We have restudied two kindreds that formed the basis of the original report of autosomal recessive chronic granulomatous disease (CGD) associated with leukocyte glutathione peroxidase deficiency. Case 1 from the original study and the surviving brother of the originally reported case 2 both have severe CGD, with no detectable respiratory burst activity in purified intact neutrophils. However, their leukocytes exhibit normal glutathione peroxidase enzyme activity and gene expression. Examination of phagocyte nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase components known to be defective in CGD reveals no detectable cytochrome b558 nor any membrane activity in a cell-free NADPH oxidase assay system. Molecular analysis of the genes encoding cytochrome b558 subunits shows, in case 1, a C → T substitution at nucleotide 688 of the gene encoding the gp91-phox subunit of cytochrome b558, resulting in a termination signal in place of Arginine-226. Levels of gp91-phox mRNA are markedly decreased despite normal levels of gene transcription, indicating a post-transcriptional effect of the nonsense mutation on mRNA processing or stability. The X-linked form of CGD developed in this cytogenetically normal female due to the uniform inactivation of the normal X chromosome in her granulocytes, indicated by the expression in her granulocyte mRNA of only one allele of a glucose-6-phosphate dehydrogenase polymorphism for which she is heterozygous in genomic DNA. Case 2 (of the present study) has distinct mutations in each allele of the p22-phox gene. Thus, the CGD phenotype in both kindreds now appears to be caused not by leukocyte glutathione peroxidase deficiency, but rather by mutations of the same subunits of phagocyte NADPH oxidase that are involved in all other cases of cytochrome b-negative CGD. Glutathione peroxidase deficiency should no longer be considered a possible cause of the CGD phenotype.

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complex includes multiple protein components, four of which have been identified as sites of mutations leading to the CGD phenotype. A low midpoint potential, membrane-bound cytochrome b_{558}, unique to phagocytes, serves as the terminal electron donor of the oxidase, and its absence marks the "cytochrome b negative" forms of CGD. All X-linked CGD results from defects in a 91 kDa glycoprotein (designated gp91-phox, for glycoprotein, 91 kDa, of phagocyte oxidase) component of the cytochrome b_{558} heterodimer. Approximately 30% of autosomal recessive CGD cases (15% of overall CGD prevalence) lack the cytochrome b_{558} as a result of defects in the other cytochrome b_{558} component, a 22 kDa nonglycosylated polypeptide (designated p22-phox) encoded by a gene located on chromosome 16. The remainder of autosomal recessive CGD cases result from defects in the p47-phox or p67-phox cytosolic components of the oxidase and are all cytochrome b positive.

In very rare cases, mutations in genes other than those encoding oxidase components can lead to a CGD phenotype. For example, severe glucose-6-phosphate dehydrogenase (G6PD) deficiency causes a CGD-like defect in superoxide production because phagocytes fail to maintain an adequate concentration of NADPH, the substrate for the oxidase.

In 1968, when CGD was thought to be purely X-linked, two females with CGD were described who thus constituted the first evidence of alternative, presumably autosomal recessive, forms of inheritance of the disease. They were two females with CGD were described who thus constituted the first evidence of alternative, presumably autosomal recessive, forms of inheritance of the disease. They were two females with CGD were described who thus constituted the first evidence of alternative, presumably autosomal recessive, forms of inheritance of the disease. They were two females with CGD were described who thus constituted the first evidence of alternative, presumably autosomal recessive, forms of inheritance of the disease.

In 1968, when CGD was thought to be purely X-linked, two females with CGD were described; who thus constituted the first evidence of alternative, presumably autosomal recessive, forms of inheritance of the disease. They were subsequently reported to have a severe deficiency of leukocyte glutathione peroxidase. Leukocyte G6PD and glutathione reductase activities were normal, as was NADH oxidase, a soluble enzyme believed at that time to be the respiratory burst oxidase. The membrane-bound NADPH oxidase (now known to be responsible for the respiratory burst) was not examined. The investigators hypothesized that the CGD phenotype in these two females was due to GSH-Px deficiency with a secondary inhibition of oxidase activity caused by the resultant accumulation of peroxides. A subsequent case of a male child with clinical CGD with normal NADPH oxidase activity and mild leukocyte GSH-Px deficiency (enzyme activity 30% of normal) was reported from Japan.

Because of the recent advances in our understanding of the molecular basis of CGD and of GSH-Px gene regulation, we restudied the cases that were originally reported to show an association of GSH-Px deficiency with CGD and that continue to be cited as examples of CGD due to defects in nonoxidase genes.

