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Genetic background of people in the Dominican Republic with or without obese type 2 diabetes revealed by mitochondrial DNA polymorphism

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Abstract People in the Dominican Republic are considered to be genetically heterogeneous owing to the post-Colombian admixture of Native American, African, and European populations. To characterize their genetic background, nucleotide sequences of the D-loop region of human mitochondrial DNA (mtDNA) were examined in 33 healthy women and 50 gender-matched patients with obese type 2 diabetes (OD) from the Dominican Republic. Phylogenetic analysis of 198 mtDNA lineages including Native Americans, Africans, and Europeans enabled us to assess relative genetic contributions of the three ancestral fractions to the two groups in the Dominican Republic. In the OD group, the majority (64.0%) of the mtDNA lineages were from African ancestry, whereas the Native American fraction was predominant (51.5%) in the healthy group, with both showing smallest amounts (14.0% and 9.1%, respectively) of European contribution. This difference in maternal genetic background between the two groups was similarly demonstrated by phylogenetic analysis at the population level based on net nucleotide diversities between populations. These findings may imply ethnic-specific predisposition to OD, a possible association of an unidentified factor from African ancestry with OD in the Dominican Republic population.

Keywords Mitochondrial DNA · D-loop region · Sequence polymorphism · Dominican Republic · Population structure

The nucleotide sequence data reported are available in the DDBJ/EMBL/GenBank databases under the accession numbers AB174901–AB174983.

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Introduction

The Dominican Republic, the second-largest nation in the Caribbean, occupies the eastern two-thirds of the islands of Hispaniola in the Caribbean Sea. On the basis of historical records, people in the Dominican Republic must be genetically heterogeneous in origin because of the post-Colombian admixture of Native Americans, Africans, and Europeans. However, there has been little attempt to characterize genetic background of the Dominican Republic population, and hence we have no valid information on relative genetic contributions of three ancestral groups to their contemporary gene pool. Such genetic information is generally useful to identify gene(s) involved in ethnic-specific predisposition to complex diseases such as obesity and type 2 diabetes, the prevalence of which continues to increase in several Caribbean populations (Forrester et al. 1996; Hennis et al. 2002; Abate and Chandalia 2003). Thus, it is of interest to determine the relative frequencies of three genetic fractions from different continents in the Dominican Republic population.

For this purpose, we examined sequence polymorphism in the D-loop region of mitochondrial DNA (mtDNA) in 83 women in the Dominican Republic, which consisted of 33 healthy volunteers and 50 patients diagnosed with obese type 2 diabetes (OD). Together with other D-loop sequences from Native Americans, Africans, and Europeans, a phylogenetic analysis of nucleotide sequences from the Dominican Republic revealed the extent of matrilineal genetic contributions of the three ancestries in the healthy and OD groups. Moreover, genetic implications of the observed difference in the relative frequencies between the two groups are discussed.

Materials and methods

Subjects and DNA samples

We examined 83 women in the Dominican Republic comprised of 33 healthy volunteers ranging from 20 to 70 years (mean 39.1 ± 16.1) and 50 patients diagnosed with OD ranging from 36 to 77 years (mean 55.2 ± 10.1). The subjects were employees or outpatients of the Centro de Gastroenterologia and the Instituto Nacional de Diabetes, Endocrinología y Nutrición (INDEN) in the city of Santo Domingo in the Dominican Republic. The patients with OD had a body mass index (BMI) (kg/m^2) that ranged from 30.0 to 54.3 (mean 36.0 ± 5.6) and at the same time were diagnosed as type 2 diabetics according to the criteria of the American Diabetes Association (Report of the Expert Committee) (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997). Clinical characteristics of these two groups are summarized in Table 1. Total DNA was extracted from blood samples by the DNA extraction kit (Dr GenTLE, Cat. no. 9081, TAKARA BIO, INC., Ootsu, Japan). This study was approved by the Ethics Committee of the Graduate University of Advanced Studies (Sokendai), Hayama, the Human Genome Committee of Oita University Faculty of Medicine, and the Ethical Committees of the Centro de Gastroenterologia and the INDEN. All ethical assessments were in accordance with the principles of the Declaration of Helsinki II. Written informed consent, as approved by the Human Genome Committee, was obtained from all subjects.

