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# The Human Chitotriosidase Gene

## NATURE OF INHERITED ENZYME DEFICIENCY*

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**The human chitinase, named chitotriosidase, is a member of family 18 of glycosylhydrolases. Following the cloning of the chitotriosidase cDNA (Boot, R. G., Renkema, G. H., Strijland, A., van Zonneveld, A. J., and Aerts, J. M. F. G. (1995) *J. Biol. Chem*. 270, 26252–26256), the gene and mRNA have been investigated. The chitotriosidase gene is assigned to chromosome 1q31-q32. The gene consists of 12 exons and spans about 20 kilobases. The nature of the common deficiency in chitotriosidase activity is reported. A 24-base pair duplication in exon 10 results in activation of a cryptic 3′ splice site, generating a mRNA with an in-frame deletion of 87 nucleotides. All chitotriosidase-deficient individuals tested were homozygous for the duplication. The observed carrier frequency of about 35% indicates that the duplication is the predominant cause of chitotriosidase deficiency. The presence of the duplication in individuals from various ethnic groups suggests that this mutation is relatively old.**

A chitinase in man has been documented only recently (1–3). The enzyme was detected initially on the basis of its capacity to hydrolyze artificial chitotrioside substrates and was therefore named chitotriosidase (1). Chitotriosidase is synthesized by activated macrophages (1). In plasma and tissues of patients suffering from Gaucher disease, chitotriosidase activity is markedly elevated because of its massive production and secretion by the lipid-laden macrophages that accumulate in these patients (1, 4). Purification of chitotriosidase and cloning of the corresponding cDNA have revealed that the enzyme belongs to the family 18 of glycosylhydrolases and shares significant sequence identity to chitinases from various nonmammalian species (3, 5). The enzyme is capable of degrading chitin and should be considered as the human chitinase analogue (2). The established antifungal action of homologous chitinases in plants and the tightly regulated expression of chitotriosidase in phagocytes suggest that chitotriosidase might fulfill a role in degradation of chitin-containing pathogens (6, 7). Recently, a number of other human members of the chitinase protein family have been identified: oviductin (human oviduct specific glycoprotein), human cartilage glycoprotein 39 (HCgp-39), and YKL39 (8–10). In contrast to chitotriosidase, these proteins have no known enzymatic activity, and their function is so far not precisely understood.

Chitotriosidase in human tissue is heterogeneous with respect to its isoelectric point and molecular mass. The major isoform shows a molecular mass of 50 kDa and a heterogeneous pI of 5.0–7.2. The minor isoform has a mass of 39 kDa and a discrete pI of 8.1 (2). The amino termini of both isoforms are identical and exactly as predicted by the cloned chitotriosidase cDNA that codes for a 50-kDa protein. Metabolic labeling experiments with cultured macrophages have revealed that a 50-kDa chitotriosidase is initially synthesized and subsequently undergoes posttranslational modification. First, *O*-linked glycosylation of the carboxyl-terminal part of the protein generates the heterogeneity in charge. Second, whereas most 50-kDa enzyme is secreted, part of it is intracellularly converted by carboxyl-terminal proteolytic processing into the 39-kDa isoform that accumulates in lysosomes (11). It has recently been noted that the carboxyl-terminal domain of 50-kDa chitotriosidase mediates the strong binding of this enzyme to chitin, a feature that is not shown by the 39-kDa enzyme (11).

A recessively inherited deficiency in chitotriosidase activity is frequently encountered (1). For example, in The Netherlands about 1 in 20 individuals is completely deficient in enzymatically active chitotriosidase in all materials tested, including plasma, urine, cultured macrophages, leukocytes, and tissues (1, 12). Because there are no indications for the presence of enzyme inhibitors in these individuals, it is most likely that some mutation in the chitotriosidase gene underlies the enzyme deficiency.

To obtain further insight in the molecular basis of the chitotriosidase deficiency, we have investigated the composition of the chitotriosidase gene and the corresponding mRNA of control and enzyme-deficient individuals. The results of this study are here described and discussed.

### MATERIALS AND METHODS

*Reagents and Cells*—All reagents were obtained from Sigma unless indicated otherwise. Peripheral blood monocytes were isolated and cultured for a prolonged time in the presence of human AB serum during which process spontaneous differentiation into macrophages occurs (1). After 7–14 days of culture, cells were used in experiments.

*Metabolic Labeling*—Cultured macrophages or transfected COS cells were incubated for 10 min in the presence of radioactive methionine and subsequently chased in the presence of excess unlabeled amino acid. Radiolabeled chitotriosidase was specifically visualized using a rabbit anti-(chitotriosidase) antiserum exactly as described before (2, 11). Binding of chitotriosidase to chitin was analyzed by incubation of enzyme containing samples with chitin particles (Sigma), followed by centrifugation.

