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The chemokine network. II. On how polymorphisms and alternative splicing increase the number of molecular species and configure intricate patterns of disease susceptibility

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Summary

In this second review on chemokines, we focus on the polymorphisms and alternative splicings and on their consequences in disease. Because chemokines are key mediators in the pathogenesis of inflammatory, autoimmune, vascular and neoplastic disorders, a large number of studies attempting to relate particular polymorphisms of chemokines to given diseases have already been conducted, sometimes with contradictory results. Reviewing the published data, it becomes evident that some chemokine genes that are polymorphic have alleles that are found repeatedly, associated with disease of different aetiologies but sharing some aspects of pathogenesis. Among CXC chemokines, single nucleotide polymorphisms (SNPs) in the CXCL8 and CXCL12 genes stand out, as they have alleles associated with many diseases such as asthma and human immunodeficiency virus (HIV), respectively. Of CC chemokines, the stronger associations occur among alleles from SNPs in CCL2 and CCL5 genes and a number of inflammatory conditions. To understand how chemokines contribute to disease it is also necessary to take into account all the isoforms resulting from differential splicing. The first part of this review deals with polymorphisms and the second with the diversity of molecular species derived from each chemokine gene due to alternative splicing phenomena. The number of molecular species and the level of expression of each of them for every chemokine and for each functionally related group of chemokines reaches a complexity that requires new modelling algorithms akin to those proposed in systems biology approaches.

Keywords: chemokines, human, polymorphisms, splicing, variability

Increasing chemokine variability: polymorphisms and alternative splicing

In a first review [1] we examined data which indicated that, during evolution, the variability of the chemokine superfamily grew in complexity, and we took advantage of the conservation of physiological functions among chemokines located in the different genetic clusters and miniclusters to improve our perspective of their functions. As for many gene families, the main mechanism that has generated this diversity of chemokines is gene duplication, which is particularly evident in the chemokine clusters. However, another important mechanism by which variation has been increasing at the genomic level is the existence of single nucleotide polymorphisms (SNPs), which are the most common form of DNA sequence variation. SNPs are highly abundant, stable and distributed throughout the genome. SNPs are an increasingly important tool for the study of the structure and history of human genome and they are also useful polymorphic markers to investigate genetic susceptibility to disease or to pharmacological sensitivity [2]. Other types of polymorphisms such as deletion/insertion polymorphisms (DIPs), copy number polymorphisms (CNPs) or those due to repeated elements (as minisatellites and microsatellites) also contribute importantly to the genomic variation but their distribution is more restricted. In addition to DNA sequence variation, alternative mRNA splicing is becoming recognized increasingly as an important mechanism for the generation of structural and functional variability in proteins. Several studies indicate that alternative splicing in humans is more the rule than the exception: primary transcripts from more than 50% of all human genes undergo alternative splicing, with a bias towards genes that are expressed in the nervous and immune systems [3,4].

In this second review, we focused upon the polymorphisms and disease associations of chemokine genes as well as variations in splicing which should be taken into account in order to understand these disease associations more clearly. We proceeded by collecting all information available in public databases, organized it by families following the systematic nomenclature [5], and finally we highlighted the cases in which disease association is stronger. In the course of this process we also analysed data available on isoforms generated by differential splicing. Even though we can expect that new data will still be produced on the polymorphisms and isoforms of chemokines, we now have a picture of their complexity and we can begin to discern patterns of disease association; this is also the subject of this review.

Polymorphisms and disease in the human chemokine superfamily

Polymorphisms in the genes of the immune system can influence the immune response markedly, human leucocyte antigen (HLA) genes being the paradigm. After the HLA genes, chemokine genes are probably one of the most polymorphic sets of genes in the immune system and it is becoming increasingly clear that chemokine polymorphisms influence the immune response to a remarkable extent. As the genome project progressed and the abundance of SNPs became evident, databases began to record SNPs and now millions of them are registered. However, the quality of the initial data contained in these databases had been questioned, because a considerable proportion of the initial SNPs may simply represent sequencing errors. Fortunately, the validation status of SNPs is improving and in this review we have included only well-documented and functionally relevant SNPs. The number of reports on disease-associated SNPs including members of the chemokine superfamily is increasing and will probably continue to rise during the next few years, as the importance of chemokines in the immune response gains recognition. As has already been documented for cytokines, the majority of SNPs found in the chemokines genes or their receptors are not located in the coding sequence but either in the promoter, the introns or the 3′ untranslated regions, and they can affect all aspects of gene expression and mRNA levels. Interestingly, most polymorphisms associated with disease in the chemokine superfamily affect their inflammatory members, thus confirming that they are the genes under stronger evolutionary pressure (Fig. 1).

