Effect of Chemokine Receptor Mutations on Heterosexual Human Immunodeficiency Virus Transmission

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To assess the effect of mutations at the CCR-2 and CCR-5 loci on heterosexual human immunodeficiency virus (HIV) transmission, 144 persons heterosexually exposed to HIV (infected and uninfected [EU]) and 57 HIV-positive index partners were genotyped. A significantly higher frequency of 64I heterozygotes at CCR-2 was observed in HIV-positive than in EU women ($P = .02$, relative risk = 1.6). The allele frequency of 64I in women was 8% in HIV-positive contacts and 1% in EUs ($P < .02$). At CCR-5, no difference in the frequency of Δ32 was seen between groups, and the CCR-5 genotypes did not differ in accumulated "at-risk" exposure in EUs. Combining the analysis of the Δ32 and 64I mutations in index partners suggested an additive effect on transmission ($P = .10$). Thus heterozygosity for 64I at CCR-2 acts as a risk factor for HIV infection of women after heterosexual contact but heterozygosity for Δ32 at CCR-5 has no detectable effect.

Until the last few years, genetic factors shown to affect the rate of progression to disease in human immunodeficiency virus (HIV)–infected persons was limited to human leukocyte antigen (HLA) alleles [1–3]. However, no HLA type has clearly correlated with protection from infection [4], although some genetic component conveying resistance is possible. This was confirmed after the discovery of the coreceptors for HIV entry. The predominant coreceptor used by non–T cell line–adapted strains of virus is the C-C chemokine receptor-5 (CCR-5) [5–8], and several highly exposed uninfected (EU) persons were found to be homozygous for a 32-bp deletion (Δ32) in the CCR-5 gene [9], resulting in a lack of functional receptor expression. The deletion is present in white populations at an allele frequency of ≈10% [9–11] but absent in persons of all other ethnic backgrounds.

Extensive screening of HIV EU and infected cohorts revealed that homozygosity for the deletion in CCR-5 conferred significant protection against infection in homosexuals and hemophiliacs and was present almost exclusively in the uninfected persons [10, 12–15]. In addition, the studies showed that heterozygosity was associated with a slower progression to AIDS in HIV-positive homosexuals, although it conferred no protection against infection after homosexual contact.

A further genetic polymorphism in the chemokine receptor CCR-2 has been reported [16–18]. This polymorphism corresponds to a single base change (G→A position 190), resulting in the conservative change of valine to isoleucine at amino acid position 64 (64I). The 64I mutation, which causes the first transmembrane loop of CCR-2 to become identical to the corresponding domain in CCR-5, was present in all ethnicities studied and was associated with slower disease progression [16–18]. However, this mutation was not protective for infection in EU homosexuals; homozygotes for the mutation 64I/64I were seen in both HIV-positive and uninfected cohorts [16–18]. Kostrikis et al. [17] have since suggested a mechanism by which this conservative mutation, in a receptor rarely used by HIV, exerts an effect. They showed the mutation to be in 100% linkage disequilibrium with a mutation in the promoter region of CCR-5. More recently, several other polymorphisms in the CCR-5 promoter region have been reported, and Martin et al. [19] showed that homozygosity for 1 of 10 haplotypes correlated with accelerated disease progression.

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The risk of acquiring HIV after heterosexual exposure is less than for homosexual contact [20], presumably reflecting differences between the mucosal surfaces of the rectum and vagina. We investigated a cohort of heterosexual couples discordant for HIV serostatus and selected subjects repeatedly exposed to HIV who remained uninfected. In order to assess the effect of genetic variation at the chemokine receptor loci on heterosexually acquired infection, these EUs were screened for the presence of the CCR-5 deletion and the CCR-2 mutation. The frequency obtained was then compared with those in heterosexual HIV-infected subjects (HIV positives) and in low-risk uninfected controls. HIV-positive index partners who transmitted virus heterosexually and the nontransmitting index partners were also screened to ascertain whether these mutations have any effect upon the transmission of HIV during heterosexual exposure. In addition to analyzing the effects of each locus separately, we also performed a joint analysis to evaluate whether they were acting independently. The CCR-5 promoter genotypes were also determined in EUs, heterosexually exposed HIV-postivies, and low-risk controls to determine whether they affect the risk of HIV transmission heterosexually.