*cDNA Cloning and Sequence Analysis*—A macrophage cDNA library, was screened using the partial chitotriosidase cDNA probe (3). Positive cDNA clones were sequenced double-stranded using the dideoxy-nucle-

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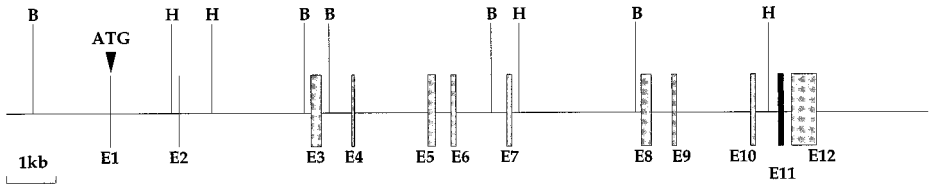


FIG. 1. **The genomic organization of the chitotriosidase gene.** Shown is the genomic structure. E1–E12 are exons defined on the basis of the cloned cDNAs. The location of the *Hin*dIII sites within the gene are indicated with "*H*" and the *Bam*HI sites with "*B*." The translation initiation site is indicated (*ATG*). The alternative exon is indicated in *black* (see "Results").

otide chain termination method with T7 DNA polymerase (Amersham Pharmacia Biotech, Uppsala, Sweden) and appropriate primers as described (3, 13, 14).

*RNA Isolation and Northern Blot Analysis*—Total macrophage RNA was isolated using the RNAzol B RNA isolation kit according to the instructions of the manufacturer (Biosolve, Barneveld, The Netherlands). For Northern blot analysis, 15-$\mu$g samples of total RNA were electrophoresed in 10 mM Hepes, 6% formaldehyde-agarose gels, transferred to Hybond N nylon membranes (Amersham, UK) by the capillary method and immobilized by UV cross-linking. The following probes were used: total chitotriosidase cDNA and mouse glyceraldehyde-3-phosphate dehydrogenase as an RNA control. The probes were radiolabeled with $^{32}$P using the random priming method (13). Hybridization conditions were exactly as described before (3).

*Isolation of a Genomic Chitotriosidase Clone*—A human genomic library (Imperial Cancer Research Fund (ICRF), Reference Library (library no. 700 P1 Human, host NS3145, vector pAd10SacBII (15)) was screened using the full-length $^{32}$P-labeled chitotriosidase cDNA probe. Duplicate filters were used in the screening procedure to avoid false results. The hybridization conditions were exactly as described before (3). The coordinates of the positive spots on the filters were determined, and the genomic clone (ICRFP700O0516) containing the chitotriosidase gene was obtained from the ICRF. The genomic clone was digested with different restriction enzymes, and the smaller fragments, containing exons, were subcloned in the plasmid vectors pGEM-7Zf(+) (Promega, Madison, WI) or pUC19 (New England Biolabs Inc.). Sequence analysis on the plasmid subclones was performed as described above, using several primers specific for the chitotriosidase cDNA.

*Localization of Chitotriosidase Gene*—The obtained genomic chitotriosidase clone of about 110 kb was biotinylated by nick translation according to the specifications of the manufacturers (Life Technologies, Inc.). High resolution chromosomes from peripheral blood lymphocytes were obtained according to standard techniques. Fluorescent *in situ* hybridization (FISH) was performed using the biotinylated genomic clone and fluorescein isothiocyanate-conjugated avidin, exactly as described before (16). For chromosome identification and probe localization, metaphases were simultaneously Q-banded using DAPI[1]/actinomycin D, as described (17).

*RNase Protection Analysis*—To generate the desired RNase protection probe, a polymerase chain reaction (PCR) fragment of chitotriosidase (216 bp), was ligated into the *Eco*RV site of pGEM-5Zf(+) (Promega). To determine the orientation and to exclude the existence of PCR artifacts, this construct was sequenced. The plasmid was then linearized with *Nde*I and size fractionated on an agarose gel, and the appropriate fragment was isolated. The protection riboprobe (298 nucleotides) was generated using this DNA template, T7 RNA polymerase (Stratagene), and [$^{32}$P]UTP. After *in vitro* transcription, the RNA was purified by electrophoresis on a 5% polyacrylamide, 7 M urea, TBE (0.09 M Tris borate, 0.002 M EDTA, pH 8.0) gel, and the appropriate band was isolated (13, 18).

