

KERATIN 8 MUTATIONS IN PATIENTS WITH CRYPTOGENIC LIVER DISEASE

NAM-ON KU, PH.D., ROBERT GISH, M.D., TERESA L. WRIGHT, M.D., AND M. BISHR OMARY, PH.D., M.D.

ABSTRACT

Background About 10 percent of patients who undergo liver transplantation have cryptogenic liver disease. In animal models, the absence of heteropolymeric keratins 8 and 18 or the presence of mutant keratins in hepatocytes causes or promotes liver disease. We have previously described a mutation in the keratin 18 gene in a patient with cryptogenic cirrhosis, but the importance of mutations in the keratin 8 and keratin 18 genes in such patients is unclear.

Methods We tested for mutations in the keratin 8 and keratin 18 genes in purified genomic DNA isolated from 150 explanted livers and 89 peripheral-blood specimens from three groups of patients: 55 patients with cryptogenic liver disease; 98 patients with noncryptogenic liver disease, with causes that included alcohol use, autoimmunity, drug use, and viral infections; and 86 randomly selected inpatients and outpatients who provided blood to the hematology laboratory.

Results Of the 55 patients with cryptogenic liver disease, 3 had glycine-to-cysteine mutations at position 61 (a highly conserved glycine) of keratin 8, and 2 had tyrosine-to-histidine mutations at position 53 of keratin 8. These mutations were not detected in the patients with other liver diseases or in the randomly selected patients. In transfected cells, the glycine-to-cysteine mutation limited keratin-filament reorganization when the cells were exposed to oxidative stress. In contrast, the tyrosine-to-histidine mutation destabilized keratin filaments when transfected cells were exposed to heat or okadaic acid stress.

Conclusions Mutations in the keratin 8 gene may predispose people to liver disease and may account for cryptogenic liver disease in some patients. (N Engl J Med 2001;344:1580-7.)

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KERATINS comprise a group of intermediate-filament cytoskeletal proteins with more than 20 members. They are expressed in epithelial cells and are classified as type I (keratins 9 through 20) or type II (keratins 1 through 8).^{1,2} Epithelial cells express at least one type I keratin and one type II keratin, which form noncovalent heteropolymers in a 1:1 ratio. Keratins 8 and 18 are found primarily in single-layered epithelia (e.g., within the liver, pancreas, and intestine), whereas heteropolymers of keratins 5 and 14 are found in basal keratinocytes of stratified epithelium and heteropolymers of keratins 1 and 10 are found in suprabasal keratinocytes of stratified epithelium.¹⁻⁴

Of the “soft” keratins (those other than the “hard” keratins of epidermal appendages), there are 13 (keratins 1 through 6 and keratins 9, 10, 12, 13, 14, 16, and 17) in which mutations have been associated with primarily autosomal dominant epidermal, oral, and ocular diseases.^{3,5-8} Studies in animal models suggest that the liver and intestine are likely targets of disease in the presence of mutations in the genes for keratin 8 and keratin 18.^{4,9,10} For example, in one strain of mice that lack expression of the keratin 8 gene, most of the mice have extensive liver hemorrhage and die before birth.¹¹ In another strain, some mice have rectal prolapse, colitis, mild elevations in aminotransferase levels,¹² hepatocyte fragility,¹³ and marked susceptibility to hepatotoxin-induced liver injury.¹³⁻¹⁵ These various phenotypes highlight the importance of other modifier genes in the manifestation of a disease phenotype. Similarly, transgenic mice that overexpress mutant keratin 18 have mild chronic hepatitis, fragile hepatocytes, and marked susceptibility to drug-induced liver injury.^{14,16,17}

These findings led to the hypothesis that mutations in the genes for keratin 18, keratin 8, or both may cause or predispose people to acute or chronic liver disease that would otherwise be classified as cryptogenic.⁹ We previously described a mutation in the keratin 18 gene (the substitution of leucine for histidine at position 127) in 1 of 28 patients with cryptogenic cirrhosis.¹⁸ We report the identification of five additional, unrelated patients who had mutations in the keratin 8 gene that may have predisposed them to cryptogenic cirrhosis.

METHODS**Patients**

We analyzed specimens of 150 explanted livers, which were obtained from the liver-transplantation units at the University of California–San Francisco and California Pacific Medical Center, and peripheral-blood samples from 1 patient with cryptogenic cirrhosis, 2 patients with neonatal hepatitis, and 86 other randomly selected (control) patients seen at the Palo Alto Veterans Affairs Medical Center or Stanford University Medical Center, according to protocols approved by the human-subjects committees at these institutions. All the patients or their parents gave written informed consent.

