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A Mitochondrial DNA Mutation Cosegregates with the Pathophysiological U Wave

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In a family with long QT syndrome (LQT2), some individuals who did not harbor the *HERG* mutation had a prolonged QTU interval on electrocardiograms after exercise. It may be determined or modified by other gene(s) or factor(s). The sequence analysis of mtDNA in these individuals of this family showed a candidate pathogenic mutation at 3394 in the ND1 gene. The cybrids (mutation at 3394) showed significantly reduced NADH-CoQ reductase (complex I) activity and O_2 consumption to normal levels. These inhibitory effects on respiratory function may result in the depletion of ATP and could possibly produce an increase in Ca^{2+} concentration in cytosol, and it may lead to the prolongation of the QTU intervals on electrocardiograms. Therefore, we stated that the 3394 mutation in the ND1 gene is pathogenic and could be the cause of prolongation of the QTU intervals or modification of the phenotypes of not only congenital but also so-called "acquired drug-induced long QT syndrome."

Congenital long QT syndrome (LQTS) is an inherited disorder characterized by a prolonged cardiac action potential. Mutations in the human *ether-a-go-go*-related gene (*HERG*), which is responsible for the rapid component of the delayed rectifier current (I_{Kr}), cause this disease which is linked to chromosome 7 (LQT2) (1, 2). Mutant HERG subunits may cause altering currents, due to gene dosage, dominant negative effects, or reduced expression levels (3), thereby affecting potassium channel properties (4). Also some published reports described the congenital and acquired LQTS represent pathophysiological states characterized by the appearance in the electrocardiograms (ECGs) of prolongation of QT intervals, notched T waves, prominent U waves and an atypical polymorphic ventricular tachycardia known as torsade de pointes (TdP) (5). Previously, we found a missense mutation (G601S) of *HERG* in a LQTS family (LQT2) (6). However, some affected individuals from this family who did not harbor the *HERG* mutation, often showed a prolonged QT interval associated with U wave (QTU) on ECGs during recovery after exercise and episodes of syncope attack. This observation suggests that development of an altered QTU interval may be determined or modified by some other gene(s) or factors. These could possibly be genes in mitochondrial DNA (mtDNA), since the mtDNA mutations have been shown to produce cardiac abnormalities, including arrhythmias (7). In this study, we performed mtDNA sequence analysis, and influence of the mtDNA mutations on respiratory function was examined.

MATERIALS AND METHODS

LQT2 family. Phenotypic identification of individuals was based on their medical history and on QT(QTU) intervals which was defined in each of ECG 12 leads between the onset of the QRS complex and the point at which the line of maximal downslope of the T wave crossed the baseline before the isoelectric UP interval (8). QT intervals corrected for heart rate (QTc) was calculated by the Bazett formula (9). Subjects were considered to be affected when abnormally prolonged QTc intervals was ≥0.46 seconds (10). QT(QTU) interval adaptation to heart rate changes was derived from the treadmill exercise test or Holter recording in children who could not perform the exercise stress test.

DNA sources. Genomic DNA was prepared from peripheral blood lymphocytes for the sequencing of mtDNA and to confirm the mtDNA mutation.

mtDNA analysis. mtDNA sequence analysis was performed according to a method reported previously (11). The PCR products of mtDNA (nt 41-3460) from all individuals and family members were digested with HaeIII and were separated on a 20% polyacrylamide gel.

