Journal of Human Genetics (2015) 60, 501–507

www.nature.com/jhg

ORIGINAL ARTICLE

Clinical outcomes and the mutation spectrum of the *OTC* gene in patients with ornithine transcarbamylase deficiency

Jin-Ho Choi[1,6], Beom Hee Lee[1,6], Ja Hye Kim[1], Gu-Hwan Kim[2], Yoo-Mi Kim[3], Jahyang Cho[1], Chong-Kun Cheon[3], Jung Min Ko[4], Jung Hyun Lee[5] and Han-Wook Yoo[1]

Ornithine transcarbamylase (OTC) deficiency is an X-linked inborn error of the urea cycle that leads to the accumulation of ammonia, resulting in neurological deficits. This study was performed to describe the clinical outcomes, biochemical features and molecular spectra of patients with OTC deficiency. A total of 49 patients from 47 unrelated Korean pedigrees were included who were diagnosed with OTC deficiency based on biochemical findings and molecular analyses. Patient clinical features, biochemical findings and molecular data were analyzed retrospectively. Males with neonatal-onset phenotype presented with seizure or altered mentality ($n=20$). Biochemical findings showed high blood ammonia ($1132.5 \pm 851.6\ \mu mol\ l^{-1}$) and urine orotic acid ($1840.7 \pm 1731.3\ mmol\ mol^{-1}$ Cr) levels. There were also five males with late-onset disease who presented with vomiting, irritability and seizure at age 8.2 ± 9.4 years old (range, 0.6–20 years). Symptomatic females presented with vomiting, seizure, and altered mentality at age 3.5 ± 3.5 years (range, 0.2–12.8 years; $n=24$). More males with the late-onset form and symptomatic females displayed mild hyperammonemia and orotic aciduria compared with those showing a neonatal phenotype ($P<0.05$). Molecular analysis identified 37 different mutations (22 missense, 5 large deletions, 4 small deletions, 1 insertion, 3 nonsense and 2 splice sites) from all 49 patients; the mutations were dispersed throughout all coding exons. In Korean patients with OTC deficiency, mutations in *OTC* are genetically heterogeneous. Male patients with the neonatal-onset phenotype showed poor outcomes because of severe hyperammonemia. Early diagnosis and interventions for hyperammonemia can provide more favorable prognosis.

Journal of Human Genetics (2015) **60,** 501–507; doi:10.1038/jhg.2015.54; published online 21 May 2015

INTRODUCTION

Ornithine transcarbamylase (OTC) deficiency (OMIM #311250) is the most common urea cycle disorder caused by mutations in the *OTC* gene located on chromosome Xp21, resulting in hyperammonemia that causes neurological deficits.[1] OTC catalyzes the formation of citrulline and inorganic phosphate from carbamyl phosphate and ornithine in the urea cycle.[2] Its clinical signs and symptoms are caused by the toxic effects of hyperammonemia on the brain, leading to coma, cerebral edema and, in severe cases, death.[2] When clinically suspected, the diagnosis of OTC deficiency can be made biochemically based on a low blood citrulline level and increased urinary excretion of orotic acid.[1]

OTC deficiency can be classified into two groups: a neonatal-onset phenotype that completely abolishes enzyme activity resulting from null alleles and a later onset phenotype with residual enzyme activity.[1] Hemizygous males with the neonatal-onset form present with a hyperammonemic coma that often leads to death in the first week of life; those individuals who recover from coma can still be neurologically handicapped as a consequence of hyperammonemic encephalopathy.[1] By contrast, most heterozygous females and some male patients manifest milder late-onset phenotypes with partial enzyme activity.[3] Mutations that result in residual enzymatic activity confer a milder phenotype, with the first clinical presentation occurring sometime between infancy and adulthood.

As of March 2015, 457 *OTC* mutations have been reported (http://www.hgmd.org/). Most of the mutations that cause OTC deficiency consist of single-base pair substitutions that cause missense or nonsense mutations (301/457, 65.9%), whereas a smaller proportions consist of small deletions or insertions (57/457, 12.5%), splice site mutations (50/457, 10.9%), large deletions (41/457, 9.0%), complex rearrangements (6/457, 1.3%) or regulatory mutations (2/457, 0.4%).

[1]Department of Pediatrics, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Korea; [2]Medical Genetics Center, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Korea; [3]Department of Pediatrics, Pusan National University Children's Hospital, Pusan National University School of Medicine, Yangsan, Korea; [4]Department of Pediatrics, Seoul National University Children's Hospital, Seoul, Korea and [5]Department of Pediatrics, Kosin University Gaspel Hospital, Pusan, Korea
[6]These authors contributed equally to this work.
Correspondence: Dr H-W Yoo, Department of Pediatrics, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, 88, Olympic-Ro 43-Gil, Songpa-Gu, Seoul 138-736, Korea.
E-mail: hwyoo@amc.seoul.kr
Received 14 March 2015; accepted 11 April 2015; published online 21 May 2015

Our research group has previously reported clinical features, biochemical findings and molecular analyses of *OTC* in a total of 33 patients with OTC deficiency in Korea.[4–7] These studies included a small number of patients and did not include the long-term outcomes of all subjects. Hence, this present study was undertaken to analyze the clinical outcomes and biochemical features of Korean patients with OTC deficiency and to better characterize the molecular spectrum of mutations of the *OTC* gene among these cases.