For RNase protection, 5 $\mu$g of total macrophage RNA was hybridized overnight at 42 °C with $10^5$ cpm of labeled probe in 20 $\mu$l of 80% formamide, 400 mM NaCl, 40 mM PIPES, pH 6.4, 1 mM EDTA. Following hybridization, single-stranded RNA was digested in 350 $\mu$l of 10 mM Tris-HCl, pH 7.5, 300 mM NaCl, 5 mM EDTA, 1 $\mu$g/ml RNase T1 (1300 units/$\mu$g, Life Technologies, Inc.) for 60 min at 37 °C. Next, RNA was purified by Proteinase K digestion, phenol/chloroform/isoamylalcohol extraction, and ethanol precipitation. Finally, RNA was visualized by autoradiography after electrophoresis on a 5% polyacrylamide, 7 M urea, TBE gel (13, 18).

*Computer Analysis*—Sequence analysis of cDNA clones, genomic clone and subclones, was performed using Mac Vector 4.1.1 and the Genetics Computing Group package (version 7.0). Sequence comparisons and multiple sequence alignments were performed using the sequence alignment programs PILEUP, BESTFIT, and GAP.

*Activity Measurement and Immunotitration of Recombinant Chitotriosidase*—Transient transfection of COS-1 cells by the DEAE-dextran method was performed essentially as described in Ref. 19. The activity of chitotriosidase was measured using the fluorogenic substrate 4-methylumbelliferyl $\beta$-D-*N*,*N*′,*N*″-triacetylchitotriose exactly as described before (1). A rabbit antiserum was raised against 39-kDa chitotriosidase purified from human spleen. This antiserum is able to inhibit human chitotriosidase in enzymatic activity (2). Immunotitrations were carried out as follows. Enzyme-containing samples were preincubated for 30 min with different amounts of antiserum, and subsequently enzyme activity was determined in the samples. For these experiments, medium from transfected COS cells was collected and cells were harvested by trypsinization. Cells were homogenized by sonication in 0.25% (v/v) Triton X-100, 50 mM potassium phosphate buffer (pH 6.5). The homogenate was centrifuged and the supernatant collected. As a control enzyme preparation, an extract of Gaucher spleen was prepared by addition of 5 volumes of $H_2O$ and homogenization with an Ultraturrax (Janke & Kunkel, Staufen, Germany). The supernatant obtained after centrifugation was collected.

*RNA Extraction and Reverse Transcription-PCR*—From harvested macrophages, total RNA was isolated and single-stranded cDNA was prepared from the total macrophage RNA using reverse transcriptase and oligo(dT) as described before (3). To obtain the complete open reading frame of chitotriosidase, PCR was carried out using the single-stranded cDNA as template as described before (3). The primers used are: Chs1, 5′-CTGCATCATGGTGCGGTC-3′; and Chas1, 5′-GAAGGCAAGGCTGAGAGC-3′ (see Fig. 3*B*). The PCR products were subsequently cloned in the pGEM-T vector (Promega). Sequence analysis of the cloned PCR products was performed exactly as described above.

*Amplification of Genomic DNA*—Genomic DNA was isolated from leukocytes, and 300 ng of this genomic DNA was used as template in subsequent PCR reactions. The following primers were employed: Chs8, 5′-TACATCTTCCGGGACAAC-3′; and Chas9, 5′-TCAGTTCCTGCCGTAGCGTC-3′. PCR fragments were cloned in the pGEM-T vector and sequenced as described above.

*Duplication Mutation Analysis*—Using specific primers (Chs9, 5′-AGCTATCTGAAGCAGAAG-3′; and Chas8, 5′-GGAGAAGCCGGCAAAGTC-3′, see Fig. 4) fragments of 75 and 99 bp are amplified from the normal and mutant chitotriosidase gene, respectively, when present in genomic DNA. Electrophoresis in a native 10% acrylamide gel allows the detection of both fragments. In the case of carriers for the duplication, a mixture of both fragments is detected.

## RESULTS

*Characterization of the Human Chitotriosidase Gene*—A genomic clone containing the chitotriosidase gene was isolated (clone ICRFP700O0516 of the Imperial Cancer Research Fund Reference Library (15)), as described under "Materials and Methods." To allow sequence analysis, the genomic clone of about 110 kb was subcloned into smaller fragments using *Hin*dIII and *Bam*HI restriction enzymes.

The exon/intron composition of the chitotriosidase gene was determined by sequencing of genomic plasmid subclones and comparison of the information with the sequence of the chitotriosidase cDNA. As shown in Fig. 1, the gene was found to be composed of 12 exons and spans about 20 kb of genomic DNA. Exon 1 was defined as containing the first nucleotide of the longest chitotriosidase cDNA; the exact transcription start site has not been accurately mapped. The sizes of the exons range

[1] The abbreviations used are: DAPI, 4,6-diamidino-2-phenylindole; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase(s); PIPES, 1,4-piperazinediethanesulfonic acid.