Material and Methods

Subjects. The Edinburgh Heterosexual Partner Study (EHPS) recruited heterosexual partners (contacts) of HIV-positive patients (indexes), whose only risk factor for HIV infection was sexual contact with an index patient. The majority of the index patients were infected after an epidemic of HIV-1 subtype B among injecting drug users (IDUs) [21]. At the initial interview, the length of the relationship and frequency and nature of sexual contacts were determined and an HIV test was offered along with counseling. If the interview revealed evidence of continued needle-sharing, those persons were excluded from this analysis. If discordant for HIV serostatus at recruitment, couples were followed at ~6-month intervals at the clinic or by home visit, and HIV status, sexual behavior, and contraceptive use were reassessed. Interviews were done by a trained research nurse. A summary of information about the cohort is given in table 1 [22].

We identified 58 (44 women, 14 men) heterosexual EUs for whom DNA samples were available from the EHPS on the basis of their continued seronegative status despite high-risk exposure to HIV-1. We also analyzed DNA samples from 86 persons (65 women, 21 men) infected with HIV by heterosexual contact. Of these samples, 62 (45 women, 17 men) were obtained from the MRC Molecular Epidemiology Repository, Edinburgh [21]. The remaining 24 samples (20 women, 4 men) were from HIV-infected contacts from the EHPS. We also analyzed control samples from 50 persons selected for a study of polycystic kidney disease without regard for risk of HIV infection.

HIV-positive heterosexual partners of the EUs (nontransmitting indexes [NTRIs]; $n = 38$; 10 women, 28 men) and persons who transmitted HIV by heterosexual contact (transmitting indexes [TRIs]; $n = 19$; 3 women, 16 men) were also selected from the EHPS cohort. All subjects were white; 95% resided in central Scotland.

Deduction of exposures for EUs. Initial interview data were assessed and the number of sexual episodes deduced from the frequency of "at-risk" sexual activity reported for the 5 years preceding the interview or the duration of the relationship if<5 years. Any periods of abstinence during this time were subtracted as were the number of protected sexual episodes estimated from the reported frequency of condom usage. Follow-up data were analyzed similarly and added to the value obtained for the initial interview. Further details of risk assessment have been published [22, 23].

Table 1. Study cohort summary.

	Women	Men	Total
Contacts[a]	180	66	246
Mean age ± SD	26 ± 6	30 ± 6	
Mean duration of relationship (months)[b]	52	42	
Non-IDU risk contacts[c]	118	34	152
HIV-negative at recruitment[d]	93	27	120
Seroconversions during follow-up	1	1	2
EUs for whom DNA sample was available[e]	44	14	58

NOTE. These data have been published [22]. Further details of cohort are outlined in Material and Methods.

[a] Total contacts recruited into study.

[b] Did not differ significantly when those with injecting drug use (IDU) risk were excluded.

[c] Persons deemed at risk of infection by IDU were identified at interview and excluded.

[d] Persons who were found to be discordant for HIV serostatus (HIV-negative) at recruitment.

[e] Exposed uninfected persons (EUs) with DNA sample available for research purposes.

Polymerase chain reaction (PCR) analysis of CCR-5 gene. Genomic DNA was extracted from either Epstein-Barr virus–transformed B cell lines derived previously from some subjects or archived cryopreserved peripheral blood mononuclear cells (PBMC) as described previously [24]. Where only plasma samples were available, we used a more sensitive extraction method as outlined by Boom et al. [25].

We determined genotypes by PCR amplification of the region of the CCR-5 gene in which the deletion site is located by using the following primers: sense, 5′-CAAAAAGAAGGTCTTCATTACACC-3′ and antisense, 5′-CCTGTGCCTCTTCTTCTCATTTCG-3′, as described by Huang et al. [26]. The resulting fragments, 189 bp (wild type, WT/WT), 157 bp (mutant, Δ32/Δ32), or both (heterozygotes, WT/Δ32) were resolved on a 3% Metaphor gel (FMC BioProducts, Rockland, ME). In addition, selected samples from each experiment were amplified with the sense primer 5′-CTCGGATCCGGTGGAACAAGATGGATTAT-3′ [7] and the antisense primer from the above reaction using identical conditions. The resulting 706-bp product was digested with *Bgl*II (Boehringer Mannheim, Indianapolis) resulting in fragments of 511 and 195 bp for WT/WT, 511 and 163 bp for Δ32/Δ32, and 511, 195, 163 bp for WT/Δ32.

