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The Genetics of HIV Coreceptors and Coreceptor Ligands

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Abstract

CCR5 is the main coreceptor used by macrophage (M)-tropic strains of human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2), and is therefore essential for transmission of the disease. CXCR4 is the coreceptor for (T)-tropic strains. CCR5 binds a number of CC-chemokines, including MIP-1α, MIP-1β, RANTES, MCP-2 and MCP-3, while CXCR4 has only a single ligand, SDF-1. A number of genetic variants of genes encoding coreceptors and their ligands have been described, and some of these variants have been associated with resistance to HIV infection and/or disease progression. We review here the data accumulated so far concerning the variants of the CCR5, CCR2, SDF-1 and RANTES genes. For some of these variants, there is strong experimental evidence linking the modification of gene function with the phenotype of HIV resistance. In other cases, no functional alteration of the encoded proteins has been found, and the link between genotype and associated phenotype has not been demonstrated so far. The best characterized mutant is the Δ32 deletion mutant of the CCR5 gene, resulting in a non-functional protein that is not transported to the cell surface. Homozygotes for the Δ32 allele exhibit a strong, although incomplete, resistance to HIV infection. Heterozygotes were shown to display retarded progression to AIDS in most studies. Many other mutations of CCR5 have been described, some of which lead to non-functional receptors. These variants are, however, relatively rare and are incompletely characterized so far. Sequence variants in the CCR5 gene promoter have been reported, but the link with CCR5 expression is not clearly established, and the influence of these variant alleles on HIV infection and AIDS progression will require confirmation. A variant allele of CCR2 (CCR2-64I) was associated to delayed AIDS progression. This association was confirmed in several (but not all) studies, but the link between a fully functional variant of a minor coreceptor and the observed phenotype is presently unclear. A variant of the SDF-1 gene affecting a single nucleotide in the 3' non-coding region of the transcript was associated with delayed progression in homozygotes. This association was, however, not found in several other studies, and no modification of gene function could be demonstrated so far. A recently reported variant of the RANTES gene promoter, providing retarded progression, will also require confirmation in independent studies. As a rule, care must be taken before taking an apparent association between a genotype and a phenotype for granted, until functional data supporting a causality link between the variables is clearly established.

Key words

Chemokine receptors. HIV coreceptors. Genetic variants. CCR5. CCR2. SDF-1.

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HIV coreceptors

Members of the G protein-coupled receptor family act as coreceptors for HIV-1, HIV-2 and SIV[1,2]. The gp120 envelope protein of the virus interacts first with CD4, promoting a conformational change that unmasks the gp120 binding site for the coreceptor. The resulting trimolecular interaction between gp120, CD4 and the coreceptor further induces shedding of gp120, releasing the gp41 subunit of the envelope protein that mediates the ill-defined membrane fusion process itself. Many chemokine receptors and related G protein-coupled receptors, including CCR2b, CCR3, CCR8, CCR9, CX3CR1, ChemR23, APJ, Bonzo/STRL33, Bob/GPR15 and GPR1, have been described as coreceptors on the basis of *in vitro* fusion and infection assays[1,2]. However, most pathophysiological data concerning the viral life cycle *in vivo* are consistent with the use of two major coreceptors, CCR5 and CXCR4. CCR5 is the coreceptor used by macrophage (M)-tropic, non syncytium-inducing (NSI) HIV-1 strains. These strains are recovered during the first years following seroconversion, and are therefore considered as responsible for disease transmission. CXCR4 is used by T-tropic, syncytium-inducing (SI) strains and primary isolates that predominate during the late stages of the disease. On the basis of coreceptor usage, viral tropism has been redefined as R5 and X4 for respectively CCR5- and CXCR4-using strains[3].

