BRIEF REPORT

Mutations in the Iodotyrosine Deiodinase Gene and Hypothyroidism

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SUMMARY

DEHAL1 has been identified as the gene encoding iodotyrosine deiodinase in the thyroid, where it controls the reuse of iodide for thyroid hormone synthesis. We screened patients with hypothyroidism who had features suggestive of an iodotyrosine deiodinase defect for mutations in *DEHAL1*. Two missense mutations and a deletion of three base pairs were identified in four patients from three unrelated families; all the patients had a dramatic reduction of in vitro activity of iodotyrosine deiodinase. Patients had severe goitrous hypothyroidism, which was evident in infancy and childhood. Two patients had cognitive deficits due to late diagnosis and treatment. Thus, mutations in *DEHAL1* led to a deficiency in iodotyrosine deiodinase in these patients. Because infants with *DEHAL1* defects may have normal thyroid function at birth, they may be missed by neonatal screening programs for congenital hypothyroidism.

IODINE IS AN ESSENTIAL COMPONENT OF THYROID HORMONE. TO ENSURE that iodine is available for thyroid hormone biosynthesis, two highly specialized systems evolved in the thyroid gland. One, the sodium–iodide symporter, accumulates iodide in thyroid cells by active membrane transport.[1] The other recycles iodide through the deiodination of monoiodotyrosine and diiodotyrosine, the main iodinated by-products of the synthesis of thyroid hormone.[2-4] This enzyme activity is known to exist in the thyroid, liver, and kidneys.[5] Three decades ago, the partial purification of a flavoprotein from bovine thyroid was reported and found to deiodinate iodotyrosines.[6] However, the molecular nature of this enzyme remained unknown. The use of serial analysis of gene expression on human thyroid tissue led to the identification of *DEHAL1*,[7,8] which encodes a nitroreductase-related enzyme capable of deiodinating iodotyrosines.[8,9] Nitroreductases are proteins that can reduce a wide range of nitroaromatic compounds with the use of flavin mononucleotide as a cofactor.[10]

Hypothyroidism is the most common congenital endocrine disorder, occurring in 1 of 3000 newborns because of complete or partial failure of thyroid development or thyroid hormone production.[11] Thyroid hormones (triiodothyronine and its precursor, thyroxine) are essential for the correct development and maturation of the brain, a process that starts in utero but that extends into postnatal life.[12]

Several genetic defects are known to be associated with both permanent and transient congenital hypothyroidism.[13] In many countries, newborns are screened

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for congenital hypothyroidism, and detected cases are immediately treated, which safely prevents the development of mental retardation.[14] Here we report the identification of defects in *DEHAL1* that produce a phenotype of severe hypothyroidism, goiter, excessive levels of iodotyrosine in serum and urine, and variable mental deficits derived from unrecognized hypothyroidism.

METHODS

PATIENTS

We selected patients for screening for *DEHAL1* mutations on the basis of the presence of hypothyroidism and goiter with additional features consistent with iodotyrosine deiodinase deficiency, including elevated serum diiodotyrosine levels (in Patients 1 and 4), a distinct iodide uptake profile by the thyroid gland (in Patient 4), and a documented family background (in Patients 2 and 3). Genetic screening was performed after written informed consent was obtained from the patients or their parents.

SCREENING FOR MUTATIONS

The six coding exons of *DEHAL1* (National Center for Biotechnology Information accession number, AY259176.1) were amplified by polymerase-chain-reaction (PCR) assay from genomic DNA with the use of primers flanking intron–exon junctions. (Primer sequences are listed in the Supplementary Appendix, available with the full text of this article at www.nejm.org.) The PCR products were purified with the use of a PCR purification kit (Roche) and were directly sequenced with the use of the BigDye Terminator V1.1 cycle sequencing kit and an automated ABI 3100 sequencer (PerkinElmer).