PCR analysis of the CCR-2 gene. We obtained genomic DNA as above and amplified a 128-bp fragment of the CCR-2 gene using the following primers [16]: sense, 5′-TTGTGGGCAACATGATGG-3′, and antisense, 5′-GAGCCCACAATGGAGAGTA-3′. Amplification was performed as for CCR-5, but the annealing temperature was raised to 60°C for all cycles. The sense primer contains a mismatch base (C→A position 184), which in the presence of the mutation (G→A position 190) generates a restriction site for the enzyme *Bsa*BI in the amplified product. The resulting 128-bp prod-

Table 2. CCR-5 and CCR-2 genotypes and allele frequencies in controls, exposed uninfected persons, and human immunodeficiency virus (HIV)–positive groups.

	CCR-5[a]				CCR-2[b]			
	WT/WT	WT/Δ32	Δ32/Δ32	Δ32 allele frequency (95% CI)[c]	WT/WT	WT/64I	64I/64I	64I allele frequency (95% CI)[c]
Exposed seronegative, $n = 58$	40 (69)	17 (29)	1 (2)	16 (10–24)	54 (93)	4 (7)	0	3 (1–8)
HIV-positive, $n = 86$	63 (73)	23 (27)	0	13 (8–19)	72 (84)	13 (15)	1 (1)	9 (5–14)
Population controls, $n = 50$	38 (76)	10 (20)	2 (4)	14 (8–22)	43 (86)	7 (14)	0	7 (3–14)

NOTE. Data are no. (%) or % allele frequency (95% confidence interval [CI]).
[a] WT/WT, homozygous wild type CCR-5 genotype; Δ32/Δ32 for homozygous mutant for 32-bp deletion in CCR-5 gene and WT/Δ32 for heterozygote.
[b] WT/WT, homozygous wild type CCR-2 genotype; 64I/64I for homozygous mutant for valine-to-isoleucine a-a change and WT/64I for heterozygote.
[c] 95% binomial CIs.

uct was ethanol precipitated and digested with *Bsa*BI (New England BioLabs, Beverly. MA). The samples were then resolved on a 3% Metaphor gel. When the mutation was present, the mismatch base in the sense primer allowed the digestion of the 128-bp PCR product to 110- and 18-bp fragments. Homozygous wild type (WT/WT) persons showed only the uncut 128-bp product; homozygous mutant persons (64I/64I) showed the digested 110-bp fragment (since the 18-bp fragment was too small to be resolved); heterozygous persons showed both the 128- and the 110-bp fragments.

CCR-5 promoter genotyping. CCR-5 promoter genotypes were determined as outlined elsewhere [19] for the heterosexual EUs ($n = 51$), HIV positives ($n = 70$), and population controls ($n = 48$).

Statistical analysis. Exposure levels for the EU group were analyzed by t test after log transformation. Genotype frequencies were tested by χ^2 (rare homozygous mutant persons were pooled with heterozygotes to avoid low expected values) and by Fisher's exact test. Binomial confidence intervals were interpolated from table W of Rohlf and Sokal [27], and probability values for the differences between means were obtained from the standardized normal deviate.

Results

Genotype and Allele Frequencies in Heterosexual HIV EUs

CCR-5. We observed 3 homozygotes for the Δ32 mutation. All were HIV negative: 2 were from the control group and 1 was an EU (table 2). There was no difference in genotype frequencies among EUs, heterosexually infected, and control subjects ($\chi^2 = .69$, $P = .71$ [homozygous mutant persons combined with heterozygotes because of the few homozygotes]) or significant departure from Hardy-Weinberg predicted frequencies. Direct comparison of heterosexually exposed infected and uninfected persons revealed neither significant difference in CCR-5 genotype frequencies nor significant differences in the mean allele frequencies between groups (table 2: 16% EUs, 13% HIV positive subjects, 14% control subjects). When all groups were combined, the overall frequency of the Δ32 allele in this population was 14% (range, 9%–23%). Because male-to-female transmission has a greater relative risk [RR] than female-to-male transmission [23, 28], women contacts were analyzed separately (table 3: EUs $n = 44$, HIV-positives $n = 65$); however, there was still no significant difference. In view of the low number of men contacts, no separate analysis is included.