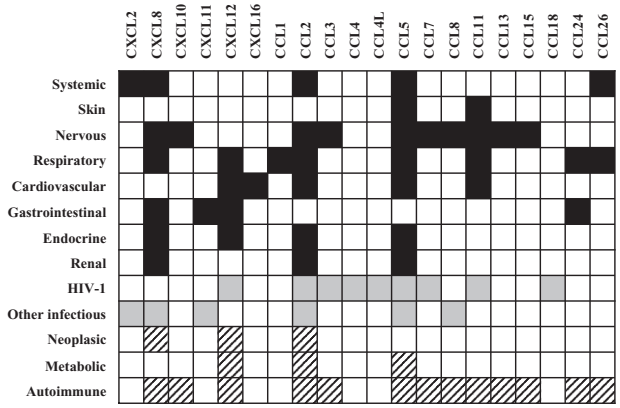

Fig. 1. Overview of chemokine polymorphisms and disease. Columns represent chemokines with disease-involved polymorphisms. Rows represent different disease categories: upper lines refer to diseases grouped by systems (black squares show polymorphism involvement), middle lines refer to infectious diseases (grey squares) and lower lines refer to other interesting physiopathological groups of diseases (dashed squares).

Human CXC chemokines

Several interesting polymorphisms affecting both inflammatory and homeostatic CXC ligands have been described (Table 1), CXCL8 [interleukin (IL)-8] and CXCL12 stromal cell-derived factor (SDF-1) being the chemokines that accumulate most of them.

CXCL8, a proinflammatory chemokine, is a potent chemoattractant for neutrophils, basophils and T lymphocytes. High levels of CXCL8 have been detected in biofluids from various acute inflammatory diseases, which is in keeping with neutrophilic infiltration into inflammatory sites as one of the hallmarks of acute inflammation. Among all CXCL8 described SNPs, the presence of −251A/T in the transcription start site is known to exert a strong influence on protein synthesis. The distribution of this SNP shows a remarkable heterogeneity among world populations [6] and has been associated with a spectrum of diseases (for references see Table 1): (a) airway diseases such as asthma, respiratory syncytial virus (RSV) infection and oral squamous cell carcinoma. Anecdotally, the inflammatory prone allele A may influence the initiation or characteristics of the smoking habit [7]; (b) gastrointestinal diseases such as *Clostridium difficile* and enteroaggregative *Escherichia coli* diarrhoea, *Helicobacter pylori*-induced gastric ulcer, atrophic gastritis, severe acute pancreatitis and gastrointestinal tract cancer; (c) central nervous system (CNS) diseases such as Parkinson's, multiple sclerosis (MS) and multiple system atrophy; and (d) a miscellany of diseases such as AIDS-related Kaposi's sarcoma and acute pyelonephritis has also shown to be influenced by the −251A/T CXCL8 polymorphism. However, in spite of the numerous studies on this polymorphism, data are still far from clear. Since the publication of the original association of the CXCL8 −251A/T polymorphism [8,9], there have been reports showing higher CXCL8 production by the A allele [8,10,11] while others showed higher production by allele T [12]. These contradictory data have their counterpart in association studies: the −251 T allele frequency has been increased significantly in asthma [13] and reduced significantly in RSV bronchiolitis [8]. In fact, it

Table 1. Polymorphisms and disease in the human CXC subfamily.

