Am. J. Hum. Genet. 62:1320–1331, 1998

X-Linked Chronic Granulomatous Disease: Mutations in the *CYBB* Gene Encoding the gp91-*phox* Component of Respiratory-Burst Oxidase

Julie Rae,[1] Peter E. Newburger,[2] Mary C. Dinauer,[3] Deborah Noack,[4] Penelope J. Hopkins,[5] Ryoko Kuruto,[6] and John T. Curnutte[1,4]

[1]Department of Immunology, Genentech, Inc., South San Francisco; [2]Departments of Pediatrics and Molecular Genetics/Microbiology, University of Massachusetts Medical School, Worcester; [3]Herman B. Wells Center for Pediatric Research and Departments of Pediatrics and Medical and Molecular Genetics, Indiana University Medical Center, Indianapolis; [4]Department of Molecular and Experimental Medicine, The Scripps Research Institute, and [5]Sequana Therapeutics, La Jolla; and [6]Laboratory of Microbiology and Host Defenses, University of Shizuoka School of Food and Nutritional Sciences, Shizuoka, Japan

Summary

Chronic granulomatous disease (CGD) is a hereditary disorder of host defense due to absent or decreased activity of phagocyte NADPH oxidase. The X-linked form of the disease derives from defects in the *CYBB* gene, which encodes the 91-kD glycoprotein component (termed "gp91-*phox*") of the oxidase. We have identified the mutations in the *CYBB* gene responsible for X-linked CGD in 131 consecutive independent kindreds. Screening by SSCP analysis identified mutations in 124 of the kindreds, and sequencing of all exons and intron boundary regions revealed the other seven mutations. We detected 103 different specific mutations; no single mutation appeared in more than seven independent kindreds. The types of mutations included large and small deletions (11%), frameshifts (24%), nonsense mutations (23%), missense mutations (23%), splice-region mutations (17%), and regulatory-region mutations (2%). The distribution of mutations within the *CYBB* gene exhibited great heterogeneity, with no apparent mutational hot spots. Evaluation of 87 available mothers revealed X-linked carrier status in all but 10. The heterogeneity of mutations and the lack of any predominant genotype indicate that the disease represents many different mutational events, without a founder effect, as is expected for a disorder with a previously lethal phenotype.

Received January 7, 1998; accepted for publication April 14, 1998; electronically published May 20, 1998.

Address for correspondence and reprints: Dr. Peter E. Newburger, Department of Pediatrics, University of Massachusetts Medical School, 373 Plantation Street, Worcester, MA 01605. E-mail: peter.newburger@ummed.edu

0002-9297/98/6206-0009$02.00

Introduction

Chronic granulomatous disease (CGD) is a hereditary disorder of host defense due to defective activity of a phagocyte NADPH oxidase that generates superoxide and related toxic oxygen metabolites necessary for microbial killing (Curnutte et al. 1994). Patients usually present early in life, with multiple, sometimes fatal, pyogenic infections (Berendes et al. 1957; Forrest et al. 1988; Mouy et al. 1989; Finn et al. 1990); in less severe cases the diagnosis may become apparent only in adolescence or adulthood (Schapiro et al. 1991).

The NADPH-oxidase enzyme system responsible for superoxide generation forms a small transmembrane electron-transport system that results in the oxidation of NADPH on the cytoplasmic surface and the generation of superoxide on the outer surface of the membrane, which in turn becomes the inner surface of the phagosome when invagination occurs during phagocytosis. Analysis of the defects responsible for CGD has helped to define many of the biochemical and molecular features of this complex system. Individual protein constituents and their genes have been identified and cloned (Royer-Pokora et al. 1986; Nunoi et al. 1988; Volpp et al. 1988; Dinauer et al. 1990; Leto et al. 1990); analysis of protein and membrane interactions are now beginning to provide a picture of oxidase organization and assembly during phagocyte activation (DeLeo and Quinn 1996; Leusen et al. 1996).

The terminal electron donor to oxygen is a unique, low-midpoint–potential flavocytochrome, termed "cytochrome b_{558}" because of its spectral peak at 558 nm. The heterodimeric molecule consists of a 91-kD glycoprotein, termed "gp91-*phox*" (*ph*agocyte *ox*idase), and a 22-kD nonglycosylated polypeptide, termed "p22-*phox*" (Royer-Pokora et al. 1986; Parkos et al. 1988; Curnutte 1993). The *CYBB* gene (GenBank accession number X04011), which encodes gp91-*phox,* was one of the first to be identified by positional cloning (Royer-Pokora et al. 1986), following chromosomal localization

Table 1

Deletion Mutations in the *CYBB* Gene

Kindred Number	Mutation: Position	Mutation: Size/Affected Amino Acid(s)	CGD Phenotype[a]	X-CGDbase Number
1	Multigene	≫30 kb	$X91^0$ + DMD + McLeod	A0119
2	Multigene	≫30 kb	$X91^0$ + McLeod	A0034
3	Multigene	≫30 kb	$X91^0$ + McLeod	
4	Multigene	≫30 kb	$X91^0$ + McLeod	A0169
5	Multigene	≫30 kb	$X91^0$ + McLeod	A0201
6	Multigene	≫30 kb	$X91^0$ + McLeod	A0167
7	Multigene	≫30 kb	$X91^0$ + ?McLeod	
8	Exons 11–13	⩾4.3 kb/439 Ile–570 Phe deleted	$X91^0$	A0172
9	Exon 4	2 kb	$X91^0$	
10	Exon 1	ND	O_2^- production = 0; cytochrome *b*, ND	
11	Exon 13	>123 nt/530 Thr–570 Phe deleted[b]	$X91^0$	A0203
12	Exon 13 (nt 1694–1724)	31 nt	$X91^0$	A0155
13	Exon/intron 5 (nt 494 to intron)	6 nt	$X91^0$	A0181
14	Exon/intron 9 (nt 1162 to intron)	4 nt (AAgt)/splicing defect	$X91^0$	A0156
15	Exon 9 (nt 954–6)	3 nt/315 Lys deleted	$X91^-$	A0163

NOTE.—ND = not determined.

[a] "X" indicates the X-linked mode of inheritance; "91" indicates that the gp91-*phox* component of the phagocyte oxidase is affected; and the superscript symbols indicate an undetectable ("0") or diminished ("−") level of that component protein (Curnutte et al. 1994). "DMD" denotes Duchenne muscular dystrophy; "McLeod" denotes the McLeod phenotype (Redman and Marsh 1993); and "?McLeod" denotes possible McLeod phenotype, not rigorously evaluated.

[b] Previously published by Royer-Pokora et al. (1986).

to Xp21.1 (Baehner et al. 1986); it encompasses 13 exons spanning ~30 kb of genomic DNA (Skalnik et al. 1991). CGD kindreds with defects in the gp91-*phox* component thus show X-linked inheritance, and in most cases cytochrome b_{558} is reduced or absent from their phagocytes (Segal et al. 1983). Recent studies have probed indirectly the structure of the glycoprotein molecule and its molecular interactions (DeLeo and Quinn 1996; Leusen et al. 1996), but the locations of its functional domains have been inferred mostly by sequence homology rather than by direct demonstration.