CCR-2. Only 1 homozygote for the 64I mutation was seen (in the HIV-positive group; table 2). As for CCR-5, no significant heterogeneity was observed among the genotype frequencies of the 3 groups (table 2; $\chi^2 = 2.79$, $P = .25$ [homozygous mutant persons were combined with heterozygotes because there were few homozygotes]). However, there were more heterozygotes among HIV-positive subjects than among the EUs; a difference that was near significance ($P < .08$, exact test, one-tailed); when women contacts were considered separately, this difference was significant ($P = .02$, exact test, one-tailed). The 64I mutant allele thus appears to be a risk factor for heterosexual infection of women (RR = 1.6; range, 1.3–2.1). The allele frequency of 64I was also increased in HIV-positive groups compared with EUs (table 2; $z = 1.77$, $P = .08$, both sexes; table 3; $z = 2.33$, $P < .02$, women only).

Combined CCR-2/5 analysis. Subjects WT at both the CCR-2 and CCR-5 loci were compared with those of all other possible genotypic combinations (WT/Δ32, 64I/WT, 64I/Δ32, Δ32/Δ32, and 64I/64I), but no significant difference in frequency was observed among the 3 groups ($\chi^2 = .38$, $P = .83$). The difference between heterosexually exposed groups only (EUs and HIV-positive subjects) also was not significant.

CCR-5 promoter genotypes. The CCR-5 promoter genotypes (as described in [19]) were determined for a proportion of subjects screened for CCR-2 and CCR-5 genotypes for whom appropriate material was available (table 4). Of the 10 alleles described, only P1, P2, and P4 were seen in this cohort.

The heterosexually exposed subjects (EUs or HIV positive subjects) had a similar distribution of both haplotypes (data not shown) and genotypes (table 4); no significant differences were seen among the 2 groups. However, the P2 haplotype was higher in the control group (14%) than in EUs (6%; $z = 1.83$, $P = .067$) and HIV positive subjects (6%; $z = 1.85$, $P = .064$), a difference that approached significance. Genotypes that contain the P2 allele (table 4) were more common in the control group, and the difference for P1/P2 was significant (21% of control subjects, 8% of EUs, 7% of HIV positive subjects; table 4; $\chi^2 = 6.19$, $P = .045$). The significance of this difference in the control group is unclear; however, the heterosexually ex-

Table 3. CCR-5 and CCR-2 genotypes and allele frequencies in exposed uninfected persons and human immunodeficiency virus (HIV)–positive women.

	CCR-5[a]				CCR-2[b]			
	WT/WT	WT/Δ32	Δ32/Δ32	Δ32 allele frequency (95% CI)[c]	WT/WT	WT/64I	64I/64I (%)	64I allele frequency (95% CI)[c]
Exposed seronegative, $n = 44$	31 (71)	12 (27)	1 (2)	16 (9–25)	43 (98)	1 (2)	0[d]	1[e] (0–6)
HIV-positive, $n = 65$	48 (74)	17 (26)	0	13 (8–20)	55 (85)	9 (14)	1[d] (2)	8[e] (4–14)

NOTE. Data are no. (%) or % allele frequency (95% confidence interval [CI]).

[a] WT/WT, homozygous wild type CCR-5 genotype; Δ32/Δ32 for homozygous mutant for 32-bp deletion in CCR-5 gene and WT/Δ32 for heterozygote.

[b] WT/WT, homozygous wild type CCR-2 genotype; 64I/64I for homozygous mutant for valine-to-isoleucine a-a change and WT/64I for heterozygote.

[c] 95% binomial CIs.

[d] $P = .02$, exact test (1-tailed), comparing CCR-2 genotype frequencies in 2 groups and pooling classes to avoid low expected values.

