BRIEF REPORT

Immunodeficiency Associated with *FCN3* Mutation and Ficolin-3 Deficiency

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SUMMARY

Ficolin-3, encoded by the *FCN3* gene and expressed in the lung and liver, is a recognition molecule in the lectin pathway of the complement system. Heterozygosity for an *FCN3* frameshift mutation (rs28357092), leading to a distortion of the C-terminal end of the molecule, occurs in people without disease (allele frequency among whites, 0.01). We describe a patient with recurrent infections who was homozygous for this mutation, who had undetectable serum levels of ficolin-3, and who had a deficiency in ficolin-3–dependent complement activation.

The complement system has a pivotal role in the innate immune response to infections, and there is compelling evidence that complement deficiency is associated with susceptibility to infections and autoimmunity.[1] Three pathways, the classical pathway, the lectin pathway, and the alternative pathway, activate the complement system. In humans, there are four recognition molecules in the lectin pathway: mannose-binding lectin (MBL), ficolin-1 (also called M-ficolin or ficolin/P35-related protein), ficolin-2 (also called L-ficolin, hucolin, EBP-37, or ficolin/P35) and ficolin-3 (also called H-ficolin, Hakata antigen, thermolabile β-2 macroglycoprotein, or thermolabile substance).[2] The ficolins bind surface structures of various classes of microorganisms and of various acetylated compounds, and they are involved in sequestration and removal of dying host cells.[2] They have homologous sequences and share certain structural elements, including an N-terminal domain that contains cysteine residues, a collagen-like domain with typical Gly-Xaa-Yaa repeats of varying length, and a fibrinogen-like binding domain. The ficolins assemble into large multimeric structures of several hundred kilodaltons that consist of four or more trimers joined by N-terminal disulphide bonds to form a C-terminal globular domain that enables the complex to bind molecular patterns, such as acetylated compounds and sugars.[3,4] The collagen-like stalk domains interact with cellular receptors and are associated with serine proteases called MBL-associated serine proteases (MASPs) — MASP-1 and MASP-2, which activate the complement system, as well as MASP-3 and the small MBL-associated protein (sMAP), the functions of which are unknown.[5] Ficolin-3, the predominant lectin-pathway recognition molecule in plasma, has a median concentration of 25 μg per milliliter in healthy white people, followed by ficolin-2 (5 μg per milliliter) and MBL (1 μg per milliliter).[6-8] In contrast, ficolin-1 is normally found in very low concentrations in plasma (<0.1 μg per milliliter).[9] The gene encoding ficolin-1 (*FCN1*) is expressed in bone marrow–derived cells, that encoding ficolin-2

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(*FCN2*) is expressed in the liver, and that encoding ficolin-3 (*FCN3*) is expressed in the liver and the lung.[10]

Genetically determined deficiency and defects of MBL are common and well described,[11] but inherited deficiency of a ficolin is rare. Polymorphisms that may influence the concentration and function of ficolin-2 are, however, relatively common.[10,12] In the coding part of the *FCN3* gene (on chromosome 1p36.11), a frameshift mutation (*FCN3+1637delC,* rs28357092) in exon 5 has been observed in the heterozygous state among healthy subjects, with an allele frequency of 0.01.[6] The mutation causes a shift in the reading frame and alters the amino acid composition of the C-terminal end of the protein spanning from amino acid position 117 to position 180, where the nonsense protein sequence is terminated as the result of a stop codon. In principle, this mutation causes a truncated ficolin-3 variant (Leu117fs) lacking the entire fibrinogen-like domain. Nevertheless, experiments with recombinant Leu117fs-containing ficolin-3 show that the variant protein cannot be expressed and would therefore most likely lead to complete ficolin-3 deficiency in vivo.[6] Given these considerations, we hypothesized that homozygosity for the *FCN3+1637delC* frameshift mutation could be responsible for a novel complement deficiency syndrome.