FUNCTIONAL ANALYSIS OF MUTANTS

Wild-type *DEHAL1* complementary DNA was subcloned in the pcDNA3.1 expression vector and subsequently underwent site-directed mutagenesis (Stratagene) for the three changes identified in patients. Wild-type and mutant alleles were expressed in mammalian cells, which were homogenized and subjected to an iodotyrosine deiodinase assay for both monoiodotyrosine and diiodotyrosine substrates with the use of cation-exchange chromatography.

To test the responsiveness of mutants to flavin mononucleotide, increasing concentrations of the flavin were added to the cell-culture medium after transfection, and the homogenates were

analyzed for their monoiodotyrosine deiodination capacity after 24 hours. The protein stability of mutants was studied by quantification of protein signals in Western blots with the use of polyclonal peptide antibodies against DEHAL1 (Eurogentec). To investigate the effect of local amino acid changes that were identified, different artificial mutants were generated at amino acid positions that had mutations in the patients and were functionally tested in the iodotyrosine deiodinase assay. (For further details concerning the experimental procedures, see the Methods section of the Supplementary Appendix.)

RESULTS

PHENOTYPES OF PATIENTS

Patient 1, a 26-year-old woman who had been born in Germany to consanguineous parents of Turkish origin, had a normal thyrotropin level during neonatal screening.[15] However, she presented at 18 months of age with typical signs of hypothyroidism, including an enlarged tongue, puffy and dry skin, and a coarse cry. She had short stature (<3rd percentile for her age) and a delayed bone age of 9 months. On the Denver Developmental Scale for infants, she had a delay of 6 to 8 months. Thyroid function tests were consistent with profound primary hypothyroidism, and an enlarged thyroid gland was evident clinically and on ultrasonography (Table 1). Thyroid uptake of iodide-123 was rapid and high, and administration of perchlorate did not release radioiodine from the gland, which ruled out the existence of an iodide organification defect. The serum diiodotyrosine level was elevated. Despite immediate treatment and strict hormonal control during childhood, the patient had psychomotor retardation, with a low IQ (85 at 9 years of age) and poor school performance. Her parents were euthyroid and without goiter.

Patients 2 and 3, who were 47 and 43 years of age, respectively, were sisters who had received the diagnosis of hypothyroidism during infancy and had been born before the era of routine screening for congenital hypothyroidism (Table 1). Their parents, who were first cousins, were members of a group of Scottish traveling families who had a high incidence of consanguinity and in whom iodotyrosine deiodinase deficiency was clinically described in pioneering reports by Hutchison and McGirr and by Murray et al.[18-20] As a consequence of the family's itinerant life-

Table 1. Characteristics of the Patients.*

Variable	Patient 1	Patient 2	Patient 3	Patient 4	Normal Range†
Demographic					
Sex	F	F	F	M	
Country of origin	Germany	Scotland	Scotland	France	
Year of birth	1979	1958	1962	1996	
Neonatal screening for congenital hypothyroidism‡					
Age (days)	5	NA	NA	4	
Thyrotropin (mU/liter)	15	NA	NA	1.57	<20
Diagnosis of hypothyroidism					
Age	18 mo	Infancy§	Infancy§	8 yr	
Thyrotropin (mU/liter)	390	NA	NA	139	0.4–4.3
Triiodothyronine					
Total (ng/dl)	70	NA	NA	NA	98–195
Free (ng/dl)	NA	NA	NA	0.14	0.2–0.5
Thyroxine					
Total (µg/dl)	1.4	NA	NA	NA	9.3–17.1
Free (ng/dl)	NA	NA	NA	<0.2	0.7–2.1
Uptake of iodide-123 (%)	Elevated	NA	NA	74	2–12¶
Serum diiodotyrosine (pmol/liter)	1470	NA	NA	2500	20–550‖
Urinary iodide (µg/liter)	NA	NA	NA	16	>60
Goiter**	Yes	Yes (compressive)††	Yes	Yes (compressive)‡‡	
Mental retardation§§	Yes	Yes	No	No	
DNA mutation	301C→T	315–317delCAT	315–317delCAT	347T→C	
Amino acid substitution¶¶	R101W	F105–I106L	F105–I106L	I116T	