From the Gastroenterology Section, Palo Alto Veterans Affairs Medical Center and Stanford University School of Medicine, Palo Alto, Calif. (N.-O.K., M.B.O.); the Department of Transplantation, California Pacific Medical Center, San Francisco (R.G.); and the Gastroenterology Section, San Francisco Veterans Affairs Medical Center, San Francisco (T.L.W.). Address reprint requests to Dr. Ku at the Palo Alto Veterans Affairs Medical Center, Mail Code 154J, 3801 Miranda Ave., Palo Alto, CA 94304.

In 55 patients cryptogenic cirrhosis was diagnosed according to the following criteria: the absence of serologic markers of hepatitis B and hepatitis C and of mitochondrial, nuclear, and smooth-muscle antigens; the presence of normal iron, ceruloplasmin, and alpha₁-antitrypsin levels; and the absence of ingestion of alcohol or other hepatic toxins. Of the 98 patients with noncryptogenic liver disease, 20 had alcohol-related disease, 10 hepatitis C, 27 autoimmune hepatitis, 21 acute fulminant hepatitis, 7 drug-induced disease, 3 neonatal hepatitis, 2 primary biliary cirrhosis, 2 Wilson's disease, 2 alpha₁-antitrypsin deficiency, 1 chronic rejection, 1 hepatitis B, 1 hemochromatosis, and 1 veno-occlusive liver disease. Among the 153 patients with cryptogenic or noncryptogenic liver disease were 120 previously described patients.¹⁸ In addition, peripheral-blood specimens from a control group consisting of 86 randomly selected inpatients and outpatients, all of whom provided blood to the hematology laboratory on a randomly chosen day, were analyzed for mutations. Specimens from the control group were analyzed only by restriction-enzyme digestion for the presence of two mutations in the keratin 8 gene: the substitution of cysteine for glycine at position 61 (G61C) and the substitution of histidine for tyrosine at position 53 (Y53H).

Molecular Methods

NIH 3T3 cells (which do not express keratins) were transfected with both DNA encoding wild-type human keratin 18 and one of three human keratin 8 constructs: constructs encoding wild type, Y53H, or G61C keratin 8.^{16,18} Transfection of both keratin 8 and keratin 18 stabilizes the keratins and allows the formation of filaments.^{19,20} Genomic DNA was prepared with a tissue kit (DNeasy, Qiagen, Chatsworth, Calif.). (The sequences of the primers used in the polymerase chain reaction [PCR] are available with the full text of this article at <http://www.nejm.org>.) The products of PCR amplification were analyzed (Mutation Detection Enhancement gels with 5 percent glycerol, FMC BioProducts, Rockland, Me.) and sequenced when necessary (ABI 377 sequencer, Applied Biosystems, Foster City, Calif., and the GeneScan Analysis Program, version 2.0.2, Applied Biosystems). Genomic DNA samples with shift patterns according to gene scanning were used to generate a 521-bp species corresponding to the keratin 8 head domain for subsequent sequencing and *Nco*I and *Eco*T221 restriction-enzyme digestion.

Biochemical Methods

Tissue specimens from explanted livers were homogenized in phosphate-buffered saline containing 1 percent n-dodecyl-N,N-dimethylglycine (Empigen BB, Calbiochem-Novabiochem, San Diego, Calif.), EDTA (5 mmol per liter), and protease inhibitors. The specimens were centrifuged at 16,000×g for 30 minutes, and the clarified supernatant was used for precipitation.¹⁸ Immunoprecipitates of the keratin 8-keratin 18 complex were obtained from the explanted livers with the use of anti-K8-K18 antibody L2A1¹⁶ and then analyzed by one of the following methods: denaturing polyacrylamide-gel electrophoresis under reducing or nonreducing conditions (i.e., with or without mercaptoethanol) followed by staining with Coomassie blue; immunoblotting; or two-dimensional gel analysis (isoelectric focusing in the horizontal direction and polyacrylamide-gel electrophoresis in the vertical direction)²¹ followed by immunoblotting. Extracts were obtained by solubilizing transfected cells with Laemmli²² sample buffer or by disrupting cells by freezing and thawing, centrifuging (to obtain a cytosolic fraction), and then solubilizing the pellet with sample buffer. Antibodies (Neomarkers, Union City, Calif.) that are specific for keratin 8 alone (antibody M20) or keratin 18 alone (antibody DC10) were used for immunoblotting.

