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## Phenotypic Expressions of CCR5- $\Delta$ 32/ $\Delta$ 32 Homozygosity

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## Phenotypic Expressions of *CCR5*- $\Delta$ 32/ $\Delta$ 32 Homozygosity

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**Objective:** As blockade of CC-chemokine receptor 5 (*CCR5*) has been proposed as therapy for HIV-1, we examined whether the *CCR5*- $\Delta$ 32/ $\Delta$ 32 homozygous genotype has phenotypic expressions other than those related to HIV-1.

**Design:** Study subjects were white homosexual men or men with hemophilia who were not infected with HIV-1. In this study, 15 *CCR5*- $\Delta$ 32/ $\Delta$ 32 homozygotes were compared with 201 *CCR5* wild-type (+/+) subjects for a wide range of clinical conditions and laboratory assay results ascertained during prospective cohort studies and routine clinical care. *CCR5*- $\Delta$ 32 genotype was determined by polymerase chain reaction, followed by single-stranded conformational polymorphism analysis.

**Results:** Hypertension and conditions attributable to hemophilia were the only diagnoses frequently found in clinical records of *CCR5*- $\Delta$ 32/ $\Delta$ 32 study subjects. Based on blood pressure measurement and treatment history, *CCR5*- $\Delta$ 32/ $\Delta$ 32 homozygotes had a 2.8-fold higher prevalence of hypertension than age-matched *CCR5*-+/+ study subjects (95% confidence interval [CI], 1.2-6.4;  $p = .01$ ); none of the homozygotes had severe hypertension. Hematologic measures were generally similar across the genotypes, but total lymphocyte counts were ~20% higher in *CCR5*- $\Delta$ 32/ $\Delta$ 32 study subjects than in *CCR5*-+/+ study subjects ( $p < .05$ ). Among patients with hemophilia who were infected with hepatitis C virus (HCV), mean alanine aminotransferase levels were 117% higher among *CCR5*- $\Delta$ 32/ $\Delta$ 32 homozygotes ( $p < .05$ ), but serum HCV levels did not differ by *CCR5*- $\Delta$ 32 genotype. *CCR5*- $\Delta$ 32/ $\Delta$ 32 homozygous study subjects had a lower prevalence of antibodies to measles virus than those with other genotypes, but this association was not confirmed in a group of blood donors. The

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prevalence of antibodies to nine other common viruses, HBV, and HCV was not related to *CCR5* genotype.

**Conclusions:** *CCR5-Δ32/Δ32* homozygotes are generally similar to wild-type persons. Confirmatory investigations are required to determine whether hypertension, increased lymphocyte counts, and higher hepatic enzyme levels in the presence of HCV infection represent true phenotypic expressions of this genotype. *CCR5-Δ32/Δ32* homozygosity does not provide broad protection against viral infections.

**Key Words:** AIDS—*CCR5*—Chemokine—Chemokine receptors—Epidemiology—Genetics—HIV-1—Hemophilia—Hepatitis C virus—Hypertension—Lymphocytes—Therapy.

Chemokines (chemoattractant cytokines), chemokine receptors, and host genetic factors are at the forefront of HIV-1 research (1). The  $\beta$ -class (CC) chemokines play a critical role in the inflammatory process through recruitment or activation of lymphocytes, monocytes, mast cells, and eosinophils (2). By binding with CC-chemokine receptor 5 (*CCR5*),  $\beta$ -chemokines RANTES, macrophage inhibitory protein (MIP)-1 $\alpha$ , and MIP-1 $\beta$  can also prevent in vitro infection of CD4<sup>+</sup> cells (3). *CCR5* is the major coreceptor for macrophage-tropic HIV-1 strains that predominate during early infection (4–8).

A 32-bp deletion ( $\Delta 32$ ) of the *CCR5* gene results in a nonfunctional receptor for  $\beta$ -chemokines and HIV-1 (9–12). This allele has a frequency of ~10% in white persons but is rarer or absent in other populations (9–13). *CCR5-Δ32/Δ32* homozygotes (~1% of white persons) are highly resistant to infection by HIV-1 (9–12,14), although this resistance is not absolute (15–17). HIV-1-infected *CCR5* heterozygotes (*CCR5+/\Delta 32*) have delayed progression to AIDS (10,12,18).