METHODS

PATIENTS

Our study included all 1282 patients referred to our department over a period of 12 years for routine immunologic investigation of various immunodeficiencies (not related to the human immunodeficiency virus). DNA and serum from patients were stored. In addition, control samples from the index patient and family members were collected. Standard laboratory assays were used to assess the activity of the classical, MBL, and alternative complement pathways; MBL and ficolin-2 levels; leukocyte count; distribution of lymphocyte surface markers; and lymphocyte proliferation in response to mitogens and microbial antigens. Vaccination responses and measurements of immunoglobulin classes and subclasses were obtained from medical records.

Methods used for pyrosequencing, polymerase-chain-reaction assay, DNA sequencing, measurement of serum levels of ficolin-3, sodium dodecyl sulfate–polyacrylamide-gel electrophoresis (SDS-PAGE) and Western blotting, assessment of ficolin-2 depletion from serum, and assessment of ficolin-3–dependent complement activation are described in the Supplementary Appendix, available with the full text of this article at NEJM.org.

STATISTICAL ANALYSIS

The Mann-Whitney test was used to compare ficolin-3 serum levels. Only a two-sided test was used.

RESULTS

FREQUENCY OF THE *FCN3+1637delC* VARIANT ALLELE

Among the 1282 patients, 23 were heterozygous and 1 was homozygous for the *FCN3+1637delC* variant allele, causing a frameshift in the amino acid sequence (Fig. 1) (allele frequency among all 1282 patients, 0.01). The frequency of heterozygosity (1.8%) is the same as that previously observed in healthy white controls.[6] Both parents of the homozygous patient were healthy and were heterozygous for the *FCN3+167delC* variant allele, as was one healthy sister. Another healthy sister had two wild-type alleles. A third healthy sister was living abroad and was not available for this investigation.

CLINICAL FEATURES OF THE INDEX PATIENT

The index patient, a 32-year-old man, was the son of unrelated parents of Macedonian and Albanian origin. He had had repeated lower respira-

Figure 1 (facing page). Structure of Ficolin-3 and the Effect of the *FCN3+1637delC* Frameshift Mutation.

A model of the ficolin-3 oligomerization into higher-order multimers is shown (top). Ficolin-3 becomes oligomerized into trimer-based hexamers to form a functional molecule. Below the multimer, the eight exonic regions of *FCN3* are shown, as are the monomeric-domain structures, of both the wild type and the *L117fs* mutant. The ficolin-3 polypeptide contains a signal peptide followed by an N-terminal cysteine-rich region, a collagen-like domain, a short linker region, and a C-terminal fibrinogen-like domain (FBG). The *FCN3+1637delC_(L117fs)* frameshift mutation is indicated by a red dot. In the bottom half of the figure, sequencing electropherograms for the part of *FCN3* exon 5 containing the *FCN3+1637delC_(L117fs)* mutation are shown for both a wild-type and a mutant homozygote. The amino acids listed in white boxes are those affected by the deletion of the C nucleotide.