* NA denotes not available. To convert the values for total triiodothyronine to nanomoles per liter, multiply by 0.01536; to convert the values for free triiodothyronine to picomoles per liter, multiply by 15.36; to convert the values for total thyroxine to nanomoles per liter, multiply by 12.87; to convert the values for free thyroxine to picomoles per liter, multiply by 12.87.
† The values listed are those used in the institutions in which the patients were treated, if not otherwise indicated.
‡ Neonatal screening programs for congenital hypothyroidism were implemented in the mid-1970s in Western countries.
§ Early medical records of Patients 2 and 3 were not available, and they declined to undergo further endocrine investigations that would have required the cessation of thyroxine therapy.
¶ The normal range for healthy adults 2 hours after the administration of radioiodide is from McDougall and Cavalieri.[16]
‖ Values are from Meinhold et al.[17]
** The presence of goiter was determined by physical examination, thyroid ultrasonography or scintigraphy, or a combination of techniques.
†† Surgical removal (hemithyroidectomy) was required.
‡‡ Thyroxine therapy resulted in rapid regression of the goiter.
§§ Mental retardation was determined by developmental tests for children (Denver Scale), clinical assessment, or both.
¶¶ One-letter amino acid codes are used for the designation of mutations.

style, their early medical records were not available. Adherence to thyroxine treatment may have been erratic. Patient 2 had undergone thyroidectomy for compressive symptoms from goiter and had a history of moderate learning disability. Patient 3 had a goiter but no history of learning disability. Their ancestry, history of hypothyroidism since infancy, and goiter made them candidates for the presence of an iodotyrosine deiodinase defect and prompted the molecular screening of *DEHAL1*.

Patient 4 was a 9-year-old boy who had been

born in France to parents of Moroccan descent who were first cousins. The results of screening for congenital hypothyroidism were normal (Table 1). His infancy and childhood were unremarkable, with good physical growth and psychomotor development. At the age of 8 years, he presented with asthenia and an enlargement in neck volume, and his condition was diagnosed as nonautoimmune hypothyroidism and goiter. Ultrasonography of the thyroid showed homogeneous and hypervascularized goiter, and scintigraphy showed a very rapid and high uptake of iodide-123 (74% at both 20 and 50 minutes after administration), which suggested increased iodide turnover in the gland.[21] The patient's serum diiodotyrosine level was elevated. His parents were euthyroid and did not have goiter.

IDENTIFICATION OF *DEHAL1* MUTATIONS

The complete open reading frame of *DEHAL1* was amplified and sequenced in all four patients. Patient 1 had a homozygous C→T mutation in exon 2 of *DEHAL1* (Fig. 1A and 1B), resulting in the replacement of arginine with tryptophan at position 101 (designated Arg101Trp) at the 10th position of the catalytic nitroreductase domain of the DEHAL1 protein, between two predicted flavin mononucleotide–binding amino acids[22] (Fig. 1C).

Patients 2 and 3 were both homozygous for a three-base-pair in-frame CAT deletion in exon 2 of the gene, causing the replacement of both phenylalanine at position 105 and isoleucine at position 106 by leucine (designated Phe105–Ile106Leu) (Fig. 1A and 1B). The change was localized within the nitroreductase domain in close vicinity to another putative flavin mononucleotide–binding amino acid (Fig. 1C).

Patient 4 was homozygous for a T→C mutation in *DEHAL1* that resulted in a substitution of threonine for isoleucine at position 116 (Ile116Thr), located in the nitroreductase domain but outside the flavin mononucleotide–binding pocket (Fig. 1).

For controls, we analyzed 118 chromosomes in DNA from healthy white subjects, since all four patients were also white.