[e] $z = 2.33$, $P < .02$, difference between allele frequencies established from standardized normal deviate.

posed infected and uninfected groups did not differ in the distribution of the CCR-5 promoter alleles and genotypes. This was not altered by separate consideration of men and women (data not shown).

Effect of exposure on genotype distribution. The availability of quantitative data on exposure levels within the EU group allowed us to investigate whether CCR-5 genotypes differed with respect to the average exposures accumulated without seroconversion. Because few EUs expressed the CCR-2 mutation (table 2), this analysis is presented only for the CCR-5 genotypes.

The level of at-risk exposure ranged from <50 sexual contacts to >1000 (figure 1). The range of exposures for both WT/WT and WT/Δ32 was evenly spread, with no difference with respect to genotype. Median values of 131 and 118 for the WT/WT and WT/Δ32, respectively, were obtained. The accumulated mean exposure for WT/WT and WT/Δ32 heterozygotes did not differ significantly ($t = .14$, $P = .89$).

When female contacts were analyzed separately, the median accumulated exposures were WT/WT, 100, and WT/Δ32, 129, but the difference was not significant. We concluded there was no difference among CCR-5 genotypes in total sexual contacts between uninfected women and their infected partners.

Genotype and Allele Frequencies in HIV-Positive Partners

CCR-5. HIV-positive index partners were grouped according to whether they transmitted the virus to their partner during the study (TRIs, $n = 19$; 3 women, 16 men) or did not (NTRIs, $n = 38$; 10 women, 28 men). No significant difference was observed between CCR-5 genotypes in the 2 groups (table 5), even when only men subjects were examined (data not shown). No homozygous Δ32/Δ32 persons were identified in either group, and the allele frequencies did not differ significantly (table 5: $z = .93$, $P = .35$, both sexes; $z = .59$, $P = .56$, men only; data not shown).

CCR-2. Genotype frequencies for CCR-2 did not differ between TRIs and NTRIs when both sexes were included (table 5) or for men only (data not shown). Allele frequencies also did not differ (table 5: TRIs, 3%; NTRIs, 11%; $z = 1.47$, $P = .14$, both sexes; $z = 1.27$, $P = .20$, men only; data not shown).

Combined CCR-2/5 analysis. When we compared the frequency of persons WT at both the CCR-2 and CCR-5 loci with those with all other genotypic combinations (as outlined above) (TRIs, 14 WT:WT, 5 non-WT:WT; NTRIs, 20 WT:WT, 18 non-WT:WT) the difference between the 2 groups increased ($P = .10$, exact test, one-tailed). Thus the two mutations appeared to have an additive effect on the probability of heterosexual transmission by HIV-positive index partners.

Discussion

We studied the distribution of mutations in genes encoding the chemokine receptors CCR-2 and CCR-5 in a cohort of heterosexually exposed subjects and population controls. An overall frequency for the mutant CCR-5 allele of 14% (range, 9%–23%) was obtained, which is higher than reported for continental European white populations (9.2%, $n = 704$; 9.8%, $n = 122$) [9, 11] and significantly higher than the 8% found in an American population (95% white; $n = 637$ [26]).

Previous studies established that homozygosity for the CCR-5 mutant allele substantially protected subjects from HIV-1 infection after homosexual contact [10, 26], with EUs showing an increased frequency of Δ32/Δ32 homozygotes and departing from Hardy-Weinberg expected frequencies. We, too, found Δ32/Δ32 persons only in our HIV-negative groups, but found no departure from Hardy-Weinberg expected frequencies. Although the lack of observed effect may be due to smaller sample size, the increased allele frequency in this Scottish population would have made any effect easier to detect.

Although persons heterozygous for the Δ32 mutant in CCR-5 have shown a slower rate of progression in several US cohorts [10, 29–31], no effect of heterozygosity on transmission has been seen, including transmission by heterosexual sex [32]. In a small-scale study of a racially mixed group, Hoffman et al. [33] found that heterosexual but not homosexual couples had increased WT/Δ32 heterozygotes in the exposed uninfected partners compared with HIV-positive partners. In our study group, which was substantially larger than Hoffman's and carefully assessed for exposure, there was no significant difference in the CCR-5 Δ32 genotype distribution of the heterosexual

Table 4. Frequency of CCR-5 promoter mutation genotypes in exposed uninfected (EUs), human immunodeficiency virus (HIV)–positive, and control groups.