Diverse molecular defects producing X-linked CGD have been identified within the coding region and introns of the *CYBB* gene (De Saint–Basile et al. 1988; Frey et al. 1988; Dinauer et al. 1989; Bolscher et al. 1991; Schapiro et al. 1991; de Boer et al. 1992; Rabbani et al. 1993; Ariga et al. 1994*a,* 1994*b,* 1995; Leusen et al. 1994; Newburger et al. 1994*a,* 1994*b;* Azuma et al. 1995; Bu-Ghanim et al. 1995; Cross et al. 1995); most have been reported in single cases or small series. Roos et al. (1996*b*) have reviewed a large collection of mutations associated with both X-linked and autosomal recessive forms of CGD. Forms of mutations causing X-linked CGD have included large multigene deletions, smaller deletions and insertions, missense and nonsense substitutions, and splicing defects. This study reports the mutations identified in 131 consecutive X-linked CGD kindreds studied at the participating institutions.

Subjects, Material, and Methods

Subjects

Blood samples were obtained from CGD patients and family members who were referred to the investigators or who were sent to the CGD reference laboratories at the Scripps Research Institute or Stanford University Medical School. Procedures and consent forms were approved by the Committees on the Protection of Human Subjects in Research at the Scripps Research Institute, Stanford University Medical School, Indiana University Medical School, and University of Massachusetts Medical Center. Most (103/131) of the mutations have been entered into the X-CGDbase database (Roos et al. 1996*a*); accession numbers are indicated in tables 1–6.

The X-linked nature of CGD was established in these

kindreds by an algorithmic series of biochemical and molecular tests of both patients and available maternal relatives, as described below. This approach, which included genomic DNA sequencing, was designed to minimize (but could not eliminate completely) false-negative results for carrier detection. In male patients with unavailable or noncarrier mothers, X-linked CGD was demonstrated by biochemical and molecular identification of a defect in the gp91-*phox* glycoprotein and the *CYBB* gene.

In all 38 non–X-linked CGD kindreds studied during the same time period, defects in the genes encoding p47-*phox,* p67-*phox,* or p22-*phox* were identified positively by western blotting for cytosolic oxidase components, complementation in cell-free oxidase assays (Curnutte et al. 1987), and DNA sequencing of the affected genes. Thus, it is unlikely that any X-linked defect was not detected because of misclassification as an unidentified form of autosomal recessive CGD.

NADPH Oxidase

Cytochrome b_{558} content was measured by dithionite reduction and difference spectroscopy, as described elsewhere (Woodman et al. 1995). NADPH-oxidase activity was determined by spectrophotometric assay of superoxide dismutase–inhibitable cytochrome *c* reduction or by flow cytometry using dichlorofluorescein or dihydrorhodamine indicators, as described elsewhere (Woodman et al. 1995).

SSCP Analysis

Genomic DNA was extracted from peripheral blood (Maniatis et al. 1990) and was analyzed by SSCP, as described elsewhere (Orita et al. 1989*a,* 1989*b*), by use of two-stage PCR utilizing 18–27-bp oligonucleotide primers (sequences available by request) with 0.1 mg genomic DNA, followed by nested PCR with primers end labeled with ^{32}P or ^{33}P. The resultant labeled PCR products were visualized by PAGE and autoradiography. SSCP analysis was performed on the 5′ flanking region and on all 13 exons of the gp91-*phox* gene. Only 7 of the 131 kindreds had mutations undetectable by SSCP; these were identified subsequently by sequencing of the complete set of PCR amplicons.

DNA Sequencing

Genomic DNA was isolated from peripheral blood leukocytes by use of standard methods (Maniatis et al. 1990) and was amplified by PCR using synthetic oligonucleotide primers designed to amplify the 5′ flanking region or each of the 13 exons of the *CYBB* gene. Alternatively, coding-region segments were amplified from cDNA prepared by reverse transcription (Pharmacia First-Strand cDNA Synthesis Kit) of mononuclear-cell RNA, prepared by differential precipitation from guanidine HCl (Ginsburg et al. 1985). Amplified segments were sequenced directly or were subcloned into pUC19 or related plasmids. Bidirectional dideoxy sequencing was performed by standard methods (Maniatis et al. 1990). For sequence alterations that were not self-evident as causes of a CGD phenotype, such as missense and splicing mutations, we compared SSCP patterns or DNA sequences from 200 normal X chromosomes, to diminish the likelihood that the change represented a polymorphism.

Results

Mutations in the gene encoding gp91-*phox* were detected in all 131 kindreds, with abnormal migration during SSCP analysis evident in 124 kindreds. Tables 1–6 present the findings, organized by type of mutation. For most cases, the source of DNA for study was the presenting CGD patient; in several families, the propositus was no longer living, and the mutation was detected by analysis of genomic DNA from the carrier mother or from an affected brother. All available members of each family were studied (412 individuals altogether), and the mutations were found to segregate with CGD disease or carrier status, in all cases. For extended kindreds with several families (or families with multiple members affected), only a single representative is listed. Thus, the "shared genotype" notation indicates no known relationship between the affected kindreds.

Deletions of $\geqslant$3 nt were found in 15 (11%) of the 131 kindreds and formed a very heterogeneous group (table 1). Several of these deletions removed the entire gene; shared genotypes cannot be ruled out, because their termini have not been mapped. The phenotype in kindred 1 included CGD, McLeod syndrome, and Duchenne muscular dystrophy, as described elsewhere in other kindreds (De Saint–Basile et al. 1988; Frey et al. 1988), and indicated a very large multigenic deletion sufficient in size to be visualized by chromosome-banding studies. At least five other patients also showed the McLeod phenotype (Densen et al. 1981). This linkage is consistent with the known X-chromosome map positions of the X-linked CGD and McLeod loci (MIM 306400 and 314850, respectively) (Nagaraja et al. 1997). At the other end of the spectrum of these mutations, very small in-frame deletions caused interstitial removal of from 1 (number 15) to 132 (number 8) amino acids. In the former patient, the deletion of a single amino acid, lysine at position 315, resulted in the retention of partial oxidase function, with 19% normal superoxide-generating activity and 13% normal levels of cytochrome *b,* which is an example of the X91$^-$ phenotype in the biochemical classification of CGD (Curnutte 1988). As was expected, all others tested had no