MATERIALS AND METHODS

CGD patients and families. Case 1 from the original studies is now 40 years old, married, and in generally good health. However, between 1968 and 1978 she was hospitalized 10 times for pneumonia, abdominal and liver abscesses (due to Staphylococcus aureus), and prolonged fevers of unknown etiology.

Case 2 from the prior studies died in 1969, at 11 years of age, of Aspergillus pneumonia. Her parents subsequently had a son with CGD, henceforth referred to as case 2 of the present study (Fig 1). His leukocytes, like those of his sister, showed no detectable reduction of nitroblue tetrazolium (NBT) dye after endotoxin stimulation, and they were severely deficient in killing of S aureus. In his first six months of life he had meningitis (considered aseptic), staphylococcal sepsis, interstitial pneumonia, and Pseudomonas osteomyelitis. He had no major problems until age 15 years, but since then he has experienced several episodes of pneumonia, recurrent sinusitis and soft tissue infections of the head and neck, acute cholecystitis requiring cholecystectomy, epididymitis and inguinal lymphadenitis from which cultures grew both Torula yeast and acid-fast bacilli, and hydronephrosis due to granulomas and strictures of both ureters requiring right nephrectomy and continued steroid treatment.

There is no historical evidence for CGD among the 50 relatives of case 2 shown on the pedigree (Fig 1). The patient's nephew (IV-6) died at age 3 months of sudden infant death syndrome. All relatives evaluated by laboratory testing showed normal endotoxin-stimulated

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**Fig 1.** Case 2 family pedigree. The arrow indicates case 2 of the present study; ♂ and ♀, CGD; and N, normal respiratory burst function assessed by NBT reduction. No other family member had a history suggestive of CGD. Years of birth are indicated for the central kindred, as well as years of death (D) where applicable.
CGD AND GLUTATHIONE PEROXIDASE

NG. reduction (Fig 1, ‘‘N’’). This pattern provides strong evidence for classical genetic autosomal recessive inheritance.

Blood samples were obtained in accordance with human subjects procedures of the institutions at which the phlebotomies were performed.

**Cell preparation.** Neutrophils or mixed mononuclear cells were isolated from whole blood (anticoagulated with acid-citrate-dextrose) by dextran sedimentation, hypotonic lysis of erythrocytes, and Ficoll-Hypaque density gradient centrifugation (Pharmacia, Piscataway, NJ) as previously described.25-26 Neutrophils used for cell-free activation experiments were obtained by leukapheresis, and the membrane and cytosol fractions were prepared as previously described.27,28

Cytochrome b. Spectrophotometric determinations of cytochrome b556 contents of cell pellets (107 cells total) were performed by dithionite difference spectroscopy.18 Immunoreactive gp91-phox and p22-phox quantitation was performed by Western blot, using previously reported antisera and methodology.29,30

**Superoxide generation.** Superoxide generation was determined histochemically by the reduction of NBT dye to blue formazan particles.32-33 NBT tests were performed on glass slides, where adherent neutrophils were stimulated either by coating the slides with bacterial endotoxin32 or by addition of phorbol myristate acetate (PMA).33 Neutrophils were examined by light microscopy for the presence of blue granules, and the percentage positive and intensity of staining were recorded for at least 200 cells in each sample.

**Quantitative rate of superoxide production by intact neutrophils and by the cell-free NADPH oxidase system were measured as superoxide dismutase-inhibitable ferricytochrome c reduction, as previously described.27,33-34** Intact neutrophils were activated by PMA (200 ng/mL) or chemotactic peptide formyl-methionyl-leucyl-phe- nylalanine (FMLP; 10-7 M); the cell-free system, by sodium dodecyl sulfate (SDS; 40 μmol/L). Cytosol activity in the cell-free system was determined by assaying varying amounts (allotted as cell equivalents) of partially purified cytosol from the patients’ neutrophils in the presence of 6.25 × 107 cell equivalents of deoxycholate-solubilized plasma membrane in each 150 μL reaction volume. Membrane activity in the cell-free system was determined by assaying varying amounts of membrane from patients’ neutrophils in the presence of 18 × 107 cell equivalents of cytosol. Normal control values were highly consistent between experiments, as previously reported.27

**GSH-Px enzymatic activity.** GSH-Px activity was measured spectrophotometrically using the coupled peroxidase-reductase method of Butler.35 Both sample and reference cuvettes contained whole cells or neutrophil cytosol fraction, plus 0.05% Triton X-100, 0.2 mmol/L NADPH, 2 mmol/L GSH and 1 U/mL glutathione reductase. The oxidation of NADPH after addition of H2O2 or t-tuyl hydroperoxide to the sample cuvette was followed spectrophotometrically at 340 nm.