Ligand	Polymorphism	Location	Symbol	Disease involved	References
CXCL2	Tandem repeat -665(AC)n	Promoter		Severe sepsis	[28]
CXCL8	Microsatellite		D4S2641	Diffuse parabronchiolitis	[64]
	SNP -845 (C/T)	Promoter	rs2227532	SLE nephritis	[16]
	SNP -251 (A/T)	Promoter	rs4073	Asthma	[13]
				RSV infection	[8, 9, 14]
				Smoking behaviour	[7]
				Oral squamous cell carcinoma	[65]
				EAEC diarrhoea	[10]
				Clostridium difficile diarrhoea	[66]
				Helicobacter pylori-induced gastric diseases	[11, 67–70]
				Acute pancreatitis severity	[71]
				Gastric cancer	[12, 72, 73]
				Colorectal cancer	[74]
				Prostate cancer	[75]
				Parkinson's disease	[76]
				Multiple sclerosis	[77]
				Multiple system atrophy	[78]
				AIDS-related Kaposi's sarcoma	[79]
				Acute pyelonephritis	[17]
	SNP +781 (C/T)	Intron 1	rs2227306	Asthma	[13, 15]
	SNP +1633 (C/T)	Intron 3	rs2227543	Asthma	[13]
	SNP +2767 (A/T)	3′UTR	rs1126647	Asthma	[13]
				Nephritis in cutaneous vasculitis	[18]
				Acute pyelonephritis	[17]
	Haplotypes			Behcet's disease	[80]
CXCL10	Haplotypes			Multiple sclerosis	[81]
CXCL11	DIP -599del5	Promoter		Hepatitis C virus (HCV) infection	[29]
CXCL12	SNP +801 (G/A)	3′UTR	rs1801157	HIV-1 infection	[20, 22–24]
				Atherosclerosis in HIV patients	[82]
				Breast and lung cancer	[83–85]
				Acute myeloid leukemia	[86]
				Lymphoma	[87]
				Chronic myeloproliferative disease	[88]
				Liver transplantation	[89]
				Type 1 diabetes	[90]
CXCL16	SNP +599 (C/T)	Exon 4	rs2277680	Coronary artery stenosis	[30]

seems that the differences in CXCL8 expression are not linked directly to the −251A/T polymorphism. In one haplotype-based association study, Hacking *et al.* [14] have shown that there are two main CXCL8 haplotypes including six SNPs (−251A/T, +396G/T, +781T/C, +1238delA/insA, +1633T/C, +2767T/A), constituting the so-called haplotype 2 (A/G/T/delA/T/T), the haplotype associated with significantly higher CXCL8 transcription levels relative to the mirror haplotype 1. Strikingly, the −251A allele present on the high producer haplotype had no significant effect on the allele-specific level of transcription when analysed in reporter gene experiments. This indicates that the functional allele might be in linkage disequilibrium (LD) with haplotype 2 and that the −251A/T is not the functional SNP. Four SNPs of the previously described haplotypes (−251A/T, +781T/C, +1633T/C and +2767T/A) have been found to be associated with asthma in different studies [13,15]. These multiple SNPs associations are due probably to the existence of a very tight LD among them. The −845C/T SNP in the CXCL8 promoter region has been associated with severe systemic lupus erythematosus (SLE) nephritis [16]. Another distant SNP in 3′UTR (+2767A/T) has been associated with acute pyelonephritis [17] and nephritis in cutaneous vasculitis [18].

CXCL12 is a homeostatic CXC chemokine widely expressed which possesses a broad range of actions (from attraction of mature T and B cells to migration of haematopoietic progenitor cells from the bone marrow). CXCL12 plays an especially important role in two non-related diseases such as human immunodeficiency virus (HIV) and cancer, because its receptor (CXCR4) is also the co-receptor used by HIV T-tropic strains and because it is the most

widely expressed chemokine receptor in many different types of cancers [19]. The +801G/A SNP, located in 3′UTR, is the best-studied polymorphism in CXCL12 gene. It has been associated extensively with clinical features of HIV infection but, as in CXCL8–251A/T polymorphism, there are some contradictory reports. The published effect of the mutated allele (−801A) ranges from strong protection of HIV infection progression to AIDS [20,21] to enhanced progression to AIDS and shorter survival [22,23]. Even though it was proposed originally that the −801A allele was associated with higher CXCL12 production [20], later studies indicated the opposite [24], and other reports claimed that there were no differences in the CXCL12 production by the A or G alleles [25,26]. A recent haplotype-based study [27] demonstrated that other polymorphisms in LD with the CXCL12 + 801G/A SNP, rather than CXCL12 + 801G/A itself, are responsible for the different transcription levels. Therefore, the discrepancy among the previous epidemiological studies may be attributed to the haplotype structures and frequencies in the studied populations. CXCL12 + 801G/A has also been associated with many different types of cancers such as breast and lung cancer, acute myeloid leukaemia, lymphoma and chronic myeloproliferative disease.

