Siiyama (Serine 53 (TCC) to Phenylalanine 53 (TTC))

A NEW α1-ANTITRYPSIN-DEFICIENT VARIANT WITH MUTATION ON A PREDICTED CONSERVED RESIDUE OF THE SERPIN BACKBONE*

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α1-antitrypsin (α1AT), a plasma serine protease inhibitor, increases the risk of precocious pulmonary emphysema in individuals when deficient. Although more than 25 years have passed since a deficiency in the serum level of α1AT was reported, it is only recently that the consequence of the amino acid replacement which leads to the deficient state has been discussed in terms of the crystallographic structure of α1AT and the amino acid residues conserved in the superfamily to which it belongs. Our case involved a 38-year-old Japanese male with α1AT deficiency which was analyzed and identified as a new deficient variant. The serum α1AT of the proband migrated to the position of the reference serum which is more cathodal than M1, the predominant normal variant, when isoelectric focusing (pH 4.2-4.9) is performed by a combination of Western blotting and crossed immunoelectrophoresis. The new deficient variant is designated as Siiyama after his birthplace. Although liver biopsy specimen showed no apparent pathological findings, PAS-positive with diastase-resistant inclusion bodies and immunoreactive aggregates were detected in several hepatocytes. In addition, similar α1AT mRNA transcript levels were observed in peripheral blood leukocytes from the proband and healthy subjects by Northern analysis. All the coding exons (exon Ic, II, III, IV, and V) of the α1AT gene of the proband and his family were amplified by polymerase chain reaction and followed by direct sequencing. A single missense mutation, Ser53 (TCC) to Phe53 (TTC) was identified in exon II of the proband's α1AT gene. All his family examined were heterozygous at this base. Ser53 is one of the most conserved residues as predicted by Huber and Carrell (Huber, R., and Carrell, R. W. (1989) Biochemistry 28, 8951–8966) and is thought to contribute to the organization of the internal core element of the α1AT molecule. The mutational matrix number of Ser to Phe substitution is -3, indicating that this change is evolutionally rare. In this regard, a possible explanation for the deficient state in α1AT Siiyama is that the change from an uncharged polar to a nonpolar amino acid imposed on the conserved serpin backbone exerts severe effects on the integrity of the molecule, and hence alters the intracellular processing of α1AT.

It is well recognized that reduced serum levels of α1-antitrypsin (α1AT)† increase the risk of early-onset pulmonary emphysema in individuals with this deficiency (2–5). α1AT is highly pleomorphic and plays a physiological role in the inhibition of neutrophil elastase, a powerful serine protease (2–5). Crystallographic analysis of the molecule has provided an understanding of structure and function relationships in α1AT and other proteins belonging to the serpin superfamily (1, 6, 7). Among these, α1AT has been extensively studied and researched because of its association with human disorders. α1AT is coded by a single-copy gene spread over 12.2 kilobases on chromosome 14q 31 to 32.3 (3, 8, 9). Serum α1AT, a 52-kDa glycoprotein, is thought to diffuse into the alveolar space and irreversibly inhibit neutrophil elastase that digests elastin and most tissue matrix proteins (3, 4, 10). In the normal alveolar space with sufficient α1AT molecules, the effects of neutrophil elastase are neutralized, and the lower respiratory tract is protected from proteolytic attack. In the α1AT-deficient state, the affected individuals are faced with the risk of developing pulmonary emphysema, usually between the age of 30 to 40 and with occasional liver damage (6, 8, 9, 10).

More than 30 α1AT genes of both normal variants and abnormal variants with clinical consequences have been analyzed at the level of nucleotide alteration (2–4). Among them are 11 clinical variants with amino acid substitutions. Utilizing an interpretation of the crystal structure of α1AT, Huber and Carrell (1) recently reported on critical amino acid residues which are conserved in the serpin superfamily. When they examined natural serpin variants including α1AT, antithrombin III, CI-inhibitor, heparin cofactor II, and antiplasmin, six clinically significant α1AT variants were found at predicted conserved residues or on residues close to the predicted locations.

While the gene frequency for the Z-type α1AT variant is relatively high (0.01–0.02) among Caucasians (2–4), deficient variants among Orientals are rare, with only one deficient variant elucidated at the gene level (12). In this study, we analyzed a new α1AT deficient case in Japan and found that the mutation occurs at one of the residues that Huber and Carrell (1) regard as conserved and predict the effect of the substitution in the crystalline structure.