SUBJECTS AND METHODS

Patients

A total of 49 patients (25 males, 24 females) from 47 independent pedigrees were included in the current investigation. All patients were born at term with an uneventful delivery. The diagnosis of OTC deficiency was based on the biochemical findings and molecular analyses of the *OTC* gene. The clinical phenotypes of all patients, such as age at diagnosis, presenting symptoms and developmental outcomes, as well as biochemical findings, including plasma ammonia, serum glutamate, glutamine, citrulline and urine orotic acid levels, were reviewed retrospectively. Molecular defects in the *OTC* gene were also analyzed (see below). Written informed consent was obtained from all subjects or from their parents. The study protocol was approved by the Institutional Review Board of Asan Medical Center.

Molecular analysis of the *OTC* gene

Genomic DNA was extracted from peripheral blood leukocytes using a QuickGene DNA blood kit (Fujifilm, Tokyo, Japan). A total of 10 coding exons and exon–intron boundaries of the *OTC* gene were amplified by PCR with customized primers. PCR products were directly sequenced with the same primers using a BigDye Terminator v3.0 Cycle Sequencing Ready Kit (Applied Biosystems, Foster City, CA, USA). Sequencing results were compared with the established human *OTC* sequences (National Center for Biotechnology Information Accession No. NM_000531.5).

Multiplex ligation-dependent probe amplification analysis was performed for patients in whom no *OTC* mutations were identified by direct sequencing using the *OTC* MLPA kit (P079; MRC Holland, Amsterdam, The Netherlands) in accordance with the manufacturer's protocol. The products were separated by electrophoresis and the data were analyzed using an ABI3130*x1* Genetic Analyzer (Applied Biosystems). The peak height of each probes was analyzed using GeneMarker software version 1.70 (SoftGenetics, State College, PA, USA).

Statistical analyses

Statistical analyses were performed using SPSS for Windows version 21.0 (SPSS, Chicago, IL, USA). The age at onset and biochemical parameters in neonatal-onset males, late-onset males and symptomatic females were analyzed using the Kruskal–Wallis test. Clinical outcomes were classified as normal development, developmental delay or death; the outcomes of the three groups were analyzed using Fisher's exact test. Kaplan–Meier curves of the estimated survival rate for each group were generated. *P*-values <0.05 were considered to be statistically significant.

RESULTS

Clinical characteristics of OTC-deficient cases at the time of presentation

The clinical phenotypes and biochemical findings at presentation, and molecular analysis of the *OTC* gene are summarized in Tables 1–3 according to neonatal-onset males, late-onset males and symptomatic females. All 20 males in our cohort with a neonatal-onset phenotype presented with seizure or altered mental function at the age of 4.3 ± 2.1 days (range, 2–10 days). Only one patient (subject 14) was diagnosed immediately after birth in the presymptomatic period for screening of high-risk family members because his two male siblings died during the neonatal period. Mutations in *OTC* were verified in the mothers in 14 (70%) of these patients. Biochemical findings revealed high blood ammonia ($1132.5 \pm 851.6\ \mu mol\ l^{-1}$), serum glutamine ($2009.8 \pm 963.5\ \mu mol\ l^{-1}$; normal 246–1182) and glutamate ($625.0 \pm 1175.2\ \mu mol\ l^{-1}$; normal 32–140) levels and profuse urine orotic acid excretion ($1840.7 \pm 1731.3\ mmol\ mmol^{-1}$ Cr; normal 0.3–6.0) levels (Table 3).

The five males from four independent pedigrees with a late-onset phenotype presented with vomiting, irritability, seizure or drowsy mental status resulting from hyperammonemia at the age of 8.2 ± 9.5 years old (range, 0.6–20 years). These cases were confirmed to have OTC deficiency at the age of 8.6 ± 9.5 years old (range, 0.8–20 years). An *OTC* mutation was documented in the mothers of three (3/5, 60%) of these cases. Biochemical findings of subjects with a late-onset phenotype demonstrated mild hyperammonemia ($163.8 \pm 100.4\ \mu mol\ l^{-1}$; range, 40–258), moderately high serum glutamine ($706 \pm 231.7\ \mu mol\ l^{-1}$; range, 462–923) and glutamate ($435.7 \pm 537.6\ \mu mol\ l^{-1}$; range, 88–1331) levels and orotic aciduria ($128.1 \pm 266.8\ mmol\ mmol^{-1}$ Cr; range, 0–605) compared with those cases showing a neonatal-onset phenotype ($P<0.05$), except for serum glutamate levels (Table 4). Among the five late-onset male patients in our series, two patients (subjects 24 and 25 in Table 2) were siblings who had the same mutation, but the older brother (subject 25) had no clinical abnormalities; the other sibling (subject 24) manifested altered consciousness and hyperammonemia ($258\ \mu mol\ l^{-1}$) at the age of 18 years old and recovered after continuous renal replacement therapy, suggesting that phenotypic heterogeneity existed within this family.[5] Subject 25 was diagnosed in the presymptomatic stage by screening for OTC deficiency in a high-risk family. Prophylactic sodium benzoate and sodium phenylbutyrate were initiated when he was confirmed to have OTC deficiency.