**Northern blot.** Total cell RNA was extracted from mixed mononuclear cells by the guanidine-EDTA method46 and analyzed by Northern blots performed according to standard procedures.37 Hybridization probes were full-length cDNAs for the human gp91-phox48 and p22-phox genes,50 and a 600-bp EcoRI fragment of human cellular GSH-Px cDNA containing most of the coding and 3'-untranslated regions. Equal loading of lanes was demonstrated by examination of gels after ethidium bromide staining.

**Nuclear run-on transcription assay.** Incubation of nuclei and subsequent RNA isolation were performed with modifications of previously described procedures.59 Nuclei were prepared from lymphoblastoid B cell lines derived from case 1 and a normal donor. These cells normally express all oxidase components and serve as cellular and molecular models for CGD.50,61 Pilot experiments showed identical ratios of oxidase gene transcript levels in fresh peripheral blood mononuclear cells and in B cell lines from cases 1 and 2 and multiple normal controls. Cell samples (107 cells) were washed with cold Hanks’ Balanced Salt Solution and counted. Cell numbers ranged from 0.3 × 107 to 8.7 × 107. Nuclei were isolated by incubating for 5 minutes in lysis buffer (10 mmol/L Tris-HCl pH 7.4, 10 mmol/L NaCl, 3 mmol/L MgCl2, 0.01% Nonidet P40 (NP40) on ice, pelleted (900g for 6 minutes at 4°C), washed once with lysis buffer (as above) and once with lysis buffer without NP40, and resuspended in 200 μL of nuclei suspension buffer (50 mmol/L Tris pH 8.3, 40% (vol/vol) glycerol, 5 mmol/L MgCl2, 0.1 mmol/L EDTA). Freshly prepared nuclei were incubated for 30 to 45 minutes at 30°C with an equal volume of reaction buffer containing 10 mmol/L Tris-HCl pH 8.0, 5 mmol/L MgCl2, 250 mmol/L KCl, 0.8 mmol/L MnCl2, 0.83 mmol/L each of adenosine triphosphate, cytidine triphosphate, and guanosine triphosphate; 250 μCi of [α-32P] uridine triphosphate (3,000 Ci/mmol); and Inhibit-ACE RNase inhibitor (5Prime→3Prime). The reaction mixture was digested with DNase I and proteinase K and the 32P-labeled RNA isolated by extraction in guanidine thiocyanate and phenol and then ethanol precipitation. Equal counts of incorporated label from each group (3 × 107 cpn) were hybridized to saturating amounts of plasmid, with or without cDNA inserts, and immobilized on nitrocellulose filters by slot blotting. Probes included the same GPX1, gp91-phox, and p22-phox cDNAs used for Northern blots; a 200-bp cDNA (obtained from Dr P. Dobner, University of Massachusetts Medical School, Worcester for the 3'-untranslated region of the constitutively expressed Kα tubulin gene52; and a pBluescript (Stratagene, La Jolla, CA) plasmid negative control. Hybridization to each probe was determined by direct measurement of 32P decay on a Betascope 603 (Betagen Co, Waltham, MA) blot analyzer. Data for bands of interest were normalized to the signal for the tubulin mRNA to correct for nonspecific differences between preparations of nuclei.

**Single-strand conformation polymorphism (SSCP) analysis.** Genomic DNA was extracted from peripheral blood27 and analyzed by SSCP as previously described43,44 using two-stage nested primer polymerase chain reaction (PCR) utilizing outer 20 bp oligonucleotide primers with 0.1 μg of genomic DNA, then inner primers end-labelled with 32P with the first PCR products as template. The resultant labelled PCR products were visualized by polyacrylamide gel electrophoresis and autoradiography. SSCP analysis was performed on the 5' flanking region and all 13 exons of the gp91-phox gene.