Immunofluorescence Staining

NIH 3T3 cells transfected with DNA encoding both keratin 8 and keratin 18 were fixed in 100 percent methanol after exposure to control conditions, heat (42°C for 18 hours), okadaic acid (1 μg per milliliter for 1 hour), or hydrogen peroxide (0, 20, or 50 mmol per liter for 1 hour) before fixation (total culture time, 54 hours).

The keratins were stained first with M20 and then with Texas Red-conjugated goat antimouse antibodies.¹⁶ Images were obtained with a confocal scanner (MRC 1024, Bio-Rad, Hercules, Calif.) with a microscope (Eclipse TE300, Nikon, Melville, N.Y.). The transfected cells were counted and categorized, according to the type of keratin filament they contained, as having normal filaments, collapsed filaments (visible as perinuclear collapse or thick bundles), or disassembled filaments (visible as dots or short filaments).

Statistical Analysis

Fisher's exact test (two-tailed)²³ was used to analyze the proportion of patients with keratin 8 mutations in the group with cryptogenic liver disease in comparison with the proportion of those with keratin 8 mutations in the group with noncryptogenic disease and in comparison with the proportion of those with keratin 8 mutations in the group of randomly selected patients (the control group). Pearson's chi-square test²³ was used to compare the filament phenotypes of transfected cells; 194 to 225 cells per construct were counted in two independent experiments.

RESULTS

Identification of Mutations in the Keratin 8 Gene

We examined DNA extracted from specimens of explanted livers or peripheral blood for the presence of mutations in the keratin 8 or keratin 18 gene. Initially, DNA from two groups of patients — 55 with cryptogenic liver disease and 98 with noncryptogenic chronic or acute liver disease — was analyzed (Table 1). Isolated genomic DNA was used as a template to amplify exonic regions (a diagram is available with the full text of this article at <http://www.nejm.org>). Subsequent analysis of the amplified regions revealed the presence of a heterozygous mutation in three patients, consisting of a nucleotide change (G to T at codon 62), resulting in the replacement of glycine with cysteine at amino acid position 61 (G61C). Another heterozygous mutation was found in the DNA of two other patients (a change in nucleotide from T to C at codon 54), resulting in the replacement of tyrosine with histidine at position 53 (Y53H) (Fig. 1A). The G61C mutation predicted a new restriction site for the enzyme *Eco*T221, and Y53H predicted a new restriction site for *Nco*I, predictions that were confirmed in additional experiments (data not shown). None of the 86 control patients without liver disease had either mutation, according to this analysis (data not shown). The mutations in the keratin 8 gene were found in 3 of 34 white patients and in 5 of 55 patients with cryptogenic cirrhosis, a significantly higher frequency than in the other groups of patients, who had no detectable mutations (Table 1).

There were no significant associations between sex and the presence of mutations in the keratin 8 gene, and there were no specific histologic features associated with these mutations in the explanted-liver specimens (or pretransplantation liver-biopsy specimens that were available) that contained them. Two of the patients with the G61C mutation had autoimmune features. One patient with a history of multiple-substance abuse had mixed lymphocytic and plasma-cell infiltrates in her liver-biopsy specimen and explanted

TABLE 1. RACIAL OR ETHNIC BACKGROUND AND SEX OF THE PATIENTS.*

CHARACTERISTIC	PATIENTS WITH CRYPTOGENIC LIVER DISEASE (N=55)	PATIENTS WITH NONCRYPTOGENIC LIVER DISEASE (N=98)	RANDOMLY SELECTED PATIENTS (CONTROLS) (N=86)	KERATIN 8 MUTATION DETECTED	P VALUE†	P VALUE‡
	number of patients					
White	34	62	64	3	0.04	0.04
Male	14	26	56	2		
Female	20	36	8	1		
Black	1	10	6	1	0.09	0.14
Male	0	3	5	0		
Female	1	7	1	1		
Hispanic	10	18	3	1	0.36	1.00
Male	3	5	3	0		
Female	7	13	0	1		
Asian	2	5	7	0	—	—
Male	1	3	6			
Female	1	2	1			
Native American	0	1	0	0	—	—
Male		0				
Female		1				
Pacific Islander	1	0	0	0	—	—
Male	1					
Female	0					
Middle Eastern	3	0	0	0	—	—
Male	2					
Female	1					
Unknown	4	2	6	0	—	—
Total	55	98	86	5	0.005	0.008

*The patients' sex and racial or ethnic background were determined from a review of patients' records. Records were not available for 12 patients. All five mutations were found in unrelated patients with cryptogenic liver disease. Two white patients (one male and one female) and one black female patient had a G61C mutation; one white male patient and one Hispanic female patient had a Y53H mutation. Dashes indicate that no comparisons were performed because no mutations were detected.