The role of *CCR5* in HIV-1 infection has potential therapeutic applications (19), but before attempts are made to block or down regulate *CCR5* in humans it is important to determine whether *CCR5-Δ32/Δ32* homozygotes suffer deleterious effects (20). Such effects might result directly from the absence of *CCR5* or from increased levels of  $\beta$ -chemokines. Lymphocytes from *CCR5-Δ32/Δ32* homozygotes produce elevated levels of *CCR5* ligand  $\beta$ -chemokines in vitro (8), and these proteins can bind chemokine receptors other than *CCR5* (2). Studies in genetically altered mice indicate that the absence of *CCR5* or higher levels of  $\beta$ -chemokines may have phenotypic expressions. Mice lacking *CCR5* had impaired macrophage function and an enhanced T-cell-dependent immune response (21). Higher levels of MIP-1 $\alpha$  are associated with Coxsackievirus-induced myocarditis in mice (22).

Homozygosity for the *CCR5-Δ32* allele may also have beneficial effects. The high allele frequency in white

persons may reflect positive genetic selection, perhaps through protection against an infectious agent other than HIV-1 (23).

By screening patients with hemophilia and homosexual men enrolled in studies of HIV-1 incidence, we identified 15 men not infected with HIV-1 who were homozygous for the *CCR5-Δ32* allele. These study subjects have been observed during follow-up for up to 14 years in these studies; study subjects with hemophilia have also undergone regular thorough clinical evaluations. To examine phenotypic expressions of the *CCR5-Δ32/Δ32* genotype, we compared clinical and laboratory data from *CCR5-Δ32/Δ32* homozygotes with that from other enrolled study subjects and reference populations.

## METHODS

### Study Subjects

Subjects were enrolled in the Multicenter Hemophilia Cohort Study (MHCS) (24) or the Washington and New York Men's Research Study (DCG) (25). MHCS is a prospective cohort study of 2117 patients enrolled in the United States and Europe beginning in 1982. At 6 to 12 month intervals, clinical status information is recorded, a physical examination is performed, and blood is collected. DCG is a study of 245 homosexual men enrolled in 1982 at primary care physicians' offices. Patients' blood was collected and interviews were conducted at enrollment and about every 12 months subsequently, but follow-up of most subjects not infected with HIV-1 was discontinued in 1991. Both studies were reviewed and approved by the relevant institutional review boards, and all study subjects provided informed consent to participate. The present analysis is limited to study subjects not infected with HIV-1.

Demographic and clinical (including hematologic) data from the studies were recorded on standardized forms and entered into a computer database. Clinical conditions that were assessed were primarily those associated with hemophilia, liver disease, HIV infection, or sexually transmitted infections. In addition, records of clinical care were available for 11 *CCR5-Δ32/Δ32* homozygotes; we systematically abstracted information from these records. To examine a possible association between *CCR5-Δ32/Δ32* homozygosity and hypertension, we also abstracted information on blood pressure and hypertension treat-

ment from clinical records of CCR5-Δ32/Δ32 homozygotes and selected CCR5 wild-type (+/+) comparison study subjects.

### Laboratory Methods

Hematologic measures were determined directly with an automated cell counter, or by an automated count of white blood cells and a manual differential count. The percentage of CD4<sup>+</sup> lymphocytes was measured by flow cytometry using the whole blood lysate method (26) or methods appropriate for frozen lymphocyte specimens (27). Assays for antibodies to HIV-1, hepatitis B virus (HBV), and hepatitis C virus (HCV), as well as for HBV surface antigen and HCV RNA (by the branched chain DNA method) were performed as previously described (28). For CCR5-Δ32 homozygotes, we measured antibodies to ten common viruses (Mayo Medical Laboratories, Rochester, MN, U.S.A.) if adequate volumes of archived serum or plasma were available. These antibodies were measured by immunofluorescence assay or enzyme-linked immunoassay (cytomegalovirus and rubella). Measles antibody results were confirmed by a measles neutralization assay (29).