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† The abbreviations used are: crlAT, crl-antitrypsin; IEF, isoelectric focusing; PCR, polymerase chain reaction; PAS, periodic acid Schiff.
MATERIALS AND METHODS

Study Group—A 38-year-old male was admitted to the Hokushin General Hospital because of exertional dyspnea. Chest x-ray films and CT scans showed overinflated lungs and bullous changes especially in the lower lung fields. Obstructive ventilatory impairment and CT scans showed overinflated lungs and bullous changes especially in the lower lung fields. Laboratory data showed no apparent liver dysfunction. The family members of the proband available for evaluation included his parents, elder sister, and daughter (Fig. 1). Their α1AT levels in serum were 106 mg/dl (father, III-3), 119 mg/dl (mother, III-14), 87 mg/dl (sister, IV-2), and 140 mg/dl (daughter, V-4), respectively, all showing about half the normal level. Consanguinity was recorded in the patient’s history; his parents are cousins (Fig. 1).

Proteins were loaded using paper (Whatman 3”) immersed in 2.5 μl of diluted or undiluted serum. Separation was carried out at constant voltage of 2000 V for 6 h was applied to the gel with barbital buffer as the electrode strip (70 g/l). After rinsing three times for 10 min, it was incubated with 1/500 diluted anti-human α1AT antibody (Bio-Rad) for 1 h. After rinsing three times, the membrane was stained in 50 mM Tris-HCl, pH 7.4, containing 0.40 mg/ml 3,3’-diaminobenzidine tetrahydrochloride and 0.04% H2O2. Phosphate-buffered saline containing 0.25 M glycine as a catholyte. Proteins were loaded using a buffer containing 25 mM Tris, 192 mM glycine, 20% aminoethanesulfonic acid, then polymerized by the addition of 0.5 mM/ml ammonium persulfate. Electrode strips were applied using a buffer containing 25 mM Tris, 192 mM glycine, 20% aminoethanesulfonic acid, then polymerized by the addition of 0.5 mM/ml ammonium persulfate.

C3753-2) were kindly provided by Dr. D. W. Cox (Toronto, Canada). Purity was confirmed for 25 cycles of the same thermal cycle setting to generate single-stranded DNA suitable for sequencing. Sequencing was carried out in both directions with Sequenase® (United States Biochemical) as instructed by the supplier.

RNA Analysis of Peripheral Blood Leukocytes—α1AT mRNA transcripts of leukocytes were evaluated by Northern analysis. Leukocytes

Immunohistochemical Staining of the Liver—Formalin-fixed, paraffin-embedded liver tissues were cut into 3-μm thick sections, deparaffinized, and rehydrated. Immunoperoxidase staining was performed with an avidin-biotin complex as described by Hsu et al. (13).

Polymerase Chain Reaction (PCR) and Sequencing of α1AT Gene—Genomic DNA was obtained from peripheral blood leukocytes according to the method described by Jeffreys and Flavell (14). Synthetic oligonucleotide primers were prepared so as to cover all the coding exons of the α1AT gene (Applied Biosystems DNA synthesizer 341A). Each exon of the α1AT gene was amplified separately by PCR with Taq polymerase (Perkin-Elmer-Cetus) using a Thermal cycler (Perkin-Elmer-Cetus) under the recommended conditions of the supplier. Amplification was performed with 30 cycles of denaturation (94°C, 1 min), annealing (55°C, 1 min), and extension (72°C, 2.0 min). Amplified products were electrophoresed in 2% low melting agarose gel (SeaPlaque®, FMC Bioproducts), excunecled and extracted with phenol/chloroform, and precipitated with ethanol. The purified DNA was used as a template in the next PCR with an asymmetrical primer ratio (50 pmol versus 1 pmol) which was performed for 25 cycles of the same thermal cycle setting to generate single-stranded DNA suitable for sequencing. Sequencing was carried out in both directions with Sequenase® (United States Biochemical) as instructed by the supplier.