†P values are for the comparison between the proportion of patients with keratin 8 mutations in the group with cryptogenic liver disease and the proportion of those with keratin 8 mutations in the group with noncryptogenic liver disease.

‡P values are for the comparison between the proportion of patients with keratin 8 mutations in the group with cryptogenic liver disease and the proportion of those with keratin 8 mutations in the group of randomly selected patients (the control group).

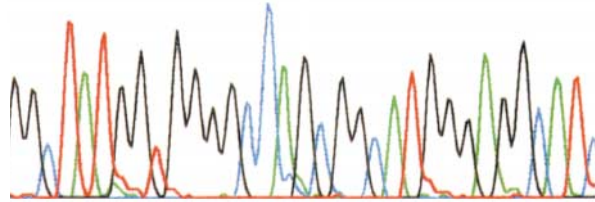
Figure 1 (facing page). Identification of Mutations in the Keratin 8 Gene and Expression of Mutant Keratin 8.

In Panel A, exonic regions corresponding to amino acids 50 through 107 and 341 through 401 of keratin 8 and 67 through 131, 223 through 273, and 322 through 389 of keratin 18 were amplified by PCR followed by DNA sequencing (shown in color). Five patients had heterozygous mutations in the keratin 8 gene that resulted in substitutions in the head domain. The normal (wild-type) products were also amplified and had a normal sequence, since the mutations were heterozygous. The encoded amino acids are denoted by their single-letter code; N indicates the site of a nucleotide substitution. As shown in Panel B, keratin 8 and keratin 18 were immunoprecipitated from detergent extracts of explanted livers from the three patients with the G61C mutation (Patients 1, 2, and 3) and from two histologically normal liver specimens (Normal 1 and Normal 2). Immunoprecipitates were analyzed by polyacrylamide-gel electrophoresis followed by staining with Coomassie blue. As shown in Panel C, keratin 8 and keratin 18 precipitates were analyzed by gel separation under nonreducing and reducing conditions followed by immunoblotting and then enhanced chemiluminescence. Under nonreducing conditions, an approximately 100-kd cross-linked form of keratin 8 (approximate weight of keratin 8 monomers, 53 kd) was found in patients with the G61C mutation but not in those with normal liver specimens or other patients with cirrhosis (data not shown). This cross-linked form disappeared under reducing conditions. The intense antibody band (>200 kd) under nonreducing conditions disappeared under reducing conditions, as expected, because of generation of the smaller antibody light and heavy chains. The asterisks denote degraded keratin 8 and antibody heavy chain. As shown in Panel D, keratin 8 and keratin 18 immunoprecipitates were obtained from normal liver specimens or specimens with the Y53H mutation. Precipitates were analyzed in the horizontal direction by isoelectric focusing and then by polyacrylamide-gel electrophoresis in the vertical direction. Separated samples were subjected to immunoblotting with anti-keratin 8 antibody M20. Histograms of the isoforms for normal keratin 8 (Normal 1 and Normal 2) and for keratin 8 with the Y53H mutation (Mutant 1 and Mutant 2) are shown. The Normal 2 and Mutant 2 isoforms represent the phosphorylated keratin 8 species.²¹ The "Normal 2" isoforms in the histograms of Y53H keratin 8 and of the mixture of Y53H and normal keratin 8 are more intense than that in the histogram for normal liver, since keratin 8 becomes hyperphosphorylated on liver injury (irrespective of the presence or absence of G61C and Y53H mutations [data not shown]).