DEHAL1 MUTATIONS IN VITRO

To evaluate the functional consequences of the mutations that were identified, we introduced each mutation into the *DEHAL1* complementary DNA. Wild-type and mutant proteins were tran-
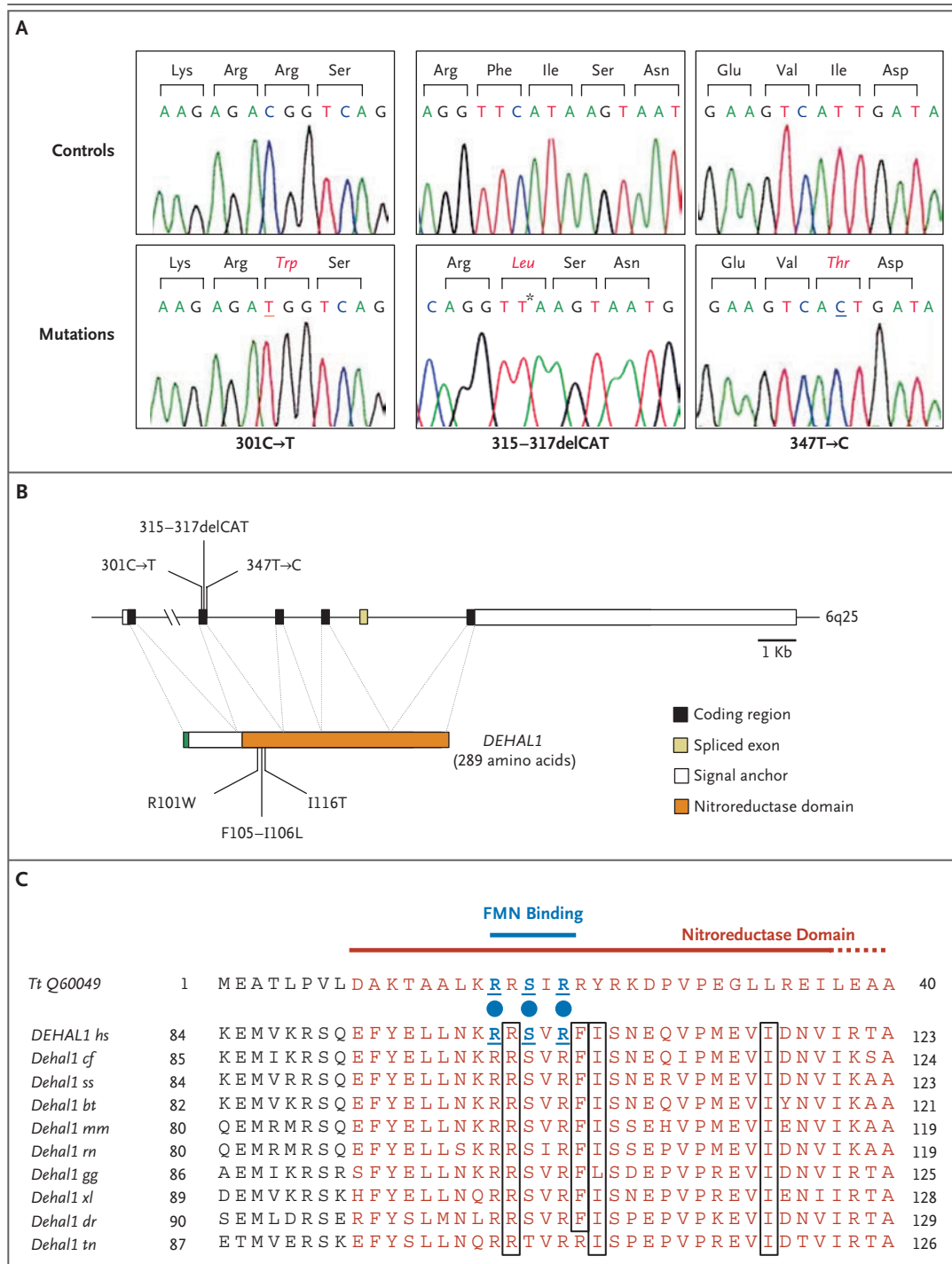
Figure 1 (facing page). Mutations in *DEHAL1* Associated with Iodotyrosine Deiodinase Deficiency.