Table 2

Frameshift Mutations in the *CYBB* Gene

Kindred Number	Mutation		CGD Phenotype[a]			Shared Genotype	X-CGDbase Number
	Position	Nucleotide Change	Termination Site	Superoxide Production (% Normal)	Cytochrome *b* Content (% Normal)		
1	Exon 3	169 C insertion	Exon 5	0	0		A0160
2	Exon 3	207 G insertion	Exon 4	0	0		A0196
3	Exon 4	307 A deletion	Exon 4	0	0		A0097
4	Exon 5	366 A deletion	Exon 5	0	ND		A0103
5	Exon 5	454–5 CA→T	Exon 5	0	ND		A0111
6	Exon 5	455 A insertion	Exon 5	0	0		A0139
7	Exon 6	607 A deletion	Exon 6	ND	0		
8	Exon 7	713–4 AG deletion	Exon 7	0	ND		A0120
9	Exon 7	728–32 deletion	Exon 7	0	0		A0117
10	Exon 7	749 A insertion	Exon 8	NL	NL	A	
11	Exon 7	749 A insertion	Exon 8	0	0	A	A0121
12	Exon 7	749 A insertion	Exon 8	0	0	A	A0126
13	Exon 7	749 A insertion	Exon 8	0	ND	A	A0146
14	Exon 7	749 A insertion	Exon 8	0	0	A	A0162
15	Exon 7	749 A insertion	Exon 8	NL	NL	A	A0170
16	Exon 7	749 A insertion	Exon 8	0	0	A	A0189
17	Exon 7	764 G deletion	Exon 8	NL	NL		A0195
18	Exon 7	768 A insertion	Exon 8	0	0		A0128
19	Exon 9	935 CCTTTCA insertion, 947–9 deletion	Exon 9	0	ND		
20	Exon 9	975 G deletion	Exon 9	0	0		A0118
21	Exon 9	1050 T deletion	Exon 10	0	0	B	A0129
22	Exon 9	1050 T deletion	Exon 10	0	ND	B	A0161
23	Exon 10	1246 G insertion	Exon 10	ND	ND		A0144
24	Exon 10	1283 G deletion	Exon 10	NL	NL		
25	Exon 10	1325–6 AG→T	Exon 12	0	0		A0091
26	Exon 11	1361 C deletion	Exon 11	NL	NL		A0192
27	Exon 11	1426 G deletion	Exon 12	0	0		A0092
28	Exon 11	1467 G deletion	Exon 12	NL	NL		A0165
29	Exon 11	1467 T insertion	Exon 12	ND	ND		A0184
30	Exon 12	1475 C deletion	Exon 12	0	ND		A0114
31	Exon 12	1539–40 TT deletion	Exon 12	0	ND		A0143
32	Exon 13	1634 insertion of 4 nt	Exon 13	0	0		A0183

[a] ND = not determined; NL = patient not living.

detectable cytochrome *b* protein or oxidase activity, termed the "$X91^0$" phenotype. In the $X91^+$ phenotype, found in patients with other mutations, described below, granulocytes have normal levels of cytochrome *b* but do not have NADPH-oxidase enzyme activity.

Smaller, usually 1-nt deletions and insertions disrupted the normal reading frame for translation in 32 (24%) of the 131 kindreds studied. As noted in other series (Roos et al. 1996*b*), such mutations are a quite common cause of CGD. The frameshift mutations (table 2) created nonsense codons within two exons downstream of the mutation and, hence, $X91^0$ phenotypes due to nonfunctional, truncated proteins that are undetectable by spectroscopic or immunological methods of analysis. Seven kindreds shared the same frameshift mutation due to insertion of adenosine at nt 749, which is one of six consecutive adenosine nucleotides that may be particularly subject to errors in recombination. The precise position of a mutational event within a tract of identical nucleotides cannot be determined; thus, all of these mutations were assigned to the last nucleotide of the series, where the functional frameshift occurs.

Direct mutation from a coding to a nonsense codon (table 3) also caused an $X91^0$ phenotype in all patients tested. These nonsense mutations also were common: they were found in 30 (23%) of the 131 kindreds. Interestingly, many kindreds shared their mutation with at least one other family. Additional genetic analysis would be necessary to determine whether these kindreds share a more extensive genetic background, owing to common founders, or whether they represent coincidental parallel mutations. Of the seven patients (numbers 1, 6, 7, 13, 19, 23, and 28; table 3) analyzed by northern blot for *CYBB* transcripts, four patients (numbers 13, 19, 23, and 28) showed reduced or undetectable levels of mRNA, consistent with the destabilizing effects of non-

Table 3

Nonsense Mutations in the *CYBB* Gene

Kindred Number	Mutation			Shared Genotype	X-CGDbase Number
	Position	Nucleotide Change	Termination Site[a]		
1	Exon 1	24 G→A	4 Trp		A0108
2	Exon 3	229 C→T	73 Arg	A	A0188
3	Exon 3	229 C→T	73 Arg	A	
4	Exon 4	283 C→T	91 Arg	B	A0149
5	Exon 4	283 C→T	91 Arg	B	A0178
6	Exon 5	400 C→T	130 Arg		A0113
7	Exon 5	468 T→A	152 Tyr		A0124
8	Exon 5	481 C→T	157 Arg	C	A0095
9	Exon 5	481 C→T	157 Arg	C	A0098
10	Exon 5	481 C→T	157 Arg	C	A0152
11	Exon 5	481 C→T	157 Arg	C	A0177
12	Exon 6	567 C→A	185 Cys		A0182
13	Exon 7	688 C→T[b]	226 Arg	D	A0132
14	Exon 7	688 C→T	226 Arg	D	A0150
15	Exon 7	688 C→T	226 Arg	D	
16	Exon 7	748 C→T	246 Gln	E	A0122
17	Exon 7	748 C→T	246 Gln	E	A0186
18	Exon 8	827 G→A	272 Trp		A0099
19	Exon 8	880 C→T	290 Arg	F	A0145
20	Exon 8	880 C→T	290 Arg	F	A0159
21	Exon 8	880 C→T	290 Arg	F	A0194
22	Exon 8	880 C→T	290 Arg	F	A0198
23	Exon 9	1018 G→T	336 Glu		A0087
24	Exon 9	1151 G→A	380 Trp		A0086
25	Exon 9	1151 G→A	380 Trp		A0174
26	Exon 11	1332 C→A	440 Tyr		A0151
27	Exon 11	1341 G→A	443 Trp	G	
28	Exon 11	1341 G→A	443 Trp	G	A0168
29	Exon 11	1370 G→A	453 Trp		
30	Exon 12	1531 C→T	507 Gln		A0116

[a] Former amino acid.

[b] Previously published by Newburger et al. (1994*a*).

sense mutations (Maquat 1995). The locations of these mutations were all 3′ to the positions of the mutations that did not affect mRNA levels, unlike the predominantly upstream positions of destabilizing nonsense codons in transcripts encoding β-globin, adenine phosphoribosyltransferase, and triose phosphate isomerase (Maquat 1995; Kessler and Chasin 1996).

Missense mutations (table 4) resulted from 1- or 2-nt changes that altered the DNA sequence to encode a different amino acid and were found in 30 (23%) of the 131 kindreds. As expected, several showed $X91^-$ phenotypes, indicating partial preservation of both cytochrome *b* levels and oxidase function. Two unrelated kindreds (numbers 23 and 24; table 4) had the same mutation, which led to substitution of histidine for proline at position 415, resulting in $X91^+$ phenotypes with complete preservation of cytochrome *b* but absence of oxidase-enzyme activity. Thus, the 415 proline residue appears to be essential for function but not for protein stability. The substitution of arginine for cysteine at residue 537 (number 30; table 4) produced a similar phenotype, indicating that this cysteine is functionally, but not structurally, essential. Four other mutations, found in five unrelated kindreds (numbers 6, 7, 14, 16, and 18; table 4), produced an amino acid substitution that greatly reduced both cytochrome *b* content and oxidase activity. Such $X91^-$ phenotypes indicate positions in the gp91-*phox* molecule that may be slightly permissive for structural alterations, but these changes greatly destabilize the protein molecule. One patient (number 18; table 4) had an additional base change (1102G→C) that also has been found in an unaffected individual and therefore appears to be a known rare polymorphism (Kuribayashi et al. 1996).