A Sequence Analysis

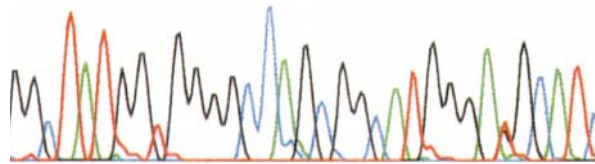
Normal

GGC TAT GGT GGG GCC AGC GGC ATG GGA GGC ATC
 G Y G G A S G M G G I



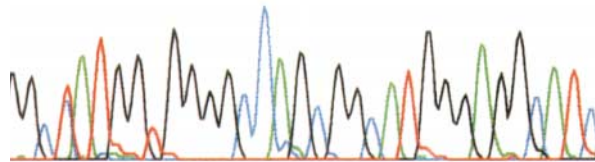
G61C mutation

GGC TAT GGT GGG GCC AGC GGC ATG GGA **NGC** ATC
 G Y G G A S G M G G/C I

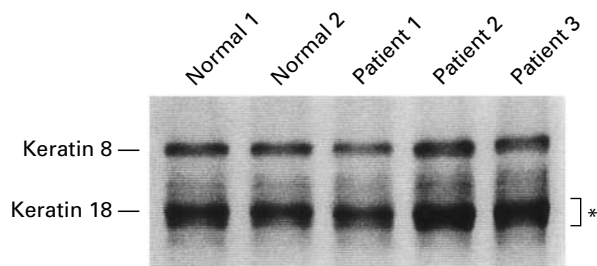


Y53H mutation

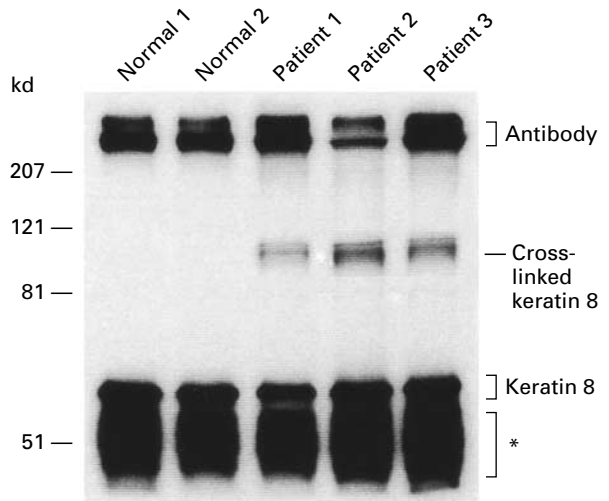
GGC **NAT** GGT GGG GCC AGC GGC ATG GGA GGC ATC
 G Y/H G G A S G M G G I



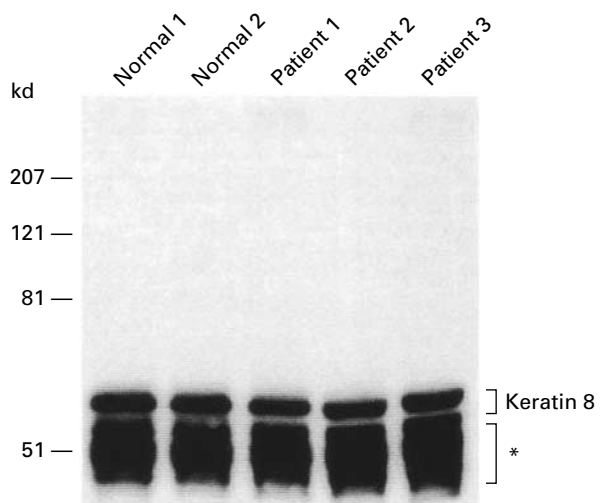
B Keratin 8 and 18 Immunoprecipitates



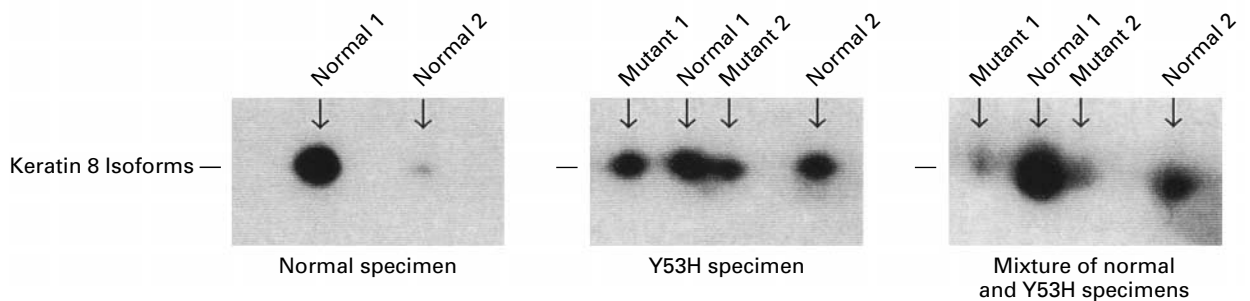
C Immunoblots of Precipitates
 Nonreducing Conditions



Reducing Conditions



D Two-Dimensional Gel Analysis



tissue, although serologic tests for autoimmunity were negative and she had no response to therapy with prednisone and azathioprine. One patient had elevated antinuclear antibodies (titer, 1:640) and anti-smooth-muscle antibodies (titer, 1:20), but her biopsy specimens did not contain plasma cells. One patient with the Y53H mutation had Mallory bodies but no history of alcohol abuse. None of the patients with a mutation in the keratin 8 gene had a history of diabetes or hyperlipidemia.