CCR5 was first described as a receptor for the three related CC-chemokines MIP-1α, MIP-1β and RANTES[4]. More recently, it was found that MCP-2 also constitutes a high affinity agonist, and MCP-4 a weaker agonist[5-7], while MCP-3 acts as a natural antagonist of the receptor[7]. Its role as HIV coreceptor was suggested following the identification of MIP-1α, MIP-1β and RANTES as major HIV-suppressive factors[8], and this role was rapidly confirmed[9-13]. The essential role of CCR5 in HIV pathogenesis was demonstrated by the strong resistance to infection of individuals carrying two copies of a non-functional allele of the coreceptor gene[14,15]. As other receptors for inflammatory chemokines, CCR5 is involved in the recruitment of various leukocyte populations to inflammatory sites. CCR5 is expressed at the surface of T cells with a memory/effector phenotype, macrophages and immature dendritic cells, and microglial cells in the central nervous system[16-19]. CCR5 was shown to play a major role in a number of inflammatory diseases such as liver injuries associated with graft versus host disease, multiple sclerosis and rheumatoid arthritis[20-23]. However, given the redundancy of the chemokine system, a mouse knock out model for CCR5 was shown to display only a modest alteration of macrophage function, as illustrated by the reduced efficiency of Listeria infection clearance[24], and homozygotes for non-functional alleles of CCR5 in the human population do not appear so far to be prone to infectious diseases or immune dysregulations[25]. Various molecules interacting with CCR5 have been shown to interfere with viral infection. These molecules include the natural CCR5 ligands, such as MIP-1α, MIP-1β, RANTES and MCP-2[6,8], chemokine analogs such as aminooxypentane (AOP)-RANTES[2-68,26] and N-nonanoyl (NNY)-RANTES[2-68,27], monoclonal antibodies directed at various regions of the receptor[16,28,29], and small molecular weight ligands such as TAK-779[30]. A great part of the inhibition of viral entry mediated by molecules acting at CCR5 is due to receptor internalisation. The high efficiency of AOP-RANTES in HIV entry inhibition is attributed to its potent agonistic activity, and the profound phosphorylation and irreversible internalisation of the receptor it promotes[31,32].

CXCR4 was reported first as LESTR[33], an orphan receptor related to chemokine receptors, and later recloned as fusin, on the basis of a fusion screening assay, becoming the first coreceptor identified as such[34]. SDF-1 was subsequently reported as the ligand of CXCR4, and it is so far the only ligand known for this receptor[35,36]. As deduced from the phenotype of the CXCR4 and SDF-1 knock out models, SDF-1 is essential for lymphopoiesis, myelopoiesis, migration of cerebellar neurons and vascularisation of the gastrointestinal tract[37-39]. As for CCR5 ligands, molecules binding CXCR4 are able to prevent entry of strains using CXCR4 as coreceptor. SDF-1, peptides derived from SDF-1, monoclonal antibodies, as well as small molecular weight antagonists have been described[40-43].

The Δ32 mutation of CCR5

The search for mutations within the CCR5 coding sequence was stimulated by the identification of CCR5 as a major coreceptor for M-tropic HIV strains. Sequencing of the CCR5 coding region in individuals belonging to the general population, or to groups of exposed uninfected individuals, allowed the identification of a mutant allele of the CCR5 gene bearing a 32 bp deletion in a region corresponding to the second extracellular loop of the receptor[14,15,44]. This mutant encodes a receptor with only four transmembrane segments. As expected from a truncated structure, the Δ32 mutant is not functional as a chemokine receptor. The variant is not expressed at the cell surface, neither in natural cells such as T lymphocytes or macrophages, nor on cell lines transfected with the mutant cDNA. Antibodies directed at the N-terminal region of CCR5 can, however, detect an intracellular immunoreactivity that presumably corresponds to the improperly folded truncated receptor which is retained in the endoplasmic reticulum[45].

The Δ32 mutant was also defective as a coreceptor. When cotransfected with CD4 into cell lines, it did not allow entry of M-tropic HIV-1 strains[14]. Peripheral blood mononuclear cells (PBMC) prepared from individuals homozygous for the mutation turned out to be uninfectable with M-tropic HIV-1 strains, while entry and infection by T-tropic strains was unaffected[14,45]. In a first set of studies, no Δ32 homozygotes were found in large cohorts of seropositive individuals, suggesting that this mutation might protect completely from HIV infection[44].

The strong protection of homozygotes was confirmed in subsequent studies, including situations of massive parenteral contact with the virus[46], but a few seropositive individuals were reported as homozygous for Δ32, demonstrating that protection is incomplete[47-51]. In one of these cases, the HIV strain was characterised as using CXCR4 as coreceptor[52]. As mentioned above, no phenotype was found in individuals homozygous for the Δ32 mutation[25].