Mutations in or near splice-junction sites (table 5) produced CGD by interfering with mRNA processing and were found in 22 (17%) of the 131 families. Most occurred at the splice junction and, in those tested, resulted in an $X91^0$ phenotype with no NADPH-oxidase expression, as in most previously reported splicing mu-

Table 4

Missense Mutations in the *CYBB* Gene

Kindred Number	Position	Mutation		CGD Phenotype[a]		Shared Genotype	X-CGDbase Number
		Nucleotide Change	Amino Acid Change	Superoxide Production (% Normal)	Cytochrome *b* Content (% Normal)		
1	Exon 2	70 G→C	20 Gly→Arg	0	0		A0136
2	Exon 3	174 G→C	54 Arg→Ser	0	100		A0133
3	Exon 3	187 T→C	59 Cys→Arg	0	0		A0175
4	Exon 5	368 A→G	119 His→Arg	0	ND	A	
5	Exon 5	368 A→G	119 His→Arg	0	ND	A	A0153
6	Exon 5	478 G→A	156 Ala→Thr	3	ND	B	A0137
7	Exon 5	478 G→A	156 Ala→Thr	2	3	B	A0187
8	Exon 6	639 T→A	209 His→Gln	0	0		A0125
9	Exon 6	676 C→A	222 His→Asn	ND	ND		
10	Exon 6	676 C→T	222 His→Tyr	ND	ND		
11	Exon 6	677 A→G	222 His→Arg	0	ND		
12	Exon 6	679–80 GG→TT	223 Gly→Leu	ND	ND		A0109
13	Exon 7	742 T→C	244 Cys→Arg	0	ND		A0171
14	Exon 9	937 G→A	309 Glu→Lys	4	17		A0101
15	Exon 9	977 G→A	322 Gly→Glu	NL	NL		
16	Exon 9	985 A→T	325 Ile→Phe	4	5		
17	Exon 9	1009 T→C	333 Ser→Pro	NL	NL		A0200
18	Exon 9	1028 C→A	339 Pro→His	0	20		A0096
19	Exon 9	1079 G→C	356 Arg→Pro	ND	ND		
20	Exon 10	1226 T→G	405 Met→Arg	0	ND		A0142
21	Exon 10	1234 G→A	408 Gly→Arg	0	ND		A0157
22	Exon 10	1235 G→A	408 Gly→Glu	0	ND		A0190
23	Exon 10	1256 C→A[b]	415 Pro→His	0	100	C	A0107
24	Exon 10	1256 C→A	415 Pro→His	0	100	C	
25	Exon 10	1256 C→T	415 Pro→Leu	ND	ND		A0112
26	Exon 10	1276 T→C	422 Ser→Pro	NL	NL		A0202
27	Exon 11	1369 T→C	453 Trp→Arg	0	ND		
28	Exon 12	1560 G→T	516 Trp→Cys	0	ND		A0094
29	Exon 13	1613 T→A	534 Val→Asp	NL	NL		A0147
30	Exon 13	1621 T→C	537 Cys→Arg	0	100		A0199

[a] ND = not determined; NL = patient not living.
[b] Previously published by Dinauer et al. (1989).

tations in X-linked CGD (de Boer et al. 1992). However, in four kindreds (numbers 1, 8, 9, 10, 11, and 14; table 5), retention of a small proportion of normal splicing allowed limited preservation of oxidase activity (authors' unpublished data). One of these kindreds (number 1; table 5) has proved uniquely responsive to treatment with interferon-γ, with virtually complete restoration of superoxide-generating activity in vitro and in vivo, at least in part owing to increased levels of normal transcripts (Ezekowitz et al. 1987, 1988; Condino-Neto et al. 1997). The exon 5 base change in kindred 14 also predicts the missense substitution 161Lys→Arg; the other exonic splicing mutations (numbers 8–11, shared genotype A; table 5) do not change the translation of the affected alanine codon. The mutation at −8 nt from the 3′ end of intron 3 creates an alternative AG dinucleotide splice-acceptor site; cDNA sequencing revealed incorporation of the downstream nucleotides into exon 4, leading to a frameshift with termination in that exon.

As previously reported, two kindreds with neighboring regulatory-region mutations (table 6) have an unusual form of X91$^-$ CGD with absent oxidase activity in 90%–95% of their neutrophils but normal levels in the remainder (Newburger et al. 1994*b;* Woodman et al. 1995). The mutations disrupt the binding site for a newly identified transcription factor, HAF-1 (Eklund and Skalnik 1995), and indicate its probable importance for expression of the *CYBB* gene.

All available maternal relatives were screened for X-linked CGD carrier status, either by genomic DNA analysis (SSCP or sequencing of the exon known to be affected) or by examination of peripheral blood granulocytes for a subpopulation of cells with the CGD biochemical phenotype due to random inactivation of the normal X chromosome. Of 87 mothers tested, only 10 (11%) did not reveal X-linked carrier status. Ten of 25 maternal grandmothers and one of three maternal great-grandmothers were not carriers. There were no

Table 5

Splicing Mutations in the *CYBB* Gene

Kindred Number	Mutation			CGD Phenotype[a,b]	Shared Genotype	X-CGDbase Number
	Position	Nucleotide Change	Splicing Product[a]			
1	Intron 1, 5′ splice site	gtaagt→gtaagc	Decreased mRNA	X91$^-$		A0134
2	Intron 2, 3′ splice site	ag→gg	Exon 3 deletion	X91^0		A0105
3	Intron 2, 5′ splice site	gt→tt	ND	X91^0		A0154
4	Intron 2, 5′ splice site	gt→gg	ND	NL		
5	Intron 2, 5′ splice site	gt→at	ND	X91^0		A0131
6	Intron 2, 5′ splice site	gt→tt	Exon 2 deletion	X91^0		A0138
7	Exon 3, −2 nt from 3′ splice	263 C deletion	Multiple	X91^0		A0110
8	Exon 3, −1 nt from 3′ splice	264 G→A	ND	X91$^-$	A	A0100
9	Exon 3, −1 nt from 3′ splice	264 G→A	Exon 3 deletion + normal	X91$^-$	A	
10	Exon 3, −1 nt from 3′ splice	264 G→A	Exon 3 deletion	X91$^-$	A	A0193
11	Exon 3, −1 nt from 3′ splice	264 G→A	ND	X91$^-$	A	A0127
12	Intron 3, −8 nt from 3′ splice	aatccaaag→agtccaaag	Alternative splicing	X91^0		
13	Intron 3, 5′ splice site	gtaag→gtaac	ND	X91^0		A0148
14	Exon 5	494 A→G	Multiple	X91$^-$		A0179
15	Intron 5, 5′ splice site	gt→gc	ND	X91^0		A0164
16	Intron 5, 5′ splice site	gt→tt	ND	X91^0		A0115
17	Intron 5, 5′ splice site	gtaag→gtaaa	ND	ND		A0140
18	Intron 6, 5′ splice site	Deletion: gtgagt→gt___	ND	X91^0		A0106
19	Intron 6, 5′ splice site	gtgagt→gtgaga	ND	X91^0		
20	Intron 7, 3′ splice site	ag→aa	NL	NL		
21	Intron 10, 3′ splice site	tcag→tcac	ND	X91^0		
22	Intron 10, 5′ splice site	gt→at	NL	NL		

[a] ND = not determined; NL = patient not living.

[b] "X" indicates the X-linked mode of inheritance; "91" indicates that the gp91-*phox* component of the phagocyte oxidase is affected; and the superscript symbols indicate an undetectable ("0") or diminished ("−") level of that component protein (Curnutte et al. 1994).

significant differences in carrier-detection rates between types of mutations.