CCR5 genotype was determined by polymerase chain reaction (PCR) amplification of DNA, followed by single-stranded conformational polymorphism analysis as previously described (10). Some study subjects could not be genotyped because no DNA specimen was available for technical reasons.

### Data Analysis

Because all HIV-1-uninfected CCR5-Δ32/Δ32 study subjects were white or white Hispanic U.S. men, we limited the comparison group to similar subjects. For repeatedly measured variables (e.g., lymphocyte count), we determined the mean value for each study subject and then calculated the grand mean for each genotype. Measurements taken before the study subject was 18 years old were excluded. For comparisons of hepatic enzyme levels among study subjects with hemophilia infected with HCV, measurements before the initial positive HCV antibody test were excluded, as were measurements taken when the hepatitis B surface antigen result was positive. Study subject characteristics and results from laboratory assays were compared using the two-sided Mann-Whitney-Wilcoxon rank sum test for continuous measures (30) and the  $\chi^2$  test for categorical data (31). We used Fisher's exact test (Epi-Info, version 6.04a, Stone Mountain, GA, U.S.A.) and the exact binomial test to compare serologic assay results for the CCR5-Δ32 homozygotes with results expected in a normal healthy population; expected values were calculated using seroprevalence data obtained from the commercial laboratory which performed the assays (A.D. Wold, personal communication, March 4, 1997).

In a substudy of hypertension prevalence, we abstracted information from clinic records of CCR5-Δ32/Δ32 homozygotes and selected CCR5-+/+ study subjects matched by age and site of enrollment for the period January 1, 1985 through August 1, 1997. We considered a study subject hypertensive if the mean of his last two systolic pressure readings was  $\geq 140$  mm Hg, if the mean of his last two diastolic pressure readings was  $\geq 90$  mm Hg, or if he had ever been treated for hypertension. These data were analyzed as a series of strata each comprised of a CCR5-Δ32/Δ32 homozygote and up to 5 CCR5-+/+ study subjects. The common relative risk was determined by the Mantel-Haenszel method, the 95% confidence interval (95% CI) by test-based methods, and the *p* value by the Cochran-Mantel-Haenszel statistic (31).

### RESULTS

Among 219 HIV-1-uninfected white patients with hemophilia genotyped for CCR5-Δ32, there were 175

(79.9%) CCR5-+/+ subjects, 32 (14.6%) CCR5-+Δ32 heterozygotes, and 12 (5.5%) CCR5-Δ32/Δ32 homozygotes. Among 97 white or white Hispanic homosexual men there were 73 (75.3%) CCR5-+/+ study subjects, 21 (21.6%) CCR5-+Δ32 heterozygotes, and 3 (3.1%) CCR5-Δ32/Δ32 homozygotes. The median age at study entry was 25 years for hemophiliacs and 33 years for homosexual men. Although most hemophiliacs, regardless of CCR5 genotype, had factor VIII deficiency (hemophilia A), CCR5-Δ32/Δ32 study subjects had received greater amounts of blood products. For example, 7 of 10 CCR5-Δ32/Δ32 hemophilia patients with available blood product exposure information had received  $>20,000$  U of non-heat-treated factor VIII concentrate between 1978 to 1985, compared with 8% of study subjects with other genotypes (*p* = .001). The elevated frequency of CCR5-Δ32/Δ32 homozygosity in this study population and the greater blood product exposure among such hemophiliacs reflect the resistance of CCR5-Δ32/Δ32 homozygotes to HIV-1 infection.

