American Journal of Medical Genetics 71:378–383 (1997)

Invited Review Article

The Ornithine Transcarbamylase (OTC) Gene: Mutations in 50 Japanese Families With OTC Deficiency

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Mutations in the OTC gene in 50 Japanese families with OTC deficiency were reviewed in relation to the phenotype of the patients and predicted structure of the mutant enzyme. Similar to other X-linked diseases, mutant alleles in OTC deficiency are highly heterogeneous. Mutations observed in male patients with neonatal onset of the disease included base insertion/deletion, exon skipping, and nonsense and missense mutations in exon 4, 5, 6, or 7. OTC activity was essentially undetectable in this group of patients. These mutations possibly resulted in unstable mRNA or truncated protein, or involved the active site or core domain of the enzyme leading to structural changes. In male patients with late onset, abnormalities observed were missense mutations in exons 2, 4, 8, 9, and 10, and missense mutations plus donor site errors involving exons 4, 5, and 6. OTC activity in these patients was 8.1 ± 6.3% of the control and most mutations occurred on the surface of the protein. In female patients, age at onset ranged from 19 months to 7 years, depending on residual OTC activities (4.5 to 33% of the control). Most mutations in this group were similar to those seen in male patients with neonatal onset, i.e., nonsense and missense mutations in exons 5 and 6, and exon skipping, leading to null enzyme activity. These collective data can serve for genetic counseling and monitoring in prenatal care. Am. J. Med. Genet. 71:378–383, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: ornithine transcarbamylase (OTC); OTC deficiency; gene mutation; genotype-phenotype correlation

INTRODUCTION

Ornithine transcarbamylase (OTC, EC2.1.3.3) is expressed in the liver and synthesizes citrulline from carbamyl phosphate and ornithine. The enzyme is a trimer composed of identical polypeptide subunits with a molecular mass of about 36,000 daltons. A larger precursor protein with about 4,000 daltons is synthesized in the cytosol and then transported to the mitochondrial matrix, where it is immediately processed to the mature enzyme. The OTC gene maps to Xp21.1 and is 73 kb long with 10 exons [Horwich et al., 1984; Hata et al., 1988; Brusilow and Horwich, 1995]. OTC deficiency is an X-linked semidominant, common urea cycle disorder (MIM *311250) with an estimated frequency of 1 in 80,000 in the Japanese population [Nagata et al., 1991b]. Enzymatic diagnosis of OTC deficiency can be made by assaying the enzyme activity in liver specimens [Matsuda et al., 1971]. In a group of male patients with neonatal onset (≤30 days), OTC activity in the liver is often undetectable and they usually die neonatally. Another group, male patients with late onset (>30 days) have mild clinical symptoms and show decreased but detectable OTC activity in the liver [Matsuda et al., 1991; Nagata et al., 1991a; Brusilow and Horwich, 1995]. Female heterozygotes have a wide clinical spectrum, ranging from asymptomatic to moderate or severe manifestations. The clinical variation is related to the random pattern of X-chromosome inactivation in hepatocytes and to allelic heterogeneity. Mutations of the OTC gene are highly heterogeneous [Brusilow and Horwich, 1995]; the phenotype of the patients depends not only on allelic heterogeneity but also on environmental factors, including the daily intake of protein [Brusilow and Horwich, 1995; Matsuda et al., 1996]. Thus, environmental factors are important in a discussion of the genotype–phenotype correlation for this disease. Since socio-economic status plays an important role in environmental factors and is quite homogeneous in Japan, the present review of Japanese patients with OTC deficiency will provide

Contract grant sponsor: Ministry of Education, Science, Sports and Culture, Japan; Contract grant sponsor: Ministry of Health and Welfare, Japan; Contract grant sponsor: National Center of Neurology and Psychiatry (NCNP) of the Ministry of Health and Welfare of Japan.