Discussion

We have identified mutations in the *CYBB* gene that are responsible for X-linked CGD in 131 consecutive kindreds examined in a multicenter study. Screening by SSCP identified abnormalities, defined by genomic DNA sequencing, in 124 of the kindreds. In an additional 7 kindreds, sequencing of all exons and intron boundary regions revealed mutations not detected by SSCP. Within the 131 kindreds, we detected 103 different specific mutations; no mutation appeared in >7 independent kindreds, and most shared genotypes occurred in only 2 or 3 kindreds. The mutations reported in this study, along with those identified by an international group of investigators, recently have been compiled by Roos et al. (1996*a*) into a computerized database, X-CGDbase.

The range of mutations identified in the present study spanned nearly all types of mechanisms by which genetic change can disrupt gene expression. The most common forms of mutation were small deletions or insertions resulting in frameshifts (32 kindreds) and single-base changes resulting in nonsense (30 kindreds) or missense (30 kindreds) codons. Of the nonsense mutations, nearly two-thirds were C→T transitions, of which 16 of 19 occurred at CG dinucleotides. The predominance of such mutations has been reported in the factor VIII gene (Youssoufian et al. 1988) and others; these C→T mutations probably represent spontaneous deamination of methylated cytosines in genomic DNA. Unlike previous studies of the factor VIII gene, our data do not show a similar distribution of base changes in missense mutations.

At least 6 of the 15 deletion mutations were large enough to affect the adjacent McLeod gene (*XK*). Clinically, the phenotype includes compensated hemolysis, acanthocytic erythrocyte morphology, and eventual neurological symptoms, including areflexia, dystonia, and choreiform movements (Redman and Marsh 1993). The

Table 6

Regulatory-Region Mutations in the *CYBB* Gene

Kindred Number	Mutation		CGD Phenotype[a]	X-CGDbase Number
	Position	Nucleotide Change		
1	5′ flank	−57 a→c	X91$^-$	A0089
2	5′ flank	−55 t→c	X91$^-$	A0166

[a] Variant phenotype with heterogeneous distribution of oxidase activity (see text).

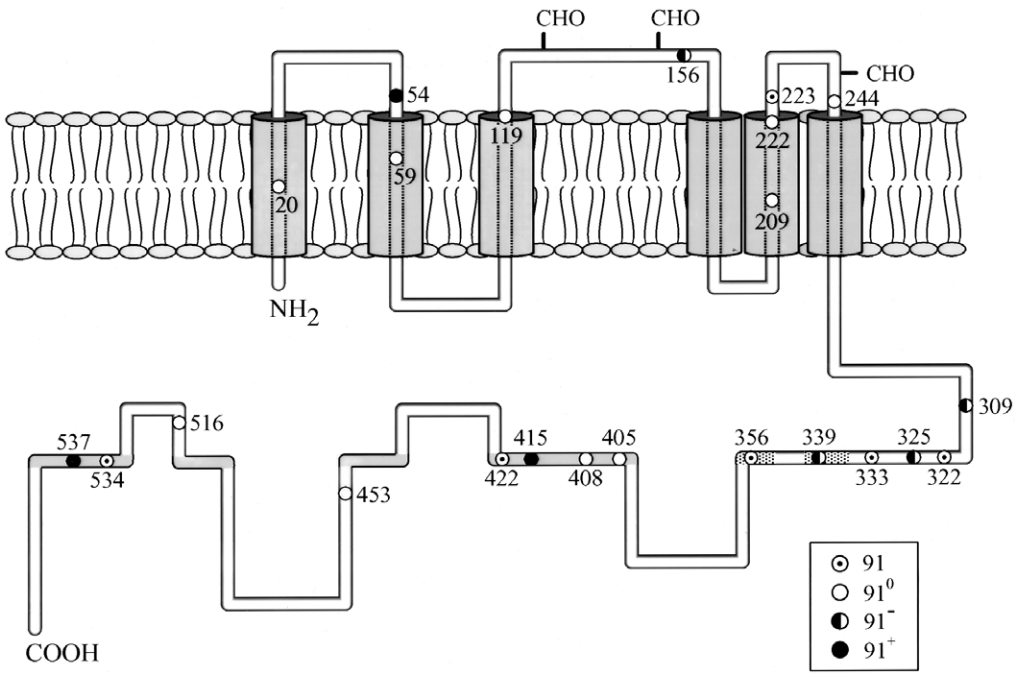

Figure 1 Schematic representation of the gp91-*phox* protein molecule. Sites of missense mutations are identified by the number of the affected amino acid, and types of mutation are indicated by symbols (as defined in the key [*bottom right*]). The shaded regions between amino acids 403 and 546 represent the putative NADPH-binding site, and the stippled region between amino acids 335 and 360 represents the putative FAD-binding site (Segal et al. 1992). "CHO" indicates a glycosylation site.

McLeod phenotype is defined hematologically by absent erythrocyte Kx protein and diminished levels of Kell–blood-group antigens, including k (Cellano), Kp^b (Rautenberg), and Ku (Peltz or antitotal Kell) (Redman and Marsh 1993). Importantly, the phenotype is completely distinct from the very common Kell-negative and the extremely rare Kell-null blood groups. When transfused, McLeod-phenotype patients may respond with anti-Kx and anti-Km antibodies, rendering future transfusion extremely difficult. For this reason, X-linked CGD patients, particularly those with deletion mutations, should be screened for the McLeod phenotype by quantitative determination of k, Kp^b, and Ku expression.

The missense mutations are shown in figure 1, mapped onto a representation of the gp91-*phox* molecule and its putative functional domains. The transmembrane domains and orientation of the amino terminus are based on current and past mutation data, hydropathy plots, and previous (Imajoh-Ohmi et al. 1992; Burritt et al. 1995) and current (A. R. Cross, M. C. Dinauer, and J. T. Curnutte, unpublished data) studies with blocking peptides and antipeptide antibodies; putative flavine-adenine dinucleotide (FAD)– and NADPH-binding regions and glycosylation sites are based on gp91-*phox* structural analyses by A. W. Segal's laboratory (Segal et al. 1992; Wallach and Segal 1997). Three regions of the molecule show clusters of mutations. Seventeen of the 30 missense mutations are located within three short (15–31 amino acids) sequences (table 4 and fig. 1): five kindreds have mutations at amino acids 209–223 (encoded in exon 6); five kindreds show mutations at amino acids 309–339 (encoded in exon 9); and seven mutations are located at amino acids 405–422 (encoded in exon 10). Similarly, a recent review of CGD mutations from multiple sources located 15 of 30 independent missense mutations in exons 9 and 10 of *CYBB* (Roos et al. 1996*b*). However, as discussed below, these exons do not constitute hot spots for other types of mutations.

Three of the histidines that constitute potential heme-binding sites are altered by missense mutations. Those tested for biochemical function showed no residual oxidase activity or cytochrome *b* protein, as was expected from the critical role of heme incorporation in the formation and stability of the cytochrome *b* heterodimer (Yu et al. 1997). In contrast, several mutations of proline moieties showed partial or complete preservation of cytochrome *b* protein (but no function), despite the presumed role of this amino acid in the formation of bends in secondary structure. In fact, a surprising 40% (7 of 17) of the missense mutations evaluated for biochemical function showed some preservation of either superoxide generation or cytochrome *b* protein. Thus, the gp91-*phox* molecule is highly susceptible to mutational damage, but not every change is a knockout.