The small number of deaths precluded a meaningful analysis of genotype-specific mortality: 5 CCR5-+/+ individuals died during the follow-up period and one CCR5-Δ32/Δ32 hemophilia patient died as a result of trauma. To assess morbidity, we abstracted diagnostic information from the study records of the 15 HIV-1-uninfected CCR5-Δ32/Δ32 study subjects and, for 11 study subjects, from clinical records covering 10 to 26 years of follow-up (Table 1). As expected, many men with hemophilia had clinical evidence of arthropathies (10 of 12), bleeding complications (8 of 12), and hepatitis or hepatomegaly (5 of 12). Of the homosexual men, 2 of 3 had reported at least one sexually transmitted infection. Of the 15 CCR5-Δ32/Δ32 study subjects, 6 had lymphadenopathy (any enlarged lymph node) noted during at least one study visit, but lymphadenopathy was similarly common among study subjects with other CCR5-Δ32 genotypes. Most other health conditions were limited to only 1 or 2 study subjects, but the diagnosis of hypertension was found for 6 of 11 CCR5-Δ32/Δ32 homozygotes with available clinical records.

To examine the possible association between CCR5-Δ32/Δ32 homozygosity and hypertension, we abstracted data on antihypertensive medications and the two most recent blood pressure readings from clinic records of the 11 evaluable CCR5-Δ32/Δ32 homozygotes and age-matched CCR5-+/+ study subjects from the same study site. This review confirmed the diagnosis of hypertension for 6 CCR5-Δ32/Δ32 homozygotes and revealed a seventh CCR5-Δ32/Δ32 homozygote who met our criteria. Two CCR5-Δ32/Δ32 homozygotes were hypertensive on the basis of treatment, 2 were hypertensive on the basis

**TABLE 1.** Clinical conditions, year of birth, and length of study observation and clinical review for 15 men with the CCR5-Δ32/Δ32 genotype

Subject	Year of birth	Length of study enrollment	Length of clinical record review	Reported or observed conditions <sup>a</sup>
Persons with hemophilia				
1	1974	8 years	16 years	Arthropathy, bleeding complications, hepatitis or hepatomegaly, lymphadenopathy, splenomegaly, and migraine headaches
2	1954	14 years	11 years	Arthropathy and hypertension
3	1951	6 years	16 years	Arthropathy and diverticulitis
4	1960	8 years	n/a	Arthropathy and colitis
5	1961	9 years	14 years	Arthropathy, hepatitis or hepatomegaly, lymphadenopathy, splenomegaly, cardiovascular abnormalities, nasal septal deviation, and allergies
6	1932	8 years	12 years	Arthropathy, bleeding complications, hypertension, lymphadenopathy, gastrointestinal problems, respiratory dysfunction, other cardiovascular abnormalities, skin disorders, benign prostatic hypertrophy, kidney stone, and depression
7	1966	12 years	25 years	Arthropathy, bleeding complications, hypertension, shingles, pneumonia, Mallory-Weiss tear, and gastroenteritis
8	1954	13 years	24 years	Arthropathy, bleeding complications, nevi, indirect inguinal hernia, and possible cartilaginous neoplasm
9	1970	9 years	24 years	Arthropathy, bleeding complications, hepatitis or hepatomegaly, hypertension, chickenpox, rosacea, herpes labialis, peptic ulcer disease, epididymitis, and learning disability
10	1942	8 years	19 years	Arthropathy, bleeding complications, hepatitis or hepatomegaly, hypertension, cellulitis, testicular pain/swelling, kidney mass, depression, and pseudotumor
11	1961	1 year <sup>b</sup>	10 years	Bleeding complications and hepatitis or hepatomegaly
12	1957	3 years	n/a	Bleeding complications and skin ulcers; study subject died at age 35 (secondary to trauma)
Homosexual men				
13	1958	1 year <sup>b</sup>	n/a	Lymphadenopathy
14	1947	9 years	n/a	Hepatitis or hepatomegaly, lymphadenopathy, gonorrhea, body lice, nongonorrheal penile discharge, nonherpetic anal problem, herpes labialis, skin rash, muscle ache, difficulty with concentration/mood, respiratory infection/dysfunction, and anal fissure
15	1947	9 years	26 years	Hypertension, lymphadenopathy, syphilis, body lice, nonherpetic anal problem, colorectal polyps, obesity, diabetes, respiratory infection/dysfunction, joint/muscle disorder, difficulty with concentration/mood, childhood impetigo, eye disorder, and neurologic disorder

<sup>a</sup> The most common conditions included: arthropathy (10 persons with hemophilia), bleeding complications (8 persons with hemophilia), hepatitis or hepatomegaly (5 persons with hemophilia, 1 homosexual), hypertension (5 persons with hemophilia, 1 homosexual), lymphadenopathy (3 persons with hemophilia, 3 homosexuals).