The 24 symptomatic females with heterozygous mutations in *OTC* presented with vomiting, seizure and altered mentality at the age of 3.5 ± 3.5 years old (range, 0.2–12.8 years). Laboratory findings demonstrated mild hyperammonemia ($350.1 \pm 146.8\ \mu mol\ l^{-1}$; range, 157–850), mild elevation of serum glutamine ($996.0 \pm 390.7\ \mu mol\ l^{-1}$; range, 334–1754), glutamate ($250.8 \pm 314.1\ \mu mol\ l^{-1}$; range, 40–1269) and urine orotic acid ($83.9 \pm 74.4\ mmol\ mol^{-1}$ Cr; range, 11.5–293) levels. Notably, in a female heterozygote (subject 28), hyperammonemia ($285\ \mu mol\ l^{-1}$) was triggered by a traffic accident at the age of 12.1 years old. Subject 45 (Table 3) was a female sibling of a male patient (subject 13 in Table 1) who showed a neonatal-onset phenotype and shared the same mutation, suggesting phenotypic heterogeneity associated with the same mutation.

The mutational spectrum of the *OTC* gene

Molecular analysis identified 37 different mutations (22 missense mutations (59.5%), 5 large deletions (13.5%), 4 small deletions (10.8%), 3 nonsense mutations (8.1%), 2 splice site mutations (5.4%) and 1 insertion (2.7%)) among the 49 patients in our series. These mutations were dispersed throughout all coding exons and did not exhibit any domain-specificity as depicted in Figure 1. The p.R26Q, p.H214Y, p.A140P, p.R141Q and complete deletion of *OTC* mutations were found in two unrelated probands, respectively. The c.664_667delinsAC (p.G222Tfs*2) mutation was identified in two siblings (subjects 13 and 45). Among the 37 mutations, six have not been reported elsewhere (p.L57P, deletion of exons 6–10, deletion of exon 10, deletion of exon 2, deletion of exon 5 and c.799_800insA). The p.L57P variant was predicted to be deleterious by PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/), and was not found in the 1000Genomes (http://browser.1000genomes.org/) and dbSNP (http://www.ncbi.nlm.nih.gov/snp/) databases.

A total of 18 different mutations were identified in males with a neonatal-onset phenotype (Figure 1). Of these, 75% (15 subjects) of

Table 1 Clinical, biochemical features and molecular analysis of the *OTC* gene in male subjects with a neonatal-onset OTC deficiency

No.	Sex	Age at onset (days)	Ammonia (μmol l^{-1})	Glutamate (5–150 μmol l^{-1})	Glutamine (254–823 μmol l^{-1})	Citrulline (44–90 μmol l^{-1})	Urine orotic acid (0.2–6 mmol-mol^{-1} Cr)	Nucleotide change	Amino-acid change	Domains	Maternal status
1	M	2	430	NA	NA	33.5	532	c.25T>G	p.L9*	Leader	Carrier
2	M	5	511	64	2473.7	4.7	18.2	c.77G>C	p.R26P	Leader	Carrier
3	M	5	1260	NA	NA	0	3685	c.77G>A	p.R26Q	Leader	Carrier
4	M	10	735	82	1662	0	47.75	c.77G>A	p.R26Q	Leader	Carrier
5	M	6	879	NA	NA	11	3750	c.274C>T	p.R92*	α-helix	Not done
6	M	3	487	NA	NA	NA	NA	c.422G>A	p.R141Q	CPS/β-sheet	Carrier
7	M	3	1779	310	1568	19	NA	c.548A>G	p.Y183C	CPS/α-helix	Carrier
8	M	7	860	NA	NA	5.7	NA	c.571del	p.L191Sfs*15		Carrier
9	M	3	3307	122	2714	2	2448.7	c.583G>A	p.G195R	ONT/β-sheet	Non-carrier
10	M	4	980	NA	NA	0	4382.8	c.614T>C	p.M205T	ONT/α-helix	Carrier
11	M	4	790	NA	NA	6.8	256	c.640C>T	p.H214Y	ONT/β-sheet	Carrier
12	M	3	3157	NA	NA	NA	NA	c.640C>T	p.H214Y	ONT/β-sheet	Carrier
13	M	3	1000	3513	NA	0	70.1	c.664_667delinsAC	p.G222Tfs*2		Carrier
14	M	2	166	134.4	290.8	13.6	23	c.846A>G	p.D249G	ONT/joint	Not done
15	M	6	639	NA	NA	9.3	2546	c.796_805del	p.I265_G268 delinsDfs*19		Carrier
16	M	7	463	NA	NA	7.3	2547.2	c.842T>C	p.F281S	ONT/β-sheet	Not done
17	M	3	856	NA	NA	18.9	3677.4	c.853del	p.F284fs*38		Not done
18	M	3	782	NA	NA	5.3	3618	c.958C>T	p.R320*		Carrier
19	M	5	2000	310	3267	1	NA	c.387-?_1065+?del	Deletion of exons 6–10		Carrier
20	M	2	1568	464.5	2093.2	7.1	8.8	c.1006-?_1065+?del	Deletion of exon 10		Carrier

Abbreviations: CPS, carbamyl phosphate binding domain; Joint, a middle location between the α-helix and β-sheet domain; Leader, the leader sequence that directs the localization of OTC into mitochondria; NA, not available; ONT, ornithine binding domain; OTC, ornithine transcarbamylase.