**DNA sequencing.** Genomic DNA was isolated from peripheral blood leukocytes and PCR-amplified using standard methods.37 Di-deoxy sequencing of the amplified segments was performed by standard methods.37

**RESULTS**

We first reconfirmed the diagnosis of CGD in both patients: case 1 from the original study27 and case 2, the brother of the originally reported case 2 (Fig 1). Both patients have the cytochrome b-negative form of CGD, based on intact neutrophil studies (Table 1) that exhibited no positively stained cells on PMA- or endotoxin-stimulated NBT slide tests, virtually no superoxide production in response to PMA or the chemotactic peptide FMLP, and undetectable levels of cytochrome b556 by visible spectroscopy or in immunoblots for the gp91-phox and p22-phox subunits. Family studies of relatives of case 2 are essentially diagnostic of an autosomal recessive pattern of inheritance: CGD in a cytogenetically normal female and her brother, normal respiratory burst function25 in five siblings and in female members of the two
Northern blot analysis (Fig 3, top panel).

The respiratory burst oxidase can be activated in a cell-free system in which membranes and cytosol from unstimulated neutrophils are incubated in the presence of Mg²⁺ and a lipid-perturbing agent such as arachidonic acid or SDS. The cytosol fractions had normal activity (Fig 2A; data from case 2 not shown). In contrast, the membrane fractions from both cases were severely defective when mixed with normal cytosol (Fig 2B). These findings indicate a profound lesion in the membrane-bound component of the oxidase and are typical of patients with either X-linked or autosomal recessive, cytochrome b-negative CGD.

We next measured levels of cellular GSH-Px enzymatic activity in each patient’s neutrophils. Intact neutrophil homogenates (as well as purified neutrophil cytosol from case 1) were analyzed using two different GSH-Px substrates: H₂O₂ and t-butyl hydroperoxide. Under both assay conditions, each patient demonstrated normal levels of GSH-Px activity (Table 2). As expected from the normal levels of cytochrome b component of the oxidase and not GSH-Px, we reasoned that the decrease in GSH-Px mRNA appears to be a post-transcriptional event. In both cases studied; thus, as expected for a termination codon, the mutation did not appear to be a polymorphism of the gp91-phox gene.

To determine the effect of this mutation on gp91-phox gene transcription, nuclear run-on assays were performed on nuclei from Epstein-Barr virus-transformed lymphoblastoid B cell lines derived from case 1 and a normal control donor. Nuclei from case 1 B cells demonstrated normal levels of transcription for both oxidase components, as well as for GSH-Px (Fig 5). Ratios of transcription rates for case 1 relative to normal were 1.09 for gp91-phox, 1.19 for p22-phox, and 1.12 for GSH-Px (all normalized to the constitutively-expressed Kæ1 tubulin control in the lowest lane). Thus, the decrease in gp91-phox mRNA appears to be a post-transcriptional event.

Possible explanations for the presence of an X-linked disease in a phenotypic female include a sex chromosome abnormality (ie, Turner Syndrome or XY female), an extremely skewed Lyon distribution of X-inactivation, or mutations in both X chromosome alleles. Multiple peripheral blood

<table>
<thead>
<tr>
<th>Patient</th>
<th>% Positive by NBT</th>
<th>PMA (pmol/min/10^7 cells)</th>
<th>FMLP (pmol/min/10^7 cells)</th>
<th>Spectroscopy (pmol/10^7 cells)</th>
<th>Immunoblot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>98 - 100 (n = 35)</td>
<td>149.2 ± 33.8 (n = 14)</td>
<td>44.2 ± 6.5 (n = 7)</td>
<td>73.0 ± 30.9* (n = 42)</td>
<td>Present</td>
</tr>
<tr>
<td>Case 1</td>
<td>0 (n = 3)</td>
<td>0.8 ± 0.2 (n = 4)</td>
<td>0 (n = 2)</td>
<td>0 (n = 5)</td>
<td>Undetectable</td>
</tr>
<tr>
<td>Mother</td>
<td>100 (n = 3)</td>
<td></td>
<td></td>
<td></td>
<td>Undetectable</td>
</tr>
<tr>
<td>Father</td>
<td>100 (n = 3)</td>
<td></td>
<td></td>
<td></td>
<td>Undetectable</td>
</tr>
<tr>
<td>Case 2</td>
<td>0 (n = 3)</td>
<td>0.1 ± 0.1 (n = 5)</td>
<td>0 (n = 2)</td>
<td>0 (n = 2)</td>
<td>Undetectable</td>
</tr>
<tr>
<td>Mother</td>
<td>100 (n = 3)</td>
<td></td>
<td></td>
<td></td>
<td>Undetectable</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SD or a range (for NBT) for the indicated number (n) of determinations.