Relatively few reports deal with the effect of polymorphisms of other CXC chemokines on disease. A short tandem repeat (STR) in CXCL2 may contribute to the development of severe sepsis [28], one haplotype in CXCL10 possibly contributes to reduce the rate of progression in MS patients, a 5-base pairs (bp) deletion in the promoter of CXCL11 may favour hepatitis C virus (HCV) infection to evolve towards chronicity [29] and, finally, a SNP in exon 4 of CXCL16, leading to an amino acid change (V200A), seemed to influence the severity of coronary artery stenosis [30].

Human CC chemokines

As many as 14 of the 26 members of CC chemokine subfamily have polymorphisms associated with disease, and most affect the inflammatory chemokines (Table 2). CCL2 [monocyte chemoattractant protein (MCP)-1] attracts specifically monocytes and memory T cells and tissue expression is found in a large variety of diseases characterized by mononuclear cell infiltration, with an essential role in atherosclerosis and multiple sclerosis. CCL2 has a SNP located in the 5′ distal regulatory region (−2518G/A) and it seems clear that the −2518G allele is associated with an increased CCL2 production (at both mRNA and protein levels) [31–34]. This −2518G/A polymorphism has been associated with a large variety of diseases: (a) systemic inflammatory diseases such as systemic lupus erythematosus (SLE), juvenile rheumatoid arthritis, systemic sclerosis and HLA-B27-associated acute uveitis; (b) conditions affecting the kidney such as renal transplantation, long-term haemodialysis and IgA nephropathy; (c) heart diseases such as myocardial infarction, coronary artery disease and the cardiomyopathy of Chagas' disease; (d) CNS diseases such as Alzheimer's and major depression; (e) endocrine diseases such as type 1 and type 2 diabetes: (f) infectious diseases such as those caused by HIV-1, HCV, HBV and *Mycobacterium tuberculosis*; and (g) other diseases such as breast cancer and asthma. It is important to note that there are also many negative reports showing a lack of association of this SNP with various diseases (including some of those cited previously as associated diseases).

CCL3 [macrophage inflammatory protein (MIP)-1α], CCL4 (MIP-1β), CCL4L [lymphocyte activation gene (LAG)-1] and CCL5 [regulated upon activation normal T cell expressed and secreted (RANTES)] have a diversity of polymorphisms that have an important impact on susceptibility to HIV-1 infection. This is not surprising, as they are ligands of the CCR5 receptor, which is the co-receptor used by HIV M-tropic strains to enter into the cells. Haplotypes defined on the region containing the genes CCL18, CCL3 and CCL4 (chromosome 17 q11–q21) have been found to be associated with HIV infection susceptibility and progression [35]. Although CCL18 has not yet been implicated in HIV-1/AIDS pathogenesis and its receptor is not known, this genetic analysis points to this gene as a candidate for modulating HIV-1 pathogenesis. CCL5 haplotypes have also been shown to influence the clinical progression of HIV infection [36,37]. Two interesting SNPs in the CCL4L gene have been associated with different aspects of HIV-1 infection: (a) the +590A/G is located at the intron 2 acceptor splice site. The G allele disrupts the original acceptor splice site and provokes a new complex transcription pattern. This allele modifies susceptibility to HIV-1 infection [38]. (b) The +59C/T is located in exon 2, leading to an amino acid change (R22H). The H variant has been associated with a lower overall survival of HIV-1 infected individuals [39]. Three CCL5 individual SNPs have also been associated with HIV-1 infection, two of them located in the promoter region (−403G/A and −28C/G) and the other in intron 1 (In1.1T/C). It has been demonstrated clearly that the −403A and −28G alleles enhance CCL5 production [40–43] and, conversely, the In1.1C allele reduces CCL5 gene transcription [37]. The two CCL5 promoter polymorphisms, −403G/A and −28C/G, have also been associated with a variety of other diseases such as allergic diseases (i.e. asthma, atopy, allergic rhinitis and atopic dermatitis), inflammatory diseases [i.e. SLE, MS, rheumatoid arthritis (RA), sarcoidosis and polymyalgia rheumatica] and infectious diseases (i.e. HIV-1 and HCV). Additionally, the −403G/A polymorphism has been found to be associated with metabolic risk-related conditions such as hypercholesterolaemia, coronary arteriosclerosis and cardiac mortality in type 2 diabetes.

CCL11 (eotaxin-1), CCL24 (eotaxin-2) and CCL26 (eotaxin-3) are CCR3 ligands and potent eosinophil chemoattractants, playing a fundamental role in asthma and other allergic diseases and in eosinophil-associated

Table 2. Polymorphisms and disease in the human CC subfamily.