In Panel A, direct sequencing of the *DEHAL1* gene from the probands and from normal controls shows a homozygous C→T transition at nucleotide 301 (in Patient 1), a homozygous CAT deletion at position 315 to 317 (in Patients 2 and 3), and a homozygous T→C transition at nucleotide 347 (in Patient 4). In Panel B, the locations of mutations in the gene and protein are shown for Patient 1 with a substitution of tryptophan for arginine at position 101 (R101W), for Patients 2 and 3 with a substitution of leucine for both phenylalanine at position 105 and isoleucine at position 106 (F105–I106L), and for Patient 4 with the substitution of threonine for isoleucine at position 116 (I116T). In Panel C, amino acid alignment of vertebrate *DEHAL1* homologues shows high conservation of the residues found in patients with mutations (in black boxes). The crystal structure of the bacterial nitroreductase from *Thermus thermophilus* shows that the highly conserved Arg17, Arg21, and Ser19 (corresponding to Arg100, Arg104, and Ser102 in human *DEHAL1* [hs]) are bound by flavin mononucleotide (FMN).[22] *Tt* denotes *T. thermophilus*, *cf* dog, *ss* pig, *bt* cow, *mm* mouse, *rn* rat, *gg* chicken, *xl* frog, *dr* zebrafish, and *tn* pufferfish. Q60049 refers to a bacterial nitroreductase of *T. thermophilus*. The human DEHAL1 amino acid sequence has the NCBI (National Center for Biotechnology Information) accession number AAP22072.1.

siently expressed in mammalian cells and subjected to an in vitro iodotyrosine deiodination assay. All three mutations dramatically reduced the capacity of DEHAL1 for deiodination of monoiodotyrosine and diiodotyrosine (Fig. 2A and 2B). Although the Arg101Trp and Phe105–Ile106Leu mutations virtually abolished monoiodotyrosine and diiodotyrosine deiodination, Ile116Thr showed some residual activity (4% for monoiodotyrosine and 2.5% for diiodotyrosine).

We examined the deiodinating capacity of the protein product of these mutations in response to the addition of increasing concentrations of flavin mononucleotide in the cell-culture medium. In agreement with the nitroreductase nature of the DEHAL1 protein, wild-type–transfected cells showed an increase in the rate of monoiodotyrosine deiodination that was dependent on the flavin mononucleotide concentration (Fig. 2C). In contrast, Arg101Trp and Phe105–Ile106Leu mutants showed no response, and a partial increase was observed for Ile116Thr with maximal induction by a factor of 7 at 0.5 mM of flavin mononucleotide (Fig. 2C).

We also analyzed the protein abundance of

A

Controls

Lys Arg Arg Ser
A A G A G A C G G T C A G

Arg Phe Ile Ser Asn
A G G T T C A T A A G T A A T

Glu Val Ile Asp
G A A G T C A T T G A T A

Mutations

Lys Arg *Trp* Ser
A A G A G A T G G T C A G

Arg *Leu* Ser Asn
C A G G T T* A A G T A A T G

Glu Val *Thr* Asp
G A A G T C A C T G A T A

301C→T

315–317delCAT

347T→C

B

315–317delCAT

301C→T   347T→C

6q25

1 Kb

DEHAL1
(289 amino acids)

R101W   I116T

F105–I106L

■ Coding region
▢ Spliced exon
□ Signal anchor
■ Nitroreductase domain

C

FMN Binding

Nitroreductase Domain

Tt Q60049	1	M E A T L P V L D A K T A A L K R R S I R R Y R K D P V P E G L L R E I L E A A	40
DEHAL1 hs	84	K E M V K R S Q E F Y E L L N K R R S V R F I S N E Q V P M E V I D N V I R T A	123
Dehal1 cf	85	K E M I K R S Q E F Y E L L N K R R S V R F I S N E Q I P M E V I D N V I K S A	124
Dehal1 ss	84	K E M V R R S Q E F Y E L L N K R R S V R F I S N E R V P M E V I D N V I K A A	123
Dehal1 bt	82	K E M V K R S Q E F Y E L L N K R R S V R F I S N E Q V P M E V I Y N V I K A A	121
Dehal1 mm	80	Q E M R M R S Q E F Y E L L N K R R S V R F I S S E H V P M E V I E N V I K A A	119
Dehal1 rn	80	Q E M R M R S Q E F Y E L L S K R R S I R F I S S E P V P M E V I D N V I K A A	119
Dehal1 gg	86	A E M I K R S R S F Y E L L N K R R S V R F L S D E P V P R E V I D N V I R T A	125
Dehal1 xl	89	D E M V K R S K H F Y E L L N Q R R S V R F I S N E P V P R E V I E N I I R T A	128
Dehal1 dr	90	S E M L D R S E R F Y S L M N L R R S V R F I S P E P V P K E V I D N V I R T A	129
Dehal1 tn	87	E T M V E R S K E F Y S L L N Q R R T V R R I S P E P V P R E V I D T V I R T A	126

