Carnitine palmitoyltransferase 1A (CPT1A) is the key regulatory enzyme of hepatic long-chain fatty acid β-oxidation. Human CPT1A deficiency is characterized by recurrent attacks of hypoketotic hypoglycemia. We presently analyzed at both the functional and structural levels five missense mutations identified in three CPT1A-deficient patients, namely A275T, A414V, Y498C, G709E, and G710E. Heterologous expression in Saccharomyces cerevisiae permitted to validate them as disease-causing mutations. To gain further insights into their deleterious effects, we localized these mutated residues into a three-dimensional structure of the human CPT1A created from the crystal structure of the mouse carnitine acetyltransferase. This study demonstrated for the first time that disease-causing CPT1A mutations can be divided into two categories depending on whether they affect directly (functional determinant) or indirectly the active site of the enzyme (structural determinant). Mutations A275T, A414V, and Y498C, which exhibit decreased catalytic efficiency, clearly belong to the second class. They are located more than 20 Å away from the active site and mostly affect the stability of the protein itself and/or of the enzyme-substrate complex. By contrast, mutations G709E and G710E, which abolish CPT1A activity, belong to the first category. They affect Gly residues that are essential not only for the structure of the hydrophobic core in the catalytic site, but also for the chain-length specificity of CPT isoforms. This study provides novel insights into the functionality of CPT1A that may contribute to the design of drugs for the treatment of lipid disorders.
Molecular Analysis of CPT1A-deficient Patients—Informed consent was obtained from all subjects. Case report of patient 2 (29) as well as molecular analysis of patients 1 and 3 (26, 27) have previously been reported. Fibroblasts from controls and patients were cultured as previously described (30) and used to extract DNA according to standard methods and RNA using RNeasy Midi kit (Qiagen). Mutation analysis of patient 2 was performed at both cDNA and gDNA levels by sequencing approach, as previously described (26, 27).

Construction of Human CPT1A Mutants—pYeCPT1A-A275T, -A414V, -Y498C, and -G709E were constructed with the QuickChange site-directed mutagenesis kit (Stratagene) using pYeCPT1A-WT (26) as template that corresponds to the yeast expression vector pYeDP1/8-10 containing the full-length human CPT1A cDNA under control of the inducible GAL10 promoter. pYeCPT1A-A275T-A414V was constructed with a second step of mutagenesis creating the A414V mutation in pYeCPT1A-A275T. Mutations A275T, A414V, Y498C, and G709E were synthesized with pairs of mutagenized primers (sequences available upon request). cDNA of mutants were sequenced to assess the presence of the designed mutation as well as the absence of unwanted mutations. Plasmids were used to transform S. cerevisiae (haploid strain W303: MATa, his3, leu2, trp1, ural, ade2–1, and can1–100) (31).

Yeast Culture, Subcellular Fractionation, and Isolation of Yeast Mitochondria—Methods for yeast culture, subcellular fractionation, and isolation of yeast mitochondria were performed as previously described (31). Protein concentration was determined by the method of Lowry et al. (32) with bovine serum albumin as standard.

CPT Assay—CPT activity, apparent K_{m} for carnitine and palmitoyl-CoA and I_{50}, value for malonyl-CoA, defined as the malonyl-CoA concentration that produces 50% inhibition of enzyme activity, were determined using mitochondria isolated from transformed yeasts, as previously reported (31).

Assessment of the Folding State of Human CPT1A Mutants—Folding state of the human CPT1A mutants was analyzed by proteolytic digestion (10 μg/ml of trypsin) using intact or Triton X-100 (0.5% v/v) solubilized mitochondria (0.05 mg of protein/ml) as previously described for the rat protein (19, 31). Samples were analyzed by SDS-PAGE and immunoblotting.

Western Blot Analysis—Proteins were analyzed by SDS-PAGE (33) in an 8% gel and detected after blotting onto nitrocellulose as previously described (31) using the ECL detection system (Pierce) according to the supplier’s instructions.

