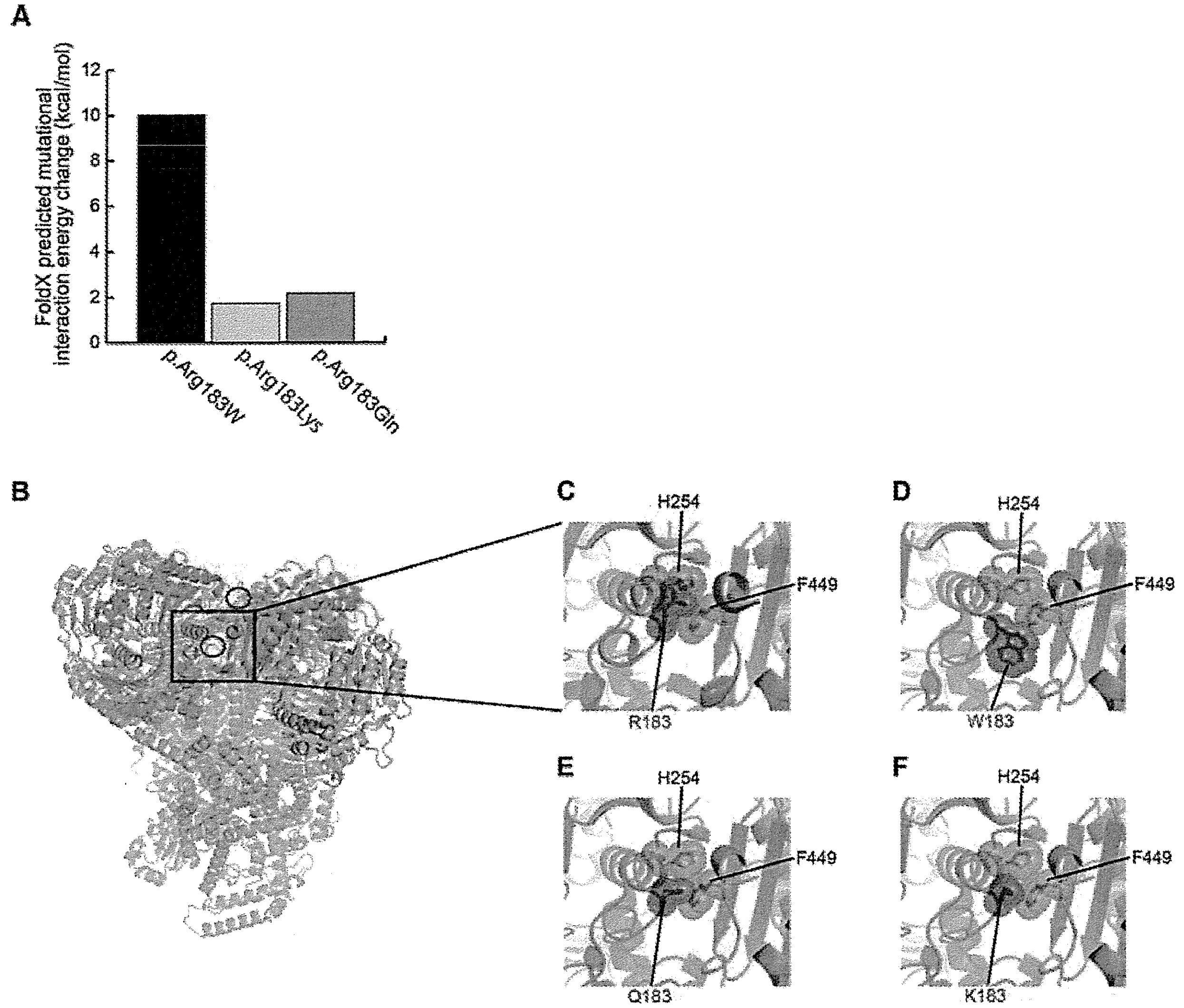

Figure 2. Molecular structural consideration of the effect on dimerization of amino acid replacement at residue position 183 in the core protein. **A:** Calculated interaction-energy change of the core protein homodimer upon replacement by the indicated amino acids at residue 183 using FoldX software. **B:** Overview of the crystal structure of the bovine mitochondrial bc1 (CIII) complex (PDB code 2A06). The core protein monomers are colored green and cyan; the other components are shown in gray. The helices, strands, and loops are shown as ribbons, arrows, and threads, respectively. The red circle indicates residue 183 in the core protein. The box corresponds to the enlarged areas shown in parts (**C**)–(**F**). **C–F:** Detailed views of the core protein homodimerization interface in the wild-type (**C**) and mutated, polymorphic, orthologous (p.Arg183Trp/Gln/Lys) (**D**, **E**, **F**, respectively) complex structures. The residues at amino acid 183 of one subunit (red), and His254 and Phe449 of the other subunit (orange) are shown as sticks with Connolly surfaces. All graphics were drawn using PyMOL (www.pymol.com).

using mitochondrial fractions prepared from primary fibroblasts derived from patient 1. With normalization to complex II activity, the CIII activity of patient 1 was decreased to 50% of that in the control subjects ($n = 10$), whereas complex I activity