Heterozygotes were found to display slower progression to clinical stages of AIDS[44]. This association was found in most cohorts[53-62], but not all[63-65]. Some studies suggested that heterozygotes could be partially protected against HIV infection[55,66,67], but this was not confirmed in numerous other studies, and this effect, if real, must be considered as mild. Since heterozygotes for the Δ32 allele represent up to 30% in some populations (see below), it may have a significant impact on the average progression to AIDS in these populations. Δ32 heterozygozity has also been associated with a lower rate of development of non-Hodgkin lymphomas in AIDS patients[68,69], although the mechanism is not yet apparent.

CCR5 levels at the surface of leukocytes were found to be reduced in Δ32 heterozygotes as compared to homozygotes for the wild-type allele, affecting *ex vivo* infection of lymphocytes by M-tropic HIV-1 strains[16]. This observation suggests that regulation of CCR5 expression does not compensate for the non-functional allele. It was also proposed that CCR5 expression in heterozygotes was decreased by more than 50%, on average, suggesting that the variant receptor could act as a dominant negative mutant. A mechanism for this hypothetical effect was proposed, in terms of dimerisation of the mutant receptor with the wild-type receptor, preventing the normal traffic of the wild-type receptor to the cell surface and its retention in the endoplasmic reticulum[70]. More extensive analysis of CCR5 surface expression in Δ32 heterozygotes has, however, revealed that expression is on average half of what is found in wtCCR5 homozygotes[71]. The partial resistance to the virus can therefore be attributed to a gene dosage effect rather than a dominant negative property of the Δ32 mutant. The slower replication of M-tropic HIV-1 strains in cells expressing functional CCR5 from a single allele was confirmed in SCID mice grafted with human leukocytes derived from Δ32 heterozygotes[72].

The frequency of the CCR5Δ32 mutation was investigated in most populations around the world. The mutation is frequent in most European populations, with a North to South downhill gradient[73-75]. The highest allele frequencies (15 to 16%) are found in Northern Russia, Finland and Sweden. Allele frequencies around 10% are found across most Western and Central Europe countries, while the lowest frequencies (4-6%) are found in Southern countries like Portugal and Greece. Allele frequencies are about 2% in Northern Africa, and the Δ32 mutant is not found in Central and Western Africa. Frequencies also drop towards the East. Low frequencies are found in the Middle East and India and this allele is absent in China and Japan. In other parts of the world, the Δ32 mutation is found as a measure of immigration and/or admixture with populations of European origin. In African Americans, an allele frequency of about 2% is attributed to admixture[44].

The restricted distribution of the Δ32 allele suggests that this mutation occurred only once in the history of human populations, and relatively recently. This hypothesis was tested by genetic analysis of polymorphic markers located in the vicinity of the CCR5 gene. The study of microsatellites located 11 kb upstream and 68 kb downstream of the CCR5 deletion allowed us to demonstrate a strong linkage dis-equilibrium, the Δ32 allele being associated at a high frequency with microsatellite alleles that were otherwise rare in the population[74]. This confirmed the single origin of the mutation and allowed one to deduct the age of the gene mutant from the number of crossing-overs and microsatellite mutations that have occurred across history on chromosomes bearing the Δ32 allele. This led to an estimate of about 2000 years since the original mutational event. Another study based on the analysis of polymorphic markers located at a greater distance from CCR5, and on a statistical analysis of haplotypes provided a similar estimate of about 700 years[76]. The recent origin of the mutation suggests a positive selective pressure in favor of the mutant allele. It is not clear, however, what the nature of this selective pressure is and whether it is still active today. The HIV pandemia is certainly too recent to have played a role. The major role of CCR5 demonstrated in diseases such as rheumatoid arthritis might suggest hypotheses for such selection, although limited protective effects of the Δ32 allele on rheumatoid arthritis symptoms were observed[77]. Recently, it was suggested that individuals carrying the CCR5Δ32 mutation had a reduced risk of developing asthma[78].

Other CCR5 mutations

Other mutations affecting the coding sequence of CCR5 have been described[79-82]. So far, 22 variants have been found, 18 of which affect the primary structure of the receptor (Fig. 1). This high ratio of non-synonymous variants has been proposed as an additional element in favor of a positive selection for non-functional alleles of CCR5[79]. The functional consequences of most of these mutations have, however, not been investigated so far. Many variants are restricted to specific populations and are relatively rare. Homozygotes were therefore not identified, and the consequences of these mutations on HIV progression or other parameters could not be investigated. Some mutations affect the N-terminal domain of the receptor and could therefore influence the binding of chemokines or gp120 to CCR5. Other mutations involve transmembrane segments and intracellular domains, with potential consequences on receptor folding or signaling properties. So far, only a few mutants have been in-