Chemicals—TaqDNA polymerase, as well as PCR and sequencing reagents were purchased from Applied Biosystems. Yeast culture media were from Difco, and Zymolase 20T was from ICN Biomedicals, Orsay, France. Others chemicals were purchased from Sigma.

RESULTS

Molecular Analysis of Patient 2 and CPT1A Expression in Fibroblasts—As previously reported, three missense mutations were identified in patient 1: A275T and A414V carried on the paternal allele, and Y498C carried on the maternal allele (27). The present molecular analysis of patient 2 permitted to identify both the heterozygous 2126G>A substitution predictive of the G709E mutation, and the 948delG deletion, which corresponds to the R316fsX328 frameshift at codon 316 (exon 9) generating a stop signal 12 codons downstream (exon 10) (Fig. 1A). This latter mutation was identified in a heterozygous state at the gDNA level, whereas it was not detected at the cDNA level (Fig. 1A), pointing out the instability of the R316fsX328 mRNA (Fig. 1B). CPT1A immunodetection in fibroblasts from patients indicated that, by contrast to a previously described patient homozygous for the G710E mutation (patient 3) (26), neither patient 1 nor patient 2 expressed CPT1A protein at a detectable level (Fig. 2).

Wild-type and CPT1A Mutants Expression in S. cerevisiae—The functional analysis of mutations A275T, A414V, Y498C, and G709E was performed using heterologous expression in yeast S. cerevisiae, an eukaryotic organism devoid of endoge-
2% of that observed for the wild-type. For mutants Y498C, A414V, and A275T-A414V this decrease in CPT1A activity may partly result from the lower level of expressed protein (Fig. 3). As previously reported for mutant G710E (26), mutant G709E was totally inactive whatever the concentration of substrate employed (Table I, Fig. 4, B and D) despite similar level of CPT1A protein expression when compared with wild-type (Fig. 3). Mutations A275T and Y498C did not alter malonyl-CoA sensitivity, their IC50 value for malonyl-CoA being similar to that of the wild-type (Table I). Due to the low residual activity in mutants A414V and A275T-A414V, it was not possible to assess their malonyl-CoA sensitivity. All mutants, except mutant G709E, exhibited normal saturation kinetics when the carnitine concentration varied relative to a fixed concentration of palmitoyl-CoA (Fig. 4, A and B) or when palmitoyl-CoA concentration varied when the molar ratio of palmitoyl-CoA/albumin was fixed at 6:1:1 (Fig. 4, C and D). Mutation A275T was previously characterized in COS cells as a functionally neutral polymorphism (25). However, analysis of its saturation kinetics, which was not performed in the study of Brown et al. (25), indicated that this mutation decreased by 25% to 43% the $V_{\text{max}}$ and catalytic efficiency ($V_{\text{max}}/K_m$) for carnitine and palmitoyl-CoA with no alteration in the apparent $K_m$ for both substrates (Table I). In comparison to wild-type, mutant Y498C had a similar apparent $K_m$ for palmitoyl-CoA but a 2-fold decrease in its apparent $K_m$ for carnitine, indicating a slight increased affinity of the enzyme to this substrate. Moreover, mutant Y498C showed a 3-fold decrease in its $V_{\text{max}}$ and catalytic efficiencies for carnitine and palmitoyl-CoA when compared with wild-type. Mutants A414V and A275T-A414V presented no alteration in the apparent $K_m$ for carnitine and palmitoyl-CoA, but at least a 98% decrease in their $V_{\text{max}}$ and catalytic efficiency whatever the substrate used (Table I). Thus, mutations A275T, Y498C, A414V, and A275T-A414V altered the $V_{\text{max}}$ and the catalytic efficiency more than the $K_m$ for carnitine and palmitoyl-CoA, whereas mutation G709E totally inactivated the enzyme.