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Cells and cell culture. mtDNA-less HeLa cells, ρ^0 HeLa cells, which are resistant to 20 mmol/L 6-thioguanine (12), the cybrid clones, Ft and Ag, which were isolated by the fusion of ρ^0 HeLa cells with enucleated fibroblasts from normal subjects (13), and fibroblast lines from the individuals were grown in glucose-rich medium RPMI 1640 supplemented with pyruvate (0.1 mg/ml) and 10% fetal bovine serum (FBS). Intercellular transfer of mtDNA was carried out by fusion of enucleated fibroblasts with ρ^0 HeLa cells, as described previously (13), and the cybrid clones were isolated in a selective medium without glucose (DM170, Kanto Kagaku, Tokyo) supplemented with 20mM 6-thioguanine and 10% FBS. Briefly, skin fibroblasts grown on round glass discs were enucleated by centrifugation (23,000 × g, at 34°C for 10 min) in the presence of cytochalasin B (Sigma; 10 mg/ml). The resulting cytoplasts were mixed with ρ^0 HeLa cells, and fusion was carried out in the presence of 50% (wt/vol) polyethylene glycol 1500 (Boehringer Mannheim). The fusion mixture was cultivated in the selective medium. The residual nonenucleated parental fibroblasts and hybrids between nonenucleated fibroblasts and ρ^0 HeLa cells were completely eliminated with 20 mmol/L 6-thioguanine. Unfused parental ρ^0 HeLa cells were removed by culture in DM170 medium, since they could not grow in the medium without glucose, due to the complete absence of mtDNA. On day 14 after fusion, cybrid colonies grown in selective medium were picked up and cloned by the cylinder method. The cybrids were cultivated in normal medium (RPMI1640 + pyruvate + 10% FBS). As a control, HeLa mtDNA was introduced into ρ^0 HeLa cells by the fusion of the ρ^0 HeLa cells with enucleated wild-type HeLa cells, which are sensitive to 6-thioguanine.

Biochemical analyses of complex I and complex IV activities. For biochemical analysis, log-phase cells were harvested and cytochrome c oxidase (complex IV:COX) activity was measured as the rate of cyanide-sensitive oxidation of reduced cytochrome c, as described previously (14). The activity of mitochondrial respiratory chain enzyme NADH-CoQ reductase (complex I) was assayed, as described previously (15).

Measurement of oxygen consumption. The cells were incubated in the medium with 0.1 mmol/L or 25 mmol/L glucose for one hour, and O_2 consumption was then measured as follows: the cells (5 × 106) were trypsinized and suspended in phosphate-buffered saline with 0.1 mmol/L or 25 mmol/L glucose, and their O_2 consumption was recorded in a polarographic cell (1.0 ml) at 37°C with a Clark-type oxygen electrode (Yellow SpringsInstruments, OH, USA) (16).

Statistics. The results were expressed as mean±SD. Statistical comparisons among the three groups were carried out by analysis of variance and Newman-Keul's Range test for multiple-sample comparisons.

RESULTS

mtDNA Analysis

We looked for the possibility that individuals of a LQT2 family with prolongation of QTU during recovery after exercise, have inherited mtDNA mutations, by sequencing the whole mtDNA (16569bp) of one individual (P8). Compared to the standard Cambridge sequence (17), this affected individual's mtDNA showed 61 mutations, including 3 missense mutations, at the evolutionary conservative sites (nt 3394 in ND1, nt 6353 in CO1, and nt 10398 in ND3 genes). Of these missense mutations, the nt 10398 (A-to-G) mutation in the ND3 gene is polymorphic, since it is frequently present (22–43%) in the natural population (18, 19). In contrast, the homoplasmic mutation at nt 3394 of mtDNA (T-to-C) (Figure 1A) converts highly conserved tyrosine to histidine in the NADH dehydrogenase subunit I of the ND1 gene product.

To determine whether the remaining mtDNA mutations, at nt 3394 in the ND1 gene and at nt 6353 in the CO1 gene, are pathogenic or polymorphic, transfer of mtDNA from the individuals to ρ^0 HeLa cells was achieved by fusing enucleated fibroblasts from the individuals (P6, P7) with ρ^0 HeLa cells. Cybrid clones containing mtDNA exclusively from the fibroblasts were then isolated in a selection medium (cf. Methods). As a control, we used the cybrid clones, Ft and Ag, which contain mtDNA from normal subjects without the mutations (12). Therefore, all cybrid clones we used in this study possessed nuclear genome from HeLa cells, whereas the mtDNA was from either normal subjects without the mtDNA mutation or the individuals with the mutation. We then examined the effect of the mutations at 3394 of the ND1 gene and at 6353 of the CO1 gene on the activity of complex I and COX, and on O_2 consumption, since ND1 and CO1 are subunits of complex I and COX, respectively. The results showed that complex I activity and O_2 consumption were significantly ($p < 0.05$) reduced only in the cybrid clones ($n = 6$) with the mtDNA from the affected individuals (Figure 1B). On the other hand, all the cybrid clones ($n = 6$) showed normal complex IV activity, irrespective of whether the mtDNA was derived from individual P6 or P7, or from the normal subjects. Accordingly, it can be concluded that the mtDNA mutation at nt 3394 in the ND1 gene, but not the mutation at nt 6353 in the CO1 gene, was responsible for reduced mitochondrial respiratory function in the individuals, without any influence from a possible defect in their nuclear genome.

