Original articles

The molecular basis of ornithine transcarbamylase deficiency: modelling the human enzyme and the effects of mutations

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Abstract

Human ornithine transcarbamylase is a trimer with 46% amino acid sequence homology to the catalytic subunit of *E coli* aspartate transcarbamylase. Secondary structure predictions, distributions of hydrophilic and hydrophobic regions, and the pattern of conserved residues suggest that the three dimensional structures of the two proteins are likely to be similar. A three dimensional model of ornithine transcarbamylase was generated from the crystal structure of the catalytic subunit of *E coli* aspartate transcarbamylase in the holoenzyme, by aligning the sequences, building in gaps, and minimising the energy. The binding sites for carbamyl phosphate in both enzymes are similar and the ornithine binding site in ornithine transcarbamylase appears to be in the same location as the L-aspartate binding site in aspartate transcarbamylase, with negatively charged side chains replaced by positively charged residues. Mutations in the ornithine transcarbamylase gene found in patients with hyperammonaemia of the "neonatal type" are clustered in important structural or functional domains, either in the interior of the protein, at the active site, or at the interchain interface, while mutations found in patients with milder "late onset" disease are located primarily on the surface of the protein. The predicted effects of all known missense mutations and in frame deletions in the ornithine transcarbamylase gene on the structure and function of the mature enzyme are described.

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Ornithine transcarbamylase deficiency (OTCD), an X linked disorder, is the most common inherited defect of the urea cycle.¹ The human ornithine transcarbamylase (OTC) gene has been localised to the short arm of the X chromosome, band p21.1,² and it has been cloned and sequenced by Horwich *et al*³ and Hata *et al*.⁴ Mature mitochondrial human OTC (ornithine transcarbamylase enzyme) was first

purified in 1978⁵ and shown to be a trimer with 322 amino acids and a molecular weight of approximately 36 000 Daltons per polypeptide chain.

Approximately 70 mutations and polymorphisms in the human OTC gene have been described^{6,7} and a number of biochemical studies of unpurified mutant human OTCases have been reported.⁸ However, for better understanding of the deleterious effects of mutations in the OTC gene, a structural model of the enzyme is essential. The mutations identified so far indicate that most patients with OTCD have "private" alterations rarely seen in other families. Even the few recurring mutations in CpG dinucleotides such as R141Q, which causes neonatal disease, and R277W, which causes late onset disease, are seen in a small fraction of patients, probably less than 10%.⁶ In a large proportion of OTC deficient families, identification of the underlying mutation may be the only tool for providing accurate carrier testing and prenatal diagnosis. Thus, a method for predicting that a mutation found in a patient is deleterious rather than an innocent polymorphism is necessary.

Although very little is known about structure/function relationships in human OTCase, bacterial and yeast OTCases have been studied in greater detail. The yeast and *E coli* enzymes have many features in common with the catalytic subunit of *E coli* aspartate transcarbamylase enzyme (ATCase). ATCase catalyses the first committed step in pyrimidine synthesis in which the carbamyl group of carbamyl phosphate is transferred to the α amino group of L-aspartate. ATCase is an extensively studied allosteric enzyme containing six catalytic and six regulatory polypeptide chains. The six catalytic chains are organised in two identical subunits, each containing three chains.⁹ Isolated catalytic subunits possess full enzymatic activity. Each catalytic polypeptide chain consists of an N-terminal polar domain, which binds carbamyl phosphate, and a C-terminal equatorial domain, which binds L-aspartate.¹⁰

As seen in *E coli* ATCase, binding of substrates and products to *E coli* OTCase is ordered, with carbamyl phosphate binding first and phosphate being released last.¹¹ *E coli* ATCase binds L-aspartate cooperatively and

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binding of Zn(II) induces cooperativity also in *E coli* OTCase by interacting with a Cys residue at the ornithine binding site and inducing a conformational change.^{12–14} Binding of carbamyl phosphate in *E coli* OTCase induces a conformational change¹⁵ as it does in *E coli* ATCase; however, in contrast to ATCase, addition of ornithine produces no detectable additional change in conformation.