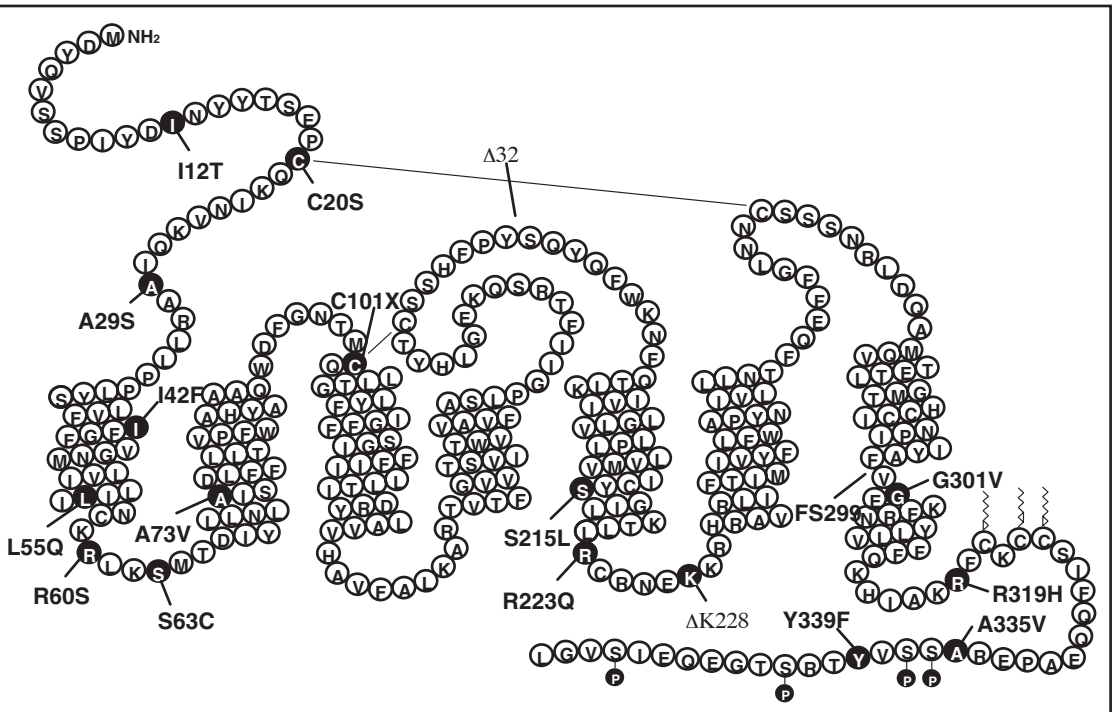

Fig. 1. *Schematic representation of CCR5.*
The putative transmembrane organization of CCR5 is represented, as well as the location of amino acid substitutions found, in natural variants of the receptor[14,15,79-82]. C101X is a mutation resulting in a stop codon. DK288 indicates the deletion of a full codon. FS299 is a frameshift that results in a truncated receptor. Putative palmitoylation and phosphorylation sites by the G protein-coupled kinases are indicated.

vestigated in terms of functional consequences[83]. The C20S mutant, that affects a disulfide bond essential for receptor function[107], is associated with poor expression at the cell surface, inability to bind or functionally respond to chemokines, and strong reduction in coreceptor function. A29S was found to result in poor chemokine binding, but kept unchanged its ability to mediate HIV entry. Other mutants, such as I42F, L55Q and A73V were associated with milder phenotypes. Among the other mutations, C101X and FS299 cause premature termination of the polypeptide chain and are therefore expected to result in non-functional receptors. The C20S, I42F and C101X alleles were found in seronegative high-risk individuals bearing the Δ32 allele on the other chromosome[79,82]. Although I42F appears as a functional HIV coreceptor, the function of C20S and C101X is strongly impaired. Individuals bearing CCR5Δ32 on one chromosome and I12T, A73V or L55Q on the other chromosome were seropositive[79], suggesting that these mutant genes encode functional coreceptors.

Variants of the CCR5 gene promoter

CCR5 expression was reported to be highly variable in individuals homozygous for the wild-type allele, and it has been shown that *ex vivo* infection efficiency can be corelated with CCR5 expression levels[16]. The fact that heterozygotes for the Δ32 mutation display slower progression demonstrated that cell surface expression of the coreceptor plays a major role in the effectiveness of viral replication. With the aim of uncovering the genetic substrate for CCR5 expression variability, various groups have investigated promoter variants and their correlation with AIDS progression.