Ligand	Polymorphism	Location	Symbol	Disease involved	References
CCL1	SNP (A/T)	Intron 2	rs2282691	Chronic obstructive pulmonary disease	[91]
CCL2	SNP -2518 (G/A)	Promoter	rs1024611	Systemic sclerosis	[92]
				Asthma	[93, 94]
				Systemic lupus erythematosus	[95–97]
				Juvenile rheumatoid arthritis	[98]
				Renal transplantation	[99]
				Breast cancer	[100]
				Long-term haemodialysis	[101]
				IgA nephropathy	[102]
				HLA-B27 associated disease	[103]
				Coronary artery disease	[104, 105]
				Myocardial infarction	[106]
				Alzheimer's disease association	[107]
				Major depressive disorder	[108]
				Type 1 diabetes	[109]
				Type 2 diabetes	[110]
				HIV-1 infection	[33]
				Pulmonary tuberculosis	[111]
				Cardiomyopathy in human Chagas' disease	[112]
				Hepatitis B virus (HBV) clearance	[113]
				Hepatitis C virus (HCV) severity	[34]
	Haplotypes			Multiple sclerosis	[114, 115]
	Haplotypes			HIV-1 infection	[116]
CCL3	Haplotypes			Multiple sclerosis	[114, 115, 117]
	Haplotypes			HIV-1 infection	[35, 36]
CCL4	Haplotypes			HIV-1 infection	[35]
CCL4L	SNP +59 (C/T)	Exon 2	rs3744595	HIV-1 infection	[39]
	SNP +590 (A/G)	Intron 2	rs4796195	HIV-1 infection	[38]
CCL5	SNP -403 (G/A)	Promoter	rs2107538	Allergic rhinitis	[118]
				Atopy and asthma	[119–121]
				Atopic dermatits	[40–42]
				Renal damage in SLE	[122]
				Rheumatoid arthritis	[123, 124]
				Multiple sclerosis	[125]
				HIV-1 infection	[36, 126, 127]
				HCV infection	[128, 129]
				Sarcoidosis	[130]
				Coronary arteriosclerosis	[131]
				Hypercholesterolaemia	[132]
				Cardiac mortality in type 2 diabetes	[133]
	SNP -28 (C/G)	Promoter	rs2280788	Allergic rhinitis	[118]
				Asthma	[134, 135]
				Nephropathy in type 2 diabetes	[136]
				HIV-1 infection	[36, 43, 127]
				Multiple sclerosis	[125]
				Systemic lupus erythematosus	[137]
				Atopic dermatitis	[42]
	SNP In1.1 (T/C)	Intron 1	rs2280789	Cardiac mortality in type 2 diabetes	[133]
				HIV-1 infection	[37, 127, 138]
	Haplotypes			HIV-1 infection	[36]
	Haplotypes			Type 1 diabetes	[139]
CCL7	Microsatellite	Promoter		Multiple sclerosis	[140, 141]
	Haplotypes			HIV-1 infection	[116]
CCL8	SNP +11 (A/C)	Exon 3	rs1133763	HCV infection	[128]
	Haplotypes			Multiple sclerosis	[114]

Table 2. *Continued*

Ligand	Polymorphism	Location	Symbol	Disease involved	References
CCL11	SNP -576 (C/T)	Promoter	rs4795896	Asthma	[44, 45]
	SNP -426 (C/T)	Promoter	rs16969415	Atopic dermatitis	[45, 142]
				Asthma	[45]
	SNP -384 (A/G)	Promoter	rs17809012	Atopic dermatitis	[44, 142]
				Asthma	[45]
	SNP +67 (G/A)	Exon 1	rs3744508	Asthma	[46, 47]
				Myocardial infarction	[143]
	Haplotypes			Multiple sclerosis	[114]
	Haplotypes			HIV-1 infection	[116]
CCL13	Haplotypes			Multiple sclerosis	[114]
CCL15	Haplotypes			Multiple sclerosis	[114, 115]
CCL18	Haplotypes			HIV-1 infection	[35]
CCL24	SNP +179 (T/C)	Intron 1	rs2302004	Asthma	[144]
				Ulcerative colitis	[145]
	SNP +275 (C/T)	Intron 1	rs2302005	Asthma	[144]
				Ulcerative colitis	[145]
	SNP +1265 (A/G)	Intron 2	rs11465310	Asthma	[47, 48]
CCL26	SNP +77 (C/T)	Intron 2	rs2240478	Asthma	[144]
	SNP +1577 (G/A)	Intron 3	rs6965556	Rheumatoid arthritis	[146]
	SNP +2497 (T/G)	3′UTR	rs2302009	Rheumatoid arthritis	[146]
				Asthma	[144]
				Allergic rhinitis	[147]