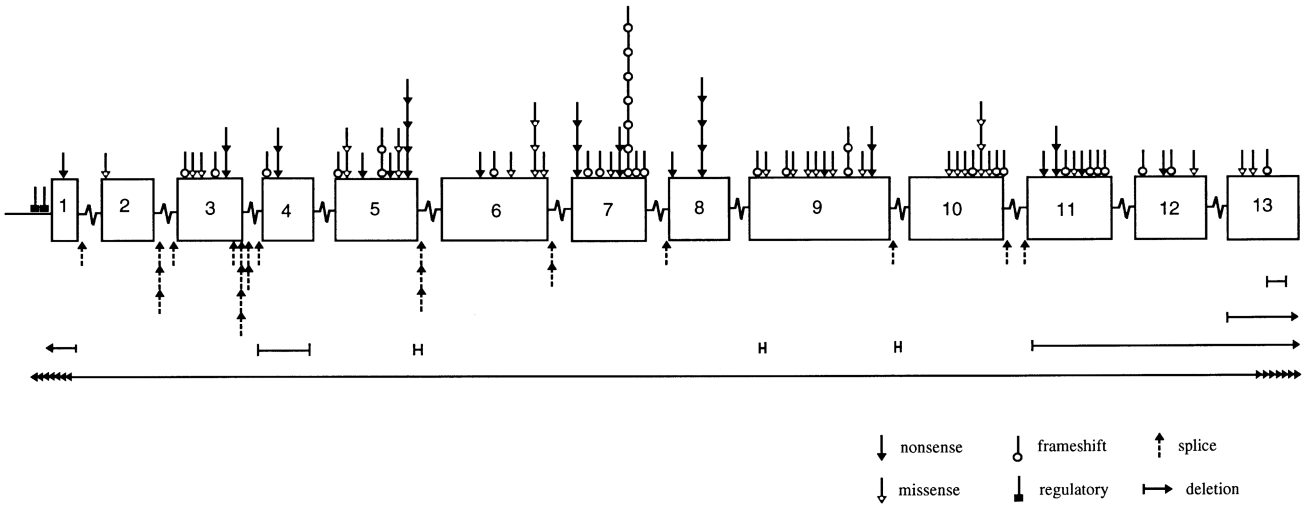

Figure 2 Heterogeneity in positions of *CYBB* mutations causing X-linked CGD. Each arrow represents a single kindred; the type of arrowhead indicates the type of mutation (as defined in the key [*bottom right*]). Stacked vertical arrows represent multiple unrelated kindreds with mutations at the same location. Solid horizontal lines indicate deletion mutations; their lengths map the sizes of the deletions. Arrowheads on the horizontal lines represent unknown deletion length in the direction of the arrow.

Mutations that interfere with normal splicing of nuclear RNA transcripts also contribute to the spectrum of X-linked CGD genotypes. Although the majority directly altered the splice junctions, several mutations occurred at locations $\leqslant 8$ nt upstream or downstream. The largely abnormal splicing of these transcripts shows, as has been noted previously in other genes (Reed and Maniatis 1986; Carothers et al. 1993), that splice-site selection can be affected by mutations of either the exon sequence just proximal to the 5′ splice junction or the intron sequence more distal to the splice site.

Only two kindreds showed regulatory-region mutations. We limited sequencing to the proximal 5′ flanking region, the exons, and the intron boundaries; therefore, potential regulatory elements in more distal upstream, downstream, or intronic locations would have escaped discovery. The detection of mutations in all 131 kindreds indicates that genotypes causing aberrant gene regulation are very rare or that the resultant phenotypes are too mild for diagnosis of CGD.

The heterogeneity of mutations detected in this study and the lack of any predominant genotype indicate that the worldwide incidence of the disease represents many different mutational events, without any evidence for a founder effect. Such a pattern is expected for a disorder with a phenotype of a severe immune-system defect and recurrent infections. From an evolutionary point of view, the originally described "fatal granulomatous disease of childhood" (Bridges et al. 1959; Holmes et al. 1966) has only very recently become a chronic disease with life expectancy extending into adulthood (Mouy et al. 1989; Finn et al. 1990).

The heterogeneity of mutations in X-linked CGD contrasts dramatically with the mutation pattern in CGD due to deficiency of p47-*phox*. Studies from the United States, Europe, and Japan (Casimir et al. 1991; Volpp and Lin 1993; Iwata et al. 1994) have demonstrated the same genetic defect, a frameshift due to a GT deletion in exon 2, in 19 alleles from 10 patients. A highly homologous p47-*phox* pseudogene contains the same defect, suggesting that recombination between the wild-type gene and the pseudogene is a common mutational event. Defects in p22-*phox* or p67-*phox* appear to arise from heterogeneous mutations (Roos et al. 1996*b*), but very few molecular analyses have been performed for these rarest forms of autosomal recessive CGD.

In addition to the wide variety of mechanisms for the observed mutations, their distribution within the *CYBB* gene also exhibits great heterogeneity. As shown in figure 2, loci are dispersed throughout the 13 exons and their intron boundaries. A similar broad distribution was reported in the review by Roos et al. (1996*b*). Thus, no mutational hot spots appear to affect this gene. In addition, the complete sequencing of >50 *CYBB* genes, in patients and carriers, showed only the one known coding-region polymorphism (Kuribayashi et al. 1996). Although many more genes will need to be sequenced to determine the true frequency of polymorphisms, their rarity in this series and in the current literature suggests that the gp91-*phox* molecule does not tolerate most minor alterations in structure. Even in the genomic DNA of the gene, there is a remarkable paucity of RFLPs. Thus, most mutations in the *CYBB* gene appear to pro-

duce biochemically significant changes and clinical CGD.

Carrier detection for this X-linked disorder revealed the mutated gene or a functionally abnormal phagocyte population in 89% of mothers. The apparent spontaneous-mutation rate of ~11% falls well below Haldane's calculation that one-third of cases of X-linked disorders represent new mutations if the population is at equilibrium and the viability or fertility of the affected males is very low (Haldane 1935). The difference probably reflects the well-known effects of genetic drift and reproductive compensation (Lange et al. 1978), as well as some degree of selection bias due to the inclusion, in our series, of carrier mothers referred for prenatal diagnosis of CGD and to the higher likelihood that a referring physician would consider the diagnosis of CGD for a patient with a positive family history.

This molecular analysis of a large series of kindreds with X-linked CGD provides insight into the breadth and relative distribution of mutations responsible for the disease. Further detection of missense mutations and pooling of data from other studies should also permit the dissection of the gp91-*phox* molecule. These "experiments of nature," in combination with structural modeling and site-directed mutagenesis, eventually should elucidate the relationship between the structure and the function of this key component of the respiratory-burst oxidase.