This mtDNA mutation at nt 3394 has been classified as an intermediate (class I/II) risk mutation in Leber's hereditary optic neuropathy (LHON) (20), and has also been identified in non-insulin-dependent diabetes mellitus (NIDDM) (21). It is frequently present (1–2%) in the natural population (22–25). LHON is a neurodegenerative disease of young adults (mostly males) that results in blindness due to atrophy of the optic nerve. NIDDM is characterized by disturbances in insulin action and secretion. Although our affected individuals who harboring this mtDNA mutation have not developed optic neuropathy or diabetes mellitus so far, except for one individual (P2) with myopia, we will need to monitor them carefully to observe whether these clinical phenotypes appear in the future.

Clinical Studies

Figure 2 shows the clinical features and distribution of the *HERG* and mtDNA mutations in this LQT2 family. In all individuals harboring the mtDNA nt 3394 mutation, the ECG showed prolongation of the

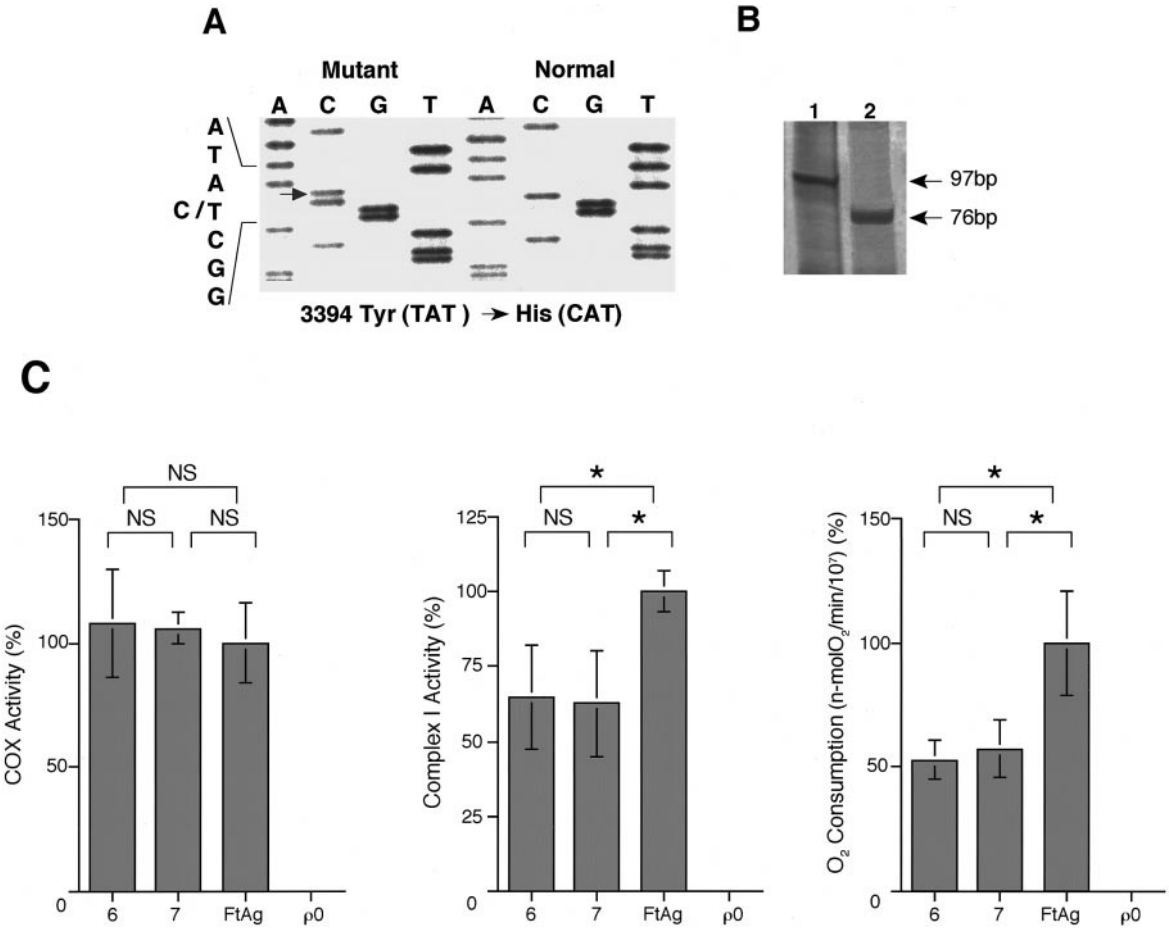
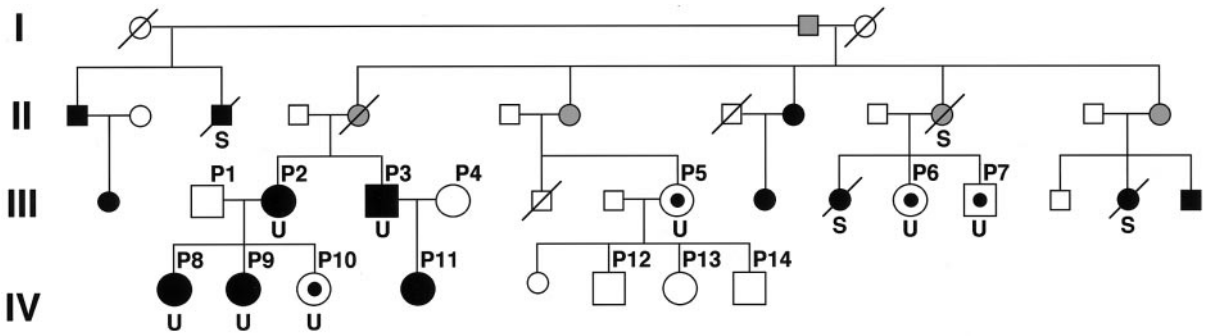