Assessment of the Folding State of CPT1A Mutants—Previous works (14, 19, 31) showed that the rat CPT1A exhibits a native functional conformation characterized by a highly folded state resistant to trypsin proteolysis. When the outer mitochondrial membrane is disrupted, such as during the swelling procedure, trypsin is able to cleave the loop connecting TM1 and -2, hence generating an 82-kDa fragment. Moreover, the catalytic C-terminal domain of the rat CPT1A has been shown to contain a highly trypsin-resistant 60-kDa folded core that could be observed when solubilized mitochondria were submitted to trypsin proteolysis (14). As shown in Fig. 5A, the human wild-type CPT1A protein also remained largely resistant to trypsin treatment in intact mitochondria. The integrity of the outer mitochondrial membrane was checked by the inaccessibility of cytochrome b2 to trypsin proteolysis (Fig. 5A). Mutants A275T and G710E, as well as mutants A414V and A275T-A414V (results not shown), exhibited the same protease resistance as the wild-type protein, whereas mutants Y498C and G709E were sensitive to trypsin proteolysis (Fig. 5A). When yeast mitochondria containing either the wild-type or the mutants A275T and G710E were solubilized by Triton X-100 in the presence of trypsin, both the 82- and 60-kDa fragments were detected (Fig. 5B, f1 and f2 fragments). These results strengthened the fact that the human CPT1A protein also contained within its catalytic C-terminal domain a highly folded trypsin-resistant core that was not affected by mutations A275T and G710E. By contrast, the generation of the f1 and f2 fragments was either less efficient or totally absent in the case of mutants Y498C and G709E (Fig. 5B), suggesting a partial unfolding of their C-terminal domain. The endogenous matrix soluble HSP70 protein (mtHSP70) was used as a positive control for trypsin proteolysis as its conformational states can be assessed by limited trypsin proteolysis (35).

Localization of Mutations in a Structure Model of Human CPT1A—To understand the possible molecular mechanism for the effects of these mutations on the catalytic activity and the conformation of the enzyme, we examined their locations in a structure model of human CPT1A. The model was created with the program MODELLER (36) based on the crystal structure of the mouse CAT (21), which shares 32% amino acid sequence identity with that of human CPT1A. Only residues 166–773 of human CPT1A have been used to build the structure model (Fig. 6A), as the first 160 residues of CPT1A do not have counterparts in CAT. This analysis indicates that residues Ala-275, Ala-414, Gly-709, and Gly-710 are in the core of the human CPT1A protein, whereas residue Tyr-498 is located in a surface loop which contains an inserted segment as compared with mouse CAT (Figs. 6A and 7).

Residues Ala-275, Ala-414, and Tyr-498 are located about 19, 24, and 43 Å from the active site, respectively (Fig. 6A). Ala-275 is in the middle of helix α6, and Ala-414 is near the end of helix α10, in a tight turn linking this helix to strand β6 (Fig. 6A). Their location at more than 20 Å away from the active site suggests an indirect mechanism for the deleterious effects of the corresponding mutations. In addition, these mutations have small effects on the $K_m$ of the enzyme (Table I), indicating that substrate binding is not significantly affected in these mutants. On the other hand, their altered $V_{\text{max}}$ (Table I) suggest that the main effect of these mutations was to decrease the stability of the enzyme-substrate complex and/or of the CPT1A protein itself (Fig. 3).

Concerning residues Gly-709 and Gly-710, the structure analysis of human CPT1A shows that these residues are located near the active site of the enzyme within the conserved strand β14 (Fig. 6A). Moreover, they have opposite location relative to the plane of strand β14, and the long-chain acyl-CoA and carnitine binding sites (Fig. 6B). Modeling studies of the mutations G709E and G710E are illustrated in Fig. 6B. Replacement of Gly-709 by a Glu residue leads to the introduction of a bulky and negatively charged group in the hydrophobic core of the enzyme. For the G710E mutation, this negatively charged Glu residue is in the vicinity of the catalytic His-473 residue (7 Å) and of the carnitine molecule (10 Å) (Fig. 6B), and causes a drastic alteration in the hydrophobic pocket of the enzyme. In conclusion, the loss of activity observed for mutants G709E and G710E can be explained by their strategic location in the catalytic machinery.
**TABLE I**