Acknowledgments

We thank Drs. Andrew Cross and Paul Heyworth, for their valuable advice, and Carolyn Padden and Constance Whitney, for their excellent technical assistance. This work was supported by National Institutes of Health grants AI24838, AI33346, DK41625, HL45635, and RR00833; by The March of Dimes; and by an award from the Howard Hughes Medical Institute, to the University of Massachusetts Medical School, under the Research Resources Program for Medical Schools.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Genbank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for *CYBB* gene [X04011])

Online Mendelian inheritance in man (OMIM), http://www.ncbi.nlm.nih.gov/htbin-post/Omim (for X-linked CGD [MIM 306400] and McLeod syndrome [MIM 314850])

X-CGDbase, http://www.helsinki.fi/science/signal/databases/x-cgdbase.html (for X-linked CGD mutations)

References

Ariga T, Sakiyama Y, Furuta H, Matsumoto S (1994*a*) Molecular genetic studies of two families with X-linked chronic granulomatous disease: mutation analysis and definitive determination of carrier status in patients' sisters. Eur J Haematol 52:99–102

Ariga T, Sakiyama Y, Matsumoto S (1994*b*) Two novel point mutations in the cytochrome b 558 heavy chain gene, detected in two Japanese patients with X-linked chronic granulomatous disease. Hum Genet 94:441

——— (1995) A 15–base pair (bp) palindromic insertion associated with a 3-bp deletion in exon 10 of the gp91-phox gene, detected in two patients with X-linked chronic granulomatous disease. Hum Genet 96:6–8

Azuma H, Oomi H, Sasaki K, Kawabata I, Sakaino T, Koyano S, Suzutani T, et al (1995) A new mutation in exon 12 of the gp91-phox gene leading to cytochrome b–positive X-linked chronic granulomatous disease. Blood 85:3274–3277

Baehner RL, Kunkel LM, Monaco AP, Haines JL, Conneally PM, Palmer C, Heerema N, et al (1986) DNA linkage analysis of X chromosome–linked chronic granulomatous disease. Proc Natl Acad Sci USA 83:3398–3401

Berendes H, Bridges RA, Good RA (1957) A fatal granulomatosus of childhood: the clinical study of a new syndrome. Minn Med 40:309–312

Bolscher BGJM, de Boer M, De Klein A, Weening RS, Roos D (1991) Point mutations in the β-subunit of cytochrome b_{558} leading to X-linked chronic granulomatous disease. Blood 77:2482–2487

Bridges RA, Berendes H, Good RA (1959) A fatal granulomatous disease of childhood: the clinical, pathological, and laboratory features of a new syndrome. Am J Dis Child 97: 387–408

Bu-Ghanim HN, Segal AW, Keep NH, Casimir CM (1995) Molecular analysis in three cases of $X91^-$ variant chronic granulomatous disease. Blood 86:3575–3582

Burritt JB, Quinn MT, Jutila MA, Bond CW, Jesaitis AJ (1995) Topological mapping of neutrophil cytochrome *b* epitopes with phage-display libraries. J Biol Chem 270:16974–16980

Carothers AM, Urlaub G, Grunberger D, Chasin LA (1993) Splicing mutants and their second-site suppressors at the DHFR locus in Chinese hamster ovary cells. Mol Cell Biol 13:5085–5098

Casimir CM, Bu-Ghanim HN, Rodaway ARF, Bentley DL, Rowe P, Segal AW (1991) Autosomal recessive chronic granulomatous disease caused by deletion at a dinucleotide repeat. Proc Natl Acad Sci USA 88:2753–2757

Condino-Neto A, Rae J, Padden C, Whitney C, Curnutte JT, Newburger PE (1997) An intronic mutation in the *CYBB* gene leading to RNA instability and variant X-linked chronic granulomatous disease. Blood Suppl 90:136A

Cross AR, Rae J, Curnutte JT (1995) Cytochrome b_{-245} of the neutrophil superoxide-generating system contains two nonidentical hemes: potentiometric studies of a mutant form of $gp91^{phox}$. J Biol Chem 270:17075–17077

Curnutte JT (1988) The classification of chronic granulomatous disease. Hematol Oncol Clin North Am 2:241–252

——— (1993) Chronic granulomatous disease: the solving of

a clinical riddle at the molecular level. Clin Immunol Immunopathol Suppl 67:S2–S15

Curnutte JT, Kuver R, Scott PJ (1987) Activation of NADPH oxidase in a cell-free system: partial purification of components and characterization of the activation process. J Biol Chem 262:5563–5569

Curnutte JT, Orkin SH, Dinauer MC (1994) Genetic disorders of phagocyte function. In: Stamatoyannopoulos G, Neinhuis AW, Majerus PW, Varmus H (eds) The molecular basis of blood diseases. WB Saunders, Philadelphia pp 493–540

de Boer M, Bolscher BGJM, Dinauer MC, Orkin SH, Smith CIE, Åhlin A, Weening RS, et al (1992) Splice site mutations are a common cause of X-linked chronic granulomatous disease. Blood 80:1553–1558

De Saint–Basile G, Bohler MC, Fischer A, Cartron J, Dufier JL, Griscelli C, Orkin SH (1988) Xp21 DNA microdeletion in a patient with chronic granulomatous disease, retinitis pigmentosa, and McLeod phenotype. Hum Genet 80:85–89

DeLeo FR, Quinn MT (1996) Assembly of the phagocyte NADPH oxidase: molecular interaction of oxidase proteins. J Leukoc Biol 60:677–691

Densen P, Wilkinson-Kroovand S, Mandell GL, Sullivan G, Oyen R, Marsh WL (1981) Kx: its relationship to chronic granulomatous disease and genetic linkage with Xg. Blood 58:34–37

Dinauer MC, Curnutte JT, Rosen H, Orkin SH (1989) A missense mutation in the neutrophil cytochrome b heavy chain leading to X-linked chronic granulomatous disease. J Clin Invest 84:2012–2016

Dinauer MC, Pierce EA, Bruns GAP, Curnutte JT, Orkin SH (1990) Human neutrophil cytochrome *b* light chain (p22-*phox*): gene structure, chromosomal location, and mutations in cytochrome-negative autosomal recessive chronic granulomatous disease. J Clin Invest 86:1729–1737

Eklund EA, Skalnik DG (1995) Characterization of a gp91-phox promoter element that is required for interferon gamma-induced transcription. J Biol Chem 270:8267–8273

Ezekowitz RAB, Dinauer MC, Jaffe HS, Orkin SH, Newburger PE (1988) Partial correction of the phagocyte defect in patients with X-linked chronic granulomatous disease by subcutaneous interferon gamma. N Engl J Med 319:146–151

Ezekowitz RAB, Orkin SH, Newburger PE (1987) Recombinant interferon gamma augments phagocyte superoxide production and X-chronic granulomatous disease gene expression in X-linked variant chronic granulomatous disease. J Clin Invest 80:1009–1016

Finn A, Hadzic N, Morgan G, Strobel S, Levinsky RJ (1990) Prognosis of chronic granulomatous disease. Arch Dis Child 65:942–945

Forrest CB, Forehand JR, Axtell RA, Roberts RL, Johnston RB Jr (1988) Clinical features and current management of chronic granulomatous disease. Hematol Oncol Clin North Am 2:253–266

Frey D, Mächler M, Seger RA, Schmid W, Orkin SH (1988) Gene deletion in a patient with chronic granulomatous disease and McCleod syndrome: fine mapping of the Xk gene locus. Blood 71:252–255

Ginsburg D, Handin RI, Bonthron DT, Donlon TA, Bruns GAP, Latt SA, Orkin SH (1985) Human von Willebrand factor: isolation of complementary DNA clones and chromosomal location. Science 228:1401–1406

Haldane JBS (1935) The ratio of spontaneous mutation of a human gene. J Genet 31:317–326

Holmes B, Quie PG, Windhorst DB, Good RA (1966) Fatal granulomatous disease of childhood: an inborn abnormality of phagocytic function. Lancet 1:1225–1228