FIG. 1. A. The DNA sequence analysis of mitochondrial DNA (mtDNA). DNA from the individual and a normal individual was analyzed. The DNA sequence shows a missense mutation of a T-to-C substitution, which results in the substitution of histidine for tyrosine in the NADH dehydrogenase subunit I of the ND-1 gene product. B. HaeIII digestion of PCR products to detect the mutation at nt 3394 of mtDNA (T-to-C). Lane 1: normal control. Lane 2: individual (right). The normal fragment is 97bp; the mutated fragment is 76bp. This mutation was homoplasmic in the individuals (P2, P3, P5, P6, P7, P8, P9, P10, P12, P13, P14). C. Comparison of the mitochondrial respiratory activity of cybrids with individual-derived mitochondrial DNA (mtDNA) and with mtDNA from normal subjects. Biochemical analysis of cytochrome c oxidase (complex IV:COX) activity (left). Biochemical analysis of NADH-CoQ reductase (complex I) activity (middle). Biochemical analysis of O2 consumption (right). P6 and P7 are cybrid clones (n = 6) containing mtDNA derived exclusively from individual P6 (sister) and individual P7 (brother), respectively. FtAgs are control cybrid clones (n = 6) containing mtDNA from a fetus subject (Ft) and from an aged (97 years old) subject (Ag) (8). Values are mean ± SD. *$p < 0.05$. NS, not significant.