Table 1 Clinical characteristics of healthy and obese diabetic subjects in the Dominican Republic

Characteristics[a]	Healthy ($n=33$)[b]	Obese diabetic ($n=50$)	P[c]
Age (years)	39.1 ± 16.1	55.2 ± 10.1	< 0.0001
BMI (kg/m^2)	24.7 ± 2.7	36.0 ± 5.6	< 0.0001
FPG (mmol/l)	4.7 ± 0.6	10.1 ± 3.7	< 0.0001
HbA_{1c} (%)	5.0 ± 0.8	8.8 ± 2.3	< 0.0001
SBP (mmHg)	120.6 ± 13.2	141.7 ± 20.9	< 0.0001
DBP (mmHg)	78.3 ± 10.5	88.5 ± 14.0	0.0024
TC (mmol/l)	5.00 ± 1.46	5.67 ± 1.46	0.049
TG (mmol/l)	1.52 ± 0.65	1.96 ± 0.93	0.030
HDL (mmol/l)	0.95 ± 0.30	0.87 ± 0.26	n.s.

[a] *BMI* body mass index, *FPG* fasting plasma glucose, HbA_{1c} hemoglobin A_{1c}, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *TC* total cholesterol, *TG* triglyceride, *HDL* high-density lipoprotein cholesterol

[b] Values are indicated as mean ± SD

[c] *P* values were calculated by unpaired *t* test; *n.s.* not significant

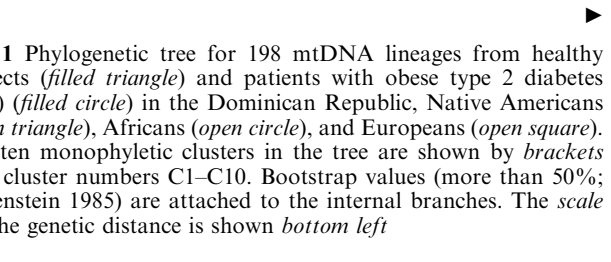

Fig. 1 Phylogenetic tree for 198 mtDNA lineages from healthy subjects (*filled triangle*) and patients with obese type 2 diabetes (OD) (*filled circle*) in the Dominican Republic, Native Americans (*open triangle*), Africans (*open circle*), and Europeans (*open square*). The ten monophyletic clusters in the tree are shown by *brackets* with cluster numbers C1–C10. Bootstrap values (more than 50%; Felsenstein 1985) are attached to the internal branches. The *scale* for the genetic distance is shown *bottom left*

Amplification and direct sequencing of the D-loop region of mtDNA

Nucleotide sequences of 482-bp fragments (positions 16129–16569 followed by 1–41 in the reference sequence of Anderson et al. 1981) were determined by direct sequencing of PCR-amplified products as described previously (Horai et al. 1996). The newly obtained sequences from the Dominican Republic have been deposited in the DDBJ/EMBL/GenBank.

Data analyses

To estimate the number of nucleotide substitutions per site between individual sequences, we used 482 nucleotide sites from the 83 Dominicans, 72 Native Americans, 23 Africans, and 20 Europeans (Horai and Hayasaka 1990; Horai et al. 1991, 1993; Vigilant et al. 1991). On the basis of the estimated number of nucleotide substitutions under the Kimura's two-parameter model (Kimura 1980), a phylogenetic tree for the 198 mtDNA sequences was constructed by using the neighbor-joining (NJ) method (Saitou and Nei 1987). We also constructed an NJ tree for human populations with net nucleotide diversities (d_A distances) between populations (Nei and Li 1979). To measure the extent of DNA polymorphisms within two groups of people from the Dominican Republic, nucleotide diversities were computed with MEGA version 2.1 (Kumar et al. 2001). Statistical comparison between the two groups was performed by using unpaired *t* test, *Z* test, or χ^2 test; $P < 0.05$ was considered significant.