<sup>b</sup> Study subject had a single study visit.

n/a, no clinical records were available for this study subject.

of blood pressure, and 3 were hypertensive on the basis of both treatment and blood pressure; no blood pressure was in the severe range (systolic  $\geq 180$  mm Hg or diastolic  $\geq 110$  mm Hg). No age- and site-matched study subject was available for 1 of 11 CCR5-Δ32/Δ32 homozygotes, a 64-year-old man on antihypertensive treatment. We compared the 10 remaining CCR5-Δ32/Δ32 homozygotes (mean age, 37.1 years) to 31 matched CCR5-+/+ study subjects (mean age, 37.9 years). Five of the CCR5-+/+ study subjects had evidence of hypertension, yielding a 2.8-fold higher prevalence of hypertension among CCR5-Δ32/Δ32 homozygotes (95% CI, 1.2–6.4;  $p = .01$ ). Compared with matched CCR5-+/+ study subjects, CCR5-Δ32/Δ32 homozygotes had more clinic visits (mean, 11.0 and 15.6 visits, respectively), more often had hemophilia A (66.7% and 88.9%, respectively), more often received  $>20,000$  U of factor VIII concentrate (37.5% and 88.9%, respectively), and less often received  $>20,000$  U of prothrombin complex con-

centrate (40.0% and 0.0%, respectively). Greater prothrombin complex concentrate use among CCR5-+/+ study subjects reflects patients with factor VIII deficiency who had inhibitors to factor VIII concentrate. Rather than receiving factor VIII concentrate, such patients were treated with prothrombin complex concentrate which, for unknown reasons, carried a lower risk of HIV-1 infection.

We compared CCR5-Δ32/Δ32 homozygotes with other study subjects with regard to hematologic and lymphocyte subset measurements (Table 2). Values for hemoglobin, erythrocytes, and platelets were similar across the genotypes. Total lymphocytes were higher for CCR5-Δ32/Δ32 study subjects than CCR5-+/+ study subjects in each cohort (the difference was not statistically significant for homosexual men), but none of the 90 separate total lymphocyte count measurements among 13 CCR5-Δ32/Δ32 homozygotes exceeded the upper limit of normal (4800 cells/mm<sup>3</sup>). When total lymphocyte counts

TABLE 2. Mean values for hematologic measures, lymphocyte subsets, hepatic enzymes, and hepatitis C virus (HCV) levels, by cohort and CCR5 genotype