QTU during recovery after exercise. Four individuals (P2, P3, P8 and P9) had both the *HERG* and the mtDNA mutations. Three of them (P2, P8 and P9) are females who had prolongation of QTc (488-593 msec at rest) and an appearance of U wave during recovery after exercise (Figure 3A), including two individuals (P2, P8) who had had episodes of syncope attack and were prescribed a β-blocker. Although the brother (P3) of individual (P2) had the same mutations, he was asymptomatic. However, in the treadmill exercise test his QTc was changed (rest, 470 msec; exercise, 442 msec; recovery, 462 msec: QTUc, 509 msec; and complete right bundle-branch block when his heart rate was over 140/min. Figure 3B). His 3-year-old daughter (P11) had the *HERG* mutation only, with prolongation of QTc but without U wave, and was asymptomatic (rest, 465 msec; exercise, 475 msec; recovery, 472 msec. Figure 3C). The other 7 family members (P5, P6, P7, P10, P12, P13, P14) carried the mtDNA nt 3394 mutation only. Four of them (P5, P6, P10, P13) are females, including two individuals (P6, P13) who had had episodes of syncope attack and one (P6) who showed the appearance of U wave following the treadmill exercise test (rest, 396 msec; exercise, 422 msec; recovery, 424 msec with U wave: QTUc, 582 msec) (Figure 3D). The younger brother (P7) of individual (P6), had the same mtDNA mutation and also showed the appearance of U wave following the treadmill exercise (rest, 422 msec; exercise, 418 msec; recovery, 388 msec: QTUc, 455 msec). Although he was asymptomatic, he felt weak or fatigued easily following exercise and needed a long time for recovery. These clinical findings revealed that



No.	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14
Age (Y)	40	36	34	28	39	27	25	13	12	6	3	15	13	8
QTc (msec)														
Rest	N	488	470	400	425	396	422	593	554	435	465	N	N	N
Exercise	N	442	442	N	424	422	418	491	482	422	475	N	N	N
Recovery	N	465	462	N	442	424	388	490	465	410	472	N	N	N
QTUc (msec)														
Recovery	N	651	509	N	568	582	455	627	561	541	−	N	N	N
U wave	N	+	+	N	+	+	+	+	+	+	−	N	N	N
Syncope	−	+	−	−	−	+	−	+	−	−	−	−	+	−
Therapy	−	BB	−	−	−	−	−	BB	−	−	−	−	−	−
HERG (G601S)	−	+	+	−	−	−	−	+	+	−	+	−	−	−
mtDNA (3394T/C)	−	+	+	−	+	+	+	+	+	+	−	+	+	+

FIG. 2. Pedigree, clinical features and mutations of the human *ether-a-go-go*-related gene (*HERG*) and mitochondrial DNA (3394) in an LQT2 family. Squares indicate males; circles, females; black solid symbols, those with clinically long QT syndrome; a dot inside solid symbols, those with a normal range of QT intervals on electrocardiograms corrected for heart rate (QTc) at rest, but the appearance of U wave during recovery after exercise; open symbols, unaffected; and gray symbols, electrocardiogram not examined but suspected of having long QT syndrome; slashed symbols, deceased; U, appearance of U wave during recovery after exercise; N, not examined; BB, β-blocker. Individuals P2 and P8 had syncope which was controlled by β-blocker; S, sudden death.

the individuals who harbor a single mtDNA mutation had a normal range of QTc at rest, but did show prolongation of QTUc during recovery, especially just after exercise. Furthermore, individuals with both the *HERG* and the mtDNA mutations, showed prolongation of QTc at rest, and prolongation of QTUc during recovery after exercise. However, a individual (P11) with the *HERG* mutation only showed prolongation of QTc without definite U wave.

DISCUSSION

The pathophysiological U wave observed in acquired or congenital LQTS is more likely to be a second component of an interrupted T wave, and argue for use of the term T2 in place of U to describe this event (5). In this study, we found two candidate pathogenic mtDNA mutations at 3394 in the ND1 gene and at 6353 in the CO1 gene. Then, to determine the involvement of these mtDNA mutations in the pathogeneses of a prolongation of QTU interval, mtDNA transfer from fibroblasts of the individuals to ρ^0 HeLa cells was carried out by the fusion of enucleated fibroblasts with ρ^0 HeLa cells. The resultant cybrid clones with imported mtDNA from the individuals showed significantly reduced complex I activity, whereas their complex IV activity was restored to normal levels. Probably, impaired respiratory function induced by the nt 3394 mutation would interrupt ATP synthesis. The class Ib antiarrhythmic agent, BRB-I-28, has concentration-dependent inhibitory effects on NADH oxidase and complex I (26). It also has significant inhibitory effects on mitochondrial ATPase activity and its inhibitory effects on mitochondrial oxidative phosphorylation may result in the depletion of ATP. This effect, in combination with concentration-dependent inhibitory effects on NADH oxidase and complex I, on Na^+, K^+-ATPase, could possibly produce an increase of Ca^{2+} concentration in cytosol (26). It also produce a dose-dependent reduction in heart rate, blood pressure and prolongation of the P-R and QaT intervals (27). Therefore, the mtDNA mutation at nt 3394 could be involved in the pathogenesis of repolarization-related arrhythmias, including the cause of the pathophysiological U wave and prolongation of QTUc associated during recovery after the exercise.