**Enzyme activity, malonyl-CoA inhibition and kinetic parameters of the wild-type and mutants CPT1A expressed in S. cerevisiae**

Mitochondria were isolated from the yeast strains separately expressing wild-type and mutants CPT1A. CPT activity was assayed with 80 μM palmitoyl-CoA and 200 μM carnitine in the absence or presence of 150 μM malonyl-CoA. Numbers in parentheses represent the percentage of catalytic efficiency ($V_{\text{max}}/K_{\text{m}}$) compared to that of the wild-type (100%). ND, not determined.

| Strain       | Activity | Malonyl-CoA | Carnitine | Palmitoyl-CoA | Catalytic efficiency |
|--------------|----------|-------------|-----------|--------------|---------------------|
|              | $V_{\text{max}}$ | $K_{\text{m}}$ | $V_{\text{max}}$ | $K_{\text{m}}$ | $V_{\text{max}}/K_{\text{m}}$ |
| Wild-type    | 6.5 ± 0.6 | 0.8 ± 0.08 | 1.83 ± 0.4 | 106.5 ± 8.4 | 147.8 ± 10.5 | 82.8 ± 9.4 | 88.6 ± 10.2 | 1.4 (100%) | 1.07 (100%) |
| A275T        | 4.8 ± 0.3 | 0.4 ± 0.08 | 1.70 ± 0.32 | 107.4 ± 16.6 | 87.0 ± 10.5 | 75.5 ± 8.3 | 61.1 ± 13.2 | 0.8 (60%) | 0.8 (76%) |
| A414V        | 0.4 ± 0.05 | 0.05 ± 0.03 | ND         | 78.0 ± 8.0 | 1.6 ± 0.1 | 54.2 ± 4.2 | 1.1 ± 0.1 | 0.02 (1.5%) | 0.02 (1.9%) |
| A275T-A414V  | 0.13 ± 0.04 | 0.04 ± 0.02 | ND         | 96.7 ± 2.9 | 0.6 ± 0.1 | 55.5 ± 3.5 | 0.4 ± 0.1 | 0.006 (0.5%) | 0.007 (0.7%) |
| Y498C        | 3.0 ± 0.2 | 0.2 ± 0.01 | 1.53 ± 0.06 | 61.3 ± 6.8 | 29.2 ± 1.3 | 66.3 ± 9.5 | 25.9 ± 5.6 | 0.5 (54%) | 0.4 (37%) |
| G709E        | Undetectable | Undetectable | ND         | ND | ND | ND | ND | ND | ND |

**DISCUSSION**

In the present study, combined functional and structural approaches allowed to validate the A275T, A414V, Y498C, and G709E substitutions as disease-causing mutations in human CPT1A and to investigate for the first time the molecular mechanisms responsible for their deleterious effects.

Substitution of the non-polar Ala-275 residue by an uncharged-polar residue could lead to more serious consequences. However, Ala-275 is not conserved among the acylcarnitine transferase family and several members, such as rat and mouse CPT1A, have a natural threonine at this codon (Fig. 7). Investigation of the protein conformational state by trypsin proteolysis experiments established that the human CPT1A protein contains within its catalytic C-terminal domain a highly folded trypsin-resistant core, as reported previously for the rat protein (12, 14). This intrinsic property of CPT1A constitutes a valuable criterion to detect important conformational change that would unmask tryptic cleavage sites. Despite that mutant A275T did not exhibit such a conformational change, its protein structure might be slightly disrupted by the replacement of a small residue into a longer one because this mutation occurs within helix α6 that is a central element essential for the protein structure. Therefore, we hypothesized that this small structure perturba-
the catalytic His residue (Fig. 6A). This would result in the destabilization of the whole catalytic core, altering both protein stability and enzymatic activity. Functional analysis of the double mutant A275T-A414V (as these two mutations were carried by the same allele in patient 1) showed that the $V_{max}$ and catalytic efficiencies were slightly more affected in comparison to mutant A414V, suggesting that mutation A275T emphasized the pathogenic character of mutation A414V.