increased by threefold and complex IV activity remained at the same level as in the control subjects (Fig. 3A). Similar results were obtained using normalization to citrate synthase activity (Fig. 3B). We also investigated the steady-state level of the respiratory complexes by blue-native polyacrylamide gel electrophoresis (BN-PAGE) using the same mitochondrial fraction used for the enzyme activity measurements. For analysis of individual complexes, mitochondria were solubilized with 0.5% (w/v) DDM. For analysis of the supercomplex (complexes I, III, and IV), mitochondria were solubilized with 1% (w/v) digitonin. After BN-PAGE, we performed immunoblotting with specific antibodies for the respiratory complexes (Fig. 3C–F, Supp. Notes, and Supp. Fig. S4). In the patient's fibroblasts, we found that CIII and supercomplex assembly were decreased to 18%–20% ≫ 22% –23% (Fig. 3C and D) and 4% ≫ 7.5% (Fig. 3E and F) of the levels in pooled control samples, respectively. These data indicate that a homozygous missense mutation (c.547C>T, p.Arg183Trp) in *UQCRC2* causes moderately impaired CIII function and severely decreased amounts of CIII and supercomplex, which would be the primary molecular pathogenesis in the patients.

Discussion

Among the genes known to cause CIII deficiency, impairment of UQCRC2, as found in our patients, leads to a similar clinical course to that reported for UQCRB defects with recurrent crises of