Results and discussion

When analyzing nucleotide sequences of the D-loop region for 33 healthy volunteers and 50 patients with OD, we found 28 and 42 sequence types in healthy subjects and OD patients, respectively. Nucleotide

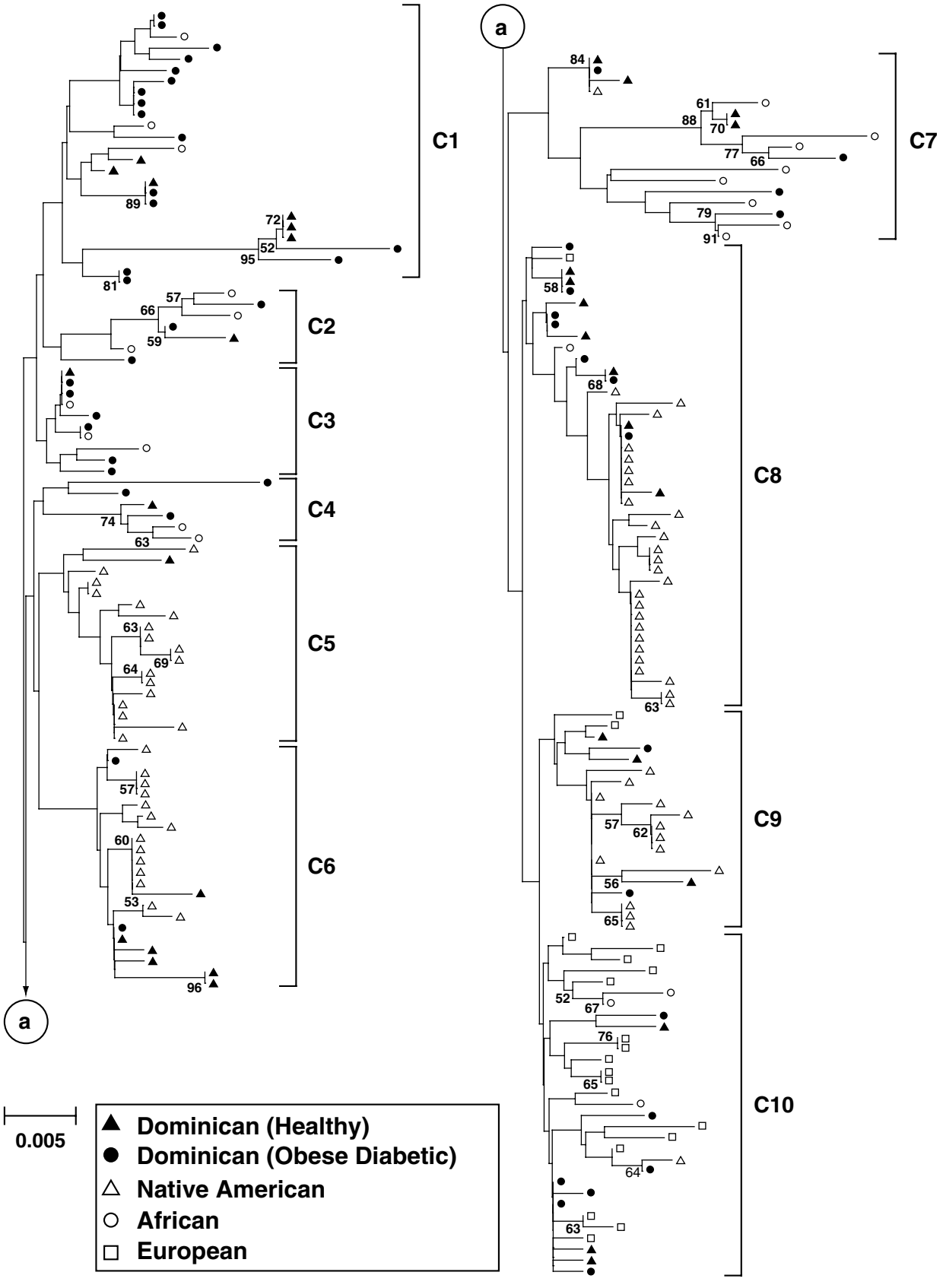
a
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
89
72
52
95
81
57
66
59
74
63
63
69
64
57
60
53
96
84
61
88
70
77
66
79
91
58
68
63
57
62
56
65
52
67
76
65
64
63
a
0.005
Dominican (Healthy)
Dominican (Obese Diabetic)
Native American
African
European