Table 2 Clinical, biochemical features and molecular analysis of the *OTC* gene in male subjects with late-onset OTC deficiency

No.	Sex	Age at onset (years)	Age at diagnosis (years)	Presenting features	Ammonia (μmol l^{-1})	Glutamate (5–150 μmol l^{-1})	Glutamine (254–823 μmol l^{-1})	Citrulline (44–90 μmol l^{-1})	Urine orotic acid (0.2–6 mmol mol^{-1} Cr)	Nucleotide change	Amino-acid change	Domains	Maternal status
21	M	0.6	0.8	Seizure, irritability	223	105	733	0	24	c.298G>C	p.G100R	CPS/α-helix	Non-carrier
22	M	1.3	2.0	Vomiting, lethargy	71	88	462	12.7	0	c.829C>T	p.R277W	ONT/α-helix	Non-carrier
23	M	2.3	2.3	Vomiting, drowsy mentality	227	103	923	9.2	605	c.967 G>A	p.V323M	CPS/α-helix	Carrier
24[a]	M	17	18	Vomiting, drowsy mentality	258	1331	NA	0	10.4	c.867-3T>C			Carrier
25[a]	M	20	20	No symptoms	40	551.3	NA	14.7	1.1	c.867-3T>C			Carrier

Abbreviations: CPS, carbamyl phosphate binding domain; Joint, a middle location between the α-helix and β-sheet domain; Leader, the leader sequence that directs the localization of OTC into mitochondria; NA, not available; ONT, ornithine binding domain; OTC, ornithine transcarbamylase.
[a]Siblings.

Table 3 Clinical, biochemical features and molecular analysis of symptomatic female subjects with mutations in the *OTC* gene

No.	Sex	Age at onset (years)	Age at diagnosis (years)	Ammonia (μmol l^{-1})	Glutamate (5–150 μmol l^{-1})	Glutamine (254–823 μmol l^{-1})	Citrulline (44–90 μmol l^{-1})	Urine orotic acid (0.2–6 mmol mol^{-1} Cr)	Nucleotide change	Amino-acid change	Domains	Maternal status
26	F	2.3	2.4	213	171	1036	32	84.9	c.77G>A	p.R26Q	Leader	Carrier
27	F	0.2	0.2	389	NA	NA	28	238	c.131C>T	p.T44I	CPS/joint	Not done
28	F	8.0	12.1	285	147	1321	21	17.4	c.140A>T	p.N47I	CPS/α-helix	Not done
29	F	2.8	2.8	284	133	789	15.2	147.09	c.170T>C	p.L57P	CPS/α-helix	Not done
30	F	1.1	1.2	200	364.7	364.7	11.5	59.3	c.386G>A	p.R129H	CPS/α-helix	Non-carrier
31	F	0.7	0.7	340	NA	NA	NA	NA	c.418G>C	p.A140P	CPS/β-sheet	Not done
32	F	2.6	2.7	200	149	971	NA	NA	c.418G>C	p.A140P	CPS/β-sheet	Non-carrier
33	F	3.4	3.9	308	65.1	603.5	15.8	156.8	c.422G>A	p.R141Q	CPS/β-sheet	Not done
34	F	7.0	7.0	547	NA	NA	NA	NA	c.422G>A	p.R141Q	CPS/β-sheet	Not done
35	F	5.0	14.0	429	434	1313	15.5	53.8	c.482A>G	p.N161S	CPS/β-sheet	Non-carrier
36	F	2.5	2.9	326	40	826	NA	53.48	c.533C>T	p.T178M	CPS/α-helix	Non-carrier
37	F	1.9	1.9	495	1269	NA	5	108.5	c.548A>G	p.Y183C	CPS/α-helix	Carrier
38	F	0.7	1.0	364	NA	NA	18	293	c.583G>C	p.G195R	ONT/β-sheet	Not done
39	F	0.8	4.0	195	69	962	18	NA	c.617T>G	p.M206R	ONT/α-helix	Non-carrier
40	F	6.2	6.2	366	70	1013	10	11.47	c.663G>T	p.K221N	ONT/joint	Non-carrier
41	F	1.9	2.0	400	114	753.3	4.9	14.4	c.710C>A	p.A237D	ONT/α-helix	Not done
42	F	2.3	2.3	494	61	334	6.8	140	c.958C>T	p.R320*		Not done
43	F	2.4	2.6	344	111	685	15	26	c.717+3A>G	Skipping of exon 7		Not done
44	F	12.8	13.6	307	401	1754	21	24.5	c.799_800insA	p.S267Kfs*26		Carrier
45	F	0.8	1.9	233	NA	NA	9.58	72.5	c.664_667delinsAC	p.G222Tfs*2		Carrier
46	F	2.3	3.3	372	64	1466	12	61	c.78-347_216+698del	Deletion of exon 2		Not done
47	F	12.5	12.5	157	98	1596	25	22.46	c.386-?_541+?del	Deletion of exon 5		Carrier
48	F	3.8	3.8	305	753.3	1066.1	15.8	61.74		Complete deletion		Carrier
49	F	1.0	1.0	850	NA	1074	4	60.1		Complete deletion		Not done

Abbreviations: CPS, carbamyl phosphate binding domain; Joint, a middle location between the α-helix and β-sheet domain; Leader, the leader sequence that directs the localization of OTC into mitochondria; NA, not available; ONT, ornithine binding domain; OTC, ornithine transcarbamylase.

the mutations were inherited from their mother (Table 1). Most have been previously reported to cause a neonatal phenotype,[2,4,5,8–11] except for the two novel large deletion mutations (deletion of exon 10 and deletion of exons 6–10). In five subjects with a late-onset phenotype from four unrelated families, four mutations were identified: p.G100R, p.R277W, p.V323M and c.867-3T>C (Figure 1). The mutations were inherited in three subjects (60%) from two families. The p.G100R and p.V323M mutations were found to have residual enzyme activities, which ranged from 17 to 23%,[4] and the p.R277W and c.867-3T>C mutations have also been reported to be associated with late-onset disease.[5,12]