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Received 12 September 1996; Accepted 21 January 1997

pathogenetic data on the development of the disease. The present review covers 50 families with OTC deficiency. All patients are of Japanese origin and all but two mutations [Satoh et al., 1992] were identified in our laboratory (Table I). Mutant alleles were analyzed as described elsewhere [Matsuura et al., 1993a, 1994b]. A three-dimensional structure of OTC was predicted from the crystal structure (1RAI in the Brookhaven Protein Data Bank) of the catalytic subunit of *E. coli* aspartate transcarbamylase [Kosman et al., 1993]. On the basis of the similarity of the two enzymes [Houghton et al., 1984; Huygen et al., 1987], homology alignment of OTC to the primary structure of the catalytic subunit of aspartate transcarbamylase was done using the Multiple Sequence Alignment program [Lipman et al., 1989] and modified manually, considering the secondary structure of aspartate transcarbamylase using GeneWorks (IntelliGenetics, USA). Both insertions and deletions required for the alignment were assigned outside the α-helix or β-sheet structure. Replacement of residues, generating a preliminary model and energy minimization, was done using NAOMI [Brocklehurst and Perham, 1993] and InsightII/Discover (Biosym/MSI, USA) programs. The resulting quaternary model is essentially the same as a previous one [Tuchman et al., 1995a].

GENE MUTATIONS IN THE NEONATAL-ONSET GROUP

Base Insertion/Deletion, Exon Skipping, and Nonsense Mutations

An insertion of single base G at codon 47 in exon 2 generates a frameshift and creates a new downstream termination of seven amino acids [Shimadzu et al., 1997], and a deletion of 6 bps AGGCTC, T178 and L179 of exon 5 [Shimadzu et al., 1996]. Six nonsense mutations, including R141X, S164X, Y167X, W193X, R320X, and W332X [Matsuura et al., 1993b, 1994b; Hoshide et al., 1993; Shimadzu et al., 1997; unpublished data]

TABLE I. Gene Mutations and Demographic Data on Japanese Patients With OTC Deficiency

Group	Exon/ Intron*	Nucleotide change	Codon change	Disease onset*	OTC activity (%)	Number of families	References
Males with neonatal onset	I1	IVS + 4, a → c		3D	0	1	Hoshide et al. [1996]
	E2	G insert at codon 47 with frame shift	54 Ter	3D	0	1	Shimadzu et al. [1997]
	I2	IVS + 1, gt → at	Possible E2 skipping	4D	0	1	Unpublished data
	E4	GAC → GGC	Asp126Gly	5D	0.9	1	Matsuura et al. [1994b]
	E5	CGA → TGA	Arg141Ter	6D	0	1	Matsuura et al. [1993b]
	E5	CGA → CAA	Arg141Gln	6D	ND	1	Matsuura et al. [1993b]
	E5	TCA → TGA	Ser164Ter	6D	0	1	Hoshide et al. [1993]
	E5	TAC → TAG	Tyr167Ter	6D	0	1	Shimadzu et al. [1997]
	E5	ATC → ATG	Ile172Met	6D	0	1	Matsuura et al. [1994b]
	E5	ACGCTC deletion	Thr178 + Lue179 deletion	6D	0	1	Shimadzu et al. [1997]
	I5	IVS + 2, gt → gc	E5 skipping	6D	0	1	Matsuura et al. [1995]
	E6	AGC → AGG	Ser192Arg	2D	0	1	Matsuura et al. [1993a]
	E6	TGG → TAG	Trp193Ter	6D	0	1	Shimadzu et al. [1997]
	E6	GAT → GTT	Asp196Val	5D/6D	7/ND	2	Matsuura et al. [1993a]
	E6	CTG → CCG	Leu201Pro	1D	0	1	Shimadzu et al. [1997]
	E6	AGC → AGA	Ser207Arg	3D	0	1	Shimadzu et al. [1997]
	E6	GCG → GTG	Ala209Val	10D	ND	1	Unpublished data
	E7	CCG → CTG	Pro225Leu	4D	2	1	Unpublished data
	E9	CGA → TGA	Arg320Ter	4D	0	1	Unpublished data
	E9	TGG → TGA	Trp332Ter	2D	6	1	Matsuura et al. [1994b]
Males with late onset	E2	CGT → CAT	Arg40His	9Y–56Y	1–12.1	6	Matsuda et al. [1996]
	E2	TAT → GAT	Tyr55Asp	46Y	1.5	1	Nishiyori et al. [1997]
	E4	CAT → CGT	His117Arg	10M	18	1	Satoh et al. [1992]
	E4	CGT → CAT (G → A at 3′ end of E4)	Arg129His +donor splicing error	1M/6M/9M	2.1/3.1/ND	2	Matsuura et al. [1994b]
	E5	CAG → CAC (G → C at 3′ end of E5)	Gln180His +donor splicing error	2Y	7.1	1	Shimadzu et al. [1997]
	E6	AAG → AAA (G → A at 3′ end of E6)	Lys221Lys +possibly generating splicing error	4Y	8.0	1	Shimadzu et al. [1997]
	E8	ACT → GCT	Thr264Ala	5Y	22	1	Matsuura et al. [1993a]
	E8	ACT → ATT	Thr264Ile	13M	2	1	Shimadzu et al. [1997]
	E8	ATG → ACG	Met268Thr	6M/13M	6.7/5.0	2	Matsuura et al. [1993a]
	E8	CGG → TGG	Arg277Trp	13M/15M/8Y	9.8/16/19	3	Hata et al. [1991] Matsuura et al. [1993a]
	E9	TTG → TTT	Leu304Phe +alternative splicing	2Y	6	1	Hoshide et al. [1993] Matsuura et al. [1995]
	E10	GTC → CTC	Val337Leu	6Y	6	1	Unpublished data
Females	E1	CGA → TGA	Arg23Ter	2Y	16	1	Kiwaki et al. [1996]
	E5	CGA → TGA	Arg141Ter	19M	4.5	1	Hata et al. [1989]
	E5	TTG → TTT	Leu148Phe	2Y	17	1	Komaki et al. [1997]
	E5	TCA → TGA	Ser164Ter	7Y	33	1	Unpublished data
	E6	CGG → AGG	Gly267Arg	4Y	6	1	Satoh et al. [1992]
	E8	AGC → CGC	Ser267Arg	8Y	21	1	Shimadzu et al. [1997]
	I8	IVS + 1, gt → at	possible E8 skipping	2Y	25	1	Hoshide et al. [1993]
	E9	CGA → TGA	Arg320Ter	2M	ND	1	Unpublished data