TABLE I
*Sequences of the exon/intron junctions of the human chitotriosidase gene*

Exon sequences are shown in uppercase and the intron sequences in lowercase letters. The reading frame of the cDNA sequence is indicated. Splice-donor and -acceptor sites are shown in bold. The length of each intron is shown in parentheses. The alternative exon (11) is underlined.

| | | |
|---|---|---|
| Exon 1 | Intron 1 | Exon 2 |
| TG.GCC.TGG.GCA.G**gt**gagccgtcgg . . . | (~1200 bp) | . . . gtgttgctcc**ag**GT.TTC.ATG.GTC.C |
| Exon 2 | Intron 2 | Exon 3 |
| TG.ATC.CCA.TGG.G**gt**aagtagcctc . . . | (~2300 bp) | . . . ccaacgggcc**ag**GC.TCT.GCT.GCA.A |
| Exon 3 | Intron 3 | Exon 4 |
| C.CTG.AAG.AAG.AT**gt**gagccaaagc . . . | (~500 bp) | . . . tgtctttccc**ag**G.AAT.CCC.AAG.CT |
| Exon 4 | Intron 4 | Exon 5 |
| C.GGC.ACT.CAG.AA**gt**tagttgactg . . . | (~1300 bp) | . . . atcacctcac**ag**G.TTC.ACA.GAT.AT |
| Exon 5 | Intron 5 | Exon 6 |
| .CCC.TGG.GTA.CAG**gt**atggctgggc . . . | (232 bp) | . . . ccactcccac**ag**GAC.TTG.GCC.AAT. |
| Exon 6 | Intron 6 | Exon 7 |
| C.AAA.ATC.GCC.CA**gt**gagtctcagg . . . | (~900 bp) | . . . tgcctcctgc**ag**G.AAC.CTT.GGA.TT |
| Exon 7 | Intron 7 | Exon 8 |
| .AGC.CTC.AAC.GTG**gt**acgtgtggca . . . | (~2500 bp) | . . . ctcttccacc**ag**GAT.GCT.GCT.GTG. |
| Exon 8 | Intron 8 | Exon 9 |
| .GCC.TAC.TAT.GAA**gt**aggaaaaccc . . . | (~350 bp) | . . . ctgctcccac**ag**GTC.TGC.TCC.TGG. |
| Exon 9 | Intron 9 | Exon 10 |
| .TTC.AAA.ACC.AAG**gt**gaggcccagc . . . | (~1300 bp) | . . . ctccctgcac**ag**GTC.AGC.TAT.CTG. |
| Exon 10 | Intron 10 | Exon 11 |
| GG.CAG.GAA.CTG.A**gt**aagtaagggg . . . | (364 bp) | . . . ttgtgcttgc**ag**AT.GGG.TAA.AGC.C |
| Exon 11 | Intron 11 | Exon 12 |
| .CTT.CAG.CTG.TAG**gt**atggctgttg . . . | (167 bp) | . . . ctgtcttccc**ag**GT.CTT.CCA.TAC.T |

from 30 to 461 bp. The sizes of the smaller introns were determined by sequencing, and those of the larger ones were estimated by electrophoresis of fragments generated either by PCR or restriction enzyme digestion (Table I). In all cases, the sequences of the intron/exon borders are compatible with consensus splice site sequences (20).

The 71-bp exon 11 can be alternatively spliced (Fig. 1). This exon is usually skipped in the splicing process, generating the predominant mRNA species encoding the 50-kDa protein. In macrophages also, a distinct mRNA is rarely formed as the result of a lack of exon 11 skipping. Since exon 11 introduces a premature stop codon, the alternatively spliced mRNA encodes a 40-kDa chitotriosidase that is almost identical to the 39-kDa isoform generated by proteolytic processing of the 50-kDa chitotriosidase (11).

Fluorescent *in situ* hybridization with the genomic clone as probe was used to assign the chromosomal locus for the chitotriosidase gene, as described under "Materials and Methods." Fig. 2 shows that the chitotriosidase gene is located on chromosome 1q31–1q32.

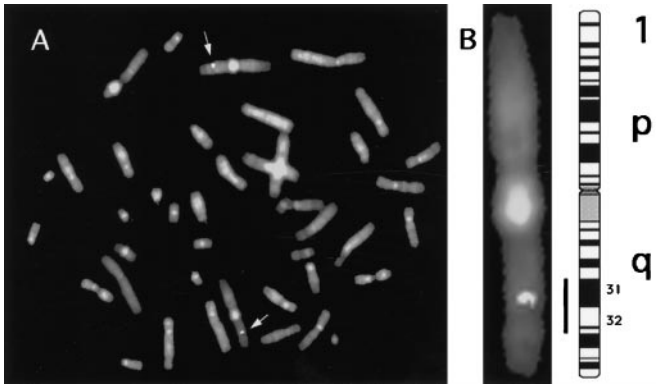


FIG. 2. **Fluorescence *in situ* hybridization of human metaphase chromosomes with the genomic chitotriosidase clone.** The genomic P1 clone containing the chitotriosidase gene was hybridized to normal metaphase chromosomes as described. *A*, specific hybridization of the probe (*arrows*) is evident on chromosome 1; *B*, shown is an ideogram of chromosome 1 with one of the labeled chromosomes indicating that the gene is located in the region of bands q31-q32.