We observed 21 different mutations in symptomatic heterozygous females in our series. Seven (29.2%) females' mutations were documented to be inherited from their mother. The p.R26Q, p.R141Q, p.Y183C, p.H214Y and p.R320* mutations were also identified in male patients with neonatal-onset phenotype in our

Table 4 Comparison of clinical and biochemical features in male subjects with a neonatal- or late-onset phenotype and symptomatic females

	Neonatal onset (n = *20*)	*Late onset* (n = *5*)	*Symptomatic females* (n = *24*)	P-*value*
Age at onset (range)	4.3 ± 2.1 days (2–10)	8.2 ± 9.5 years (0.6–20)	3.5 ± 3.5 years (0.2–12.8)	<0.001
Age at diagnosis (range)		8.6 ± 9.5 years (0.8–20)	4.4 ± 4.3 years (0.2–13.6)	
Presenting symptoms	Seizure, altered mentality	Vomiting, irritability, seizure	Vomiting, irritability, seizure	
Ammonia, μmol l^{-1} (range)	1132.5 ± 851.6 (166–3307)	163.8 ± 100.4 (40–258)	350.1 ± 146.8 (157–850)	<0.001
Glutamate (normal, 5–150 μmol l^{-1})	625.0 ± 1175.2 (64–3513)	435.7 ± 537.6 (88–1331)	250.8 ± 314.1 (40–1269)	0.618
Glutamine (normal, 254–823 μmol l^{-1})	2009.8 ± 963.5 (290.8–3267)	706 ± 231.7 (462–923)	996.0 ± 390.7 (334–1754)	0.017
Citrulline (normal, 44–90 μmol l^{-1})	8.3 ± 8.6 (0–33.5)	7.3 ± 7.0 (0–14.7)	15.2 ± 7.3 (4–32)	0.007
Urine orotic acid (normal, 0.3–6.0 mmol mmol^{-1} Cr)	1840.7 ± 1731.3 (8.8–4382.8)	128.1 ± 266.8 (0–605)	83.9 ± 7 4.4 (11.47–293)	0.007

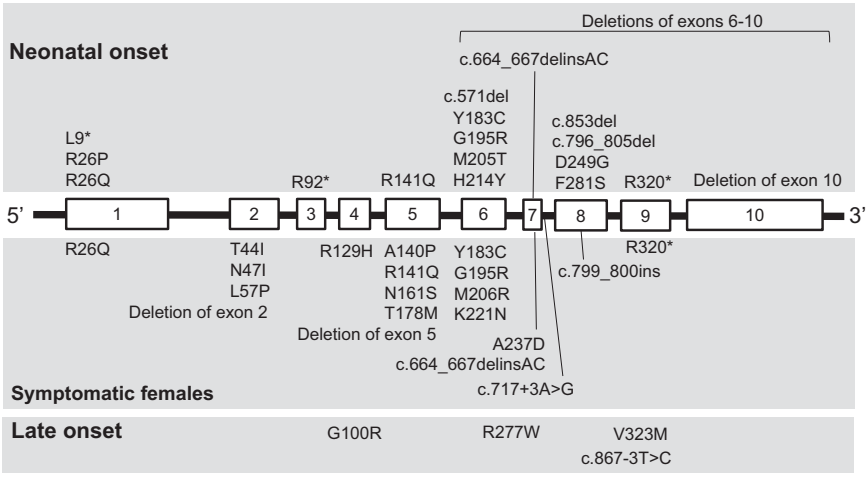

Figure 1 Mutational spectrum of the *OTC* gene in Korean patients with ornithine transcarbamylase (OTC) deficiency. Molecular analysis identified 37 different mutations among the 49 patients. These mutations were dispersed throughout all coding exons without domain-specificity.

cohort. The p.N47I, p.A140P, p.N161S, p.T178M, p.M206R, p.K221N, c.717+3A>G and complete deletion of *OTC* mutation have been previously reported in patients with neonatal onset.[2,13–17] We found only one manifesting female heterozygote who had a mutation that was associated with late-onset disease in males (p.R129H).[18] Two mutations (p.T44I and p.A237D) have only been previously reported in female heterozygotes by our group.[4] This finding suggests that most mutations found in symptomatic female heterozygotes are presumed to confer a neonatal phenotype in hemizygous males, whereas mutations conferring a late-onset type in males are rarely observed in manifesting heterozygous females.[3]

Clinical outcomes

The clinical outcomes of all subjects with OTC deficiency are summarized in Figure 2. Patients were treated with protein restriction with special formula, arginine (100–150 mg kg^{-1} per day), or citrulline (150–200 mg kg^{-1} per day) and alternative pathway therapy that included sodium benzoate (250 mg kg^{-1} per day) and sodium phenylbutyrate (250 mg kg^{-1} per day). In Figure 3, 11 (11/20, 45%) neonatal-onset male patients died because of hyperammonemic crisis in the neonatal period or infancy, and seven males exhibited severe neurological deficits. Although four of our patients with neonatal onset (subjects 9, 13, 19 and 20) underwent continuous renal replacement therapy, one (subject 20) died of hyperammonemic encephalopathy at 2 months of age, and two (subjects 9 and 13) displayed developmental delay. Subject 2 developed a cataract in the left eye and lens dislocation in the right eye at 7 months of age, and then underwent lens removal at 8 months of age. Subject 24 with late-onset disease underwent continuous renal replacement therapy at the time of diagnosis because of altered consciousness. He recovered completely without any neurological defects.