Imajoh-Ohmi S, Tokita K, Ochiai H, Nakamura M, Kanegasaki S (1992) Topology of cytochrome b_{558} in neutrophil membrane analyzed by anti-peptide antibodies and proteolysis. J Biol Chem 267:180–184

Iwata M, Nunoi H, Yamazaki H, Nakano T, Niwa H, Tsuruta S, Ohga S, et al (1994) Homologous dinucleotide (GT or TG) deletion in Japanese patients with chronic granulomatous disease with p47-phox deficiency. Biochem Biophys Res Commun 199:1372–1377

Kessler O, Chasin LA (1996) Effects of nonsense mutations on nuclear and cytoplasmic adenine phosphoribosyltransferase RNA. Mol Cell Biol 16:4426–4435

Kuribayashi F, de Boer M, Leusen JH, Verhoeven AJ, Roos D (1996) A novel polymorphism in the coding region of CYBB, the human gp91-phox gene. Hum Genet 97:611–613

Lange K, Gladstien K, Zatz M (1978) Effects of reproductive compensation and genetic drift on X-linked lethals. Am J Hum Genet 30:180–189

Leto TL, Lomax KJ, Volpp BD, Nunoi H, Sechler JM, Nauseef WM, Clark RA, et al (1990) Cloning of a 67-kD neutrophil oxidase factor with similarity to a noncatalytic region of p60c-src. Science 248:727–730

Leusen JHW, de Boer M, Bolscher BGJM, Hilarius PM, Weening RS, Ochs HD, Roos D, et al (1994) A point mutation in gp91-*phox* of cytochrome b_{558} of the human NADPH oxidase leading to defective translocation of the cytosolic proteins p47-*phox* and p67-*phox*. J Clin Invest 93: 2120–2126

Leusen JHW, Verhoeven AJ, Roos D (1996) Interactions between the components of the human NADPH oxidase: intrigues in the *phox* family. J Lab Clin Med 128:461–476

Maniatis T, Fritsch EF, Sambrook J (1990) Molecular cloning: a laboratory manual, 2d ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Maquat LE (1995) When cells stop making sense: effects of nonsense codons on RNA metabolism in vertebrate cells. RNA 1:453–465

Mouy R, Fischer A, Vilmer E, Seger RA, Griscelli C (1989) Incidence, severity, and prevention of infections in chronic granulomatous disease. J Pediatr 114:555–560

Nagaraja R, MacMillan S, Kere J, Jones C, Griffin S, Schmatz M, Terrell J, et al (1997) X chromosome map at 75-kb STS resolution, revealing extremes of recombination and GC content. Genome Res 7:210–222

Newburger PE, Malawista SE, Dinauer MC, Gelbart T, Woodman RC, Chada S, Shen Q, et al (1994*a*) Chronic granulomatous disease and glutathione peroxidase deficiency, revisited. Blood 84:3861–3869

Newburger PE, Skalnik DG, Hopkins PJ, Eklund EA, Curnutte JT (1994*b*) Mutations in the promoter region of the gene for gp91-phox in X-linked chronic granulomatous disease with decreased expression of cytochrome b_{558}. J Clin Invest 94:1205–1211

Nunoi H, Rotrosen D, Gallin JI, Malech HL (1988) Two forms of autosomal chronic granulomatous disease lack distinct neutrophil cytosol factors. Science 242:1298–1301

Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T (1989*a*) Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc Natl Acad Sci USA 86:2766–2770

Orita M, Suzuki Y, Sekiya T, Hayashi K (1989*b*) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5: 874–879

Parkos CA, Dinauer MC, Walker LE, Allen RA, Jesaitis AJ, Orkin SH (1988) The primary structure and unique expression of the 22 kilodalton light chain of human neutrophil cytochrome b. Proc Natl Acad Sci USA 85:3319–3323

Rabbani H, de Boer M, Åhlin A, Sundin U, Elinder G, Hammarström L, Palmblad J, et al (1993) A 40-base-pair duplication in the gp91-*phox* gene leading to X-linked chronic granulomatous disease. Eur J Haematol 51:218–222

Redman CM, Marsh WL (1993) The Kell blood group system and the McLeod phenotype. Semin Hematol 30:209–218

Reed R, Maniatis T (1986) A role for exon sequences and splice-site proximity in splice-site selection. Cell 46:681–690

Roos D, Curnutte JT, Hossle JP, Lau YL, Ariga T, Nunoi H, Dinauer MC, et al (1996*a*) X-CGDbase: a database of X-CGD-causing mutations. Immunol Today 17:517–521

Roos D, de Boer M, Kuribayashi F, Meischl C, Weening RS, Segal AW, Åhlin A, et al (1996*b*) Mutations in the X-linked and autosomal recessive forms of chronic granulomatous disease. Blood 87:1663–1681

Royer-Pokora B, Kunkel LM, Monaco AP, Goff SC, Newburger PE, Baehner RL, Cole FS, et al (1986) Cloning the gene for an inherited disorder—chronic granulomatous disease—on the basis of its chromosomal location. Nature 322: 32–38

Schapiro BL, Newburger PE, Klempner MS, Dinauer MC (1991) Chronic granulomatous disease presenting in a 69-year-old man. N Engl J Med 325:1786–1790

Segal AW, Cross AR, Garcia RC, Borregaard N, Valerius NH, Soothill JF, Jones OTG (1983) Absence of cytochrome b_{-245} in chronic granulomatous disease: a multicenter European evaluation of its incidence and relevance. N Engl J Med 308: 245–251

Segal AW, West I, Wientjes F, Nugent JHA, Chavan AJ, Haley B, Garcia RC, et al (1992) Cytochrome b_{-245} is a flavocytochrome containing FAD and the NADPH-binding site of the microbicidal oxidase of phagocytes. Biochem J 284: 781–788

Skalnik DG, Strauss EC, Orkin SH (1991) CCAAT displacement protein as a repressor of the myelomonocytic-specific gp91-phox gene promoter. J Biol Chem 266:16736–16744

Volpp BD, Lin Y (1993) In vitro molecular reconstitution of the respiratory burst in B lymphoblasts from p47-*phox*–deficient chronic granulomatous disease. J Clin Invest 91:201–207

Volpp BD, Nauseef WM, Clark RA (1988) Two cytosolic neutrophil oxidase components absent in autosomal chronic granulomatous disease. Science 242:1295–1297

Wallach TM, Segal AW (1997) Analysis of glycosylation sites on gp91phox, the flavocytochrome of the NADPH oxidase, by site-directed mutagenesis and translation in vitro. Biochem J 321:583–585

Woodman RC, Newburger PE, Anklesaria P, Erickson RW, Rae J, Cohen MS, Curnutte JT (1995) A new X-linked variant of chronic granulomatous disease characterized by the existence of a normal clone of respiratory burst–competent phagocytic cells. Blood 85:231–241

Youssoufian H, Antonarakis SE, Bell W, Griffin AM, Kazazian HH Jr (1988) Nonsense and missense mutations in hemophilia A: estimate of the relative mutation rate at CG dinucleotides. Am J Hum Genet 42:718–725

Yu L, Zhen L, Dinauer MC (1997) Biosynthesis of the phagocyte NADPH oxidase cytochrome b_{558}: role of heme incorporation and heterodimer formation in maturation and stability of gp91(phox) and p22(phox) subunits. J Biol Chem 272:27288–27294