	P1/P1	P1/P2[a]	P1/P4	P2/P2	P2/P4	P4/P4
EU, $n = 51$	19 (37)	4 (8)	21 (41)	1 (2)	0	6 (12)
HIV-positive, $n = 70$	29 (41)	5 (7)	26 (37)	1 (1)	2 (3)	7 (10)
Controls, $n = 48$	16 (33)	10 (21)	13 (27)	0	3 (6)	6 (13)

NOTE. Promoter haplotypes outlined elsewhere [19]. Data are no. (%).

[a] $\chi^2 = 6.19$, $P = .045$, comparing genotype frequencies of this genotype among 3 groups.

EUs compared with heterosexually infected persons. Because some of the HIV-positive subjects studied by Hoffman et al. [33] became infected by other modes of transmission, they were unable to directly assess the effect of heterozygosity on heterosexually acquired infection.

In order to address the effects of heterozygosity at the CCR-5 locus on infection more sensitively, we analyzed the distribution of WT/Δ32 heterozygotes with regard to the level of at-risk exposure. Increased exposure confers an enhanced risk of infection [22, 23]; however, we found no evidence for any increase in the mean exposure accumulated for WT/Δ32 heterozygous EUs. Indeed, the highest levels of exposure (estimated unprotected sexual contacts >1000) were in persons who were wild type at the CCR-5 locus. Thus we found no evidence that heterozygosity for the Δ32 mutant CCR-5 provides significant protection against heterosexual HIV transmission. As the frequency of homozygotes for the mutant CCR-5 does not appear to be >1% in any white population and is absent in nonwhite populations, the Δ32 mutation may not contribute substantially to variation in susceptibility to heterosexual infection.

The more recently discovered point mutation in CCR-2, which causes a single amino acid change (V64I), also affects the rate of progression in seroconversion cohorts [16–18]. We analyzed the distribution of the CCR-2 mutation in our groups and found that, whereas the frequency of 64I in HIV-positive persons was similar to that of population controls, there were significantly fewer persons heterozygous for the 64I mutation in CCR-2 among EUs than in heterosexually infected HIV-positive subjects, when women only were analyzed. Thus, CCR-2 64I acted as a risk factor for heterosexual infection of women in this cohort (RR = 1.6). HIV transmission is less frequent from women to men than from men to women [20, 23, 28], so it is appropriate to analyze the sexes separately; more men

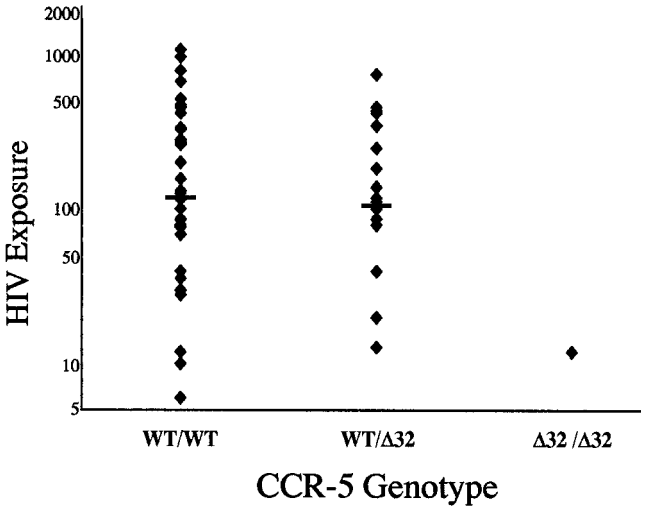

Figure 1. Human immunodeficiency virus (HIV) exposure among seronegative partners of HIV-positive persons related to CCR-5 genotype. HIV exposure levels (plotted on log scale) are no. of at-risk exposures for each contact person (estimated as outlined in Materials and Methods). Horizonal bars, median values. CCR-5 genotypes are identified as WT/WT for homozygous wild type, Δ32/Δ32 for homozygous mutant for 32-bp deletion in CCR-5 gene, and WT/Δ32 for heterozygote. Mean exposure values were 279.9 (WT/WT) and 215.9 (WT/Δ32) ($t = .14$, $P = .89$).

contacts would be required to detect any possible effect of genotype on the infection of men. The majority of contacts in this study were women, and the significance of the difference between genotypes was reduced ($P < .08$) when men contacts were included in the analysis. No difference in infection risk for 64I heterozygotes has been seen among homosexual contacts [16, 17, 34], which may reflect contrasting mechanisms of transmission in these risk groups.