mutants, as compared with that of wild-type DEHAL1, in 24-hour transfected cells. As detected by an antibody against carboxy-terminal epitopes of DEHAL1, the Arg101Trp and Phe105–Ile106Leu mutants showed protein levels similar to those of wild-type DEHAL1, whereas the protein signal for Ile116Thr was decreased by a factor of approximately 10 (Fig. 2D). In the absence of significant differences between messenger RNA levels for wild-type and Ile116Thr alleles on semiquantitative reverse-transcriptase PCR experiments, this finding suggested accelerated pro-
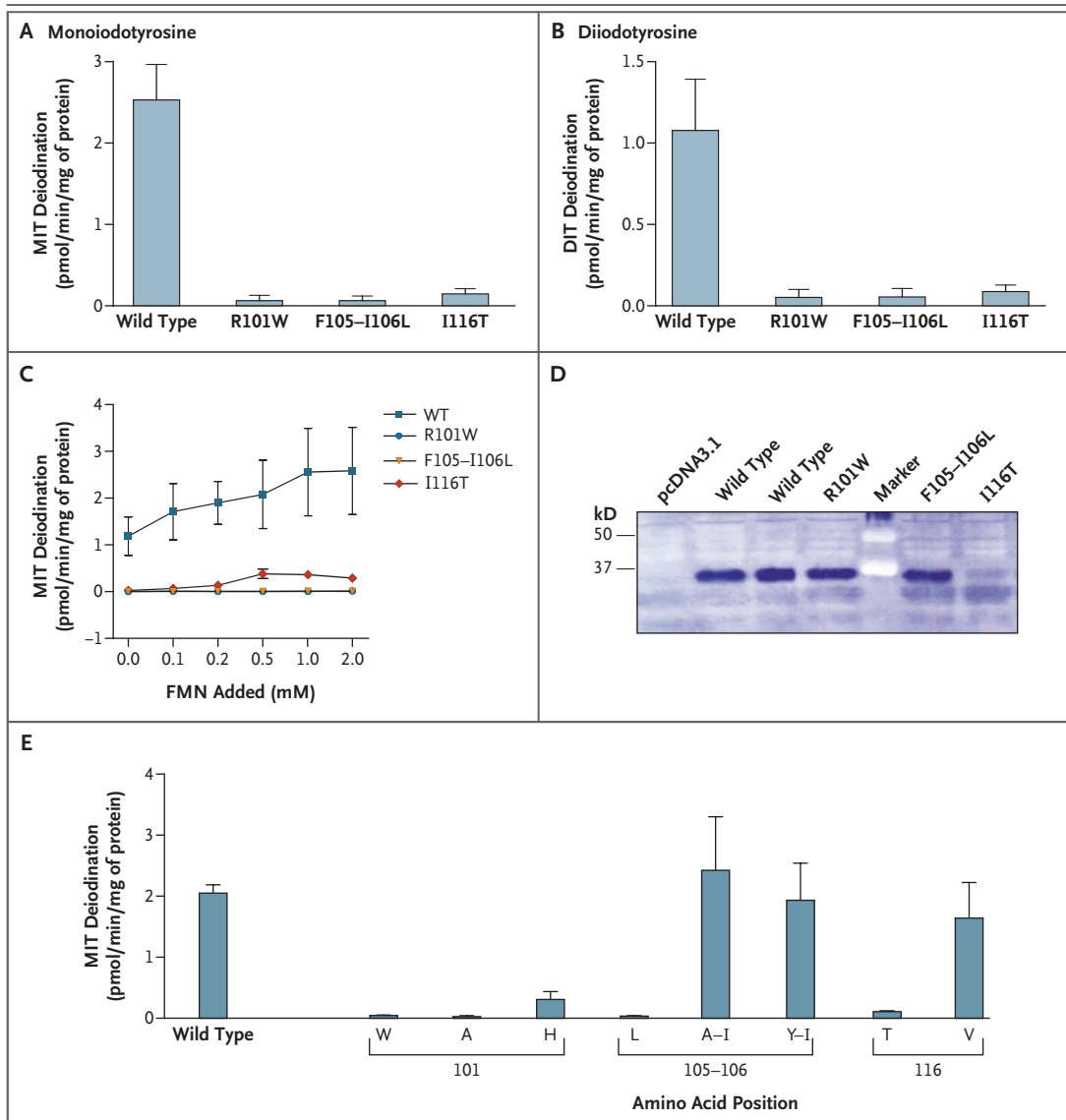
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Figure 2. Functional Characterization of Wild-Type and *DEHAL1* Mutations.