*Genetic Basis of Chitotriosidase Deficiency*—Cultured macrophages of enzyme-deficient individuals do not produce enzymatically active chitotriosidase. Metabolic labeling experiments showed that only minor amounts of a short-lived 47-kDa chitotriosidase are synthesized (not shown). Northern blot analysis revealed that these macrophages do contain some chitotriosidase RNA although in markedly reduced amounts (Fig. 3*A*). From macrophage RNA of two control subjects and two unrelated chitotriosidase-deficient individuals, chitotriosidase cDNAs containing the complete open reading frame were generated using reverse transcription-PCR. Sequencing showed that these cDNAs were completely identical with the exception of a deletion of 87 nucleotides in exon 10 of the mutant RNA (Fig. 3*B*). The abnormal mRNA codes for a protein that lacks amino acids 344–372, a highly conserved region in members of the chitinase protein family (Fig. 3*B*, and Ref. 3). Transfection of COS-1 cells with wild-type chitotriosidase cDNA resulted in secretion of enzymatically active 50-kDa chitotriosidase, whereas parallel transfection with mutant cDNA led to modest synthesis of a 47-kDa protein that was largely intracellularly degraded, as was seen for cultured macrophages. The recombinant protein that could be isolated showed no capacity to degrade chitin or artificial substrates. It was, however, still able to bind to chitin particles.

To establish the precise molecular defect, genomic DNA of control subjects and chitotriosidase-deficient individuals was studied. Relevant parts of the mutant gene were amplified by PCR using appropriate primers (Fig. 4*A*, and under "Material and Methods"). Sequence analysis revealed that in exon 10 of the mutant gene a 24-nucleotide duplication is present (Fig. 4*A*). Apparently, this duplication leads to the selection of a cryptic 3′ splice site downstream in the exon, although the authentic splice site is still intact in the mutant chitotriosidase gene. Consistent with the findings made for macrophages of control subjects and chitotriosidase-deficient individuals (shown in Fig. 3*A*), RNase protection assays revealed that macrophages of heterozygotes contain only a very small amount of mutant mRNA as compared with wild-type mRNA. These reduced levels of steady state chitotriosidase mRNA could be due to two different reasons: (i) reduced transcription of the mutant gene, and/or (ii) production of mRNA that is unstable relative to wild-type chitotriosidase mRNA. Attempts to determine half-life of mRNA following actinomycin-D treatment were so far unsuccessful because of its apparent lability.