An elegant demonstration of the structural similarity between *E coli* ATCase and OTCase was provided by Houghton *et al*¹⁶ who fused the polar domain of *E coli* OTCase with the equatorial domain of *E coli* ATCase. The resulting chimeric protein assembled into a trimer, bound carbamyl phosphate and L-aspartate, and catalysed the formation of carbamyl aspartate. This observation suggests that the structural features required for creating the proper tertiary and quaternary structure for binding carbamyl phosphate and transferring its carbamyl group to the α amino group of L-aspartate or the terminal δ amino group of L-ornithine are essentially identical in both enzymes.

Since human and *E coli* OTCase have 58.8% sequence similarity, the three dimensional structures of the two are almost certainly similar. Although *E coli* OTCase has been crystallised,¹⁷ its structure prediction did not extend beyond determining the space group. However, the sequence, structural, and functional similarities between human OTCase and the catalytic subunit of *E coli* ATCase suggest that it is possible to develop a structural model of OTCase based upon the crystal structure of *E coli* ATCase.^{18–20} We describe here a structural model of human OTCase and the results of mapping onto the model mutations found in the OTCase genes of patients with OTCD. Our results indicate that this model is useful for predicting the functional effects of various mutations on mutant OTCases.

Methods

A computerised search of crystallised proteins for regions of sequence similarity was performed using the Basic Local Alignment Search Tool (BLAST) server based at the National Center for Biotechnology Information.²¹ The search showed that of all crystallised proteins, *E coli* ATCase has the highest degree of homology to human OTCase. The human OTCase amino acid sequence was aligned with that of the ATCase catalytic chain using the Needleman-Wunsch algorithm from the gap module of the Genetics Computer Group (GCG) suite.^{22,23} Most of the gaps were located outside α helices or β strands of the ATCase structure. Frodo, a general purpose molecular modelling and display program, was used to generate a preliminary model by replacing residues in aligned regions of the sequence of the ATCase crystal structure (1RAI in the Brookhaven Protein Data Bank) with amino acid residues of human OTCase.^{24,25} Three insertions of four to five residues each were built in with conformations of similar sequences in the Brookhaven Protein Data Bank, identified through a

BLAST search. Two single amino acids were also inserted. Steep descent energy minimisation was performed by the method of Powell²⁶ with the program X-PLOR²⁷ to improve the orientation of side chains and bond geometry and the movement of the backbone was constrained with a high energy term (20 kcal) to prevent the structure from collapsing.

ATCase and OTCase sequences from other organisms were identified by querying the Predict Protein server at the European Molecular Biology Laboratory in Heidelberg with the *E coli* ATCase and human OTCase sequences.^{28–30} The alignments of these sequences with those of human OTCase and *E coli* ATCase were used to identify evolutionary invariant residues likely to be important for proper folding, assembly, and catalytic activity.

Of a total of 62 mutations found in patients with OTCD,⁶⁷ 40 mutations affecting the code of mature OTCase (38 missense mutations and two in frame deletions of three nucleotides each) do not result in large deletions, insertions, or incomplete polypeptide chains. Eighteen mutations were found in patients with neonatal onset of disease, 14 in late onset disease, and eight were found in females heterozygous for OTCD. All these mutations were mapped onto the structural model of OTCase and their effects on the structure/function of the enzyme were predicted based upon the location in the OTCase model and the chemical alteration created by the respective mutation.

Results and discussion

PRIMARY AND SECONDARY PROTEIN SEQUENCE COMPARISONS OF ALIGNMENT

Both insertions and deletions were needed to align *E coli* ATCase with human OTCase. Five insertions were needed in the ATCase sequence, two involving single residues and three involving four or five residues. Using different values for the gap and length weights did not change the positions of these insertions significantly. Two of the larger insertions occurred at the ends of secondary structure in ATCase (residues 235–238 and 261–264), while the third lies in a loop between an α helix and a β strand (residues 71–75). There were also four single residue deletions and three deletions of two residues each in the ATCase sequence. This alignment yielded a percent similarity of 46.36% and a percent identity of 26.82%.