* Range, 36.7 to 197 pmol/10^7 cells.
Table 2. Glutathione Peroxidase Activity in CGD Neutrophils

<table>
<thead>
<tr>
<th>Subjects</th>
<th>H₂O₂</th>
<th>tBHP</th>
<th>H₂O₂</th>
<th>tBHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (4)</td>
<td>19.4 ± 5.0</td>
<td>28.7 ± 6.4</td>
<td>10.4 ± 2.2</td>
<td>20.6 ± 5.4</td>
</tr>
<tr>
<td>Case 1</td>
<td>15.0</td>
<td>37.5</td>
<td>8.6</td>
<td>14.9</td>
</tr>
<tr>
<td>Proband</td>
<td>28.2</td>
<td>30.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mother</td>
<td>29.8</td>
<td>31.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Father</td>
<td>24.1</td>
<td>28.3</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Results are expressed as mU/mg protein. Assays performed as described in Materials and Methods, with H₂O₂ or t-butyl hydroperoxide substrate as indicated.

Abbreviations: tBHP, t-butyl hydroperoxide; ND, not determined.

DISCUSSION

We have restudied two kindreds that were originally reported to have autosomal recessive CGD associated with GSH-Px deficiency. Case 1 from the original studies and the brother of the originally reported case 2 (Fig 1) both have CGD by current diagnostic criteria. However, their leukocyte cytogenetic analyses showed the normal 46,XX female karyotype. As reported in a preliminary communication, examination of the nucleotide 1311 polymorphism in the G6PD gene demonstrated both the 1311-C and 1311-T alleles in the patient’s genomic DNA, but expression of only the 1311-T allele in mRNA from her peripheral blood neutrophils. Both parents have normal NBT tests (Table 1) and normal gp91-phox gene sequence at the arginine-226 codon, indicating that the patient’s CGD allele arose as a new mutation. Her mother is homozygous for G6PD 1311-C and her father hemizygous for G6PD 1311-T, so the CGD mutation appears to have occurred on the paternal X chromosome. The mutational event must have occurred within the father’s germ line, since he does not demonstrate the defect in his hematopoietic cells.

RNA from leukocytes of case 2 contains normal levels of transcripts for both gp91-phox and p22-phox (Fig 3, lower panels). The absence of cytochrome b₅₅₆ in case 2 is caused, as previously reported, by two distinct mutations in the two alleles for the p22-phox subunit. One allele contains a single nucleotide deletion (CCC → CC) at nucleotide 272 (in the codon for proline-82), resulting in a frameshift of the open reading frame with eventual termination at 189 amino acids. The other allele carries a G → A mutation at nucleotide 297, which predicts the nonconservative missense substitution of a glutamine residue for arginine-90.

Fig 2. NADPH oxidase activity in a cell-free system utilizing membrane and cytosolic fractions from CGD and normal neutrophils. A: Superoxide generation was measured in combinations of normal neutrophil membrane fraction (6.25 × 10⁵ cell equivalents per reaction) with the indicated concentrations of normal (○) or patient (△) cytosol fraction. B: Superoxide generation was measured in combinations of normal neutrophil cytosol fraction (18 x 10⁵ cell equivalents per reaction) with the indicated concentrations of normal (○) or patient (△, case 1; □, case 2) membrane fraction.

Fig 3. Northern blot analysis of GSH-Px, gp91-phox, and p22-phox transcripts in total cellular RNA extracted from peripheral blood mononuclear cells of a normal donor (NI), case 1, and case 2. Labels to the left of the autoradiograph mark the positions of the indicated transcripts, relative to the indicated position (right margin) of 18S ribosomal RNA.
granulocytes exhibit normal cellular GSH-Px enzyme activity and gene expression. Examination of phagocyte NADPH oxidase components known to be defective in other cases of CGD reveals the absence of detectable cytochrome \( b_{558} \) and of membrane activity in the cell-free oxidase assay. Molecular analysis of the genes encoding cytochrome \( b_{558} \) subunits shows disabling mutations in the gp91-phox gene in case 1 and in the p22-phox gene in case 2. Thus, the CGD phenotype in both kindreds now appears to be caused not by GSH-Px deficiency, but rather by mutations of the same subunits of phagocyte NADPH oxidase that are involved in all other cases of cytochrome \( b \)-negative CGD studied so far at the molecular level.\(^5\,\,57\)