Western blotting and crossed immunoelectrophoresis of α1AT. The PAGE was run using 3-μm filter (Whatman 3MM) immersed in 2.5 μl of diluted or undiluted serum. Separation was carried out at constant voltage of 2000 V for 6 h was applied to the gel with barbital buffer as the electrode strip (70 g/l). After rinsing three times, the membrane was stained in 50 mM Tris-HCl, pH 7.4, containing 0.40 mg/ml 3,3’-diaminobenzidine tetrahydrochloride and 0.04% H2O2. Phosphate-buffered saline containing 0.25 M glycine as a catholyte. Proteins were loaded using a buffer containing 25 mM Tris, 192 mM glycine, 20% aminoethanesulfonic acid, then polymerized by the addition of 0.5 mM/ml ammonium persulfate. Electrode strips were applied using a buffer containing 25 mM Tris, 192 mM glycine, 20% aminoethanesulfonic acid, then polymerized by the addition of 0.5 mM/ml ammonium persulfate.

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FIG. 1. A family pedigree of the Siiyama variant. Shown is a pedigree of five generations (I–V) with individuals in each generation numbered sequentially. The proband (IV-3) is indicated by an arrow. Serum α1AT levels (normal range 140 ~ 317 mg/dl) are shown below the symbols: □, males; ○, females. The inheritance of the α1AT Siiyama gene is shown as black in the individuals included in this study. There was a consanguineous marriage recorded in the patient’s history; his parents are cousins.

FIG. 2. Western blotting and crossed immunoelectrophoresis analysis of serum α1AT Siiyama after IEF. A, Western blotting of α1AT. After conventional IEF, the proteins were transferred to Immobilon® (see “Materials and Methods”). For the reference sera α1AT M1S, M1X, and M1Ysiiyama, 2.5 μl of one-seventh-diluted serum was loaded on IEF. For α1AT ZZ 2.5 μl of one-half-diluted serum, and for the proband 2.5 μl of undiluted serum were used, respectively. The anode is at the top and the cathode at the bottom. Lane 1, α1AT M1S; lane 2, α1AT M1X; lane 3, α1AT M1Ysiiyama; lane 4, α1AT ZZ; lane 5, proband. Note that in lane 5, three faint bands (arrowhead 1–3) could be observed, indicating the possibility of either phenotype S or Z. B, crossed immunoelectrophoresis of α1AT. The polycrylamide gel after IEF was unexcuclated and placed in the vertical direction (anode at the top and cathode at the bottom in the first IEF). For reference serum of M1Z, 2.5 μl of one-seventh-diluted serum was loaded on IEF, and for ZZ and the proband the amounts loaded on IEF were the same as described in the legend for Fig. 2A. The immunoelectrophoresis was performed in the horizontal direction (anode at the right and cathode at the left). Panels 1 and 2, proband; panel 3, α1AT ZZ; panel 4, α1AT M1Z. In the case of lane 1, agarose gel containing 1/960-diluted anti-human α1AT antibody was used. In the case of lanes 2–4, agarose gel containing 1/480-diluted antibody was used. Note in lanes 1 and 2 two broad precipitation peaks could be observed at the position corresponding to Z but not to Z.
were collected from blood by the dextran method (15), and their total RNA was obtained using RNAZol® (Cinna/Biotex Laboratories). Northern analysis was performed with 1% agarose gel electrophoresis followed by immunoperoxidase staining using the IEF of the proband serum showed three faint bands, indicating the possibility of the S or Z phenotype of the proband serum was determined as the α1AT diastase-resistant inclusion bodies (arrowheads) were found in the several hepatocytes. No such inclusion bodies were recognized in the hepatocytes from normal liver biopsy specimens under the same staining conditions.

**Identification of Single-base Substitution in α1AT-coding Exons**—Direct sequencing of all coding exons (1c, II, III, IV, and V) of the α1AT gene using single-stranded DNA from asymmetric PCR revealed a single-base substitution in exon II of the α1AT gene. In this context, the proband showed a homozygous C to T mutation resulting in replacement of Ser12629 (TCC) by Phe12629 (TTC) (lane 3, Fig. 4). His father (lane 2, Fig. 4), mother, sister, and daughter (not shown) were all heterozygous with both C and T at this base position. These results correlated with the fact that the serum α1AT levels detected in the family members were about half the normal serum level of healthy individuals while the level of α1AT of the proband was severely low (Fig. 1). Together with the data for serum α1AT concentration and the base substitution, it is likely that this missense mutation is responsible for the deficient state in α1AT Siiyama. When compared with the reference sequence of M1 (Ala126) (16, 17), residue 213 of Siiyama was Val, indicating that it is derived from the normal variant α1AT M1 (Val126) gene.