	Grand mean (n) (p value)					
	Male patient hemophiliacs			Homosexual men		
	+/+	+Δ32	Δ32/Δ32	+/+	+Δ32	Δ32/Δ32
Hemoglobin (g/dl)	15.0 (127)	15.4 (21) (.09)	15.2 (11) (.72)	15.5 (66)	15.0 (19) (.02)	15.3 (2) (.66)
Erythrocytes (10 <sup>12</sup> cells/L)	4.97 (127)	4.96 (21) (.82)	5.09 (11) (.33)	—	—	—
Platelets (10 <sup>3</sup> cells/mm <sup>3</sup> )	246 (126)	254 (21) (.86)	272 (11) (.58)	252 (65)	257 (19) (.72)	286 (2) (.66)
Leukocytes (10 <sup>3</sup> cells/mm <sup>3</sup> )	6.47 (128)	6.94 (21) (.24)	7.06 (11) (.18)	6.80 (73)	6.66 (20) (.69)	8.39 (3) (.12)
Lymphocytes (cells/mm <sup>3</sup> )	1809 (127)	2182 (21) (.02)	2194 (11) (.01)	2397 (64)	2361 (19) (.75)	3018 (2) (.13)
Neutrophils (cells/mm <sup>3</sup> )	3892 (127)	3922 (21) (.83)	4180 (11) (.38)	3782 (64)	3769 (19) (.82)	4218 (2) (.68)
Eosinophils (cells/mm <sup>3</sup> )	164 (118)	165 (20) (.97)	128 (11) (.25)	122 (63)	117 (19) (.47)	294 (2) (.01)
Monocytes (cells/mm <sup>3</sup> )	418 (119)	526 (20) (.07)	444 (11) (.34)	353 (63)	354 (19) (.69)	322 (2) (.78)
Basophils (cells/mm <sup>3</sup> )	43 (112)	87 (20) (.67)	33 (10) (.32)	48 (63)	39 (19) (.75)	69 (2) (.46)
Bands (cells/mm <sup>3</sup> )	25 (107)	45 (20) (.39)	21 (10) (.19)	106 (60)	60 (19) (.96)	74 (2) (.63)
CD4 <sup>+</sup> count (cells/mm <sup>3</sup> )	761 (119)	942 (20) (.11)	916 (11) (.04)	950 (73)	923 (21) (.47)	893 (3) (.73)
CD8 <sup>+</sup> count (cells/mm <sup>3</sup> )	507 (119)	634 (20) (.03)	589 (11) (.19)	609 (73)	700 (21) (.41)	751 (3) (.17)
HCV RNA (log <sub>10</sub> copies/ml) <sup>a</sup>	5.69 (94)	4.90 (17) (.08)	5.57 (8) (.37)	—	—	—
AST (U/L) <sup>a</sup>	51 (90)	52 (16) (.51)	85 (8) (.02)	—	—	—
ALT (U/L) <sup>a</sup>	72 (94)	87 (16) (.15)	155 (8) (.01)	—	—	—

For leukocyte differential counts, the number of observations vary because results for eosinophils, monocytes, basophils, and bands were not entered for some study subjects.

<sup>a</sup> HCV-infected hemophiliacs only.

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

among heterozygotes were compared with those for CCR5-+/+ study subjects, a similar increase was noted among patients with hemophilia ( $p = .02$ ), but not homosexual men. Compared with CCR5-+/+ study subjects, total CD4<sup>+</sup> lymphocytes were higher among CCR5-Δ32/Δ32 homozygotes with hemophilia, but not among homosexual men. There were no consistent differences between genotypes for other leukocyte blood cell types.

For 10 CCR5-Δ32/Δ32 homozygotes, blood chemistry results were found in the clinical record. Comparing the most recent result to the testing laboratory's reference range, hepatic enzymes and bilirubin were commonly elevated among the HCV-infected patients with hemophilia, but abnormal results for other measures (blood urea nitrogen, creatinine, uric acid, CO<sub>2</sub>, calcium, phosphorus, albumin, electrolytes, lactate dehydrogenase, alkaline phosphatase, total protein) were observed in only 1 or 2 CCR5-Δ32/Δ32 study subjects each. Hepatic enzyme results were available from study records for most patients with hemophilia. Among HCV-infected patients with hemophilia, mean aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were 67% and 117% higher, respectively, among CCR5-Δ32/Δ32 homozygotes than CCR5-+/+ study subjects (Table 2). Elevated hepatic enzymes among CCR5-Δ32/Δ32 patients with hemophilia were not explained by higher viral burden, because serum HCV RNA levels did not vary by genotype (Table 2).