Most females (14/24, 58.3%) in our series exhibited normal development without neurological sequelae. The remaining eight heterozygous female patients experienced neurological sequelae, such as developmental delay. Two female heterozygotes, subjects 27 and 42, died at 2.3 and 5.5 years of age, respectively. Interestingly, one heterozygous female (subject #26 in Table 3) was diagnosed with 21-hydroxylase deficiency at 1 month of age because of a salt-losing phenomenon and hyperkalemia. Molecular testing of the *CYP21A2* gene identified the compound heterozygous mutations c.[293-13A>G]; [955C>T] (p.[splicing];[Q319*]) in this individual. At the age of 2.2 years, she experienced recurrent vomiting and a drowsy mentality and was subsequently diagnosed with OTC deficiency by biochemical and molecular analysis. This subject has since been under treatment with sodium beozoate, sodium phenylbutyrate and arginine with a low-protein diet and has shown normal development.[19]

Liver transplantation was performed in two males in our cohort with neonatal phenotype (subjects 14 and 19) during infancy and one heterozygous female (subject 38) at age 2 years; these patients showed normal development without neurologic sequelae. Patients were classified into neonatal onset, late-onset males and symptomatic females. Their outcomes, including normal development,

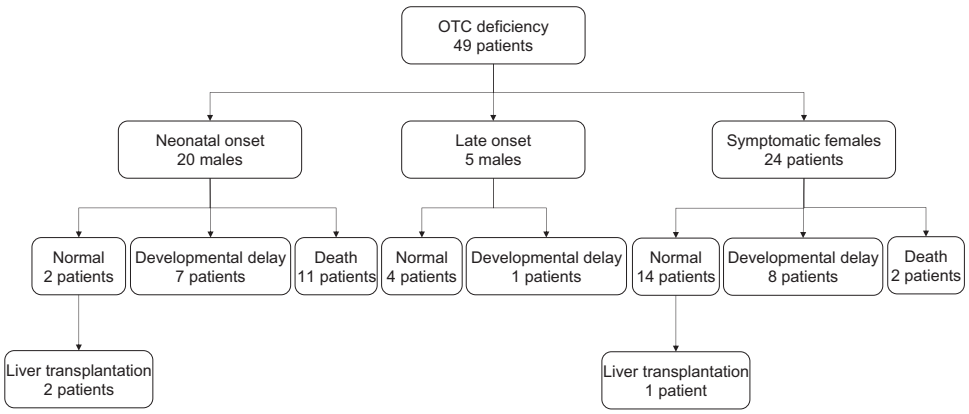

Figure 2 Clinical outcomes of the study subjects with ornithine transcarbamylase (OTC) deficiency. Male patients with the neonatal-onset phenotype showed poor outcomes with neurological deficit or death because of severe hyperammonemia, whereas most females exhibited normal development without neurological sequelae. Two males with neonatal phenotype and one heterozygous female who underwent liver transplantation showed normal development without neurologic sequelae.

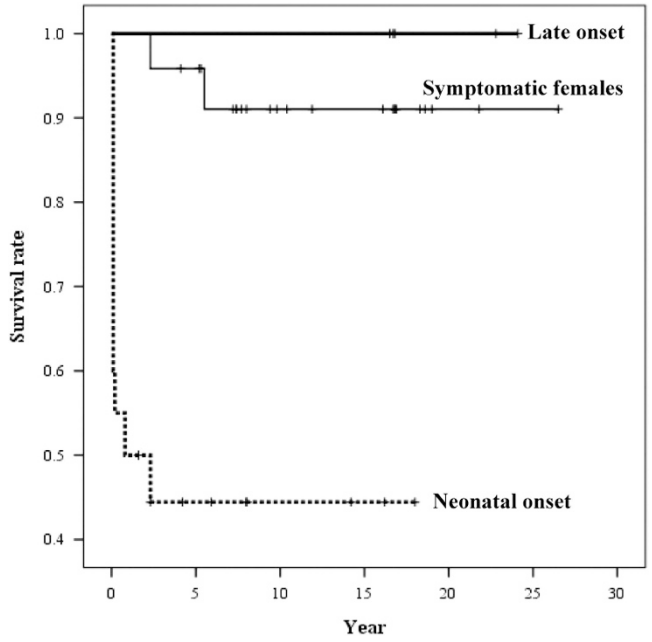

Figure 3 Survival rates of male subjects with neonatal onset or late onset and symptomatic females. The survival rate of neonatal-onset disease was significantly lower (45%) compared with those of late-onset disease (100%) and symptomatic females (91.7%; both, $P<0.001$).

developmental delay and death, were examined using a Fisher's exact test. Overall, we found that the outcomes of the three groups were significantly different ($P<0.001$), indicating that an earlier onset age was associated with poorer developmental outcomes.

DISCUSSION

We here describe 49 patients with OTC deficiency from 47 unrelated Korean pedigrees. The severity of clinical symptoms differed based on the age at onset and hyperammonemia. The age at onset of male patients with a neonatal-onset phenotype was significantly earlier than males with late-onset disease and symptomatic female heterozygotes ($P<0.001$, Table 4). Male patients with the neonatal-onset form showed poor clinical outcomes with neurological deficit or death resulting from severe hyperammonemia compared with those with late-onset and symptomatic females ($P<0.001$, Fisher's exact test). The late onset of male and female patients presented with diverse clinical features, which can hamper early diagnosis for the prevention of acute metabolic crash.