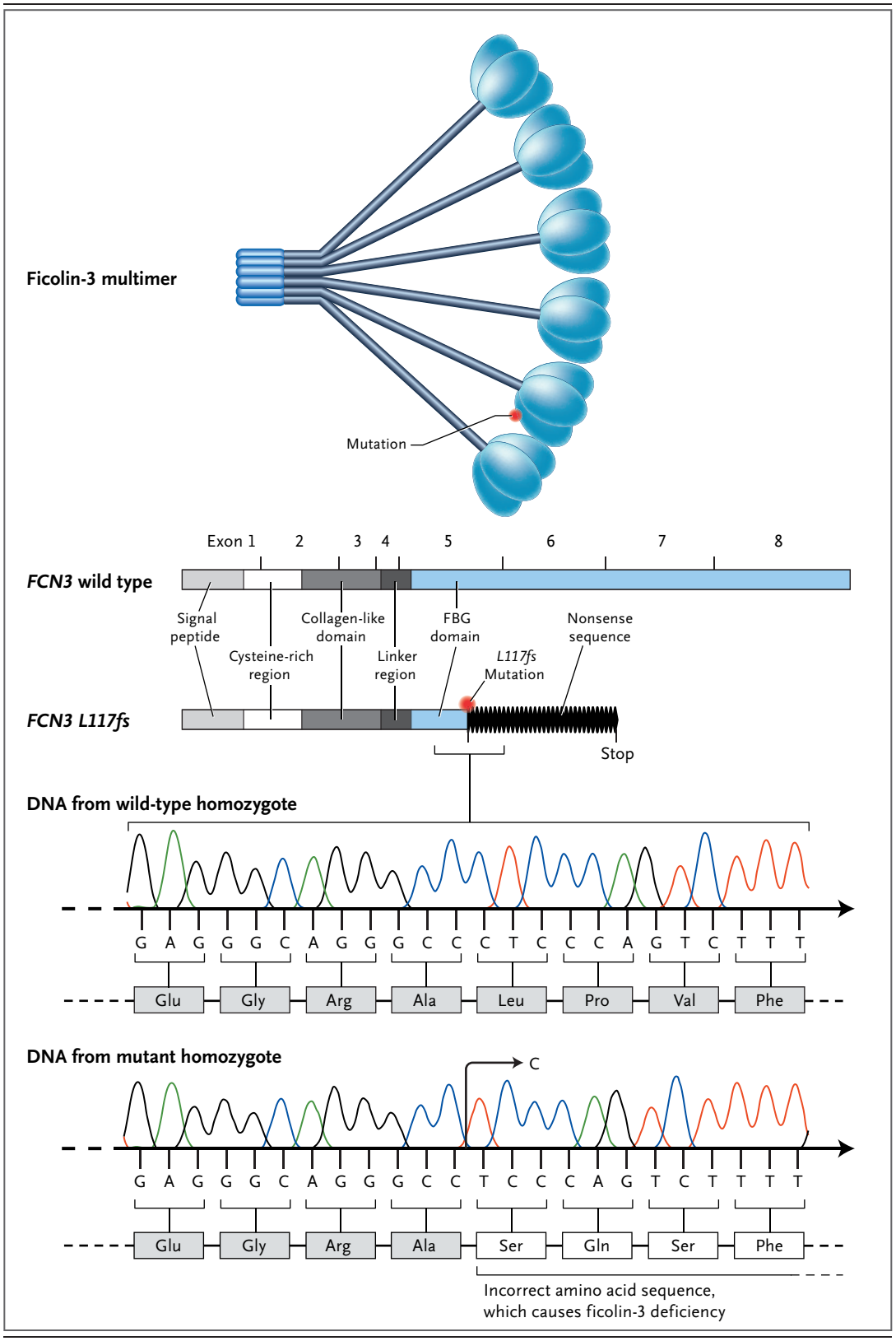

Table 1. Immunologic Characteristics of the Index Patient.

Variable	Reference Range	Index Patient
Leukocytes ($\times 10^{-6}$/ml)		
Neutrophils	1.8–7.4	4.8
Lymphocytes	0.7–4.8	3.9
T cells		
CD3	0.69–2.7	3.0
CD4	0.39–1.7	1.4
CD8	0.19–1.03	1.3
Killer cells and natural killer cells	0.08–0.56	0.46
B cells	0.09–0.57	0.11
Immunoglobulin antibodies (g/liter)		
IgG	6.8–15.8	8.0
IgA	0.65–3.55	1.98
IgM	0.29–1.98	0.24
IgG1	3.7–10.2	4.8
IgG2	1.1–5.9	2.8
IgG3	0.15–1.3	0.99
IgG4	0.06–1.9	0.09
Vaccination response		
Haemophilus influenza type b		Normal
Diphtheria and tetanus		Normal
Pneumococcal polysaccharide		Low
Complement pathway		
Classic		Normal
Mannose-binding lectin		Normal
Alternative		Normal