The iodotyrosine deiodinase activity of wild-type and mutant proteins is shown for monoiodotyrosine (MIT, Panel A) and diiodotyrosine (DIT, Panel B) substrates in samples from a normal control (wild type), from Patient 1 with a substitution of tryptophan for arginine at position 101 (R101W), from Patients 2 and 3 with a substitution of leucine for both phenylalanine at position 105 and isoleucine at position 106 (F105–I106L), and from Patient 4 with the substitution of threonine for isoleucine at position 116 (I116T). Panel C shows MIT deiodinase activity of wild-type (WT) and mutant proteins at increasing concentrations of the cofactor flavin mononucleotide (FMN) added to the cell-culture medium. Panel D shows Western blot analysis of the DEHAL1 protein content in HEK293 cell homogenates transfected with wild-type and mutant complementary DNA. Panel E shows MIT deiodinase activity of *DEHAL1* mutants generated by site-directed mutagenesis at amino acid positions (101, 105 to 106, and 116) found in patients with mutations. A denotes alanine, H histidine, I isoleucine, L leucine, T threonine, V valine, W tryptophan, and Y tyrosine.

tein degradation of the Ile116Thr mutant (Fig. 2 of the Supplementary Appendix).

Finally, we examined the effect of the introduction of various amino acids with varied hydropathic profiles, size, and structural characteris-

tics on substrate deiodination at the three mutated sites found in the patients. Replacement of the basic arginine at position 101 by a small and neutral alanine showed the same deleterious effect as the original Arg101Trp mutation (0.9% mono-

iodotyrosine deiodination) (Fig. 2E). In contrast, the introduction of histidine at this position, an amino acid more similar to the wild-type arginine in charge and size, partially rescued the activity (15%). Similarly, introduction of a nonpolar valine at position 116, a hydrophobic amino acid similar to the natural isoleucine at this position, completely recovered the normal deiodinase activity of DEHAL1 (Fig. 2E). However, even when the loss of the aromatic phenylalanine at position 105 seemed the most relevant change in the Phe105–Ile106Leu mutation, we showed that the introduction of either tyrosine (aromatic) or alanine (nonaromatic) at position 105 inferred no reduction in DEHAL1 enzyme activity (Fig. 2E). These results suggest that strong changes in hydrophobicity are involved in the pathogenic effect of the two missense mutations, even though they are not essential in the inactivation of DEHAL1 by the three-base-pair deletion.

DISCUSSION

We screened four patients who had hypothyroidism with biochemical features or a clinical history suggestive of an iodotyrosine deiodinase defect for mutations in *DEHAL1*. We identified three different mutations in exon 2; these mutations were in close vicinity to one another and were localized within the nitroreductase, catalytic domain of the protein. The mutations dramatically disrupt the capacity of DEHAL1 to deiodinate each of its natural substrates, monoiodotyrosine and diiodotyrosine.