TERTIARY AND QUATERNARY DESCRIPTION AND COMPARISONS

The positions of protein backbone atoms were constrained in the energy minimisation by a high energy term producing an overall folding of the OTCase model which is very similar to that of the catalytic chain of ATCase (fig 1). The N-terminal half of the protein forms the polar domain, while the C-terminal half forms the equatorial domain. Each domain has a core composed of a parallel β sheet enclosed by α helices. The topology of this repeating α - β - α motif is similar to a "Rossman fold", a folding

Figure 1 (Left) A view of the model of the OTCase homotrimer down the three fold symmetry axis that relates the three monomers. The active sites are at the interfaces between adjacent polypeptides, and catalytically important residues are contributed by both chains. (Right) Secondary structure illustration of the OTCase model, with α helices shown as cylinders and β sheets as arrows, positioned in the same orientation as the monomer in the upper right corner of fig 1 (left). The polar domain is to the left, the equatorial domain to the right, with helix 1 (H1) positioned between the two domains. The numbers on the structures indicate the flanking N- and C-terminal residues of each helix and sheet. The location of both substrates in the model is also shown as grey shadows.

Figure 2 Location of evolutionary conserved residues in the primary sequence of human OTCase. The sequence numbering starts with the leader peptide codes of 32 amino acids; mature human OTCase begins with amino acid 33. OTCase sequences from other organisms were identified by querying the Predict Protein server at the European Molecular Biology Laboratory in Heidelberg with the human OTCase sequence.^{28, 30} The organisms used in the OTCase alignment were: *Homo sapiens*, *Rattus norvegicus*, *Rana catesbeiana*, *Schizosaccharomyces pombe*, *Bacillus subtilis*, *Aspergillus niger*, *Emeticella nidulans*, *Streptococcus pyogenes*, *Mycobacterium bovis*, *Pachysolen tannophilus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Salmonella typhimurium*, *Pseudomonas syringae*, *Saccharomyces cerevisiae*, *Neisseria cinerea*, *Neisseria polysaccharea*, *Neisseria lactamica*, *Neisseria flavescens*, *Neisseria meningitidis*, *Neisseria mucosa*. The alignments of 26 OTCases from these species were used to identify evolutionary invariant residues which are shown as black boxes. Residues in gray boxes are conservative substitutions that do not significantly alter the chemical properties of the residue. When three or fewer residues are listed at a given position, only those alternative amino acids were found in the alignment of all OTCases. Residues in white boxes showed a higher degree of variability.

Figure 3 Positions of conserved residues, residues participating in the active site, and the location of mutations in the structural model of human OTCase. The orientation is approximately perpendicular to that shown in fig 1. The polar domain is to the left, the equatorial domain to the right. Residues 32–35 and 347–354 are shown in an extended conformation because no adequate structural models were found for these regions. (A) Location of residues that were 100% conserved in the alignment of 26 OTCases from different species. Residues in the core of the protein are required for folding and stability, residues at the surface are required for assembly of the trimer, and residues at the active site are required for catalysis. (B) Location of active site residues. The two residues at the far left (H117, V120) belong to the active site of the adjacent monomer. (C) Location of mutations found in patients with neonatal disease. Note the clustering of mutations around the active site, and in the core of the protein. (D) Location of mutations found in patients with late onset disease. Most of these mutations are located near the active site, but on the surface of the protein.

pattern found in several phosphate binding proteins.³¹ Two helices link the two domains, one formed by residues near the middle of the primary sequence (H5) and a second comprised of residues near the C-terminus (H12) which runs back to the polar domain (fig 1, right). The H5 helix appears to maintain the separation of the domains and to contribute residues that stabilise the interface between polypeptide chains in the trimer. As shown in fig 1 (left), in the assembled trimer, the tip of the polar domain of one chain interacts with the interdomain region of a neighbouring chain to form the active site.