*E, exon; I, intron; D, days; M, Months; Y, years.

were found in eight patients in the neonatal-onset group (Table I). Among them, R320X was the same mutation as the one reported from Korea [Yoo et al., 1996]. Two mutations at the 5'-splicing donor site in introns 2 and 5, GT-to-AT and GT-to-GC, respectively, were detected in two patients. The two mutations are expected to produce exon 2 or exon 5 skipping, as was confirmed in the latter [Matsuura et al., 1995]. An A-to-C transversion in intron 1, +4, was found in a high-risk family in which seven males died neonatally [Hoshide et al., 1996]. All these mutations may have led to an unstable mRNA or truncated protein, and the enzyme activity would not be detectable. Since T178 and L179 are predicted to be located to two α-helix-linking domains, deletion of the two residues may alter geometric orientation of these two domains (Fig. 1a).

Missense Mutations

Missense mutations were identified in nine males with a neonatal onset of the disease. They included D126G, R141Q, I172M, S192R, D196V, L201P, S207R, A209V, and P225L [Matsuura et al., 1993a, b, 1994b, 1995; Hoshide et al., 1993; Shimadzu et al., 1997; unpublished data] (Table I). The mutations covered exons 4–7. P225L was the same mutation reported by Hetzen et al. [1991]. Hepatic OTC activity in a patient with D196V was 7% of the control, and essentially null in other patients. Expression study of the D196V cDNA transfected to cultured COS-1 cells showed 3.7% of the wild-type cDNA product [Matsuura et al., 1994a]. The molecular structure of the mutant enzyme derived from each missense mutation is predicted as follows (Fig. 1a): mutations at the active site (R141Q and D196V), at a part of the hydrophobic active site pocket (I172M), at the subunit interface affecting assembly (D126G), at the well-packed core structure of the ornithine-binding domain affecting protein stability (S192R, L201P, and P225L), and at the interdomain cleft disrupting domain interaction (S207R). A209V is exceptional, in that the mutation is located close to the surface of the protein. Most patients listed in Table I died within several weeks of disease onset or even later, but within several months, regardless of the type of mutation.