tory tract infections since early childhood. Since the age of 17 years, he had had recurring warts on his fingers. When he was 20 years old, his spleen was removed because of unexplained thrombocytopenia, from which he fully recovered. At the age of 26 years, he was admitted to our hospital for treatment of bilateral frontal cerebral abscesses with nonhemolytic streptococci. Since then the patient has had grand mal seizures, which have been treated with antiepileptic drugs. During the past 8 years, he has had several episodes of bacterial pneumonia requiring hospital admission, with positive cultures of bronchial sputum for *Haemophilus influenzae* and *Pseudomonas aeruginosa*. A computed tomographic scan of the chest showed severe bronchiectasis and pulmonary fibrosis. Lung-function tests performed over a period of 6 years have revealed progressively decreased lung capacity and obstructive lung disease, despite intensive antibiotic treatment and experimental treatment with subcutaneously administered IgG.

Laboratory investigations for a suspected immunodeficiency after the diagnosis of cerebral abscesses showed a normal blood count and normal concentrations of B, T, and killer–natural killer lymphocytes but an inverse ratio of CD4 to CD8 T cells, which later became normal (Table 1). The patient's lymphocytes responded normally in vitro to mitogens, in a mixed lymphocyte reaction, and to microbial antigens (purified protein derivative, *Candida albicans*, cytomegalovirus, and tetanus toxoid). Serum levels of IgG, IgG subclasses, and IgA were normal, but the IgM level was slightly decreased (0.24 g per liter; reference range, 0.29 to 2.0) (Table 1). The patient had a low response to pneumococcal polysaccharide vaccine (Pneumovax 23, Sanofi Pasteur MSD) on testing against serotypes 1, 4, 7F, 14, 18C, and 19F, but his responses to *H. influenzae* type b vaccine (ActHIB, Sanofi Pasteur MSD) and to diphtheria and tetanus vaccines (Statens Serum Institut) were normal. Complement analyses revealed normal activity of the classical, MBL, and alternative pathways, as well as normal levels of MBL (2.0 μg per milliliter) and ficolin-2 (5.0 μg per milliliter).

SERUM FICOLIN-3

The median concentrations of ficolin-3 in 115 randomly selected patients with a normal *FCN3* genotype, 23 patients who were heterozygous for the *FCN3+1637delC* frameshift mutation, and the homozygous patient were 27.5 μg per milliliter (range, 3.5 to 42.6), 14.1 μg per milliliter (range, 8.0 to 31.4), and 0, respectively (Fig. 2). The ficolin-3 concentration differed significantly between patients who were heterozygous for the variant allele and those who had two wild-type alleles ($P<0.001$). SDS-PAGE, followed by Western blotting, of serum with the use of a polyclonal anti–ficolin-3 antibody showed an identical ficolin-3 banding pattern under reduced and nonreduced conditions in these two groups and in experiments with recombinant ficolin-3. These results indicated that the detected ficolin-3 in serum from heterozygous patients was represented only by the

normal wild-type allele and not the mutated allele, which would have yielded a shorter fragment. No ficolin-3 bands were observed in the serum from the homozygous patient (Fig. 3).

FICOLIN-3–DEPENDENT COMPLEMENT ACTIVATION

We found that acetylated bovine serum albumin functioned as a binding ligand for ficolin-3, whereas nonacetylated bovine serum albumin did not. We used acetylated bovine serum albumin as a ligand to determine whether ficolin-3 deficiency causes a defect in complement activation. A dose-dependent deposition of complement component C4 on acetylated bovine serum albumin was found when a pool of normal serum samples was used (Fig. 4), but when the pooled serum was used with nonacetylated bovine serum albumin, there was no deposition of C4. In samples of serum obtained from the patient with ficolin-3 deficiency at different time points, there was no C4 deposition on acetylated (Fig. 4) or nonacetylated (data not shown) bovine serum albumin. In contrast, reconstitution of the patient's serum with recombinant ficolin-3 in a physiologic concentration (20 μg per milliliter) in undiluted serum restored C4 deposition in the ficolin-3–deficient serum. Normal C4 deposition on acetylated bovine serum albumin was observed in serum from a subject with a deficiency in the complement component C1q,[13] serum from a subject with MBL deficiency,[14] and ficolin-2–depleted serum (Fig. 4).