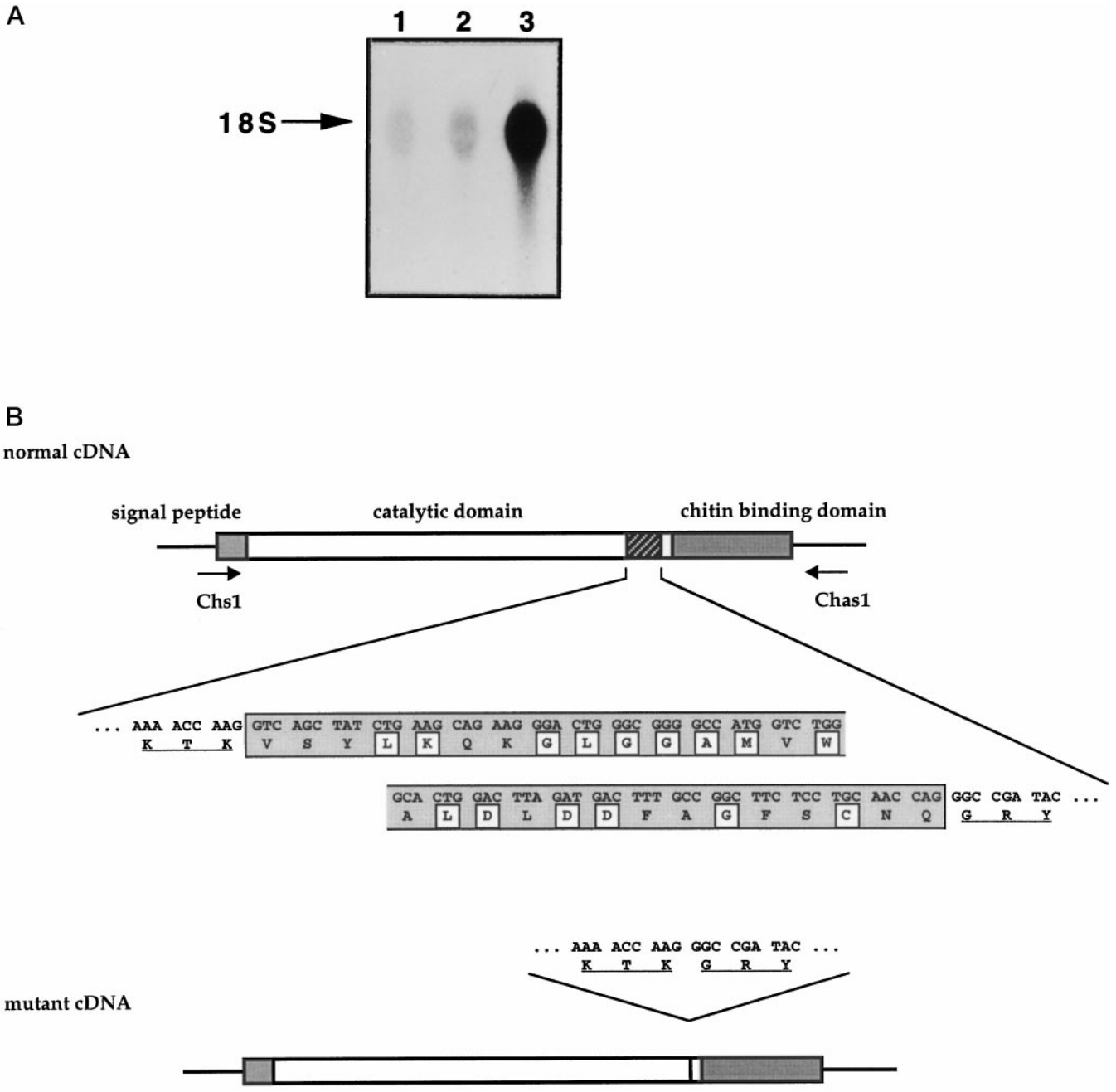


FIG. 3. **Defect in chitotriosidase mRNA in chitotriosidase-deficient individuals.** *A*, detection of chitotriosidase mRNA in macrophages. Human peripheral blood monocytes were isolated and cultured as described under "Material and Methods." Total RNA was extracted after 10 days and analyzed by Northern blotting as described under "Material and Methods." *Lane 1*, chitotriosidase-deficient individual 1; *lane 2*, chitotriosidase-deficient individual 2; *lane 3*, control individual. *B*, overview of the normal and mutant chitotriosidase cDNA. The positions of the signal peptide, the catalytic (TIM-barrel) domain, and the chitin binding domain, are shown (3). The *arrows* indicate the primers used to generate the complete coding sequence. The sequence of the deleted part in the mutant cDNA is *shaded*. The amino acids in this region that are highly conserved among members of the chitinase protein family (see Ref. 3) are *boxed*.

The presence of the 24-bp duplication in the chitotriosidase gene can be detected by PCR of genomic DNA with specific primers (Fig. 4*B,* and "Material and Methods"). Fragments of 75 and 99 bp are amplified from the normal and mutant gene, respectively. All chitotriosidase-deficient individuals examined so far ($n$ = 26) were homozygous for the 24-bp duplication in the chitotriosidase gene. Table II shows the results of the analysis of chitotriosidase genotype of Ashkenazi Jewish and Dutch individuals. It can be seen that the incidence of homozygotes and heterozygotes for the duplication was about 6 and 35%, respectively, in both populations. The same mutation has presently been detected in individuals with European (including Dutch) ($n$ = 326), Jewish ($n$ = 68), African ($n$ = 10), and Asian ($n$ = 20) ancestry. A study on Indonesian individuals has indicated that the incidence of enzyme deficiency is also about 6% in this ethnic group. Genotyping of Indonesian subjects showed that again about one in every three individuals is a carrier for 24-bp duplication in the chitotriosidase gene.

## DISCUSSION

Our investigation of the features of the chitotriosidase gene and its RNA has been informative in a number of respects. First, the established exon composition of the chitotriosidase gene points out the striking similarity of the organization of this gene