GENE MUTATIONS IN THE LATE-ONSET GROUP

Single base substitutions in exons 2, 4, 5, 6, 8, 9, and 10 were identified in this group of patients, the result including missense mutations or missense mutations plus donor splicing site errors (Table I). No nonsense mutations or base deletions/insertions were found. Some families shared identical mutations, such as R40H and R277W. Hepatic OTC activity in this group was 8.1 ± 6.3% of the control. Almost all living patients have been treated with oral administration of sodium benzoate and L-carnithine and a low-protein diet with arginine supplementation [Brusilow and Horwich, 1995; Matsuda et al., 1991].

R40H

A G-to-A transversion at exon 2 generating R40H was found in five families from the Fukuoka district of Kyushu Island [Matsuda et al., 1996] and in one family from Tokyo [unpublished data]. In the families from Kyushu, seven male hemizygotes age 6, 9, 15, 17, 17, 56, and 65 years and a putative candidate age 48 years were identified. OTC activities in autopsied liver specimens were ~12% of the control and those in COS-1 cells transfected with R40H cDNA were 10.2 ± 1.8% of the wild-type cDNA product. Symptoms ranged from death at the first attack at age 9, 15, 17, and 56 years to an asymptomatic condition until age 65 years. Amounts of protein ingested by these subjects may be a factor predisposing them to manifestation of the disease [Matsuda et al., 1996]. All patients lived a normal daily life, and attended school prior to the onset of the disease. In three families carrying R40H, there are three living hemizygotes with normal mental development (age 6 months, 2 years, and 6 years), the first two of whom were diagnosed by prenatal monitoring and have received close follow-up and care since obstetrical delivery. The same mutation was found by Tuchman [1993]

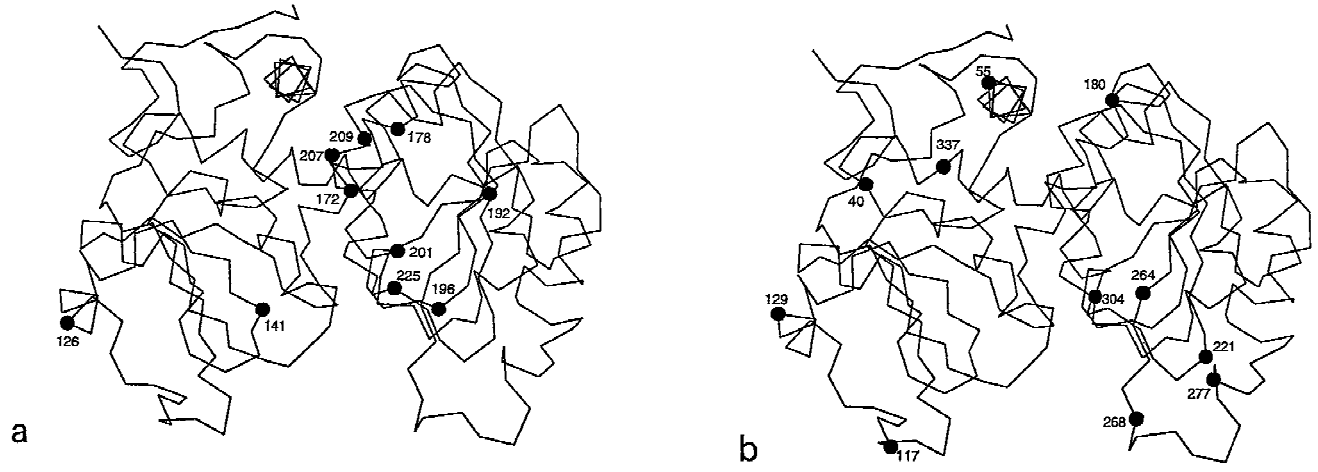

Fig. 1. Schematic drawing of α-carbon tracing of the three-dimensional structure model of ornithine transcarbamylase and sites of missense mutation found in patients with neonatal onset (**a**) and with late onset (**b**) of the disease in Japan.

in a 3-year-old patient. According to the molecular model of the enzyme, R40 forms parts of the β-sheet and the side chain is exposed to the surface (Fig. 1b). A mutation from Arg to His will not alter the net charge of the protein molecule. Thus, the asymptomatic clinical state can be explained.