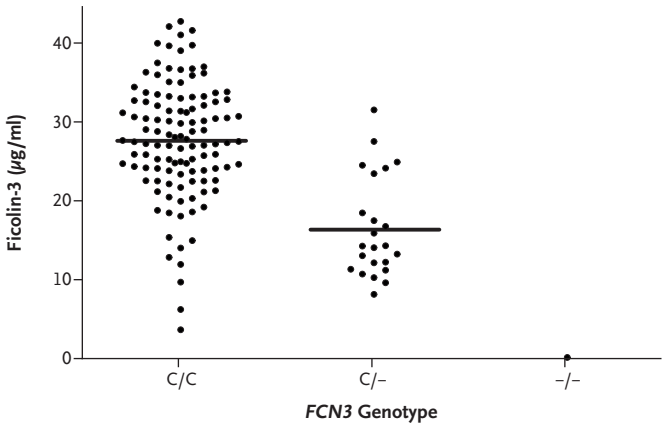

Figure 2. Individual Serum Ficolin-3 Concentrations in 115 Patients with Wild-Type *FCN3*, 23 Patients Who Were Heterozygous for the *FCN3+1637delC* Frameshift Mutation, and 1 Patient Who was Homozygous for the Mutation.

The median serum concentration of ficolin-3 in patients who were homozygous for wild-type *FCN3* (C/C) differed significantly from the concentrations in patients who were heterozygous (C/–) ($P<0.001$). Horizontal lines indicate medians.

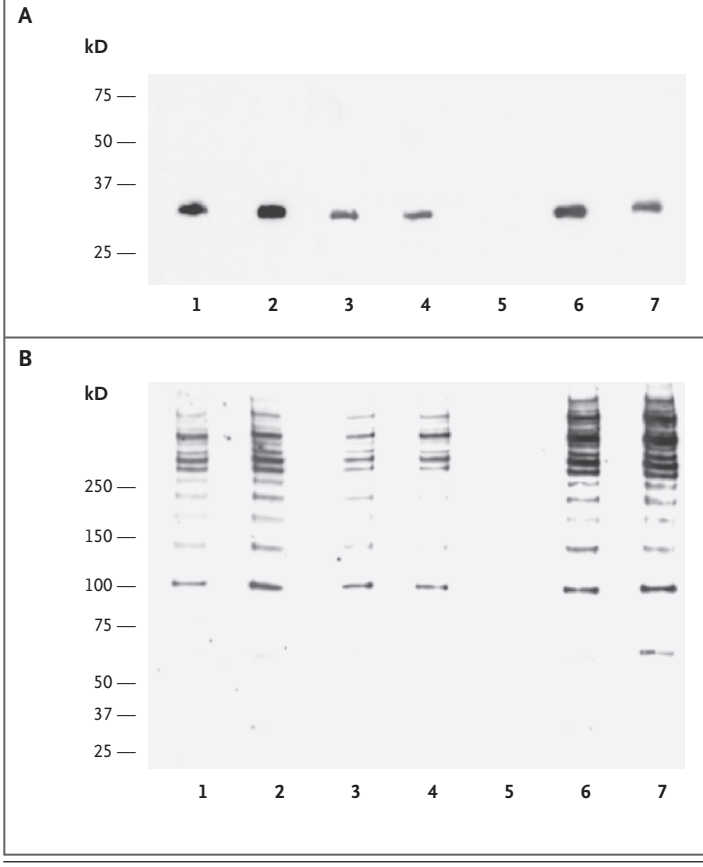

Figure 3. Western Blots of Serum Ficolin-3 from the Patient Who Was Homozygous for *FCN3+1637delC*, His Family Members, and a Control Subject.