ACTIVE SITE ALIGNMENT AND COMPARISONS BETWEEN OTCASE AND ATCASE

The carbamyl phosphate binding motif is highly conserved in the OTCase and ATCase structures. All six residues that interact with carbamyl phosphate in ATCase are conserved in OTCase (Ser 90, Thr 91, Arg 92, Thr 93, Arg 141, and His 168; OTCase sequence) and have the same relative positions in the model. However, Lys 88 in OTCase located near the carbamyl phosphate binding site is replaced by Glu in ATCase.

Since ornithine reacts with carbamyl phosphate via its δ amino group, while the reactive

Mutations in the human ornithine transcarbamylase gene, their predicted effects, and clinical phenotypes

Exon	Codon*	Mutation	Amino acid change	Location†	2° structure interactions	Postulated structure/function effect‡	Clinical/biochemical phenotype§
2	40	CGT→CAT	Arg→His	Polar S1	H1, H12	Lose interactions maintaining proper orientation of the polar and equatorial domains	Late onset, <1 y of age Neonatal disease
2	45	CTA→CCA	Leu→Pro	Polar	H6		
2	60	TCA→TTA	Ser→Leu	Polar H1	H5, H12	Stabilises orientation of the two interdomain helices	Female, 2 y
3	79	GGG→GAG	Gly→Glu	Polar		Affects assembly	Neonatal disease, 5 d, peak NH3 = 1300, OTCase activity = 0
3	81–82	ΔCTT	Deletion of Leu	Polar S2	S4, H12		Female, 7 y, peak NH3 = 248
3	87	GAG→AAG	Glu→Lys	Polar S2		Part of consensus motif for CP binding	Late onset, 7·5 mth, peak NH3 >700
3	88	AAA→AAT	Lys→Asn	Polar	S4	Breaks ionic bond stabilising core of polar domain	OTCase activity = 0
3	92	CGA→CAA	Arg→Gln	Polar H2		Part of consensus motif for CP binding	Late onset, 9 mth, peak NH3 = 200
3	93	ACA→GCA	Thr→Ala	Polar H2	H12, S4, S2	K at this position used to identify OTCase from ATCase	OTCase activity = 3% Neonatal disease
3	94	AGA→ACA	Arg→Thr	Polar H2	H2	Active site residue	Late onset, 5 mth, peak NH3 = 379
3						Affects CP binding	Late onset, 7 y, peak NH3 = 780
4	102	GCA→GAA	Ala→Glu	Polar	S2, S3	Affects assembly	OTCase activity = 1%, no change up to 100 mmol/l CP
4	117	CAT→CTT	His→Leu	Polar			Neonatal disease, <72 h, peak NH3 = 277
4	126	GAC→GGC	Asp→Gly	Polar H3		Affects assembly	OTCase activity = 0
4	129	CGT→CTT	Arg→Leu + donor splice error?	Polar H3		Possibly inefficient translation of correct message owing to splice error	Late onset, same residue affected in the spf mouse (H117N)
4	129	CGT→CAT	Arg→His + donor splice error?	Polar H3		Possibly inefficient translation of correct message owing to splice error	Neonatal disease, <72 h, OTCase activity = 0·9% Late onset
5	141	CGA→CAA	Arg→Gln	Polar S4	S1, H4	141 in direct contact with active site residues 86–93 & 168. Changes in local geometry as well as charge cause loss of activity	Late onset, 9 mth, OTCase activity = 2·1% Identical mutation to the spfASH mouse
5	161	AAT→AGT	Asn→Ser	Polar S5	S1, H4, H5	Arg makes steric intrusion into active site	Neonatal onset, <72 h, peak NH3 ~ 2000