The structure of CCR5 gene is represented in Fig. 2[85-88]. As for many other G protein-coupled receptors, the coding region is contained in a single exon. The major transcripts encoding CCR5 are under control of the so-called downstream promoter P_d. A 1.9 kb intron interrupts the 3' untranslated region of the transcripts resulting from the activity of P_d, between nucleotides –11 and –12 relative to the start of translation. Longer and rarer transcripts have been identified, involving two additional exons[86]. One of these exons is contiguous to the first exon transcribed from P_d. The other is separated by a 501 bp intron. Transcription starting at this site is dependent on the upstream promoter P_u.

A number of promoter variants have been described to date (Fig. 2), all consisting in single base substitutions in the P_d promoter region, the first exon of the major transcript, or the following intron[89-92]. A number of haplotypes was determined, and their frequencies and association with AIDS progression were analyzed. By including the Δ32 and CCR2-64I variants, Martin, *et al.*[90] identified six major haplotypes in Caucasians, one of them (CCR5P1) being associated with fast AIDS progres-

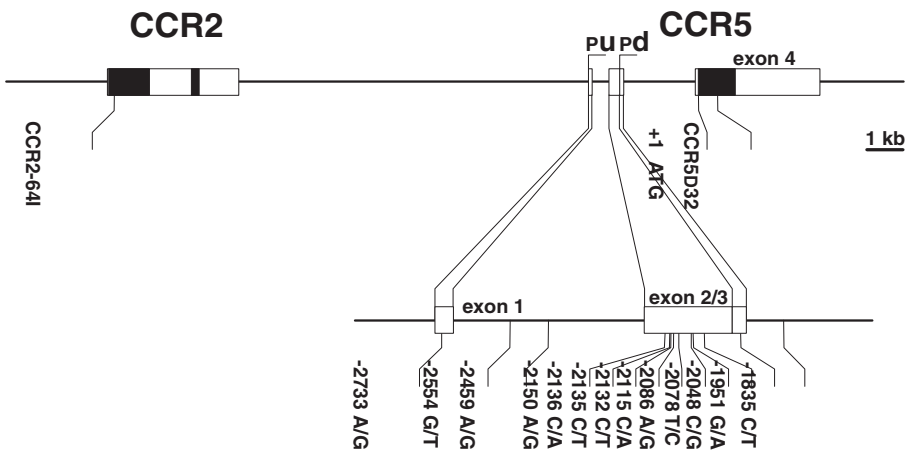

Fig. 2. *Structure of the CCR5 gene and flanking regions, including the CCR2 gene. Exons are indicated by boxes, and coding sequences are represented in black. The position of the upstream promoter (Pu) and downstream promoter (Pd) is indicated. The downstream promoter is used much more efficiently. The location of the sequence variants in the promoter region, associated or not with phenotypic variation of progression to clinical stages of AIDS, is indicated. The positions of the CCR5Δ32 and CCR2-64I mutations are indicated as well. Numbering is based on a translation start site as proposed at the CCR5-AIDS Symposium in April 1999[80].*

sion. Functional testing of the promoters, or direct measure of CCR5 expression on white blood cells from homozygotes for these haplotypes, did not allow the demonstration of a significant modification of CCR5 expression levels. An association with disease progression was also reported for a single base substitution (-2459) within the promoter[89].

Another study has identified additional haplotypes and demonstrated that the number of haplotypes frequent in Caucasians is much smaller than the number found in populations originating from Africa[92]. Analysing the correlation between CCR5 promoter haplotypes and AIDS progression, these authors found that the spectrum of CCR5 haplotypes associated with disease acceleration or retardation differs between African Americans and Caucasians. Moreover, these authors suggest a complex interaction between CCR5 haplotypes, the effect of one haplotype depending on the identity of the other allele. If confirmed, this complex interplay between CCR5 promoter alleles would hardly be consistent with a simple correlation between haplotype and CCR5 expression. Given the clustering of many CC-chemokine receptors around the CCR5 locus[100,101], some of which are of unknown function, it may well be that CCR5 promoter haplotypes corelated with AIDS progression represent no more than genetic markers for other unidentified gene variants on chromosome 3. It is also possible that the observed inter-individual variation of CCR5 expression, if genetically determined, may rely on the activity of transacting factors rather than the integrity of cis-regulatory elements.