The mechanism by which a conservative amino acid change in the transmembrane region of a receptor could affect transmission is not obvious but may be due to linkage disequilibrium with another mutation. The 64I mutation in CCR-2 is in strong linkage disequilibrium with a mutation, known as both 59673T and 927T, in the promoter region of the closely linked CCR-5 gene [17]. However, 927T is in a putative intron, and no obvious mechanism to explain these results has been forthcoming. We also assessed previously described [19] CCR-5 promoter hap-

Table 5. CCR-5 and CCR-2 genotypes and allele frequencies in transmitting indexes (TRIs) and non-TRIs (NTRIs).

	CCR-5[a]				CCR-2[b]			
	WT/WT	WT/Δ32	Δ32/Δ32	Δ32 allele frequency (95% CI)[c]	WT/WT	WT/64I	64I/64I	64I allele frequency (95% CI)[c]
TRIs, $n = 19$	15 (79)	4 (21)	0	11 (3–24)	18 (95)	1 (5)	0	3 (0–14)
NTRIs, $n = 38$	25 (66)	13 (34)	0	17 (10–27)	32 (84)	4 (11)	2 (5)	11 (5–20)

NOTE. Data are no. (%).

[a] WT/WT, homozygous wild type CCR-5 genotype; Δ32/Δ32 for homozygous mutant for 32-bp deletion in CCR-5 gene and WT/Δ32 for heterozygote.

[b] WT/WT, homozygous wild type CCR-2 genotype; 64I/64I for homozygous mutant for valine-to-isoleucine a-a change and WT/64I for heterozygote.

[c] 95% binomial CIs.

lotypes and genotypes in our cohort, but we found no haplotype or genotype differences between the 2 heterosexually exposed cohorts. One recent study [35] assessed the effects of the 64I polymorphism on coreceptor activity of CCR-2, CCR-3, CCR-5, and CXCR-4. It found that the 64I mutation had no effect on CCR-2 function or its use by HIV as a coreceptor, confirming the hypothesis that this conservative mutation does not alter CCR-2 greatly. Altered expression of CCR-5 and CXCR-4 was seen in the PBMC of WT/64I heterozygotes, although only the latter was significant. The mechanism by which such differences occur is unresolved and may reflect polymorphisms either in the CCR-5 gene or in as yet unidentified genes that may affect the level of expression of CCR-5, which correlates with infectibility [36].

Because genetic host factors influence progression [1–3, 10, 16–18, 29–31, 37] and the disease status or CD4 cell count of the transmitting index person has affected the probability of transmission in the EHPS and in other studies [23, 38], we also assessed whether the chemokine receptor genotype of the HIV-positive index patients affected the probability of transmission. No significant effect was seen for either mutation, although, when 64I and Δ32 mutations were analyzed together, an additive effect was seen that would require a larger study for confirmation. These mutations may affect progression by influencing virus load [39]. Differences in virus load could affect how much virus the contact is exposed to and hence alter the risk of transmission. However, direct assessment of virus load was not possible in this study. Viral variation is unlikely to be an important factor affecting the probability of transmission in this cohort, since viral strains from Edinburgh IDUs are closely related and fall into a single cluster within the B subtype [21].

In this analysis of the influence of genotype at CCR-5 and CCR-2 on heterosexual transmission, we found no evidence that either acts to protect the exposed person when heterozygous. More surprisingly, we found that the presence of the 64I alteration in CCR-2 increases the risk of infection in this cohort. This mutation may act as a marker for a mutation in the regulatory region of the CCR-5 gene [17], and we await further understanding of the functional effects of both mutations before this result can be more clearly defined. However, it is possible that a mutation of this kind in the regulatory region of the CCR-5 gene or other mutations in the CCR-5 gene may alter the level and expression of CCR-5 in different tissues and cell types and thus may affect the risk of heterosexual transmission.

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