5	162	GGG→AGG	Gly→Arg	Polar S5	H4		OTCase activity = 0
5	172	ATC→ATG	Ile→Met	Interdomain H5	H6	Weakens orientation of interdomain interface	Female, 2 y, peak NH3 ~ 400
5	174	GCT→CCT	Ala→Pro	Interdomain H5	H12	Prolines destabilise helices. This residue is at contact point between helix 5 and 9 which affects orientation of the two domains, weakens interdomain interface	Neonatal disease, <72 h, peak NH3 >700, OTCase activity = 0
5	180	CAG→CAC	Gln→His + donor splice error?	Interdomain H5	H1	Relative orientation between helix 1 and 5 important for domain geometry	Neonatal disease, 8 d, OTCase activity = 0
6	182	CAC→CTC	His→Leu	Equatorial H5	H12, S10	Possibly inefficient translation of correct message owing to splice error	Female, 3 y, peak NH3 ~ 300
6	183	TAT→TGT	Tyr→Cys	Equatorial H5	S6, S9, S10	Decreases stability of Orn domain by loss of ionic interaction with D297	Neonatal disease, 5 d, peak NH3 >380
6	192	AGC→AGG	Ser→Arg	Equatorial S6	S7, S9	Decreases stability of Orn domain	Neonatal disease, <72 h
6	195	GGG→AGG	Gly→Arg	Equatorial S6	S7, S9, S10	Part of beta sheet which forms core of equatorial domain. Changes disrupt structure	Neonatal disease, <72 h, OTCase activity = 0
6	196	GAT→GTT	Asp→Val	Equatorial	S9	Part of beta sheet which forms core of equatorial domain. Changes disrupt local structure	Neonatal disease, 2 d, peak NH3 = 1212, OTCase activity = 0
6	203	TCC→TGC	Ser→Cys	Equatorial H6	H5	Part of beta sheet which forms core of equatorial domain. Changes disrupt structure	Female, infancy
6	216	CAG→GAG	Gln→Glu	Equatorial S7	S6, S8	Interacts with active site residue H168, as well as interactions which orient the two domains	Neonatal disease, 5 d, peak NH3 = 497
7	225	CCG→CTG	Pro→Leu	Equatorial before H7	S7, S8	Part of beta sheet which forms core of equatorial domain. Changes disrupt structure	OTCase activity = 7%, Orn Km = 20 mmol/l
7	225	CCG→ACG	Pro→Thr	Equatorial before H7	S7, S8	Folding of equatorial domain affected by changes to the helix start position	CP Km = normal
8	247	ACA→AAA	Thr→Lys	Equatorial S8	S7	Folding of equatorial domain affected by changes to the helix start position	Female, 2 y
8	264	ACT→GCT	Thr→Ala	Equatorial S9	S6	Part of beta sheet which forms core of equatorial domain. Changes disrupt structure	brother died at 6 d (neonatal disease)
8	268	ATG→ACG	Met→Thr	Equatorial end S9		Postulated Orn binding residues: 267–269 are all conserved, correspond to ATCase residues binding aspartate alpha carboxyl	Neonatal disease, <72 h, peak NH3 ~ 2000, OTCase activity = 0
8	277	CGG→TGG	Arg→Trp	Equatorial 270's loop		Affects conformation of Orn active site	Late onset, 4 y, peak NH3 = 175, OTCase activity = 22%
8	277	CGG→CAG	Arg→Gln	Equatorial H9		Affects conformation of Orn active site	Late onset, 6 mth, peak NH3 = 164. OTCase activity = 6·7%