R129H, Q180H, and L221L Leading to Donor Splicing Errors

The first report of a G-to-A transition at the 3' end of exon 4 generating R129H concerned a 9-month-old boy identified by Matsuura et al. [1994b]. As of this writing, the boy is 9 years old with a normal daily life (IQ = 102). Two additional hemizygotes with R129H were found in another family. The propositus died of the first attack at age 6 months. The mutation in this patient was detected in the autopsied liver tissue and the information was useful for prenatal diagnosis in the second pregnancy. This second child with the mutation is under dietary control and so far has had a normal life (age 16 months). The same mutation was detected in the *Spf-ash* mouse, an animal model of OTC deficiency. The mutation resulted in two mRNAs, one with normal splicing (R129H) and the other with a 48-bp extension of mRNA due to a cryptic splicing donor site in intron 3 [Hodges and Rosenberg, 1989].

Similarly, there were two transitions, G-to-C and G-to-A at the 3' end of exons 5 and 6, in a 2-year-old and a 4-year-old patient, respectively. The splicing error, in addition to normal splicing (Q180H), was noted in the mRNA in the former case [Shimadzu et al., 1997]. In the latter case, although mRNA was not available the mutation was expected to involve the splicing error, the finding corresponding to 8% OTC activity of the control, since normal splicing resulted in a silent mutation L221L [Shimadzu et al., 1997]. The first patient died during the first attack and the second is 12 years old with normal mental development. R129 is possibly situated on the edge of a subunit interface. Q180 is located on the terminal region of two α-helix linking domains and the side chain is exposed to the surface (Fig. 1b).

M268T

M268T, due to a T-to-C transversion at exon 8, was found in a 6-month-old boy [Matsuura et al., 1993a] and in a 13-month-old boy [unpublished data], from two unrelated families. The first patient died at age 2 years and the second patient is living, with an IQ of 51. The mutation is located at the end of the β-sheet at the entrance of the cleft of the active site (Fig. 1b).

R277W

Hata et al. [1991] and Matsuura et al. [1993a] reported data on five male hemizygotes with R277W from three different families; two were from the same prefecture and with the same RFLP haplotype. In these families, propositi experienced their first attack at age 13 months, 15 months, and 8 years, respectively. There was one each asymptomatic hemizygote with the same mutation in the first two families. All five hemizygotes had a normal life until age 9–18 years, but contact with them was subsequently lost. OTC activity in the patients was ~19% of the control and K_m for ornithine at pH 7.5 was approximately 10 times higher than that for the control [Hata et al., 1991]. The same point mutation was noted by Finkelstein et al. [1990] in a patient with late onset of the disease. R277 is located on the surface of the protein, possibly forming an ionic cluster with K307 and E309. A mutation from Arg to non-polar Trp would result in a subtle change of conformation at the active site (Fig. 1b), resulting in a K_m mutation for ornithine.

L304F

In a 4-year-old boy with hyperammonemic attacks [Hoshide et al., 1993; Matsuura et al., 1995], a G-to-A transition at position +45 in exon 9 yielded three mRNAs: a normally spliced transcript (L304F), a 23-bp insertion of intron 8, and a first 50-bp missing within 9 exon. Each aberrant splicing, whose cryptic 3' splicing site is the AG dinucleotide, generates a stop codon at positions 296 and 330. Hepatic OTC activity was 6% of the control. Expression study of L304F mutant cDNA transfected to cultured COS-1 cells demonstrated 34.4 ± 4.1% of the wild-type cDNA product. Since OTC activity in hepatic cells is generally higher than that in the transfected cells [Matsuura et al., 1994a], the discrepancy of a higher activity in the latter cells (6% vs. 34.4%) can be explained by the presence of two unexpected aberrant splicing products. The patient is 8 years old with normal mental development. The mutation is the same as that found in a patient of the late-onset group in the US [Tuchman, 1993]. L304 forms a part of the active site wall, providing a hydrophobic nature to the ornithine-binding area (Fig. 1b).