The survival rates of patients with neonatal onset, late onset and manifesting female heterozygotes were 45%, 100% and 91.7%, respectively, resulting in an overall survival rate of 73.5% (Figure 3). The mortality rate of males with neonatal-onset disease was relatively higher than that reported previously[20] because most patients with neonatal-onset type manifesting severe hyperammonemia were identified at a local hospital and were transferred to a tertiary medical center late. Subsequently, therapeutic interventions, such as hemodialysis, continuous renal replacement therapy and toxic metabolite removal by alternative pathway therapy, were so delayed that most patients died before reaching 2 weeks of age, which occurred prior to commencing treatment or becoming neurologically crippled. In addition, continuous renal replacement therapy for newborns was not widely available in the early 1990s. Early diagnosis and proper intervention is critical for achieving good outcomes, as supported by the fact that two males in our present series with a neonatal-onset phenotype (subjects 14 and 19) who were diagnosed early and underwent liver transplantation showed normal development.

Our present study identified 37 different mutations in 47 independent pedigrees in patients with OTC deficiency. The mutations were evenly distributed throughout all coding exons of the *OTC* gene and were largely 'private'.[21] In patients with neonatal onset, these mutations have been predicted to abolish all enzyme activity, with late-onset cases showing residual OTC enzyme activity.[3,22,23] It is known that most *OTC* mutations found in patients with neonatal presentation involve amino-acid residues that are located within the interior of the protein or at the active site, whereas those found in patients with late-onset disease involve an amino-acid residue located far from the active site or on the surface.[21] Splice site mutations in non-consensus sequences have also been shown to cause partial loss of enzymatic activity.[2]

However, in the case of an *OTC* mutation, it is not always possible to predict phenotype based on the genotype, especially in late onset and symptomatic female groups in which a different age at onset and divergent clinical courses can be observed within a genotype.[24] Phenotypic heterogeneity can also be observed in patients within the

same family, and might result from a combination of genetic factors and environmental variables, such as infections or other causes of catabolic stress.[21] Thus, the diagnosis of OTC deficiency should be considered in patients with hyperammonemia, orotic aciduria and low plasma citrulline levels regardless of their age and gender.[1] More research is needed to better understand the genetic and environmental factors that influence the manifestation of partial deficiencies.

Most of our current symptomatic females harbored mutations that were also identified in male patients with neonatal-onset phenotype, as previously described.[3] However, female patients who are heterozygous for an *OTC* mutation present a wide spectrum of clinical manifestations, from apparently normal-to-severe neonatal onset because of random X-chromosome inactivation in hepatocytes or the severity of the mutation.[25] Female patients in one family can also exhibit different phenotypes with the same mutation of the *OTC* gene.[26] Our current data are consistent with the previous findings showing that symptomatic females were associated with *OTC* mutations that caused a severe neonatal-onset phenotype,[3] except for subject 30 in our current series with the p.R129H mutation, which had been previously identified in late-onset disease.[18] It is possible that X-chromosome inactivation in peripheral blood cells might not fully reflect the inactivation pattern in liver cells. Therefore, highly skewed X inactivation in the liver in favor of the mutant allele could cause disease manifestation in female heterozygotes with the hypomorphic mutation p.R129H.[27] As symptomatic females who were treated with medicines that activate pathways of waste-nitrogen excretion have fewer hyperammonemic episodes and a reduced risk of cognitive impairment,[28] it is recommended to treat them to improve survival and stabilizing cognitive functioning.

In summary, we here report the clinical outcomes of a series of 49 Korean patients with OTC deficiency, and the mutational spectrum in the *OTC* gene. Our data indicate that most of the mutations in this population were private and genetically heterogeneous. Our male patients with a neonatal-onset phenotype showed poor outcomes, and we found that an early diagnosis and interventions for hyperammonemia can provide a more favorable prognosis. In addition, family screening and genetic counseling can also be an important aspect of disease prevention.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by a grant from the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (grant no. 2011-0019674).

Author contributions: Yoo H-W designed research. Choi JH and Lee BH wrote the manuscript. Kim JH, Kim YM, Cho J, Cheon CK, Ko JM and Lee JH collected clinical data. Kim GH analyzed the data and performed DNA analysis.