Consistent with the high prevalence of bloodborne in-

fections among persons with hemophilia, antibody was detected in 11 of 11 CCR5-Δ32/Δ32 patients with hemophilia tested for HBV and 10 of 12 (83.3%) tested for HCV. For 9 of 10 common viruses, antibody prevalence was similar in CCR5-Δ32/Δ32 study subjects and the reference population (Table 3). Antibody to measles virus was, however, less common than expected among CCR5-Δ32/Δ32 study subjects, especially in those born before 1957 (4 of 7; 57%) who would be expected to have high levels of immunity acquired through natural exposure to measles virus. In an attempt to verify this association in a general population, we determined CCR5-Δ32 genotype for a group of U.S. blood donors born before 1957. All 14 CCR5-Δ32/Δ32 homozygotes in this group had detectable measles antibody levels. In addition, peripheral blood mononuclear cells from CCR5-Δ32/Δ32 or CCR5-+/+ individuals replicated Edmonston wild-type measles virus or the Bilthoven strain of measles virus to similar levels (data not shown), indicating that CCR5 was not required for infection with these strains.

## DISCUSSION

In this study, most clinical and laboratory parameters were similar in CCR5-Δ32/Δ32 homozygotes compared with CCR5-+/+ study subjects or reference populations. Together with previous data indicating that CCR5-Δ32 genotypic frequencies conform to the expected Hardy-Weinberg distribution (11), we conclude that the CCR5-Δ32/Δ32 homozygous genotype has no highly lethal

**TABLE 3.** Number of *CCR5-Δ32* homozygotes with antibodies against selected viral infections (cryopreserved serum from 11 men with hemophilia and 2 homosexual men)

Virus	Number of study subjects with antibody	Proportion (%) of reference population with antibody <sup>a</sup>	<i>p</i> Value <sup>b</sup>
Cytomegalovirus	5	47	.74
Epstein-Barr virus	13	85	.24
Varicella zoster virus	12	98	.46
Respiratory syncytial virus	13	95	1.00
Herpes simplex virus	9	72	1.00
Influenza A virus	13	93	.78
Influenza B virus	13	93	.78
Rubeola/measles <sup>c</sup>	9	99/105	.01 <sup>d</sup>
born before 1957 ( <i>n</i> = 7)	4	19/19	.01 <sup>d</sup>
born in or after 1957 ( <i>n</i> = 6)	5	80/86	.39
Mumps	12	93	1.00
Rubella	11	93	.46

<sup>a</sup> Data for reference population provided by commercial laboratory (Wold AD, personal communication, March 4, 1997).

<sup>b</sup> Binomial test, except for rubeola/measles (Fisher's exact test). Availability of reference population data did not permit Fisher's exact calculations for all viruses.

<sup>c</sup> Study subjects born before 1957 were less likely to have received measles vaccine (licensed in the United States in 1963) and more likely to have had natural exposure to measles (39).

<sup>d</sup> Statistically significant,  $\alpha = 0.05$ .

phenotypic expressions and that persons with this genotype are generally similar to wild-type subjects. These overall observations support the development of therapeutics designed to mimic *CCR5-Δ32/Δ32* homozygosity. We did, however, find statistically significant elevations among *CCR5-Δ32/Δ32* homozygotes in hypertension prevalence, lymphocyte counts, and, for HCV-infected patients with hemophilia, hepatic enzyme levels. These three findings require further consideration.

Although our study population was relatively young (median age at study entry, 25 years for patients with hemophilia and 33 years for homosexual men), 7 of 11 *CCR5-Δ32/Δ32* homozygotes with evaluable clinical records were hypertensive on the basis of antihypertensive therapy or blood pressure. Compared with matched *CCR5-+/+* study subjects, *CCR5-Δ32/Δ32* homozygotes were 2.8-fold more likely to be hypertensive. Although a substantial proportion of blood pressure variation may be genetically determined, the few recognized mendelian forms of hypertension result from rare alleles that cause severe hypertension by altering the renin-angiotensin system (32). It may be noteworthy that *CCR5* and *AT1*, the major angiotensin receptor, are both seven-transmembrane G protein-coupled receptors (32) and that these two receptors share ~60% amino acid homology. If the association between *CCR5-Δ32/Δ32* homozygosity and hypertension is verified in other populations, a possible interaction with *AT1* should be explored.