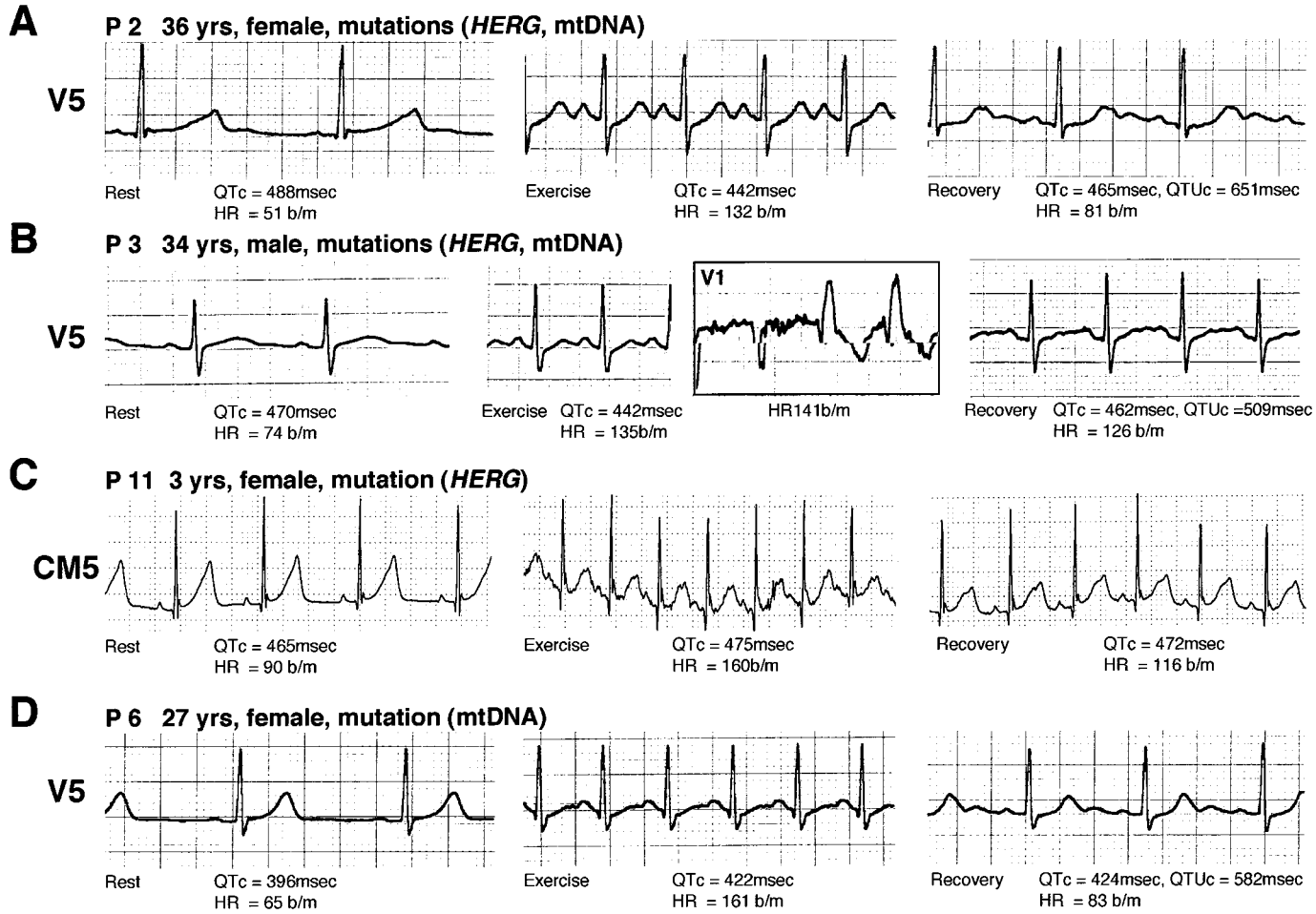


FIG. 3. Tracings showing examples of electrocardiogram changes in response to a change in heart rate during and after a treadmill exercise test or Holter recording in these LQT2 individuals. A. Individual P2 (female) had both the *HERG* and the mtDNA mutations. B. Individual P3 (male) had both the human *ether-a-go-go*-related gene (*HERG*) and the mitochondrial DNA (mtDNA) mutations. C. Individual P11 (female) had only the *HERG* mutation. D. Individual P6 (female) had only the mtDNA mutation.