**DISCUSSION**

Serpin is the name given to a superfamily of proteins that are inhibitors of specific serine proteases (1, 6, 7). Serpins though no apparent pathological findings were observed after routine hematoxylin-eosin and silver staining (not shown), inclusion bodies in the hepatocytes were observed in the proband's liver. Several hepatocytes had PAS-positive with diastase-resistant inclusion bodies (Fig. 3A) and immunoreactive substances with anti-α1AT antibody (Fig. 3B) especially in the periporal regions, suggesting the existence of aggregated α1AT molecules in hepatocytes. No such inclusion bodies were recognized in the hepatocytes from normal liver biopsy specimens under the same staining conditions.

**Histological Findings in Biopsied Liver Specimen**—Although no apparent pathological findings were observed after routine hematoxylin-eosin and silver staining (not shown), inclusion bodies in the hepatocytes were observed in the proband's liver. Several hepatocytes had PAS-positive with diastase-resistant inclusion bodies (Fig. 3A) and immunoreactive substances with anti-α1AT antibody (Fig. 3B) especially in the periporal regions, suggesting the existence of aggregated α1AT molecules in hepatocytes. No such inclusion bodies were recognized in the hepatocytes from normal liver biopsy specimens under the same staining conditions.

![Fig. 3. Histological findings of the liver obtained from the proband. A, diastase digestion followed by PAS staining (×400 magnification). PAS-positive with diastase-resistant inclusion bodies (arrowheads) were found in the several hepatocytes. B, immunohistochemical staining (×400 magnification). Aggregates in the hepatocytes which reacted with anti-α1AT antibody are stained.](image-url)

![Fig. 4. Identification of a point mutation in exon II of the α1AT gene. Shown on the top is the structure of the α1AT gene (exons I, II, III, IV, V). Hatched region is the part transcribed in mononuclear phagocytes and gray region corresponds to signal peptides. The region indicated by an arrow is shown below in the sequencing gel. Sequencing was performed by the dideoxy termination method using second PCR products as template (for details, see Materials and Methods). Analysis of all coding exons of the α1AT gene revealed the single abnormality in exon II. Shown in the sequence from Ile50 to Ser12629 in exon II. Lane 1 normal control; lane 2, the proband's father; and lane 3, the proband. His father, mother, sister, and one daughter are heterozygous in the mutated base.](image-url)
include plasma protease inhibitors such as α1AT, α-antichymotrypsin, antithrombin III, plasminogen activator inhibitor, and even ovalbumin and protein motrypsin, antithrombin 111, plasminogen activator inhibitor, are presumed to have lost their function as serine protease inhibitors.

The most researched and studied serpin is α1AT. The prime physiological role of α1AT is the inhibition of elastase derived from leukocytes. The clinical consequence of α1AT deficiency in serum is the early onset of emphysema, a destruction of lung parenchyma by neutrophil proteases accumulated in the lung by smoking (2–4, 11). Structural understanding of the serpins was accelerated by the crystallographic structure of α1AT (22). Huber and Carrell (1) recently analyzed the three-dimensional structure of α1AT and discussed the structure and function of the serpins. They listed 51 conserved amino acid residues in the serpin superfamily. Among them, four have already been reported in natural serpin variants with pathological significance, including Glu342 → Lys342 in a Z-type α1AT (16, 23) and Gly264 → Val264 of an S-type α1AT (8). The most interesting mutant which occurs naturally is Pro369 → Leu369 in α1AT Mvaries, which occurs at the beginning of sheet 4B (24). When the mutation was evaluated in the background of the serpin structure, the mutation at the identical position in the crystallographic structure was already reported in antithrombin III Utah (Pro407 → Leu407) causing reduction in the serum level and increase in the tendency to thrombosis (25). This example indicates the validity of the α1AT crystallographic structure.

The mutation found in α1AT Sivans, Ser35 → Phe63, occurs on one of the 51 residues noted as conserved by Huber and Carrell (10).