Other Mutations

Y55D, H117R, T264A, T264I, and V337L were found in six male patients in whom the first attack occurred at age 46 years, 10 months, 5 years, 13 months, and 6 years, respectively. Before the onset of the disease, their daily lives had been normal [Satoh et al., 1992; Matsuura et al., 1993a; Shimadzu et al., 1997; Nishiyori et al., 1997; unpublished data]. OTC activity of the Y55D cDNA expressed in COS-1 cells was 28.0 ± 6.5% of the wild-type cDNA products [Nishiyori et al., 1996]. The replacement of amino acid is located either on the surface (Y55D and H117R) or inside of the protein (T264A, T264I, and V337L) (Fig. 1b). Interestingly, H117 is in the vicinity of the trimmer interface. T264 is in a parallel β-sheet and V337 in two long α-helix-linking domains. These mutations may produce a slight conformational change or result in reduced stability.

GENE MUTATIONS IN FEMALE PATIENTS

Mutations in female patients are heterogenous and similar to those in male patients. The mutation differed in each family. Of the eight female patients analyzed, four had a nonsense mutant allele: R23X [Kiwaki et al., 1996], R141X [Hata et al., 1989], S164X or R320X [unpublished data], and one had a GT-to-AT substitution at the 5' splicing donor site of intron 8,

which possibly generates exon 8 skipping [Hoshide et al., 1993] (Table I). These mutations will lead to a truncated or unstable enzyme protein. There were three missense mutations in other patients: L148F [Komaki et al., 1997], S267R [Shimadzu et al., 1997], and G195R [Satoh et al., 1992]. OTC activity was undetectable by expression studies of L148F cDNA and G195R cDNA using cultured COS-1 cells, the findings being similar to those with mock transfection [Matsuura et al., 1994a; Komaki et al., 1996]. The onset of hyperammonemic attacks ranged from age 19 months to 7 years, depending on residual enzyme activities amounting 4.5% to 33% of the control. These activities are most likely attributable to skewed X-inactivation rather than to allelic heterogeneity, as seven of eight mutations were nonsense mutations, exon skipping, and missense mutation of exons 5 and 6, thereby resembling mutations found in male patients with neonatal onset and leading to undetectable OTC activity. A patient with R23X received a liver transplant from her mother and is living normally at age 4 years. A patient with G195R is severely retarded (age 15 years) but another patient with GT-to-AT substitution at intron 8, +1 (age 16 years) is normal. A patient with S267R was living normally at our last contact with her. Others died during the first attack or later. In six (75%) of the nine families, the mutation had occurred freshly [Hata et al., 1989; Satoh et al., 1992; unpublished data] as reported by Tuchman et al. [1995b]. However, the L148F mutation in a patient was inherited from her phenotypically normal father, who is a germinal mosaic with one cell-line carrying the wild-type gene and the other line carrying the mutant gene [Komaki et al., 1997].

RELATIVE FREQUENCY OF MUTANT ALLELES

One of 20 mutant alleles (5%) and 4 of 12 mutant alleles (33.3%) were found in male patients with neonatal and late onset groups from two or more families, respectively. This difference may partly explain the fact that some hemizygotes with late onset were living normally even after the age of reproduction; thus, the mutant gene was inherited in a female-to-male and then male-to-female fashion and possibly derived from a common ancestor, as was speculated in the case of R40H [Matsuda et al., 1996]. The most frequent mutation is R40H (6/50; 12.0%) and the second most frequent R227W (3/50; 6.0%) in the Japanese population. In the US and Spain, the mutation with the highest incidence was R129H (5/78; 0.4%), as noted by Tuchman et al. [1996], a mutation found in two families in our series. Thus, if mutations found in Japan, the US, and Spain are combined the most frequent is R40H (9/128; 7.0%), the second most frequent R129H (7/128; 5.5%), and the third P225L (4/128; 3.1%) or R227W (4/128; 3.1%). All four of these mutations are due to a substitution of CpG for TpG or CpA. Such a single base change was observed in 11 of 38 (28.9%) alleles in Japan and in 25 (39.0%) of 64 alleles in the US and Spain, 35.3% (36/102) in all. This supports the concept that recurrent mutations occur most often at the CpG dinucleotide [Nussinov, 1981].

ACKNOWLEDGMENTS

We are grateful to Drs. T. Matsuura, S. Komaki, and F. Endo at the Department of Pediatrics, and Dr. M. Shimadzu at the Department of Genetics, Mitsubishi Kagaku Bioclinical Laboratories, Tokyo, Japan, who worked with us on this project. We also thank M. Ohara for her helpful comments.

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