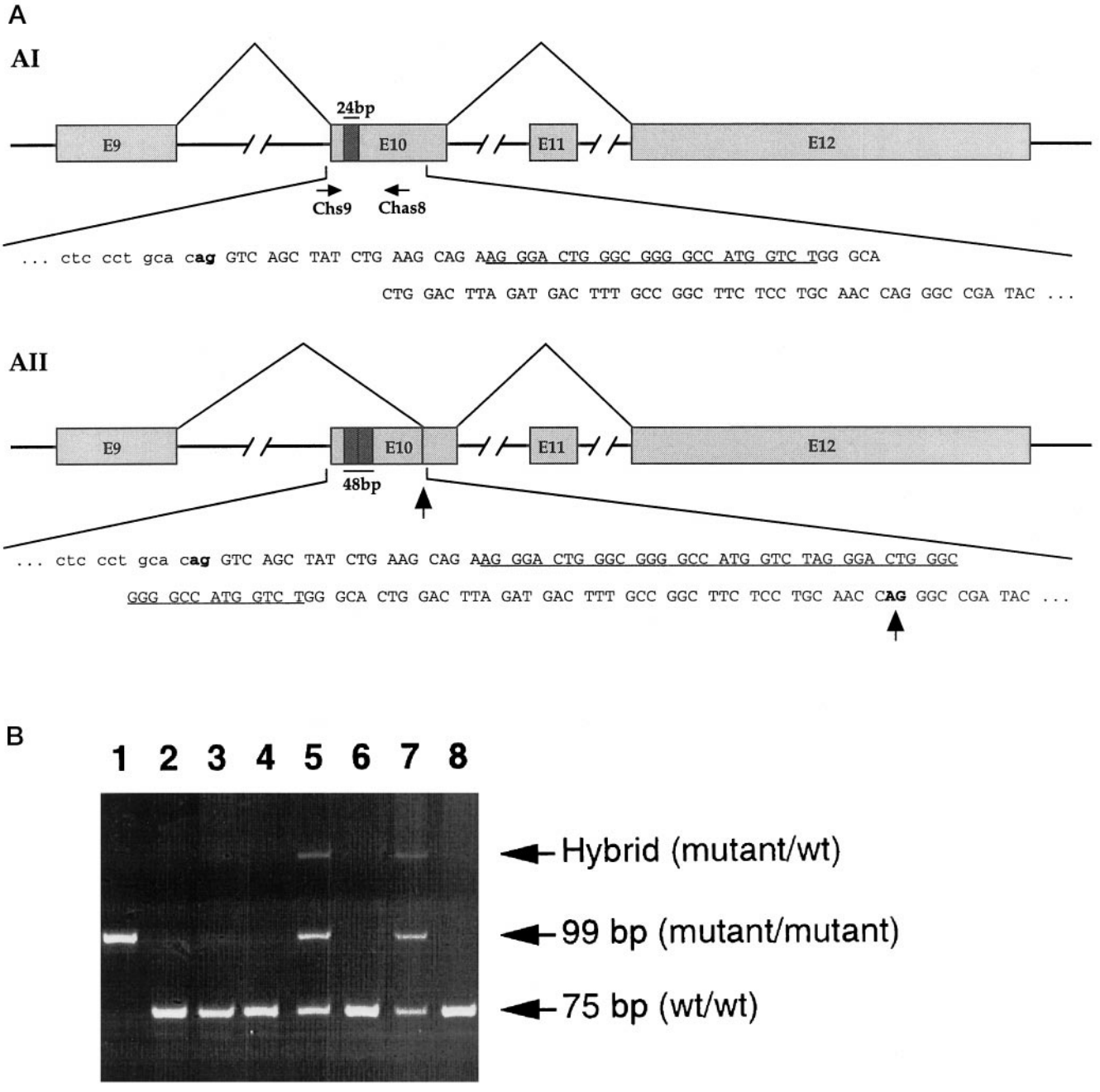


FIG. 4. **Defect in the chitotriosidase gene in chitotriosidase-deficient individuals.** *A*, overview of the relevant part of the chitotriosidase gene. *Upper part* (*AI*) shows the normal chitotriosidase gene. The *arrows* indicate the primers used for amplification of the genomic DNA. The lines above the gene show the normal splicing in which exon 11 is skipped. *Lower part* (*AII*) shows the difference in the mutant chitotriosidase gene. The mutant gene contains a 24-bp duplication in exon 10. Above the genes the difference in splicing is depicted. The relevant nucleotide sequences are shown. The *vertical arrow* indicates the activated 3′ splice site in the mutant gene. The 24-bp fragment in the normal gene and the 48-bp fragment in the mutant gene are *underlined*. *B*, detection of the 24-bp duplication in the chitotriosidase gene by PCR of genomic DNA. Amplified fragments (primers Chs9 and Chas8) were separated on a native 10% polyacrylamide gel and stained with ethidium bromide. The size of the fragments of the normal and mutant allele is 75 and 99 bp, respectively. *Lane 1*, homozygote mutant; *lanes 2-4*, *6*, and *8*, homozygote wild type; and *lanes 5* and *7*, heterozygote. The additional larger fragment that is only present in the case of heterozygotes is because of formation of a hybrid DNA molecule, consisting of a normal and a mutant strand. *wt*, wild-type

with that of other human genes coding for members of the chitinase protein family. The exon/intron boundaries in the genes of the human oviduct glycoproteins YKL-39 and of HCgp-39 are very similarly located (Refs. 8, 10, and 21, and GenBank accession numbers U58001–U58010, U58514, U58515, and U49835). This suggests that these genes have evolved by duplication events.