Table 2 Comparison of frequencies of ten phylogenetic clusters in five human populations. Percent frequencies of individuals, together with numbers of individuals (in parentheses), in respective clusters are indicated. The maximum percentage in each cluster is shown in bold face

Cluster	Specificity	Dominican (healthy)	Dominican (obese diabetic)	Native American	African	European
C1	Dominican (OD)-1	18.2 (6)	**32.0** (16)	0 (0)	13.0 (3)	0 (0)
C2	African-1	3.0 (1)	6.0 (3)	0 (0)	**13.0** (3)	0 (0)
C3	African-2	3.0 (1)	12.0 (6)	0 (0)	**13.0** (3)	0 (0)
C4	African-3	3.0 (1)	6.0 (3)	0 (0)	**8.7** (2)	0 (0)
C5	Native American-1	3.0 (1)	0 (0)	**23.6** (17)	0 (0)	0 (0)
C6	Native American-2	18.2 (6)	4.0 (2)	**19.4**(14)	0 (0)	0 (0)
C7	African-4	12.1 (4)	8.0 (4)	1.4 (1)	**34.8** (8)	0 (0)
C8	Native American-3	21.2 (7)	14.0 (7)	**36.1** (26)	4.3 (1)	5.0 (1)
C9	Native American-4	9.1 (3)	4.0 (2)	**18.1**(13)	0 (0)	10.0 (2)
C10	European-1	9.1 (3)	14.0 (7)	1.4 (1)	13.0 (3)	**85.0** (17)
No. of individuals studied		33	50	72	23	20

diversities within the two groups were estimated as $1.77 \pm 0.27\%$ and $1.61 \pm 0.28\%$, respectively. The difference in the two estimates for nucleotide diversities was not statistically significant ($P > 0.05$, Z test). These two values were slightly higher than those for Native American (1.29%; Horai et al. 1993) and East Asian (1.34%; Horai et al. 1996) populations, indicating high levels of DNA polymorphisms in the two groups from the Dominican Republic.

To examine mtDNA lineages observed in the Dominican Republic population, we constructed an NJ phylogenetic tree (Fig. 1) for 198 individuals including Native Americans, Africans, and Europeans (Horai and Hayasaka 1990; Horai et al. 1991, 1993; Vigilant et al. 1991). The tree revealed that the 198 mtDNA lineages could be classified into ten monophyletic clusters, named C1–C10, although this classification was somewhat arbitrary. The 83 lineages from the Dominican Republic were dispersed into every cluster, suggesting that their maternal gene pool comprised of diversified mtDNA lineages.

Table 2 shows frequency distribution of the 198 mtDNA lineages among the ten phylogenetic clusters. To examine the clustering patterns in detail, we assigned "specificity" for each cluster according to the population from which the maximum percentage of individuals was derived. The Native American population exhibited four dominant clusters (C5, C6, C8, and C9), which corresponded to the four distinct phylogenetic groups for Native Americans (Horai et al. 1993). We therefore assigned these four clusters as being specific to Native Americans and named them as Native American 1, 2, 3, and 4 (Table 2). Africans were predominant in four clusters (C2, C3, C4, and C7), whereas Europeans dominated in one cluster (C10). The OD group had one dominant cluster (C1), although individuals from the healthy group were not predominant in any cluster. Thus, all clusters except C1 exhibited the respective assigned specificities characteristic of three reference populations, as summarized in Table 2. Because cluster C1 contained mtDNA lineages only from the two groups in the Dominican Republic and African population, these could have been derived from African ancestry.