Compared with *CCR5-+/+* study subjects, mean total

lymphocyte counts were 20% higher in *CCR5-Δ32/Δ32* subjects; each individual count of the *CCR5-Δ32/Δ32* homozygotes, however, was within the reference range. Given that  $\beta$ -chemokines regulate lymphocyte trafficking, it is plausible that *CCR5-Δ32/Δ32* homozygotes, who may have elevated levels of  $\beta$ -chemokines, could have slightly elevated lymphocyte counts. Because lymphocytes are an important component of the hepatic infiltrates found in chronic HCV infection (33), our finding of elevated transaminase levels among HCV-infected *CCR5-Δ32/Δ32* homozygotes might be related to an increase in the number of lymphocytes or an enhanced T-cell-dependent immune response, as reported in mice that lack *CCR5* (21). Regardless, we found no evidence that the *CCR5-Δ32/Δ32* genotype was clinically deleterious or beneficial for HCV-infected patients with hemophilia: none of these men developed hepatic failure and HCV serum levels did not differ by genotype.

Although impaired macrophage function has been reported in mice lacking *CCR5* (21), we found no evidence of an increased frequency or unusual pattern of infections among the *CCR5-Δ32/Δ32* study subjects. We also found no evidence that *CCR5-Δ32/Δ32* homozygosity provides broad resistance to viral infections. Patients with hemophilia who are homozygous for *CCR5-Δ32/Δ32* had high rates of HBV and HCV infection, and the prevalence of antibodies against nine common viruses was similar in the *CCR5-Δ32/Δ32* homozygotes and in the reference population. The exception to this pattern was a lower prevalence of detectable antibodies to



measles virus in CCR5-Δ32/Δ32 study subjects than in study subjects with other genotypes, but this association was not confirmed in a population of blood donors or in infectivity studies.

The limitations of our study should be considered. Multiple comparisons raise the possibility that a statistically significant result is due to chance (type I error). Conversely, because of the limited number of CCR5-Δ32/Δ32 homozygotes, subtle manifestations of that genotype may have gone undetected (type II error). In addition to these statistical concerns, our findings are potentially confounded by associations between hemophilia type or severity and the outcomes we examined. Because CCR5-Δ32/Δ32 homozygosity confers resistance to HIV-1 infection, CCR5-Δ32/Δ32 homozygous patients with hemophilia resemble HIV-1-infected people with hemophilia in that they have a higher frequency of hemophilia A and received higher dosages of factor VIII concentrate than most HIV-1-uninfected patients with hemophilia. Although two studies suggest that people with hemophilia have a modestly elevated prevalence of hypertension compared with reference populations, neither found an association with hemophilia severity (35,36). On the basis of these studies, it is unlikely that confounding explains the higher prevalence of hypertension among CCR5-Δ32/Δ32 homozygotes. It is also unlikely that higher lymphocyte counts among CCR5-Δ32/Δ32 homozygotes reflect greater factor VIII concentrate exposure. Lymphopenia has been noted in some cohorts of hemophilia patients, (29,37,38) and these lower lymphocyte counts appears to be unrelated to hemophilia treatment status (37). Among HIV-1-uninfected CCR5+/+ and CCR5-Δ32/Δ32 participants in this study, lymphocyte counts did not vary significantly by either hemophilia type or the amount of plasma product received (data not shown).

We are unaware of any previous detailed reports examining phenotypic expressions of the CCR5-Δ32/Δ32 homozygous genotype. An initial paper reporting the CCR5-Δ32 allele described two CCR5-Δ32/Δ32 homozygous homosexual men as healthy, without obvious clinical conditions (9). A larger study, such as ours, is necessary to detect the relatively subtle differences we observed in CCR5-Δ32/Δ32 homozygotes: modestly increased lymphocyte counts, higher hepatic enzyme levels in the presence of HCV infection, and an increased prevalence of mild to moderate hypertension. It is biologically plausible that these findings result from CCR5-Δ32/Δ32 homozygosity, but confirmatory investigations are required to determine whether they are true phenotypic expressions that need to be considered in the de-

velopment of therapeutic modalities that mimic this genotype.

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