohlman MC, Bu X, Öztas S, Qiu W-Q, Arnos KS, et al. (1993). Mitochondrial ribosomal RNA mutation associated with both antibiotic–induced and non-syndromic deafness. Nat Genet 4:289–94.

Qin Y, Xue L, Jiang P, Xu M, He Y, Shi S, Huang Y, et al. (2014). Mitochondrial tRNA variants in Chinese subjects with coronary heart disease. J Am Heart Assoc 3:e000437.

Rolo AP, Palmeira CM. (2006). Diabetes and mitochondrial function: Role of hyperglycemia and oxidative stress. Toxicol Appl Pharm 212: 167–78.

Shoffner JM, Lott MT, Lezza A, Seibel P, Ballinger SW, Wallace DC. (1990). Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA mutation. Cell 61: 931–7.

Suzuki Y, Taniyama M, Muramatsu T, Atsumi Y, Hosokawa K, Asahina T, Shimada A, et al. (1997). Diabetes mellitus associated with 3243 mitochondrial tRNA G13708A mutation: Clinical features and coenzyme Q10 treatment. Mol Aspects Med 18:181–8.

Tanaka D, Nagashima K, Sasaki M, Funakoshi S, Kondo Y, Yasuda K, Koizumi A, Inagaki N. (2013). Exome sequencing identifies a new candidate mutation for susceptibility to diabetes in a family with highly aggregated type 2 diabetes. Mol Genet Metab 109:112–17.

Van den Ouweland J, Lemkes H, Ruitenbeek W, Sandkujl L, De Vijlder KS, et al. (1993). Mitochondrial ribosomal RNA mutation associated with Leber’s hereditary optic neuropathy. Science 258:370–3.

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza A, Eilers LJ, Nikoskelainen EK. (1988). Mitochondrial DNA mutation associated with Leber’s hereditary optic neuropathy. Science 224:1427–30.

Wang HW, Jia X, Ji Y, Kong QP, Zhang Q, Yao YG, Zhang YP. (2008). Strikingly different penetration of LHON in two Chinese families with primary mutation G11778A is independent of mtDNA haplogroup background and secondary mutation G13708A. Mutat Res-Fund Mol M 643:48–53.

Xu Y, Wang L, He J, Bi Y, Li M, Wang T, Wang L, et al. (2013). Prevalence and control of diabetes in Chinese adults. J Am Med Assoc 310:948–59.

Zhong S, Ng MC, Lo YD, Chan JC, Johnson PJ. (2000). Presence of mitochondrial tRNA G13708A to G 3243 mutation in DNA extracted from serum and plasma of patients with type 2 diabetes mellitus. J Clin Pathol 53:466–9.

**Supplementary material available online**

Supplementary Appendix A–E.