1 Gordon, N. Ornithine transcarbamylase deficiency: a urea cycle defect. *Eur. J. Paediatr. Neurol.* **7**, 115–121 (2003).
2 Yamaguchi, S., Brailey, L. L., Morizono, H., Bale, A. E. & Tuchman, M. Mutations and polymorphisms in the human ornithine transcarbamylase (OTC) gene. *Hum. Mutat.* **27**, 626–632 (2006).
3 McCullough, B. A., Yudkoff, M., Batshaw, M. L., Wilson, J. M., Raper, S. E. & Tuchman, M. Genotype spectrum of ornithine transcarbamylase deficiency: correlation with the clinical and biochemical phenotype. *Am. J. Med. Genet.* **93**, 313–319 (2000).
4 Kim, G. H., Choi, J. H., Lee, H. H., Park, S., Kim, S. S. & Yoo, H. W. Identification of novel mutations in the human ornithine transcarbamylase (OTC) gene of Korean patients with OTC deficiency and transient expression of the mutant proteins *in vitro*. *Hum. Mutat.* **27**, 1159 (2006).
5 Lee, J. H., Kim, G. H., Yoo, H. W. & Cheon, C. K. OTC gene in ornithine transcarbamylase deficiency: clinical course and mutational spectrum in seven Korean patients. *Pediatr. Neurol.* **51**, 354–359 e351 (2014).
6 Yoo, H. W., Kim, G. H. & Lee, D. H. Identification of new mutations in the ornithine transcarbamylase (OTC) gene in Korean families. *J. Inherit. Metab. Dis.* **19**, 31–42 (1996).
7 Yoo, H. W. & Kim, G. H. Prenatal molecular evaluation of six fetuses in four unrelated Korean families with ornithine transcarbamylase deficiency. *J. Korean Med. Sci.* **13**, 179–185 (1998).
8 Grompe, M., Caskey, C. T. & Fenwick, R. G. Improved molecular diagnostics for ornithine transcarbamylase deficiency. *Am. J. Hum. Genet.* **48**, 212–222 (1991).
9 Maddalena, A., Spence, J. E., O'Brien, W. E. & Nussbaum, R. L. Characterization of point mutations in the same arginine codon in three unrelated patients with ornithine transcarbamylase deficiency. *J. Clin. Invest.* **82**, 1353–1358 (1988).
10 Tuchman, M. Mutations and polymorphisms in the human ornithine transcarbamylase gene. *Hum. Mutat.* **2**, 174–178 (1993).
11 Demmer, L. A., Kim, J. M., de Martinville, B. & Dowton, S. B. A novel missense mutation in the exon containing the putative ornithine-binding domain of the OTC enzyme in a female. *Hum. Mutat.* **7**, 279 (1996).
12 Tuchman, M., Morizono, H., Rajagopal, B. S., Plante, R. J. & Allewell, N. M. The biochemical and molecular spectrum of ornithine transcarbamylase deficiency. *J. Inherit. Metab. Dis.* **21** (Suppl 1), 40–58 (1998).
13 Tuchman, M., Morizono, H., Rajagopal, B. S., Plante, R. J. & Allewell, N. M. Identification of 'private' mutations in patients with ornithine transcarbamylase deficiency. *J. Inherit. Metab. Dis.* **20**, 525–527 (1997).
14 Tuchman, M. & Plante, R. J. Mutations and polymorphisms in the human ornithine transcarbamylase gene: mutation update addendum. *Hum. Mutat.* **5**, 293–295 (1995).
15 Oppliger Leibundgut, E. O., Liechti-Gallati, S., Colombo, J. P. & Wermuth, B. Ornithine transcarbamylase deficiency: new sites with increased probability of mutation. *Hum. Genet.* **95**, 191–196 (1995).
16 Arranz, J. A., Madrigal, I., Riudor, E., Armengol, L. & Mila, M. Complete deletion of ornithine transcarbamylase gene confirmed by CGH array of X chromosome. *J. Inherit. Metab. Dis.* **30**, 813 (2007).
17 Carstens, R. P., Fenton, W. A. & Rosenberg, L. R. Identification of RNA splicing errors resulting in human ornithine transcarbamylase deficiency. *Am. J. Hum. Genet.* **48**, 1105–1114 (1991).
18 Tuchman, M., Plante, R. J., McCann, M. T. & Qureshi, A. A. Seven new mutations in the human ornithine transcarbamylase gene. *Hum. Mutat.* **4**, 57–60 (1994).
19 Kim, Y. M., Lee, B. H., Choi, J. H., Kim, G. H., Lim, H. H. & Yoo, H. W. A case with combined rare inborn metabolic disorders: congenital adrenal hyperplasia and ornithine transcarbamylase deficiency. *Gene* **527**, 394–396 (2013).
20 Kido, J., Nakamura, K., Mitsubuchi, H., Ohura, T., Takayanagi, M., Matsuo, M. *et al.* Long-term outcome and intervention of urea cycle disorders in Japan. *J. Inherit. Metab. Dis.* **35**, 777–785 (2012).
21 Tuchman, M., Jaleel, N., Morizono, H., Sheehy, L. & Lynch, M. G. Mutations and polymorphisms in the human ornithine transcarbamylase gene. *Hum. Mutat.* **19**, 93–107 (2002).
22 Tuchman, M., McCullough, B. A. & Yudkoff, M. The molecular basis of ornithine transcarbamylase deficiency. *Eur. J. Pediatr.* **159** (Suppl 3), S196–S198 (2000).
23 Tuchman, M. & Holzknecht, R. A. Heterogeneity of patients with late onset ornithine transcarbamylase deficiency. *Clin. Invest. Med.* **14**, 320–324 (1991).
24 Matsuda, I., Matsuura, T., Nishiyori, A., Komaki, S., Hoshide, R., Matsumoto, T. *et al.* Phenotypic variability in male patients carrying the mutant ornithine transcarbamylase (OTC) allele, Arg40His, ranging from a child with an unfavourable prognosis to an asymptomatic older adult. *J. Med. Genet.* **33**, 645–648 (1996).
25 Girgis, N., McGravey, V., Shah, B. L., Herrin, J. & Shih, V. E. Lethal ornithine transcarbamylase deficiency in a female neonate. *J. Inherit. Metab. Dis.* **10**, 274–275 (1987).
26 Legras, A., Labarthe, F., Maillot, F., Garrigue, M. A., Kouatchet, A. & Ogier de Baulny, H. Late diagnosis of ornithine transcarbamylase defect in three related female patients: polymorphic presentations. *Crit. Care Med.* **30**, 241–244 (2002).
27 Storkanova, G., Vlaskova, H., Chuzhanova, N., Zeman, J., Stranecky, V., Majer, F. *et al.* Ornithine carbamoyltransferase deficiency: molecular characterization of 29 families. *Clin. Genet.* **84**, 552–559 (2013).
28 Maestri, N. E., Brusilow, S. W., Clissold, D. B. & Bassett, S. S. Long-term treatment of girls with ornithine transcarbamylase deficiency. *N. Engl. J. Med.* **335**, 855–859 (1996).