It should be noticed that the difference in the expression of clinical phenotypes between individuals P2 and P3 and individuals P6 and P7, is biased to females. However, no difference in the extent of complex I deficiency was observed between individual P6 (female) and individual P7 (brother of P6). Some studies have shown a predominance of females in LQTS families (28, 29). The shortening of rate-corrected QT that is observed during puberty in males (in contrast with persistent juvenile sex-independent values in females) (30), attenuation in the rate of cardiac events among postadolescent males, but not females, in LQTS families (31), and experimental findings of blunted quinidine-induced QT prolongation after exposure to testosterone, but not estrogen (32), point to the QT-shortening, protective, electrophysiological action of androgens. Since similar, sex-biased expression was also observed in individuals with Leber's disease, expression of some clinical phenotypes related to mtDNA mutations could be affected by sex hormones.

The coexistence of the *HERG* and mtDNA mutations in the same long QT family suggests that nuclear gene mutation may interact with or be modified by mtDNA mutation in the phenotypes of LQTS. Furthermore, mtDNA mutations themselves are pathogenic and could be the cause of prolongation of the QTU intervals or modification of the so called "acquired (drug-induced) long QT syndrome".

Thus, mtDNA, a maternally transmitted genetic factor can contribute greatly to changes in electrocardiographic characteristics.