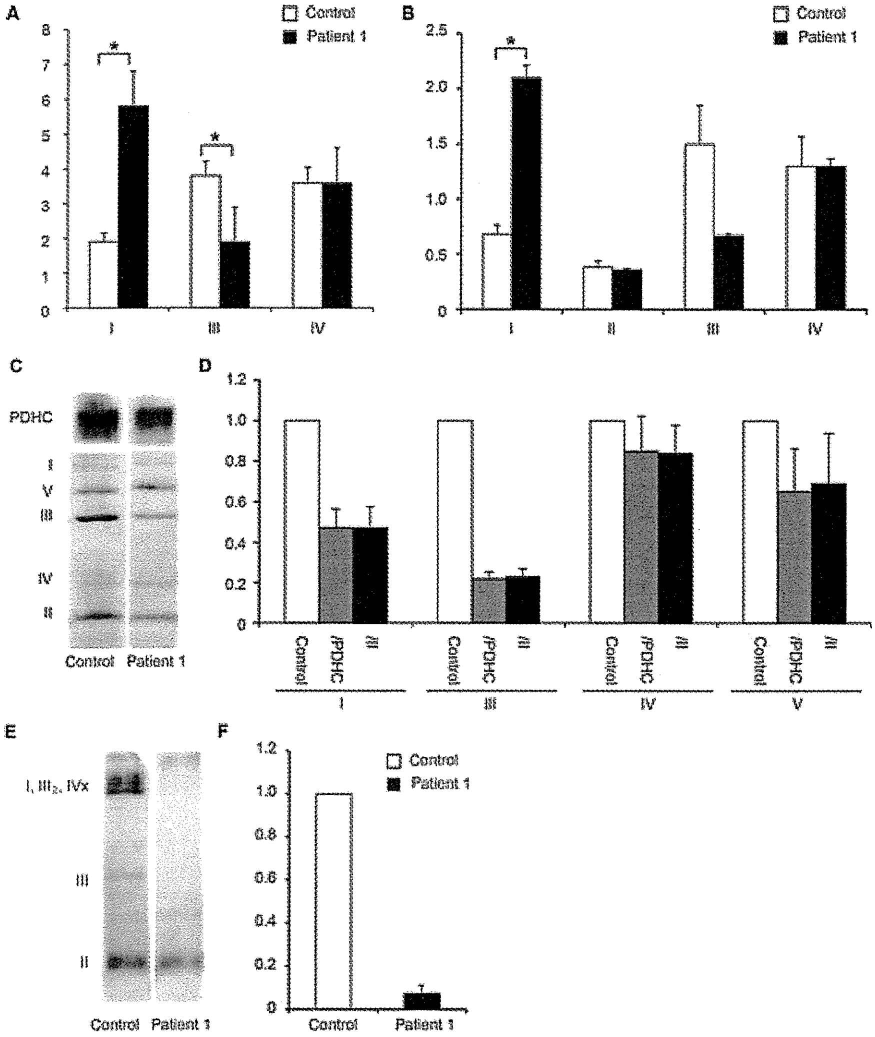

Figure 3. Mitochondrial enzyme activity and supercomplex formation. **A, B:** Enzyme activities of the mitochondrial respiratory chain complexes using mitochondrial fractions prepared from primary fibroblasts derived from patient 1 (*n* = 3) and control subjects (*n* = 10). Each measurement was performed in triplicate. The values were normalized to complex II (**A**) or citrate synthase (**B**). Error bars represent the SEM. **C, D:** Immunoblot detection of each respiratory chain complex using mitochondria solubilized with 0.5% DDM. The same amount of pooled mitochondrial protein from control subjects (*n* = 10) was loaded into the control lane. The band intensity of each respiratory complex was estimated by densitometry and normalized to that of PDHC (gray bar) or complex II (black bar). The data were obtained by three independent assays and the error bars in (**D**) represent the SEM. **E, F:** Immunoblot detection of the respiratory supercomplex using mitochondria solubilized with 1% (w/v) digitonin. The same amount of pooled mitochondrial protein from control subjects (*n* = 10) was loaded into the control lane. The band intensity of the supercomplex was estimated by densitometry and normalized to that of complex II (black bar). The data were obtained by three independent assays and the error bars in (**F**) represent the SEM.

hypoglycemia, lactic acidosis, and ketosis, although the latter did not show hyperammonemia. In contrast, impairment of BCS1L, TTC19, and UQCRQ leads to rather severe complications such as intrauterine growth retardation, liver failure, tubulopathy, sensorineural hearing loss, and abnormalities on brain MRI. The normal development in our patients, despite frequent metabolic crises, may suggest that the UQCRC2 phenotype in our family is milder than disorders of the CIII genes and that this UQCRC2 abnormality does not primarily affect the brain. However, patients 2 and 3 showed epilepsy, and developmental delay was noted in patient 3. It remains to be seen whether this clinical variability is caused by variable expressivity, unknown modifiers, or secondary to the severity of the acute metabolic crises. Interestingly, our patients showed hyperammonemia, highly abnormal urine organic acids indicative of mitochondrial dysfunction, and highly elevated plasma hydroxyl fatty acids during their crises, whereas patients with the other reported CIII impairment disorders did not [Barel et al., 2008; de Lonlay et al., 2001; Ghezzi et al., 2011; Haut et al., 2003; Hinson et al., 2007; Visapaa et al., 2002]. These observations may imply that *UQCRC2* mutations have secondary effects in other metabolic pathways including the Krebs cycle, beta oxidation, and urea cycle.

Conclusion

We have identified, for the first time, a homozygous mutation in human *UQCRC2* encoding a core protein of mitochondrial CIII. Further studies of additional patients with *UQCRC2* abnormalities are necessary to fully understand human CIII disorders.

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