The patients presented striking phenotypic variability with regard to the time of expression of the disease. Although hypothyroidism was already present during infancy in Patients 1, 2, and 3, as judged both by clinical records and evident intellectual deficits in two patients, clinical disease presented much later in childhood in Patient 4. One might speculate that differences in iodine intake in the geographic areas where the patients were born or later lived may have influenced the phenotypic expression of *DEHAL1* defects. For example, Patient 1 was exposed to high iodine levels at birth through maternal disinfection with povidone iodine, which might have masked the genetic defect.

The prevalence of iodotyrosine deiodinase defects among persons with hereditary hypothyroidism is currently unknown. We speculate that

borderline expression during the neonatal period and a possible masking of this defect as a nonautoimmune goiter (the cause of which is usually not investigated in detail) might lead to underdiagnosis of the *DEHAL1* defect. Furthermore, the general lack of sensitive assays for diiodotyrosine and monoiodotyrosine to determine these levels in urine or serum and a lack of awareness of this type of hypothyroidism may also contribute to overlooking the diagnosis. Development of alternative diagnostic means (e.g., iodotyrosine detection in urine by mass spectrometry) might facilitate the study of neonatal populations to estimate the prevalence and degree of expression of *DEHAL1* defects in various communities. Better and earlier diagnosis might prevent the mental deficits related to delayed diagnosis and treatment of hypothyroidism when the disease is expressed in infancy, at a time when the brain is still very sensitive to a lack of thyroid hormone.

We identified three different defects in *DEHAL1*, two missense mutations (Arg101Trp and Ile116Thr) and one in-frame three-base-pair deletion (Phe105–Ile106Leu). Although Arg101Trp and Phe105–Ile106Leu virtually abolish the activity of DEHAL1 protein, the Ile116Thr mutant showed significant residual iodotyrosine deiodinase activity. This finding might partially explain the more favorable phenotype of Patient 4. However, functional differences among the mutants, along with environmental factors (e.g., iodine intake), may well be involved in the phenotypic variability of the disease.

Functional analysis of *DEHAL1* mutations suggests that each mutation may lead to disease through varied pathogenic pathways. The two missense mutations involved amino acid changes that caused strong local changes in hydrophobicity. However, the characteristics of the amino acid changes that were introduced by the three-base-pair deletion were not responsible for the deleterious effect of the Phe105–Ile106Leu mutation on DEHAL1 function but rather the deletion of one amino acid at position 105 of the protein. The flavin mononucleotide–dependent increase in iodotyrosine deiodinase activity was absent in the Arg101Trp and Phe105–Ile106Leu mutants, whereas the Ile116Thr mutant retained this capacity. It is tempting to speculate that localization of mutations within or outside putative pockets of flavin mononucleotide binding of the enzyme

could be involved in this distinct effect. Finally, the Ile116Thr mutant was specifically subjected to rapid protein degradation, which did not affect the other two mutants. The rapid degradation of this mutant may have contributed to its low iodotyrosine deiodinase activity more than a decrease in catalytic efficiency. Further studies are required to elucidate the intrinsic pathway implicated in degradation of this particular genetic mutant.

In conclusion, we showed that homozygous mutations in *DEHAL1* appear to cause human iodotyrosine deiodinase deficiency, leading to hereditary hypothyroidism and goiter. There was substantial variability in the time of onset of hypothyroidism in the patients. This variability is of critical importance for the development of mental retardation, since unrecognized hypothyroidism with onset during the first years of life, but not later, will lead to disturbed maturation of the central nervous system and psycho-motor retardation. Factors that can influence this phenotypic variability may be environmental (e.g., variations in intake of iodine) but also genetic, since each individual type of *DEHAL1* defect determines the functional capacity for iodotyrosine deiodination. Our work reveals a crucial role for the DEHAL1 protein in human iodide metabolism[23] and emphasizes the importance of worldwide efforts to eradicate iodine deficiency,[24] including in industrialized countries in which mild-to-moderate iodine deficiency still exists.

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