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REFERENCES

1. Curran, M. E., Splawski, I., Timothy, K. W., Vincent, G. M., Green, E. D., and Keating, M. T. (1995) *Cell* **80,** 795–803.
2. Sanguinetti, M. C., Jiang, C., Curran, M. E., and Keating, M. T. (1995) *Cell* **81,** 299–307.
3. Furutani, M., Trudeau, M. C., Hagiwara, N., Seki, A., Gong, Q., Zhou, Z., Imamura, S., Nagashima, H., Kasanuki, H., Takao, A., Momma, K., January, C. T., Robertson, G. A., and Matsuoka, R. (1999) *Circulation,* in press.
4. Sanguinetti, M. C., Curran, M. E., Spector, P. S., and Keating, M. T. (1996) *Proc. Nat. Acad. Sci. USA.* **93,** 2208–2212.
5. Yan, G., and Antzelevitch, C. (1998) *Circulation* **98,** 1928–1936.
6. Akimoto, K., Furutani, M., Imamura, S., Furutani, Y., Kasanuki, H., Takao, A., Momma, K., and Matsuoka, R. (1997) *Hum. Mut;* Mutation in Brief #75 On-line (http://journals.wiley.com/1059-7794/).
7. Johns, D. R. (1995) *N. Engl. J. Med.* **333,** 638–644.
8. Browne, K. F., Zipes, D. P., Heger, J. J., and Prystowsky, E. N. (1982) *Am. J. Cardiol.* **50,** 1099–1103.
9. Bazett, H. C. (1920) *Heart* **7,** 353–370.
10. Maron, B. J., Moller, J. H., Seidman, C. E., Vincent, G. M., Dietz, H. C., Moss, A. J., Towbin, J. A., Sondheimer, H. M., Pyeritz, R. E., McGee, G., and Epstein, A. E. (1998) *Circulation* **98,** 1460–1471.
11. Tanaka, M., and Ozawa, T. (1992) *in* Protocols in Molecular Neurology (Longstaff and Revest, Ed.), pp. 25–53, Humana Press, Totawa.
12. Hayashi, J. I., Ohta, S., Kagawa, Y., Kondo, H., Kaneda, H., Yonekawa, H., Takai, D., and Miyabayashi, S. (1994) *J. Biol. Chem.* **269,** 6878–6883.
13. Hayashi, J. I., Ohta, S., Kikuchi, A., Takemitsu, M., Goto, Y., and Nonaka, I. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10614–10618.
14. Miyabayashi, S., Haginoya, K., Hanamizu, H., Iinuma, K., Narisawa, K., and Tada, K. (1989) *J. Inher. Metab. Dis.* **12,** 373–377.
15. Miyabayashi, S., Narisawa, K., Iinuma, K., Tada, K., Sakai, K., Kobayashi, K., Kobayashi, Y., and Morinaga, S. (1984) *Brain Dev.* **6,** 362–372.
16. Soejima, A., Inoue, K., Takai, D., Kaneko, M., Ishihara, H., Oka, Y., and Hayashi, J. I. (1996) *J. Biol. Chem.* **271,** 26194–26199.
17. Anderson, S., Bankier, A., Barrell, B. G., deBruijn, M. H. L., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J. H., Staden, R., and Young, I. G. (1981) *Nature* **290,** 457–465.
18. Prezant, T. R., Agapian, J. V., Bohlman, C., Bu, X., Oztas, S., Qiu, W., Arnos, K., Cortopassi, G. A., Jaber, L., Rotter, J. L., Shohat, M., and Fischel-Ghodsian, N. (1993) *Nature Genet.* **4,** 289–294.
19. Noer, A. S., Sudoyo, H., Lertrit, P., Thyagarajan, D., Utthanaphol, P., Kapsa, R., Byrne, E., and Marzuki, S. (1991) *Am. J. Hum. Genet.* **49,** 715–752.
20. Brown, M. D., Voljavec, A. S., Lott, M. T., Macdonald, I., and Wallace, D. C. (1992) *FASEB J.* **6,** 2791–2799.
21. Hirai, M., Suzuki, S., Onoda, M., Hinokio, Y., Ai, L., Hirai, A., Ohtomo, M., Komatsu, K., Kasuga, S., Satoh, Y., Akai, H., and Toyota, T. (1996) *Biochem. Biophys. Res. Commun.* **219,** 951–955.
22. Ishikawa, S., Ichibe, Y., Yokoe, J., and Wakakura, M. (1995) *Muscle & Nerve* Suppl 3:S85–S89.
23. Johns, D. R., Neufeld, M. J., and Park, R. D. (1992) *Biochem. Biophys. Res. Commun.* **187,** 1551–1557.
24. Brown, M., Torroni, A., Reckord, C. L., and Wallace, D. C. (1995) *Hum. Mut.* **6,** 311–325.
25. Gerbitz, K., van den Ouweland, J. M. W., and Massen, J. A. (1995) *Biochim. Biophys. Acta* **1271,** 253–260.
26. Chen, C. L., Sangiah, S., Yu, C.-A., Chen, H., Berlin, K. D., Garrison, G. L., Scherlag, B. J., and Lazzara, R. (1994) *Res. Commun. Mol. Pathol. Pharmacol.* **85,** 193–208.
27. Pugsley, M. K., Saint, D. A., Hayes, E., Berlin, K. D., and Walker, M. J. A. (1995) *Eur. J. Pharmacol.* **294,** 319–327.
28. Moss, A. J., Schwartz, P. J., Crampton, R. S., Tzivoni, D., Locati, E. H., MacCluer, J., Hall, W. J., Weitkamp, L., Vincent, G. M., Garson, A., Jr., Robinson, J. L., Benhorin, J., and Choi, S. (1991) *Circulation* **84,** 1136–1144.
29. Hashiba, K. (1992) *Ann. N.Y. Acad. Sci.* **644,** 142–156.
30. Rautaharju, P. M., Zhou, S. H., Wong, S., Calhoun, H. P., Berenson, G. S., Prineas, R., and Davignon, A. (1992) *Can. J. Cardiol.* **8,** 690–695.
31. Zareba, W., Moss, A. J., Cessie, S., Locati, E. H., Robinson, J. L., Hall, W. J., and Andrews, M. L. (1995) *J. Am. Coll. Cardiol.* **26,** 1685–1691.
32. Drici, M. D., Burklow, T. R., Haridasse, V., Glazer, R. I., and Woosley, R. L. (1996) *Circulation* **94,** 1471–1474.