The CCR2-64I variant

A mutant allele of CCR2 (CCR2-64I) was described, in which a valine within the first transmembrane segment of the receptor was replaced by an isoleucine[93]. The allele frequency does not vary much among populations, with an average frequency of 10 to 20%[94]. CCR2-64I has no influence on the incidence of HIV-1 infection, but heterozygotes for this allele were found to display slower progression to AIDS than homozygotes for the wild-type allele[93]. Some studies have confirmed the association of the CCR2-64I allele with slower disease progression[95,96], others did not[58,65,97,98]. One study reported a protective effect of the 64I variant on African Americans, but not on Caucasians[91].

As CCR2 is used as a coreceptor only by a few strains, it is unlikely that the effect of the mutation is similar to that of CCR5Δ32. Moreover, the V64I substitution in a transmembrane domain is conservative, and CCR5 contains an isoleucine at the corresponding position, so that the first α helix of the CCR2 mutant is identical to that of CCR5 (Fig. 3). CCR2-64I was shown to keep its functional properties in terms of chemokine binding, activation of intracellular cascades and coreceptor function[99]. Leukocytes express normal levels of the CCR2 mutant and the expression of CCR5 was not modified in cells coexpressing CCR2-64I[99]. It was suggested that the 64I allele could be a marker for another genetic variation on the same chromosomal segment, such as a modification of the CCR5 promoter. CC-chemokine receptor genes are clustered together on the short arm of chromosome 3[100,101]. The cod-

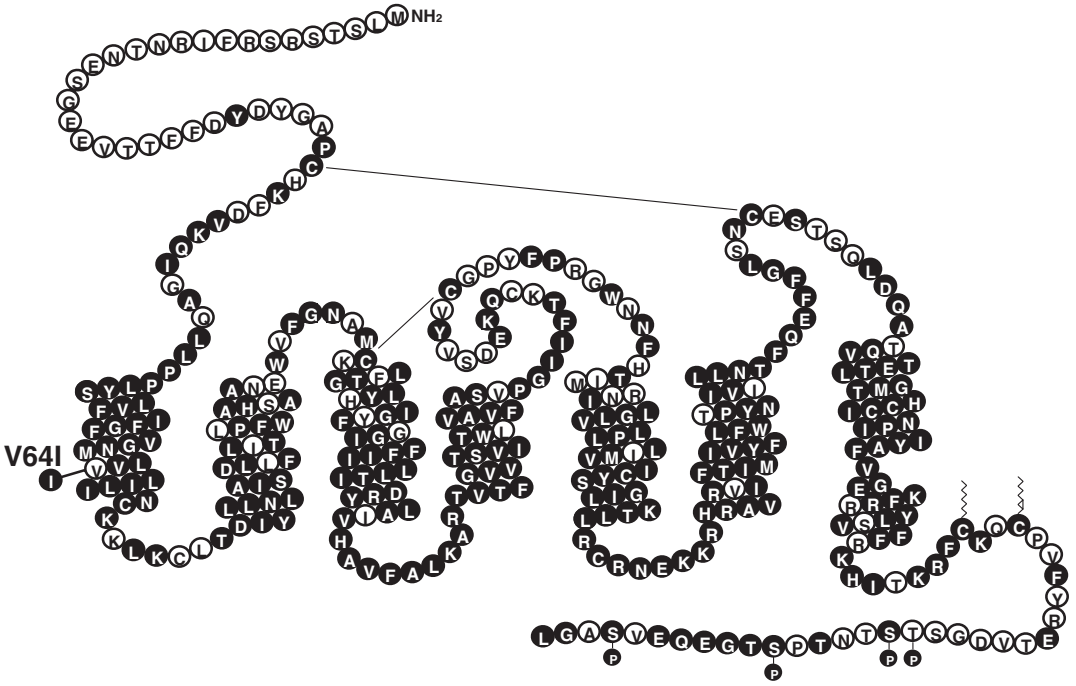

Fig. 3. *Transmembrane organization of CCR2b and location of the V64I mutation associated with delayed progression to AIDS. Amino acids identical between CCR2b and CCR5 are represented in black. Note that the substitution in the CCR2b sequence results in a first transmembrane domain which is fully identical to CCR5.*