Y498C was shown to be responsible for a slight protein instability, a 3-fold decrease in the $V_{max}$ and catalytic efficiencies for both carnitine and palmitoyl-CoA, and a 2-fold increase in the affinity for carnitine. As Tyr-498 is located at more than 40 Å from the active site (Fig. 6A), its deleterious effects are indirect and may result from an altered conformation, as indicated by the trypsin proteolysis experiments (Fig. 5). Nevertheless, it is difficult to predict the exact behavior of this mutated residue because it is located in the loop connecting helix a12 and strand $\beta$9 that contains an inserted segment in CPT1A compared with CAT (Figs. 6A and 7). Despite being on the external face of the protein, this loop is likely to play a structural role, strengthening the importance of protein conformation for the functionality of the enzyme.

Functional analysis of the G709E mutation, which behaves as in a hemizygous state in patient 2, was particularly interesting because this mutation resulted in protein instability in patient fibroblasts whereas the previously reported G710E mutation did not (Fig. 2). Both G709E and G710E mutants were totally inactive (present study and Ref. 26), but only G709E mutant exhibited a trypsin-sensitive conformation. Thus, protein unfolding of G709E mutant explained the protein instability observed in fibroblasts. Residues Gly-709-Gly-710 are conserved among all the CPT1 isoforms and are adjacent to a Gly-Phe-Gly pattern (Fig. 7), previously suggested to be involved in carnitine binding in rat CPT2 (37). They are equivalent to Val-563-Met-564 in CAT (Fig. 7) and are located near the end of strand $\beta$14, in the immediate vicinity of the carnitine molecule (Fig. 6A and B). The side chain of the Val-563 resides within the hydrophobic pocket of the catalytic core of the CAT, pointing away from carnitine. Modeling studies showed that its replacement by the small Gly residue in the CPT1A, together with several other amino acid changes in this region, would produce a cavity in the core. It is therefore likely that the position of strand $\beta$14 will move slightly, away from carnitine, to fill this void, keeping Gly-709 in the hydrophobic core of the structure. Based on this model, the large effects of the G709E mutation on the stability of CPT1A can be explained by the introduction of a bulky and negatively charged group in the hydrophobic core of the enzyme, causing atheric repulsions as well as unfavorable electrostatic interactions (Fig. 6B).

Concerning the Gly-710 residue, structural analysis showed
that this residue is in the active site, facing both the catalytic His residue and the carnitine molecule (Fig. 6B). However, our modeling studies of the G710E mutation indicated that the negatively charged Glu residue is physically unable to interfere with the catalytic His residue and/or to balance the positive charge of the trimethylammonium group of carnitine (Fig. 6B). We have suggested earlier that its equivalent in CAT, Met-564, partly fills the hydrophobic pocket, and hence allows the access to the binding site for only acetyl-CoA and not for the palmitoyl group (21). In addition to the movement of the strand β14 discussed above, the presence of a Gly residue at this position in CPT1A also contributes to create additional space for binding the long acyl chains. This is reinforced by the fact that the Gly-710 residue is conserved in all the long- and medium-chain acyltransferases (Fig. 7). Therefore, the functional inactivity of mutant G710E can be explained by the fact that the bulkier Glu side chain blocks the binding of long-chain acyl groups. Altogether, these results demonstrate that both Gly-709-Gly-710 residues are essential for enzymatic activity because they are structurally part of the hydrophobic core of the catalytic site. Moreover, this illustrates that residues located far from the catalytic residues in the primary amino acid sequence can fact be crucial elements of the catalytic core. This also explains why deletion and/or mutation within the C terminus of CPT1A so dramatically affected initial protein folding and/or catalytic activity (19, 20).