(continued)

Table—contd

Exon	Codon*	Mutation	Amino acid change	Location†	2° structure interactions	Postulated structure/function effect‡	Clinical/biochemical phenotype§
9	304	TTG→TTT	Leu→Phe	Equatorial	H12	Part of Orn binding site Interactions with other Orn active site residues and interdomain interactions with R92 in CP binding site affect activity	Late onset, 7 mth, peak NH3 ~ 400, OTCase activity = 3.7%
9	309	ΔGAA	Deletion of Glu	Equatorial H11		Structural changes affect the 100% conserved residues important for Orn binding along residues 301–305	Late onset disease, 2 y, peak NH3 ~ 200 OTCase activity = 2.4%
9	320	CGA→CTA	Arg→Leu	Equatorial	S9	Affects folding of equatorial domain	Neonatal disease
10	343	ACA→AAA	Thr→Lys	Polar		Buried charges are unfavourable Destabilises helix 9, affecting orientation of equatorial domain	Female, 8 y, peak NH3 = 250
10	345	TAC→GAC	Tyr→Asp	Polar			Female, 33 mth, peak NH3 = 525, OTCase activity = 3–16%

* Codon number from the translation start site.

† Location of the amino acid change within the OTCase structure and functional domain.

‡ The predicted alteration of structure/function as a result of the amino acid change.

§ Clinical and biochemical data reported on patients affected by the respective mutations.

The data include age of onset of clinical symptoms of hyperammonaemia, highest ammonia level documented in $\mu\text{mol/l}$, residual liver OTCase enzymatic activity in percent of normal, and other biochemical studies on the liver enzyme.

When the mutation is known only in a heterozygous female, it is so noted.

|| 100% evolutionary conserved in an alignment of 26 OTCases from various species.

CP = carbamyl phosphate, Orn = ornithine, spf = sparse fur, spfASH = sparse fur abnormal hair and skin.

group in L-aspartate is the α amino group, some differences in the binding sites of the amino acid substrates of the two enzymes are expected. Thus, Lys 84, Arg 167, Arg 229, Glu 231, and Pro 266 (ATCase sequence) in the binding site for L-aspartate of ATCase have different counterparts in OTCase. In ATCase, Arg 167 interacts with the α carboxylate of L-aspartate, Arg 229 and Gln 231 interact with its β carboxylate, and Lys 84 interacts with both.³² In OTCase, Val 120, possessing a non-polar side chain, replaces the positively charged side chain of ATCase Lys 84 and hence cannot interact with charged groups of the substrate. However, Glu 122 nearby is strongly conserved and probably interacts with the α amino group of ornithine. In addition, Met 268 and Arg 270 of OTCase which replace Arg 229 and Glu 231 of ATCase may interact with the methylene groups of the ornithine side chain.

Kuo *et al*¹² have shown that Zn(II) induces cooperativity and a conformational change in *E coli* OTCase by binding to a Cys residue at the ornithine binding site. Human OTCase also has a Cys residue at the putative ornithine binding site (Cys 303) with four His residues (His 168, 202, and 302 of one subunit and His 117 in the adjacent subunit) 2–10 Å away. Some or all of these residues could act as ligands for a bound metal ion if they were brought closer together by a small conformational change. Therefore, it is possible that binding of Zn(II) or another divalent metal ion at this site may also induce a conformational change and cooperativity in human OTCase.

EVOLUTIONARY CONSERVED RESIDUES

The pattern of conserved residues in OTCase which identifies groups that are likely to be important in folding, catalysis, or stability is illustrated in fig 2. Of the 39 highly conserved OTCase residues, 25 are also present in the alignment with ATCase used to generate this OTCase model, either in its carbamyl phosphate binding site (fig 3A) or in the cores of its domains. Thirty-eight out of the 40 amino acid alterations in OTCase that are inferred

from the mutations listed in the table are not present in any of the 26 OTC genes which have been sequenced. This observation strongly suggests that the 38 mutations have deleterious effects on folding, stability, or catalysis. However, two mutations associated with neonatal presentations, I172M and Q180H, occur normally in other species. The possible mechanisms for the deleterious effect of both mutations are discussed below.

Conserved residues were mapped on the three dimensional model, as illustrated in fig 3A. Residues that are conserved in all known OTCases are concentrated in three clusters: the interdomain crevice, the tip of the polar domain (both of which together form the active site), and the cores of these domains. Residues in the first two clusters are involved in catalysis while those in the third are required to maintain the structural integrity of the protein. Among the OTCases, there is somewhat greater sequence conservation in the polar domain, which binds carbamyl phosphate, than in the equatorial domain, which binds L-ornithine.

LOCALISATION AND EFFECTS OF AMINO ACID ALTERATION ON HUMAN OTCASE

Of approximately 70 mutations and polymorphisms affecting the human OTC gene, 40 mutations (38 missense alterations and two in frame deletions) have been identified in the sequence encoding the mature enzyme. These have been classified in terms of the age of onset and severity of OTCase deficiency. The table summarises all 40 mutations and the resulting amino acid changes, their location on the OTCase model, the expected structure/function effects, and relevant clinical information of the respective patients. The mutations described in the table are mapped on the OTCase model as illustrated in fig 3C and D. The majority of mutations were found in patients with acute neonatal hyperammonaemia and are expected to be more severe. They affect three regions: at or near the active site, at the interface between subunits, or in the cores of the domains. As expected, this is the same pattern as

seen with residues conserved between species. The postulated structural basis of a few of these mutations is discussed below in more detail to illustrate the utility of the model.