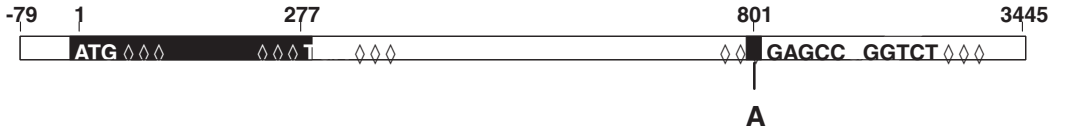

Fig. 4. *Structure of the SDF-1β cDNA and location of the 3' sequence variation (G to A at position 801) described as delaying AIDS progression for homozygous carriers[103]. The open reading frame is represented in black. Numbering is relative to the start codon.*

ing sequence of CCR2 is located some 17 kb upstream of that of CCR5 (Fig. 2), and other chemokine receptor genes, such as CCR1, CCR3 and the orphan receptor CCRL-2 are located at close proximity. The sequence of a BAC covering the CCR2 and CCR5 genes is available in the databases (GenBank accession number U95626). Although at larger distance, CCR4, CCR8, CCR9, CXCR1 and XCR1 are also located on the same chromosomal segment. A linkage between the 64I allele and a base substitution in the CCR5 gene (C to T transition at position -1835 relative to CCR5 translational start) has been described[102]. The nucleotide substitution is, however, located within an intron (Fig. 2), not in the CCR5 promoter, and careful analysis did not allow the demonstration of an association between this substitution and the level of CCR5 expression, neither *in vivo* nor *in vitro*.

The SDF1-3'A variant

The alteration of the CCR5 coding region and its relation to HIV-1 resistance has stimulated the search for other mutations in key genes encoding coreceptors or coreceptor ligands. No mutations affecting the primary structure of CXCR4 or SDF-1 have been described to date, in accordance with the essential role of these proteins during development[37-39]. A variation in a non-coding region of the transcript encoding SDF-1β was, however, described. A G to A transition (SDF1-3'A) was found at position 801 relative to the ATG start codon, within the 3526 bp transcript[103]. This allele was found to display variable frequencies in world populations, the highest frequencies being found in Asian (25-35%) and particularly Oceanian (50-70%) populations[94]. In the homozygous state, SDF1-3'A/3'A was reported to delay the onset of AIDS. Heterozygous individuals displayed progression rates similar to

homozygotes for the wild-type allele. It was suggested that increased stability of SDF-1 transcripts could explain the phenotype, resulting in higher production of SDF-1 and inhibition of the entry of CXCR4-using strains. Such an effect would however be expected to be dominant rather than recessive. Moreover, modifications of stability or translation efficiency for the variant SDF-1 transcript could not be demonstrated[104].

Another study showed an association of the SDF1-3'A/3'A genotype with accelerated disease progression rather than slower progression[91]. Accelerated progression to AIDS, but prolonged survival following AIDS diagnosis, was reported in another[105]. In still other reports, no association with progression was found[57,58,96].

RANTES promoter polymorphisms

Polymorphisms in the RANTES promoter were described recently[106]. One of these variants, called RANTES-28G, was found to be associated with reduced CD4+ lymphocyte depletion, and to increased levels of RANTES transcription. No confirmation of these findings has been reported so far.

Conclusions

A number of variant alleles in HIV coreceptors or natural ligands of these chemokine receptors have been described. Altogether, only the association between non-functional CCR5 variants and HIV infection or AIDS progression is unambiguously established. Homozygotes for the Δ32 allele of CCR5 display strong but incomplete resistance to HIV infection. Heterozygotes exhibit delayed progression to AIDS. Although incompletely characterised, it is likely that some other (and rarer) CCR5 mutants are non-functional and should also provide relative protection to their carriers. Other genetic variants will require additional studies in order to clarify their links with HIV/AIDS pathogenesis. The association between CCR5 promoter variants and disease progression is presently unclear, given the conflicting reports and the lack of evidence so far that the genetic variations actually affect CCR5 expression.
The 64I variant of CCR2 was associated in several (but not all) studies with delayed progression, but no functional consequence of this gene variation has been described so far. The reported association between the SDF1-3'A variant and delayed AIDS progression was not found in subsequent studies, and the mutation does not seem to affect SDF-1 expression. The effect of the recently described RANTES promoter variant will have to be confirmed in other cohorts.

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