### Table I

| Group      | Genotype          | No. of amino acid | Amino acid in M1(Val215) | Mutated amino acid | Mutational matrix no. | Consensus sequence | Ref. |
|------------|-------------------|-------------------|--------------------------|--------------------|-----------------------|--------------------|------|
| Deficiency | α1AT I            | 39                | Arg                      | Cys                | –4                    | +                  | 28   |
|            | α1AT Mprovida     | 41                | Leu                      | Pro                | –3                    | Leu                | 29   |
|            | α1AT Sivans       | 53                | Ser                      | Phe                | –3                    | Ser                | This study |
|            | α1AT Mvaries      | 369               | Pro                      | Leu                | –3                    | Pro                | 24   |
|            | α1AT Pnew/ill     | 256               | Asp                      | Val                | –2                    | +                  | 30, 31|
|            | α1AT S            | 264               | Glu                      | Val                | –2                    | Glu                | 8    |
|            | α1AT Mmineral springs | 67     | Gly                      | Gly                | 0                    | Gly                | 32   |
|            | α1AT Z            | 342               | Glu                      | Lys                | 0                    | Glu                | 16, 23|
|            | α1AT Wartehols    | 336               | Ala                      | Thr                | 1                    | Ala                | 33   |
| Null       | α1AT Nullbehipslen | 92                | Ile                      | Asn                | –2                    | +                  | 34   |
|            | α1AT Nullsion     | 115               | Gly                      | Ser                | 1                    | +                  | 2    |
| Dysfunction| α1AT Pittsburgh   | 358               | Met                      | Arg                | 0                    | +                  | 35   |
| Normal     | α1AT F            | 223               | Arg                      | Cys                | –4                    | +                  | 2    |
|            | α1AT Vnew/ich     | 2                 | Asp                      | Ala                | 0                    | +                  | 36   |
|            | α1AT X            | 204               | Glu                      | Lys                | 0                    | +                  | 2    |
|            | α1AT M1(Ala23)    | 213               | Val                      | Ala                | 0                    | 16, 17             |
|            | α1AT Xarteholch   | 363               | Glu                      | Lys                | 0                    | 37                |
|            | α1AT M4           | 101               | Arg                      | His                | 2                    | 38                |
|            | α1AT M2           | 101               | Arg                      | His                | 2                    | 39                |
|            | α1AT M3           | 376               | Glu                      | Asp                | 3                    | +                  | 40   |
|            | α1AT Pnew/almans  | 256               | Glu                      | Asp                | 4                    | +                  | 31   |
|            |                   | 341               | Asp                      | Asn                | 2                    | +                  |

*The mutational matrix numbers are from the mutational matrix table of Dayhoff et al. (27). Briefly, 0 means neutral, +4 means 2.5 times (10^4) as frequent, and –4 means 0.4 times (10^-4) less frequent as average.

The definition of "consensus" is after Ye et al. (20) where a residue is observed if present in half or more sequences, and a plus “+” is assigned if conserved residues are present in two-thirds or more sequences. "Conserved" means positive numbers in the mutational matrix table (27) for a given amino acid substitution using human α1AT (M1 (Val215)) as standard.
Amino acid differences between human (Ma [ValF11]) and baboon α1AT (26) were sorted in regard to the mutational matrix number.

| No. of amino acid | Amino acid residue | Mutational matrix no.* | Consensus sequence† |
|-------------------|--------------------|------------------------|--------------------|
| 230               | Gln     | Tyr                  | -4                 |
| 15                | His     | Pro                  | 0                  |
| 74                | Asp     | Ser                  | 0                  |
| 122               | Glu     | Lys                  | 0                  |
| 148               | Gly     | Glu                  | 0                  |
| 201               | Lys     | Glu                  | 0                  |
| 262               | Asp     | Ala                  | 0                  |
| 213               | Val     | Ala                  | 0                  |
| 233               | Lys     | Glu                  | 0                  |
| 14                | Ser     | Pro                  | 1                  |
| 29                | Ser     | Ser                  | 1                  |
| 105               | Gln     | Lys                  | 1                  |
| 121               | Ser     | Asn                  | 1                  |
| 123               | Gly     | Ser                  | 1                  |
| 144               | Thr     | Ser                  | 1                  |
| 285               | Ser     | Asn                  | 1                  |
| 292               | Ser     | Ala                  | 1                  |
| 301               | Ser     | Thr                  | 1                  |
| 19                | Asp     | Asn                  | 2                  |
| 23                | Phe     | Leu                  | 2                  |
| 126               | Lea     | Val                  | 2                  |
| 159               | Asp     | Asn                  | 2                  |
| 280               | Asp     | Asn                  | 2                  |
| 385               | Met     | Ile                  | 2                  |
| 222               | Lys     | Arg                  | 3                  |
| 306               | Glu     | His                  | 3                  |
| 234               | Gru     | Asp                  | 3                  |
| 92                | Ile     | Val                  | 4                  |
| 169               | Ile     | Val                  | 4                  |

* See the footnote to Table I.