In conclusion, these combined functional and structural analyses of missense mutations in CPT1A deficiency provide novel insights into the functionality of this enzyme. Firstly, this work demonstrates that disease-causing mutations in CPT1A can be roughly divided into two categories depending on whether they affect directly (functional determinant) or indirectly the active site of the enzyme (structural determinant). Mutations A275T, A414V, and Y498C clearly belong to the second class, as they are located more than 20 Å away from the active site and affected more the stability of the protein itself and/or of the enzyme-substrate complex than the $K_m$ for the substrates. By contrast, mutations G709E and G710E belong to the first category. Indeed, these small Gly residues are not only essential for the structure of the hydrophobic core in the catalytic site, but also contribute to the structural basis for the selectivity of long-chain acyl-CoA. This illustrates that significant structural differences between CAT and CPT1A are indeed underlying structural bases for their kinetic specificity. Finally, it is also clear from structural modeling studies that human CPT1A has outside its active site significant insertions in several of the surface loops, such as the one containing Tyr-498, as compared with CAT. These secondary structural elements may form additional interactions within the catalytic domain. However, the functional exploration of these secondary structural elements of CPT1A, as well as of the interactions between its N- and C-terminal domains reported to modulate the degree of malonyl-CoA sensitivity (39), requires the specific crystal structure of the CPT1A enzyme.

Acknowledgments—We are grateful to the referring physicians and F. Demaurex (INSERM U370, Paris, France) for patient tracking. We thank W. Neupert (Munich, Germany) for the antibodies against the yeast cytochrome b2 and HSP70 and N. Kadhom (INSERM U393) for cell culture.