Table 3 Relative frequencies of ethnic-specific mtDNA lineages in five human populations

Presumed ancestry[a]	Frequency (%)				
	Dominican (healthy)	Dominican (obese diabetic)	Native American	African	European
Native American	51.5	22.0	97.2	4.4	15.0
African	39.4	64.0	1.4	82.6	0
European	9.1	14.0	1.4	13.0	85.0

[a]Classification is based on the assigned specificity for the phylogenetic clusters (characterized in Fig. 1)

Indeed, all of the 25 individuals in this cluster shared a characteristic combination of three transitional mutations (16223T–16278T–16390A), which could be considered as one of the D-loop sequence motifs for African-specific mtDNA (Alves-Silva et al. 2000; Torroni et al. 2001). As shown in Table 3, each of the three reference populations (Native Americans, Africans, and Europeans) was characterized by a high frequency of its own specificity (in the range of 82.6–97.2%), when cluster C1 was regarded as African specificity. This means that the assigned specificity could discriminate among the ethnic origins of the mtDNA lineages with high accuracy, although the sample numbers from African and European populations were relatively small.

We also estimated relative frequencies of three ancestral fractions in the two groups of people from the Dominican Republic based on the assigned cluster specificity (Table 3). In the healthy group, the majority (51.5%) of mtDNA lineages were from Native American ancestry, whereas the African fraction was most frequent (64.0%) in the OD group. On the other hand, the healthy and OD groups possessed European specificity at lowest frequencies (9.1% and 14.0%, respectively). Thus, the maternal gene pool of the Dominican Republic population may mainly consist of both Native American and African ancestries. However, a statistically significant difference was observed for the frequencies of three ancestral fractions between the two groups studied using 3×2 contingency table (Table 3)

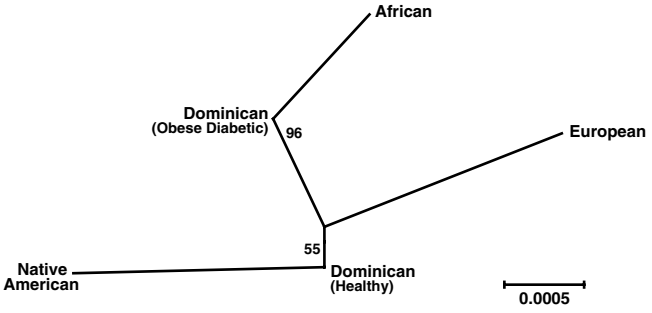

Fig. 2 Neighbor-joining (NJ) tree showing the relationships of two groups of people from the Dominican Republic and three reference populations on the basis of d_A distances with Kimura's two-parameter correction. The numbers shown for the internal branches are the bootstrap probabilities. The *scale* for the distance is shown *bottom right*

with χ^2 test ($\chi^2 = 7.75$, $P < 0.05$), although their sample sizes were not so large.

A phylogenetic analysis with d_A distances (Nei and Li 1979) between populations also indicated the difference in genetic background between the two groups in the Dominican Republic, as shown in Fig. 2. Although the smallest d_A distance (0.034%) was observed between the healthy and OD groups, there was no tight phylogenetic cluster of the two groups in this unrooted tree. Instead, the OD group and African population were neighbors in the NJ tree, and this relationship was supported by a high bootstrap probability (96%). The genetic dissimilarity between the two groups may partly account for an underlying predisposition to OD in the Dominican Republic population. Taking into account the relatively high prevalence of disease in African Caribbean populations such as Barbadians (Hennis et al. 2002), the present observations imply that unidentified genetic element(s) from African ancestry might be positively associated with OD in the Dominican Republic. A recent association study in the Dominican Republic population (Hamaguchi et al. 2004) has revealed that the Q121 allele [Q (glutamine) allele at codon 121] of membrane glycoprotein *PC-1* gene may be one of the candidates. Another explanation might be due to a potential association of nongenetic factors, e.g., diet and/or cultures characteristic of African population with the disease. Thus, future investigations into other factors (such as other genetic loci, environment, and socioeconomics) will help us attain a deeper understanding of this multifactorial disease in the Dominican Republic.

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