Of the mutations causing neonatal disease, R141Q recurs among mutations examined to date. Arg 141 forms contacts with His 168 at the carbamyl phosphate binding site. The neonatal OTCD that results when Arg 141 is replaced by Gln can be attributed to the disruption of this interaction. A different mechanism for loss of function is probably responsible for the deleterious effect of the D126G mutation causing neonatal OTCD. Here, the elimination of a salt bridge to Arg 306 of the putative ornithine binding site in the adjacent polypeptide would probably result in disruption of the tertiary and quaternary structure of the active site.

In contrast, several mutations that result in milder, late onset OTCD are often found in regions of the structure that would not be expected to be directly involved in function. Some of these mutations occur in regions that are conformationally flexible in ATCase. For example, Arg 277, the site of another recurrent mutation involving a CpG dinucleotide (R277W/Q) is part of a flexible protein loop that in ATCase swings in towards the active site when L-aspartate binds to the enzyme. Mutations at this site in OTCase may alter the conformation of this loop and propagate their effects to the equatorial domain by subtle conformational changes. Lys 88 located near the carbamyl phosphate binding site provides a second example. Its side chain is not positioned in the model in a way that would allow it to interact with carbamyl phosphate. Instead, it appears to form a salt bridge with Glu 273, and a hydrogen bond with Asn 199, in the ornithine binding domain. In ATCase, the corresponding residues are involved in closing the crevice between the two domains when L-Asp binds.³³ The charges in the OTCase salt bridge are reversed from those in ATCase, a structural modification that is probably required to accommodate the change in function. The negatively charged Glu 273 is near the ornithine binding site, while in ATCase the corresponding residue near the aspartate binding site is an Arg.

Mutations that would be compensatory if they coexist sometimes occur in neighbouring residues. For example, S192R or Q216E would each alone result in severe OTCase dysfunction since both are part of the core of the equatorial domain. In combination, however, as rare as it would be, their deleterious effects would be attenuated by the formation of a new salt bridge that would neutralise the opposite charges introduced by the mutations. Thus when assessing the deleterious effect of mutations, compensatory changes need to be sought and ruled out. A related example is provided by the I172M mutation near the active site. This is one of the mutations associated with neonatal disease that is found normally in OTCases from other species. This mutation results in a hydrophobic side chain being replaced by a similar residue with somewhat different pack-

ing characteristics. In those OTCases in which this substitution occurs, Ser 207 is replaced by either Leu or Ile, presumably to maintain favourable packing interactions. Since this does not happen when the I172M mutation occurs in human OTCase, neonatal OTCD is likely to occur.

The deleterious effects of some mutations may result from their effects on transcription or translation, rather than altering the three dimensional structure of the mature protein. This is probably the case with Q180H, a second neonatal mutation found normally in another species, suggesting that by itself it does not give rise to a loss in enzymatic activity or protein stability. A His residue is found at this position in the anabolic OTCase of *Pseudomonas aeruginosa*. The effects of this mutation may result from a splicing error, since this mutation changes the terminal nucleotide of exon 5 from G to C and all the exons of normal human OTCase have G as their last nucleotide (G is present in 78% of mammalian splice junctions while C is present in 3%³⁴). Moreover, this splice junction may be less efficient already owing to a relatively uncommon intronic sequence of GTTGG relative to the consensus sequence of GT(A or G)AG.³⁴ Thus, any additional unfavourable replacement may have a deleterious effect on correct splicing at this site.

The table summarises the predicted effects of many other mutations on the structure and function of OTCase based on the structural model described here. It is impossible to discuss them all in this publication; however, we hope that the information that was included will help investigators to determine whether a particular mutation found in the OTC gene is likely to be deleterious or an innocent polymorphism.

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