Serum proteins were separated with the use of sodium dodecyl sulfate–polyacrylamide-gel electrophoresis (SDS-PAGE) under reducing conditions, with disrupted protein oligomerization and therefore only monomers present (Panel A), and nonreducing conditions, with intact protein oligomerization (Panel B), and proteins were blotted onto a nitrocellulose membrane and probed for ficolin-3. Lane 1 shows serum from a sister of the index patient who was heterozygous for *FCN3+1637delC*; lane 2 serum from a sister who had wild-type *FCN3*; lane 3 serum from the patient's mother, who was heterozygous for *FCN3+1637delC*; lane 4 serum from the patient's father, who was also heterozygous for *FCN3+1637delC*; lane 5 serum from the index patient, who was homozygous for *FCN3+1637delC*; lane 6 serum from a control subject with wild-type *FCN3*; and lane 7 a sample of recombinant wild-type ficolin-3. The findings are representative of two individual experiments.

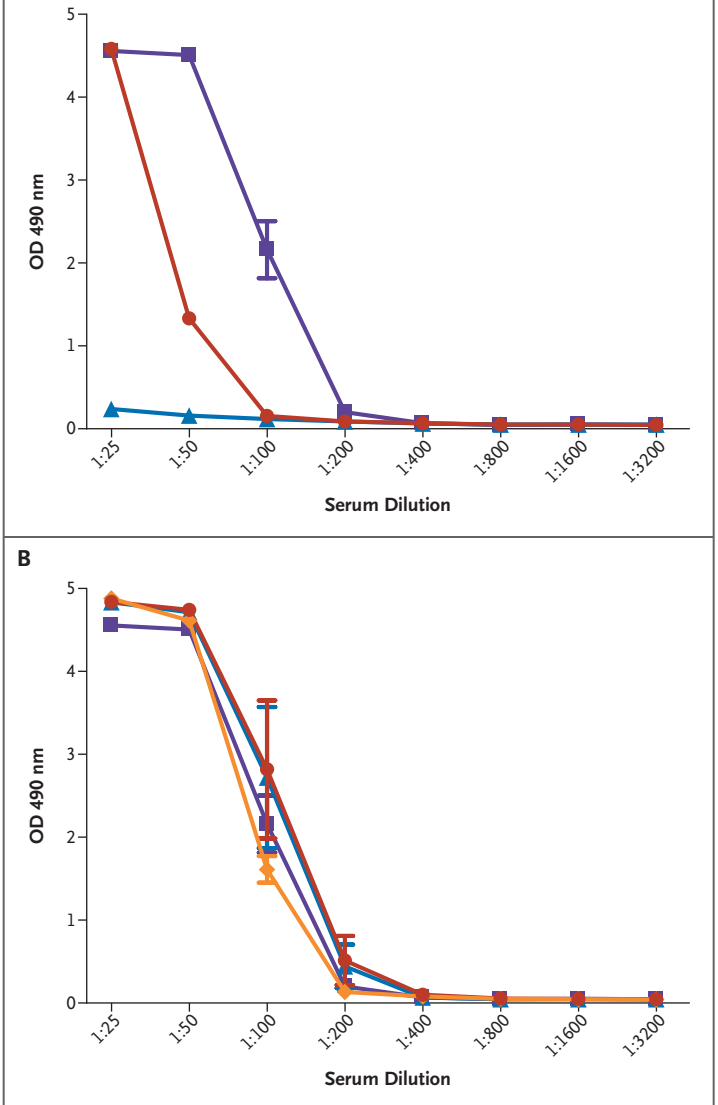

Figure 4. C4 Deposition on Acetylated Bovine Serum Albumin, as Mediated by Ficolin-3.

A coating of acetylated bovine serum albumin on microtiter plates was incubated with human serum for 30 minutes at 37°C. C4 deposition was detected with the use of an antibody against C4. In Panel A, C4 deposition is shown with the use of pooled normal serum (squares), serum from the patient with ficolin-3 deficiency (triangles), and serum from the patient with ficolin-3 deficiency, reconstituted with 20 μg per milliliter of recombinant ficolin-3 (circles). In Panel B, C4 deposition is shown with the use of pooled normal serum (squares), serum from a subject with mannose-binding lectin deficiency (triangles), serum from a subject with C1q deficiency (circles), and ficolin-2 depleted serum (diamonds). Values are means (±SE) from three individual experiments. OD denotes optical density.