gastrointestinal diseases. Not unexpectedly, SNPs in these three chemokines have been found to be associated with allergic diseases such as asthma, allergic rhinitis and atopic dermatitis. Four SNPs of the CCL11 gene have been found to be associated independently with asthma in several studies [44–47]. Three of them are located in the promoter region (−576C/T, −426C/T and −384 A/G) and the other (+67G/A) in the signal peptide (exon 1) leading to an amino acid change (T23A). Interestingly, both the −384G and +67A alleles are associated with lower CCL11 production [44,46]. The three polymorphisms of the CCL24 gene associated with asthma are intronic SNPs: +179TC and +275C/T in intron 1 and +1265A/G in intron 2. There are data indicating that the +1265A allele is associated with lower CCL24 levels than the G allele [48]). Finally, CCL26 has two SNPs affecting asthma differently: the +2497T/G (in the 3′UTR region) have been associated with susceptibility and the +77C/T (in intron 2) seem to play a critical role in attracting eosinophils and maintaining high IgE levels.

Regarding the C and CX3C subfamilies, no relevant polymorphisms in their members have so far been described.

Transcriptional variability: alternative splicing in the chemokine superfamily

The mRNA of several chemokines is known to undergo alternative splicing (Table 3), some of them with repercussions in the molecular activity and/or in the tissue distribution of the differentially spliced variants. However, to date, there are no reports on their implication in disease pathogenesis.

CXCL12 is the only CXC chemokine known to generate isoforms by alternative splicing. The two main splice forms of CXCL12 (SDF-1α and SDF-1β) have similar amino acid sequences except for the presence of four additional amino acids at the carboxy terminus of SDF-1β. Both isoforms display a similar tissue expression pattern, but SDF-1α mRNA can be detected in the adult human brain, whereas SDF-1β cannot. The two isoforms are subjected to different proteolytic processing, and this fact could explain functional differences [49]. Recently, four additional human SDF-1 isoforms derived from alternative splicing events have been identified (SDF-1γ, SDF-1δ, SDF-1ε and SDF-1ϕ), showing some differential distribution of tissue expression [50].

CCL4 and CCL4L, two closely related chemokines, have different isoforms due to alternative splicing. Both chemokines have exon 2 skipped variants that keep only the two first amino acids from the original protein due to a frameshift in the new junction between exon 1 and exon 3. Additionally, CCL4L2 (an allelic variant of CCL4L) has a nucleotide change in the acceptor splice site of intron 2 leading to a complex transcription pattern due to multiple usage of new alternative acceptor splice sites surrounding the original mutated one [38].

Two alternative splice isoforms of CCL20 have been identified, resulting from the alternative usage of two potential acceptor splice sites separated by three nucleotides in the junction of intron 1 and exon 2. The longer form

Table 3. Alternative splicing in the human chemokine superfamily.