Table II

Amino acid difference between human (M1 [ValF11]) and baboon α1AT: relation to mutational matrix number and consensus serpin backbone

Carrell (1). In this respect, α1AT Sijamn of this study is unique in the following points. First, mutation at Ser103 has a profound effect on the three-dimensional structure of the α1AT molecule because O of Ser103 initiates helix B, hydrogen bonded to N of Ser160, and stabilizes a portion of sheet 5B by bonding to O of Leu121. It also participates in the folding of the internal core which consists mainly of the most conserved secondary structural elements (helix B, sheet 3A, sheet 4B, sheet 5B). It can be assumed that the changes in properties resulting from the replacement of hydrophilic Ser103 by hydrophobic Phe160 may influence the integrity and organization of the α1AT molecule. This is related to the marked cathodal shift to S on IEF (Fig. 2) and the existence of immunoreactive aggregates in haptocyes from the proband (Fig. 3). The intracellular events caused by the altered protein structure eventually results in the reduction in the serum level of α1AT.

As summarized by Huber and Carrell (1), there is a convincing correlation between the structural changes predicted and the actual functional consequences observed. α1AT is one of the most extensively studied human proteins in regard to amino acid replacement, because of its pleomorphic nature and the clinical consequences caused by deficiency in serum. In addition, amino acid differences between human α1AT and baboon α1AT (26) are evolutionarily interesting. When these amino acid substitutions are analyzed in regard to the mutational matrix number and the substitutions found in the physiological variants (Table I) or in baboon α1AT (Table II), replacements with more negative (zero to minus) mutational matrix numbers at the positions of highly conserved residues are found in the variants of pathological importance, suggesting evolutionarily rare and functionally diverse substitutions occur in the sterically critical area. In the case of Sijamn, the mutation occurs at conserved residue Ser160 (negative mutational matrix number -3 (Ser→Phe)). In contrast, nine normal variants and 29 amino acid differences between human α1AT and baboon α1AT occur at less conserved residues with more positive (zero to plus) mutational matrix number. Although one difference (Ile177→Val160) between human and baboon occurs at a conserved position, the mutational matrix number of Ile to Val is +4, which is one of the most frequent substitutions found in evolutional change (27).