The locus of the chitotriosidase gene was assigned to 1q31–32 using fluorescent *in situ* hybridization with the genomic clone as probe. During the preparation of the manuscript, Eiberg and Den Tandt (22) independently mapped the gene to 1q31-qter by linkage analysis. The genes of all the human members of the chitinase protein family known so far are located on chromosome 1. According to the data deposited in Human Gene Map of the National Center for Biotechnology Information (NCBI), the genes of YKL-39 and the human oviduct specific glycoprotein are located on 1p13 and that of HCgp-39, like the chitotriosidase gene, is on 1q31–1q32.

TABLE II
*Chitotriosidase genotype distribution*

The chitotriosidase genotype of unrelated Ashkenazi Jewish and Dutch subjects was determined as described under "Materials and Methods."

| Population | Genotype | | |
|---|---|---|---|
| | Wild-type | Heterozygote mutant | Homozygote mutant |
| | | % | |
| Dutch ($n$ = 171) | 58.5 | 35.1 | 6.4 |
| Ashkenazi Jewish ($n$ = 68) | 60.3 | 33.8 | 5.9 |

The nature of the common deficiency in chitotriosidase activity was elucidated in this study. The defect was found to be a 24-bp duplication that activates a cryptic 3′ splice site in the same exon, causing the formation of mRNA with an in-frame internal deletion of 87 nucleotides. Similar mutations affecting splice site selection have been reported earlier for two lysosomal hydrolases, the β-subunit of β-hexosaminidase and arylsulfatase A, and for episialin (23–25).

Based on the incidence of deficiency of chitotriosidase activity in The Netherlands and among Ashkenazim of about 6%, the Hardy-Weinberg equilibrium predicts that 37% of the population will be carriers of a mutant chitotriosidase gene. This prediction corresponds very well with the observed frequency of carriers of the 24-bp duplication in the chitotriosidase gene. This and the finding that all chitotriosidase-deficient individuals so far are homozygous for the duplication indicate that this mutation must be the predominant cause of chitotriosidase deficiency. The multi-ethnic occurrence and prevalence of the 24-bp duplication in the chitotriosidase gene suggests that it probably originated before the radiation of tribal populations that have formed the present ethnic groups.

Cultured macrophages of chitotriosidase-deficient individuals contain very little mRNA and secrete almost no chitotriosidase protein. We have not been able to detect the mutant protein in plasma of chitotriosidase-deficient individuals, including Gaucher patients. Analysis of recombinant-produced mutant chitotriosidase indicated that the protein is enzymatically inactive. The mutant chitotriosidase is predicted to lack amino acids 344–372. For several homologous chitinases, the three-dimensional structure has been resolved by crystallographic analysis (26, 27). The catalytic core structure of these hydrolases is thought to be an 8-stranded α/β (TIM) barrel (26–28). On the basis of its homology with these chitinases, it can be predicted that the internal deletion in the mutant chitotriosidase will prevent the formation of a proper TIM-barrel conformation. In view of this, it is not surprising that the recombinant produced mutant protein shows no chitinolytic activity. On the basis of all our findings, it seems extremely unlikely that the mutant chitotriosidase allele renders a functional protein.

Given the high incidence of chitotriosidase deficiency, it could be argued that the enzyme is redundant in man. However, it is unattractive to assume that the enzyme does no longer fulfill any function. The role of the homologous chitinases in plants in defense against fungal pathogens has been demonstrated convincingly (6, 7, 29–34). The structural features of chitinases have been extremely well conserved in chitotriosidase. Furthermore, the expression of the enzyme in phagocytes is regulated in a remarkable manner. It seems therefore likely that chitotriosidase still fulfills a role, but that a deficiency can be somehow compensated. A comparison with the closely related lysozyme is of interest. The bactericidal function of this hydrolase is well established; nevertheless, in rabbits an inherited deficiency in lysozyme occurs that seems to have little consequence for susceptibility to infections (35). The diverse array of defense mechanisms of the immune system in mammals probably renders sufficient tolerance to defects in single enzymes such as lysozyme and chitotriosidase. In this connection, it will be of interest to determine whether the chitotriosidase genotype of immunocompressed individuals is predictive for their risk on infections with fungal pathogens.

It is intriguing to understand why the mutation in the chitotriosidase gene occurs with such high incidence in different ethnic groups. This could point to some selective advantage for carriers, but at present there are no clues for the nature of such a selective factor. In this connection, it will be essential to obtain detailed insight in the physiological role(s) of chitotriosidase and the mechanism(s) that can compensate for its absence.

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