Ligand	Splicing phenomena	Isoforms	Length	Isoforms identity	Refs
CXCL12	Exon 4 alternative splicing	SDF-1α	68 aa	All variants share the same first three exons but contain different fourth exons	[49, 50, 148]
		SDF-1β	72 aa		
		SDF-1γ	98 aa		
		SDF-1δ	119 aa		
		SDF-1ε	69 aa		
		SDF-1ϕ	79 aa		
CCL4	Exon 2 skipping	CCL4	69 aa	The Δ2 isoform keeps only the two first amino acids due to a frameshift	[38]
		CCL4Δ2			
CCL4L1	Exon 2 skipping	CCL4L1	69 aa	The Δ2 isoform keeps only the two first amino acids due to a frameshift	[38]
		CCL4L1Δ2	29 aa		
CCL4L2	Alternative acceptor splice sites in exon 3	CCL4L2	64 aa	The Δ2 isoforms keep only the two first amino acids due to a frameshift	[38]
	Exon 2 skipping	CCL4L2Δ2	24 aa	The rest of the isoforms share the same first two exons but contain different third exons	
		CCL4L2b	41 aa		
		CCL4L2bΔ2	45 aa		
		CCL4L2c	80 aa		
		CCL4L2d	73 aa		
		CCL4L2e	63 aa		
		CCL4L2f	80 aa		
CCL20	Alternative acceptor splice site in exon 2	CCL20 Ala	70 aa	100% (CCL20 Ala has 1 additional aa in N-terminus)	[51, 52]
		CCL20 Ser	69 aa		
CCL23	Alternative acceptor splice site in exon 3	CKβ8-1	116 aa	99% (CK8 lacks 17 aa before the two first cysteines)	[53, 54]
		CKβ8	99 aa		
CCL27	Alternative first exon usage	CCL27	95 aa	PESKY and canonical CCL27 differ only in the first of three exons	[56, 57, 149]
	Intron retention	PESKY	127 aa	The partially spliced and unspliced variants of CCL27 retains the intron 1 and	
		CCL27 unspliced	32 aa	intron 1 and 2, respectively	
		CCL27 partially spliced	32 aa		

(CCL20Ala) has an alanine (Ala27) as its predicted N-terminal amino acid, whereas the deletion of Ala27 leads to Ser27 as the predicted N-terminal amino acid in the short form (CCL20Ser) [51,52]. The biological activity of CCL20Ala and CCL20Ser and the tissue-specific preference of different acceptor splice-sites usages are not yet known.

CCL23 has two variants originated by alternative splicing in exon 3: the originally described CKβ8 and the splicing variant CKβ8-1, which is 17 amino acids longer. The mature proteins CKβ8 and CKβ8-1 consist of 99 and 116 amino acids, respectively. It has been shown that CKβ8 differed from CKβ8-1 in the monocyte chemoattraction and in the binding to human formyl peptide-receptor-like-1 (FPRL-1), suggesting that these two CCL23 isoforms could possibly have different a kinetic and specificity of chemotactic function *in vivo* [53,54].

Finally, CCL27 is produced as two splice variants. One of these variants encodes a classical chemokine with an associated signal peptide (CCL27), while the other variant (PESKY) maintains the sequence of the mature chemokine, but the signal peptide has been replaced by an alternative stretch of amino acids that directs this isoform to the nucleus where it modulates transcription. Surprisingly, secreted CCL27 can also reach the nucleus after CCR10-mediated internalization, and in this way directly modulates transcription and influences several cellular processes [55]. Expression studies have revealed differential tissue expression of CCL27 and PESKY. Interestingly, while CCL27 is highly expressed in the placenta, PESKY is expressed mainly in the testes and brain and weakly in the developing embryo [56]. Recently, several novel CCL27 variants have been identified in mouse but their presence in humans has not yet been demonstrated [57].

Concluding remarks

The high variability of the chemokine superfamily includes mechanisms of genomic and transcriptional variation. There is already a good number of well-described polymorphisms of chemokines with functional relevance and we made a detailed review of those involved significantly in disease. In spite of the many reports on the association of these polymorphisms to diseases, there are still confusing and contradictory data. Many factors in the epidemiological investigation could explain this phenomenon (covered widely in several reviews [58–60]), but it is clear that further studies are necessary to define more clearly the role of genetic variants of chemokines in disease. The recently developed high-throughput methods for SNP genotyping should make it easy to carry out larger association studies using a high number of SNPs, covering from one or a few genes (candidate gene approach) to the whole genome (genome-wide approach). In fact, the single SNP association studies are currently being replaced by the haplotype-based studies using tagSNPs, as this approach ensures the capture of most of the genetic variation in a relatively transferable manner among global populations [61]. With regard to the alternative splicing phenomena in the chemokine superfamily, several members with different splice variants have been identified but there are still few available data about its functional role. Molecular analyses during the last decade demonstrate that alternative splicing determines the binding properties, intracellular localization, enzymatic activity, protein stability and post-translational modifications of a large number of proteins [62,63]. Efforts are now being directed at establishing the full repertoire of functionally relevant transcript variants generated by alternative splicing, the specific roles of such variants in normal and disease physiology, and how alternative splicing is co-ordinated on a global level to achieve cell- and tissue-specific functions. Although the interaction between all these factors will probably provide us with the true key to understanding their real effect on pathology, future studies will be necessary to achieve all these goals in the chemokine superfamily.

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