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REFERENCES

1. Huber, R., and Carrell, R. W. (1989) Biochemistry 28, 8861-8866
2. Crystal, R. G. (1980) J. Clin. Invest. 65, 1343-1352
3. Brantly, M., Nukiwa, T., and Crystal, R. G. (1988) Am. J. Med. 84 (Suppl. 6A), 13-31
4. Cox, D. W. (1989) in The Metabolic Basis of Inherited Disease (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) pp. 2409-2457, McGraw-Hill, New York
5. Arnaud, F., and Chapuis-Cellier, C. (1988) Methods Enzymol. 163, 400-418
6. Carrell, R., and Boswell, D. R. (1986) in Proteinase Inhibitors (Barrett, A. J., and Salvesen, G., eds) pp. 403-420, Elsevier, Amsterdam
7. Carrell, R. W., and Travis, J. (1985) Trends Biochem. Sci. 10, 29-24
8. Long, G. L., Chandra, T., Woo, S. L. C., Davie, E. W., and Kurachi, K. (1984) Biochemistry 23, 4828-4837
9. Rabin, M., Watson, M., Kidd, V., Woo, S. L. C., Breg, W. R., and Ruddle, F. H. (1986) Somatic Cell Mol. Genet. 12, 209-214
10. Gadek, J. E., Fells, G. A., Zimmerman, R. L. Rennard, S. I., and Crystal, R. G. (1981) J. Clin. Invest. 68, 889-888
11. Brantly, M. L., Paul, L. D., Miller, B. H., Falk, R. T., Wu, M., and Crystal, R. G. (1988) Am. Rev. Respir. Dis. 138, 327-336
12. Matsunaga, E., Shikakawa, S., Nakamura, H., Maruyama, T., Tsuda, K., and Fukumaki, Y. (1990) Am. J. Hum. Genet. 46, 602-612
13. Hsu, S. N., Raine, L., and Fanger, H. (1981) J. Histochem. Cytochem. 29, 577-580
14. Jeffreys, A. J., and Flavell, R. A. (1977) Cell 12, 429-439
15. McMillan, J. A., Gallin, J. I., Nauseef, W. M., and Root, R. K. (eds) (1988) Laboratory Manual of Neutrophil Function, pp. 2-3, Raven Press, New York
16. Nukiwa, T., Satoh, K., Brantly, M. L., Ogushi, F., Fells, G. A., Courtney, M., and Crystal, R. G. (1986) J. Biol. Chem. 261, 15899-15904
17. Nukiwa, T., Brantly, M., Ogushi, F., Fells, G., Satoh, K., Stier, L., Courtney, M., and Crystal, R. G. (1987) Biochemistry 26, 5259-5267
18. Perlmuter, D. H., Cole, F. S., Killbridge, P., Rossing, T. H., and Colten, H. R. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 795-799
19. Perlino, E., Cortese, R., and Ciliberto, G. (1987) EMBO J. 6, 2767-2771
20. Ye, R. D., Wun, T.-C., and Sadler, J. E. (1987) J. Biol. Chem. 262, 3718-3725
21. Huc, N. T. and Dayhoff, M. O. (1980) Biochem. Biophys. Res. Commun. 95, 894-901
22. Loebermann, H., Tokuoka, R., Deisenhofer, J., and Huber, R. (1984) J. Mol. Biol. 177, 531-566
23. Yoshida, A., Lieberman, J., Gaudulis, L., and Ewing, C. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 1324-1328
24. Hofker, M. H., Nukiwa, T., van Puassen, H. M. B., Nelen, M., Kramps, J. A., Klaasen, E. C., Frants, R. R., and Crystal, R. G. (1989) Hum. Genet. 81, 264-268
25. Bock, S. C., Marrinan, J. A., and Radziejewska, E. (1988) Biochemistry 27, 6171–6178
26. Kurachi, K., Chandra, T., Degen, S. J. F., White, T. T., Marchioro, T. L., Woo, S. L. C., and Davie, E. W. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6826–6830
27. Dayhoff, M. O., Schwartz, R. M., and Orcutt, B. C. (eds) (1978) Atlas of Protein Sequence and Structure, pp. 345–352, National Biomedical Foundation, Washington, D. C.
28. Graham, A., Kalsheker, N. A., Newton, C. R., Bamforth, F. J., Powell, S. J., and Markham, A. F. (1989) Hum. Genet. 84, 55–58
29. Takahashi, H., Nukiwa, T., Satoh, K., Ogushi, F., Brantly, M., Fells, G., Stier, L., Courtney, M., and Crystal, R. G. (1988) J. Biol. Chem. 263, 15528–15534
30. Faber, J.-P., Weidinger, S., Goedde, H.-W., and Olek, K. (1989) Am. J. Hum. Genet. 45, 181–183
31. Holmes, M. D., Brantly, M. L., and Crystal, R. G. (1990) Am. Rev. Respir. Dis. 142, 1185–1192
32. Curiel, D. T., Vogelmeier, C., Hubbard, R. C., Stier, L. E., and Crystal, R. G. (1990) Mol. Cell Biol. 10, 47–56
33. Holmes, M. D., Brantly, M. L., Fells, G. A., and Crystal, R. G. (1990) Biochem. Biophys. Res. Commun. 170, 1013–1020
34. Fraizer, G. C., Siewertsen, M. A., Hofker, M. H., Brubacher, M. G., and Cox, D. W. (1990) J. Clin. Invest. 86, 1878–1884
35. Owen, M. C., Brennan, S. O., Lewis, J. H., and Carrell, R. W. (1983) N. Engl. J. Med. 309, 694–698
36. Holmes, M. D., Brantly, M. L., Curiel, D. T., Weidinger, S., and Crystal, R. G. (1990) Am. J. Hum. Genet. 46, 810–816
37. Brennan, S. O., and Carrell, R. W. (1986) Biochim. Biophys. Acta 873, 13–19
38. Okayama, H., Holmes, M. D., Brantly, M. L., and Crystal, R. G. (1989) Biochem. Biophys. Res. Commun. 162, 1560–1570
39. Nukiwa, T., Brantly, M. L., Ogushi, F., Fells, G. A., and Crystal, R. G. (1988) Am. J. Hum. Genet. 43, 322–330
40. Curiel, D., Laubach, V., Vogelmeier, C., Wuets, L., and Crystal, R. G. (1989) Am. J. Respir. Cell. Mol. Biol. 1, 471–477