Expression of Mutant Keratin 8 Protein

We analyzed precipitates of keratin 8 and keratin 18 (Fig. 1B) that were obtained from specimens of two normal livers and of three explanted livers with the G61C mutation by immunoblotting for keratin 8 under reducing and nonreducing conditions (Fig. 1C). This approach takes advantage of the absence of cysteine residues in keratin 8 and keratin 18, which potentially could covalently link proteins that would otherwise not be linked. The three specimens with the G61C mutation but not the normal specimens (or specimens of cirrhotic livers [data not shown]) had a keratin 8 cross-linked species of approximately 100 kd that appeared only under nonreducing conditions (Fig. 1C). The 100-kd species most likely corresponded to two keratin 8 molecules (covalently linked by two mutant cysteine residues), a finding that would be compatible with the normal tetrameric, noncovalent association of keratins (two keratin 8 molecules and two keratin 18 molecules) as heteropolymers²⁴ and with lack of reactivity of the 100-kd species with keratin 18-specific antibodies (data not shown). Although an association between keratin 8 with the G61C mutation and other molecules cannot be excluded, our findings indicate that the G61C mutant protein was expressed in these three liver specimens.

The presence of the mutant Y53H protein was confirmed by assessing the change from the acidic tyrosine (isoelectric point, 5.63) to the basic histidine (isoelectric point, 7.64) on two-dimensional gel electrophoresis. Keratin 8 in normal liver consists of two charged isoforms: one that is unphosphorylated and one that is phosphorylated.^{18,21} In contrast, keratin 8 isolated from the explanted liver of a patient heterozygous for the Y53H mutation resolved into four isoforms — the two normal isoforms and two mutant Y53H isoforms (Fig. 1D). As predicted, the mutant unphosphorylated isoform was more basic than the normal unphosphorylated isoform, because of the presence of the additional histidine. Assignment of the normal and mutant isoforms was confirmed by analysis of a mixture of normal and mutant samples (Fig. 1D).

Mutations in the Keratin 8 Gene and Cell Stress

Given the well-established cytoprotective role of hepatocyte keratins,^{4,9} we compared the effect of cell

stress on the reorganization of keratin filaments in normal keratin 8 and the mutant keratin 8. We examined stresses that induce reorganization of keratin filaments, including heat,²⁵ the phosphatase inhibitor okadaic acid,²⁶⁻²⁸ and hydrogen peroxide.²⁹ The protein with the Y53H mutation was associated with a significantly greater proportion of cells with unstable (collapsed or disassembled) filaments than was wild-type or G61C keratin 8 when cells were exposed to heat stress (20 percent, 56 percent, and 28 percent of cells transfected with wild-type, Y53H, and G61C keratin 8 DNA, respectively) or to okadaic acid (31 percent, 69 percent, and 31 percent, respectively) ($P < 0.001$ for the comparison between the wild type or G61C and Y53H) (Fig. 2). In contrast, exposure to hydrogen peroxide prompted reorganization (visible as fine dots, a marker of unstable filaments) in 82 percent and 76 percent of cells transfected with wild-type and Y53H keratin 8 DNA, respectively, whereas only 35 percent of cells transfected with G61C keratin 8 DNA had a dot pattern ($P < 0.001$ for the comparison between wild-type and G61C keratin 8) (Fig. 2). The G61C and Y53H mutations had no effect on filament organization under basal conditions in cells transfected with these mutant proteins (data not shown).

DISCUSSION

The etiologic role of mutations in keratins and other intermediate-filament proteins, including desmin³⁰⁻³² and lamins,³³⁻³⁶ in human disease is well established. Our findings add keratin 8 to the other “soft” keratins in which mutations have been associated with skin, oral, and ocular diseases.⁸ Additional mutations in the gene for keratin 8 and possibly keratin 18 may be found in association with liver disease. The five patients in whom we identified mutations in the keratin 8 gene and the patient in whom we identified a mutation in the keratin 18 gene¹⁸ all had cryptogenic cirrhosis, although two of these patients had some autoimmune features. Analysis of other patients with autoimmune disease should help clarify the role of mutations in the genes for keratin 8 and keratin 18 in autoimmune hepatitis. In addition, a greater number of patients without liver disease should be examined to estimate the prevalence of these mutations in the general population.