DISCUSSION

We found that a patient with a history of recurrent severe pulmonary infections, brain abscesses, and recurrent warts on his fingers was homozygous for an *FCN3+1637delC* frameshift mutation and had no detectable levels of ficolin-3 in his serum. Both of his heterozygous parents and three siblings were healthy; genetic and serologic analyses were performed in two siblings (one heterozygote and one with two wild-type alleles). Thus, the phenotype for ficolin-3 deficiency appears to be present only in the homozygous state, suggesting a recessive pattern of inheritance. The ficolin-3 serum concentration in heterozygotes was decreased to about 50% of the levels found in patients with wild-type *FCN3*. To ensure that the heterozygous carriers of the *FCN3+1637delC* frameshift mutation did not carry another, unknown mutation on the other chromosome, we sequenced the *FCN3* promoter region, exons, and exon–intron boundaries in all heterozygous subjects. No additional mutations were observed, a finding that is consistent with the serum levels of ficolin-3 in the heterozygous carriers. Western blots of serum samples showed no differences between the heterozygous subjects and the subjects with wild-type *FCN3*.

In the patient who was homozygous for *FCN3+1637delC*, there was a complete absence of complement deposition in serum samples when acetylated bovine serum albumin was used as the ligand; this defect was corrected with the addition of recombinant ficolin-3.[15] The patient's serum had normal activity of the classical, MBL, and alternative complement pathways, and serum concentrations of MBL and ficolin-2 were normal. Ficolin-2 has been shown to bind to acetylated bovine serum albumin.[16] However, our results show that serum depleted of ficolin-2 has a normal capacity to activate complement, suggesting that ficolin-3 is the predominant player in complement activation when acetylated bovine serum albumin is the target molecule. This result is not in accord with a recent report indicating that ficolin-2 has a higher complement-activating potential on acetylated bovine serum albumin as compared with ficolin-3.[5] However, in that study the conclusions were based on a comparison of

recombinant ficolin-2 and ficolin-3, and recombinant ficolin may have lower biologic activity than serum ficolin.

About 1.8% of the 1282 patients in our study were carriers of the *FCN3+1637delC* mutation, giving an allele frequency of 0.01, which is the same as that observed in healthy white subjects.[6] Thus, in the healthy white population, one would expect about 1 person in 10,000 to be homozygous for the mutation, making ficolin-3 deficiency similar in frequency to deficiency of complement component C2[17] but less frequent than deficiencies in MBL or MASP-2.[11,18] Screening of several hundred healthy subjects has not identified any who were homozygous for *FCN3+1637delC* (unpublished data). Moreover, all serum samples from 10,050 healthy Japanese donors and virtually all serum samples from 113,216 hospitalized Japanese patients contained ficolin-3.[19] Nevertheless, transient ficolin-3 deficiency may occur in systemic lupus erythematosus as a result of the presence of ficolin-3 autoantibodies.[19]

Since our index patient had severe respiratory and intracerebral abscesses, it is of interest that ficolin-3 is highly expressed in the lungs and released into bronchi and is also expressed by glioma cells.[15,20,21] Ficolin-3 has also been shown to be important in the sequestration of dying host cells.[22,23] Therefore, in our patient, a deficiency of ficolin-3 may have hampered removal of cellular debris, thereby aggravating ongoing infections and inflammatory processes.

The patient's antibody response to a pneumococcal polysaccharide vaccine was low, but the antibody responses to *H. influenzae*, diphtheria, and tetanus vaccines were normal, indicating a poor response to polysaccharides but not protein antigens. Whether these findings are random or are causally linked to ficolin-3 deficiency remains to be determined.

Although it seems likely that ficolin-3 deficiency was responsible for the clinical disorders in this patient, observations made in a single patient must be interpreted with caution. Nevertheless, this study shows that inherited deficiency of ficolin-3 results in a lack of complement deposition on acetylated structures and is most likely associated with chronic disabling infections and lung damage.

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