The reason for the discrepancy between these results and those previously reported\(^22\,\,25\) is not known. It is possible that the patients' medications or clinical conditions at the time of the previous study somehow affected measurements of GSH-Px activity. As GSH-Px is a selenoenzyme dependent upon availability of selenium for gene expression and enzyme activity,\(^48\,\,49\) deficiency of the trace element due to malnutrition, malabsorption, or inadequate intake could masquerade as primary GSH-Px deficiency.\(^50\,\,51\) Selenium deficiency has been reported in patients with AIDS,\(^52\) possibly due in part to such consequences of impaired host defense. In addition, case 1 lives in South Dakota, a region of endemic selenium scarcity.\(^51\)

The acquisition of the X-linked form of CGD in case 1, a cytogenetically normal female, occurred through the unfortunate coincidence of a new mutation in the gp91-phox gene, along with uniform inactivation of the normal X chromosome in her granulocytes. The development of a disease phenotype due to a lopsided distribution of X inactivation in carriers of an X-linked disorder has been reported for CGD\(^53\,\,57\) and other X-linked conditions such as hemophilia A and G6PD deficiency.\(^29\) However, these CGD carriers still have populations of normal cells, ranging from 5\% to 20\% of all phagocytes, unlike the classic CGD phenotype in the present case. Her distribution of X inactivation probably represents an extreme example of a skewed Lyon distribution. Random inactivation of one of each cell’s pair of X chromosomes occurs early in embryonic development, and the progeny of those cells continue to express only the set of genes remaining active after that event. Buescher et al\(^59\)

Fig 4. Sequence analysis of a mutation in exon 7 of the gp91-phox gene in case 1. The far right set of G-A-T-C lanes demonstrates heterozygosity at nucleotide 688 (−) for a C to T substitution and the normal "C" base at that position. Other sets of lanes demonstrate the normal sequence in the father, mother, and a representative normal control, as indicated.
determined, on the basis of the distribution of NBT-positive and -negative cells in CGD carriers, that the inactivation event takes place at time in embryogenesis when there are only about eight hematopoietic founder cells. Thus, the predicted probability of inactivation of the same X chromosome in all myeloid cells would be 1 in 256 (ie, 1:256), a level that is low but would be expected to result in occasional patients such as ours within the population of CGD carriers.

Alternatively, the coincidental acquisition of a mutation inhibitory to myeloid development on the X chromosome bearing the normal gp91-phox gene would result in exclusive expression of those cells expressing the normal allele. This conjectural mutation would be the myeloid equivalent of the X-linked immunodeficiencies in which obligate carriers express only the normal gene in their lymphocytes due to preferential survival of cells expressing the normal gene on the active X chromosome in lineages dependent on the gene product for development or function.60

The reason for the markedly decreased level of gp91-phox transcripts from a gene containing a nonsense mutation is unclear. In humans, nonsense mutations result in decreased levels of mRNA transcripts in some forms of β-thalassemia and in deficiencies of triose phosphate isomerase and dihydrololate reductase.61-64 These abnormal termination codons may interfere with nuclear mRNA processing65 or may diminish mRNA stability,62,63 probably through decreased ribosomal protection from normal degradation pathways. In Saccharomyces cerevisiae, premature termination of translation leads to mRNA degradation through the interaction of downstream sequence elements with at least one specific RNA-binding protein.66

In summary, the reevaluation of both kindreds previously reported to have CGD associated with GSH-Px deficiency23 has demonstrated instead mutations of the same phagocyte oxidase subunits as found in other cases of cytochrome b-negative CGD,24 along with normal GSH-Px enzyme activity and gene expression. Although acquired GSH-Px deficiency may affect phagocyte function, at least in animal models25,26,27,67 and in vitro,68 congenital GSH-Px deficiency should no longer be considered a possible cause of the CGD phenotype.

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We thank Dr Ernest Beutler (The Scripps Research Institute, La Jolla, CA) for advice and support; Dr O. Jones and the University of California, San Diego cytogenetics laboratory for performing the karyotypes; Drs Robert L. Harris (Bloomfield, CT), Lee Hoffman (Bloomfield, CT), and Harry L. Malech (National Institute of Allergy and Infectious Diseases, Bethesda, MD) for clinical information; Mary G. Breitenstein, Stella B. Cretella, Julie Rae, and Constance Whitney for excellent technical assistance; and most of all, the CGD patients and their families for their generous participation in these studies.

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PE Newburger, SE Malawista, MC Dinauer, T Gelbart, RC Woodman, S Chada, Q Shen, G van Blaricom, PG Quie and JT Curnutte

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