Two features of the glycine at position 61 of keratin 8 suggest that it is an important residue: it is located at a juncture between a predicted turn and a beta strand within the H1 head subdomain³⁷ (as shown in the diagram available at <http://www.nejm.org>), and it is conserved in all type II keratins. The H1 subdomain of the type II keratins is probably important in filament organization,³⁸ and several mutations within H1 (none involving the conserved glycine at position 61) in the keratin 5 gene^{39,40} and the keratin 1 gene^{37,41,42} have been identified as the cause of epi-

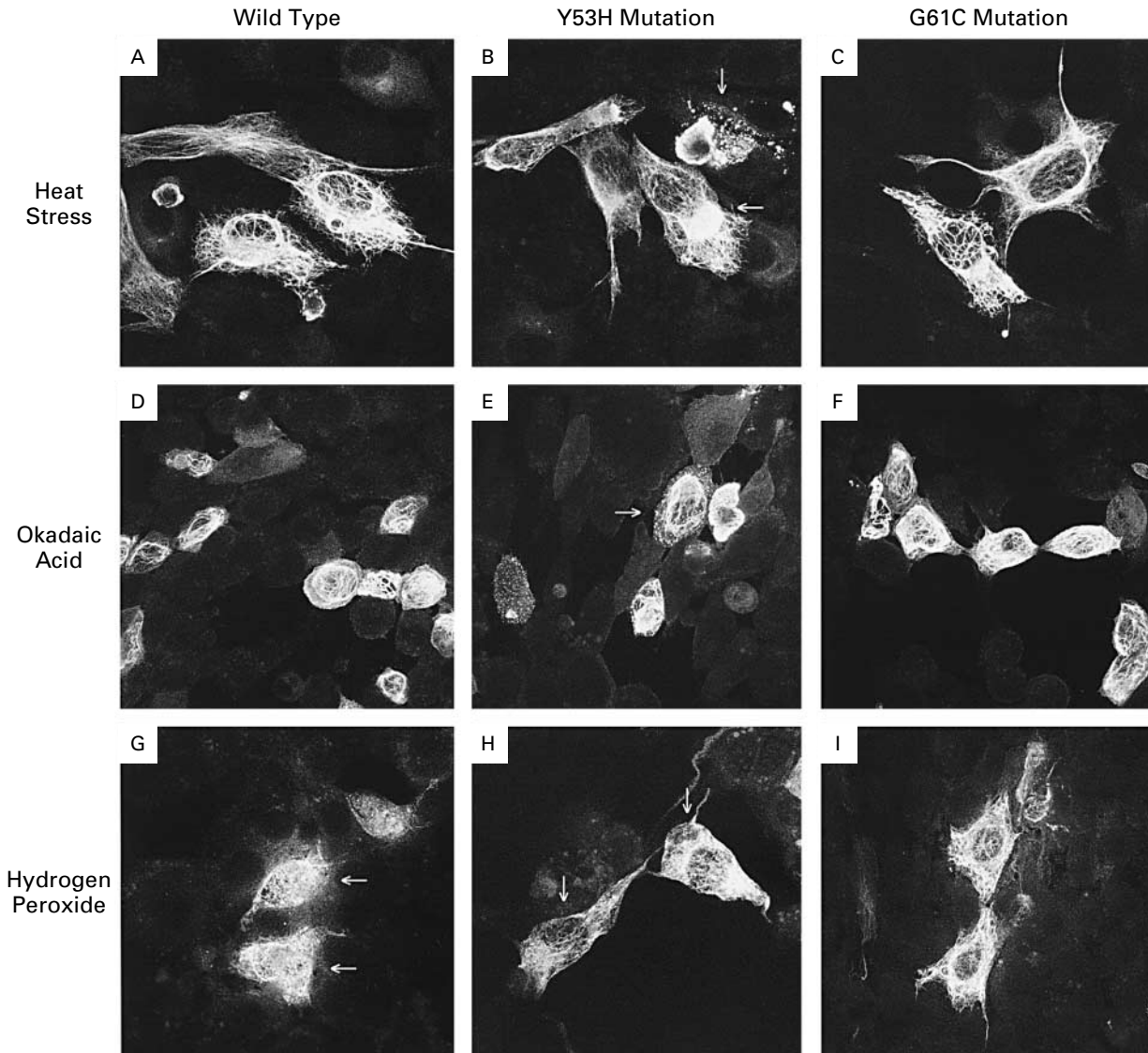


Figure 2. Immunofluorescence Staining of NIH 3T3 Cells Transfected with Wild-Type and Mutant Keratin 8 Constructs.