REFERENCES

1. McGarry, J. D., and Brown, N. F. (1997) Eur. J. Biochem. 244, 1–14
2. Ramsay, R. R., Gandour, R. D., and van der Leij, F. R. (2001) Biochim. Biophys. Acta 1546, 21–43
3. Pretkni, M., and Corkey, B. E. (1996) Diabetes 45, 273–283
4. Zammit, V. A. (1999) Biochem. J. 343, 505–515
5. Ruderman, N. B., Saha, A. K., Varvas, D., and Wittler, L. A. (1999) Am. J. Physiol. 276, E1–E18
6. Unger, R. H., and Orci, L. (2001) Faseb J. 15, 312–321
7. McGarry, J. D. (2002) Diabetes 51, 7–18
8. Britton, C. H., Schultz, R. A., Zhang, B., Esser, V., Foster, D. W., and McGarry, J. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1984–1988
9. Yamanuki, N., Shinohara, Y., Shima, A., Yamanaaka, Y., and Terada, H. (1996) Biochim. Biophys. Acta 1307, 157–161
10. Price, N., van der Leij, F., Jackson, V., Corstorphine, C., Thomson, R., Sorensen, A., and Zammit, V. (2002) Genomics 80, 433–442
11. Fraser, F., Corstorphine, C. G., and Zammit, V. A. (1997) Biochem. J. 323, 711–718
12. Cohen, I., Kohl, C., McGarry, J. D., Girard, J., and Prip-Buus, C. (1998) J. Biol. Chem. 273, 29986–29994
13. Shi, J., Zhu, H., Arvidson, D. N., and Woldegiorgis, G. (1999) J. Biol. Chem. 274, 9421–9426
14. Cohen, I., Guillerault, F., Girard, J., and Prip-Buus, C. (2001) J. Biol. Chem. 276, 5403–5411
15. Ijlst, L., Mandel, H., Oostheim, W., Ruiter, J. P., Gutman, A., and Sanders, R. J. (1990) J. Clin. Invest. 86, 527–531
16. Morillas, M., Gomez-Puertas, P., Ruhi, B., Clotej, J., Arino, J., and Hegardt, F. G., Serra, D., and Asins, G. (2002) J. Biol. Chem. 277, 11473–11480
17. Morillas, M., Gomez-Puertas, P., Bentebebel, A., Selles, E., Casals, N., Valen-
19. Pan, Y., Cohen, I., Guillerault, F., Feve, B., Girard, J., and Prip-Buus, C. (2002) J. Biol. Chem. 277, 47184–47189
20. Treber, M., Dai, J., and Woldegiorgis, G. (2003) J. Biol. Chem. 278, 11145–11149
21. Jogl, G., and Tong, L. (2003) Cell 112, 113–122
22. Wu, D., Govindasamy, L., Lian, W., Gu, Y., Kokar, T., Aghandie-McKenna, M., and McKenna, R. (2003) J. Biol. Chem. 278, 13159–13165
23. Bougnieres, P. F., Saudubray, J. M., Marsac, C., Bernard, O., Odievre, M., and Girard J. R. (1980) N. Engl. J. Med. 302, 123–124
24. Bonnefont, J. P., Demaugre, F., Prip-Buus, C., Saudubray, J. M., Brivet, M., Abadi, N., and Thuillier, L. (1999) Mol. Genet. Metab. 68, 424–440
25. Brown, N. F., Mullur, R. S., Subramanian, I., Esser, V., Bennett, M. J., Saudubray, J. M., Feigenbaum, A. S., Kohari, J. A., Macleod, P. M., McGarry, J. D., and Cohen, J. C. (2001) J. Lipid Res. 42, 1134–1142
26. Prip-Buus, C., Thuillier, L., Abadi, N., Prasad, C., Dilling, L., Klassing, J., Demaugre, F., Greenberg, C. R., Haworth, J. C., Drein, V., Kadhorn, N., Gobin, S., Kameon, P., Girard, J., and Bonnefont, J. P. (2001) Mol. Genet. Metab. 73, 46–54
27. Gobin, S., Bonnefont, J. P., Prip-Buus, C., Mugnier, C., Ferrac, M., Demaugre, F., Saudubray, J. M., Rostane, H., Djouadi, F., Wilcox, W., Cederbaum, S., Haas, R., Nyhan, W. L., Green, A., Gray, G., Girard, J., and Thuillier, L. (2002) Hum. Genet. 111, 179–189
28. Ogawa, E., Kanazawa, M., Yamamoto, S., Ohitsuaka, S., Ogawa, A., Ohtake, A., Takayamagata, M., and Kohno, Y. (2002) J. Hum. Genet. 47, 342–347
29. Schaefer, J., Jackson, S., Taromi, F., Swift, P., and Turnbull, D. M. (1997) J. Neurol. Neurosurg. Psychiatry 62, 169–176
30. Saudubray, J. M., Coude, F. X., Demaugre, F., Johnson, C., Gibson, K. M., and Nyhan, W. L. (1982) Pediatr. Res. 16, 877–881
31. Prip-Buus, C., Cohen, I., Kahl, C., Esser, V., McGarry, J. D., and Girard, J. (1998) FEBS Lett. 429, 173–178
32. Lewry, O. H., Rosebrough, N. J., Lewis Farr, A., and Randall, R. J. (1951) J. Biol. Chem. 183, 265–275
33. Laemmli, U. K. (1970) Nature 227, 680–685
34. Brown, N. F., Esser, V., Foster, D. W., and McGarry, J. D. (1994) J. Biol. Chem. 269, 26438–26442
35. Fung, K. L., Hilgenberg, L., Wang, N. M., and Chirico, W. J. (1996) J. Biol. Chem. 271, 21559–21565
36. Sali, A., and Blundell, T. L. (1993) J. Mol. Biol. 234, 779–815
37. Brown, N. F., Anderson, R. C., Caplan, S. L., Foster, D. W., and McGarry, J. D. (1994) J. Biol. Chem. 269, 19157–19162
38. Jackson, V. N., Cameron, J. M., Fraser, P., Zammit, V. A., and Price, N. T. (2000) J. Biol. Chem. 275, 19560–19566