NIH 3T3 cells were transfected with both wild-type keratin 8 and wild-type or mutant keratin 8 constructs. After 54 hours, cells were processed for immunofluorescence staining with anti-keratin 8 antibody M20. Before processing, transfected cells were cultured at 42°C for 18 hours (Panels A, B, and C) and incubated for 1 hour in the presence of okadaic acid (1 μg per milliliter) (Panels D, E, and F) or hydrogen peroxide (50 mmol per liter) (Panels G, H, and I). Heat stress and treatment with okadaic acid resulted in collapse and disassembly of a substantial proportion of the keratin filaments in cells transfected with Y53H keratin 8 (arrows in Panels B and E) as compared with cells transfected with wild-type keratin 8 (Panels A and D) or G61C keratin 8 (Panels C and F). Exposure to hydrogen peroxide prompted less filament disassembly (visible as dot formation) in cells transfected with G61C keratin 8 (Panel I) than in those transfected with wild-type or Y53H keratin 8 (arrows in Panels G and H, respectively).

dermolysis bullosa simplex and epidermolytic hyperkeratosis, respectively.

Most of the epidermal, oral, and ocular keratin diseases are well-defined autosomal dominant diseases with a high penetrance. In contrast, and aside from rare reports of familial cirrhosis (which tends to be recessive),⁴³ cryptogenic cirrhosis had been thought to

be sporadic and of late onset. Thus, environmental or other genetic modifiers may contribute significantly to the expression of the disease. Notably, cryptogenic liver disease accounts for nearly 10 percent of all cases of liver disease.^{44,45} Although some conditions — including diabetes, obesity, nonalcoholic steatohepatitis, and surreptitious viral hepatitis — have been

linked to cryptogenic liver disease,⁴⁵⁻⁴⁸ it remains poorly understood. Studies in animals¹¹⁻¹⁷ support the hypothesis that mutations in the keratin 8 and keratin 18 genes may place carriers at high risk for liver disease rather than directly cause liver disease. The mutations we describe are likely to be pathogenic factors and not polymorphisms, since they were found in the group of patients with cryptogenic cirrhosis but not in the group with noncryptogenic liver disease or the control group and since they resulted in phenotypes of resistance to filament reorganization (in the case of the G61C mutation) and filament instability (in the case of the Y53H mutation) in transfected cells exposed to stress. These findings agree well with the known cytoprotective function of keratin 8 and keratin 18 in the liver.^{4,9-17}

In the epidermal keratins, genetic and environmental modifiers may result in different diseases for a given mutation at a given site of the keratin. For example, a specific mutation of the keratin 17 gene (the substitution of cysteine for arginine at position 94) was associated with steatocystoma multiplex in one family but pachyonychia congenita type 2 in another.⁴⁹ If mouse phenotypes can be extrapolated to humans, as is true of several animal models of keratin disease,^{3,9,10} then it is possible that diseases that do not primarily involve the liver may also be associated with mutations in the keratin 8 gene. This hypothesis is supported by the finding that different inbred strains of mice that lack the keratin 8 gene have markedly different phenotypes, primarily intestinal disease or liver disease, depending on the particular strain.^{11,12}

The physiological consequences of the Y53H and G61C mutations in the keratin 8 gene are probably related to the dynamic nature and reorganization that keratins undergo during cellular events, including mitosis and stress.²⁻⁴ These mutations do not affect the organization of keratin filaments under basal conditions, but they do result in substantial abnormal reorganization of the filaments in transfected cells exposed to stress. The stresses we tested included heat, oxidative stress (hydrogen peroxide), and exposure to okadaic acid, all of which induce the hyperphosphorylation and reorganization of keratins in normal cells.⁴ The hepatotoxin and phosphatase inhibitor microcystin, which causes marked hyperphosphorylation and reorganization of keratin,^{14,26-28} accounted for several deaths due to liver failure.⁵⁰ One of our two patients with a G61C mutation in the keratin 8 gene had been repeatedly exposed to halothane, and another had used cocaine regularly and alcohol occasionally. Halothane is a known hepatic toxin,⁵¹ and mice lacking the keratin 8 gene are highly susceptible to anesthetics with hepatotoxic properties¹³; alcohol and cocaine synergistically promote oxidative stress in hepatocytes.⁵² We propose that mutations in the keratin 8 gene or the keratin 18 gene predispose patients to liver disease at least in part by interfering with the normal re-

organization of keratin filaments (and its presumed functional sequelae) that occurs in response to physiological and nonphysiological stimuli. Further studies should clarify the role of genetic and environmental modifiers in such liver disease and the potential presence of these mutations in other diseases.

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