24 Pulmonary Fibrosis

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CONTENTS

24.1 OVERVIEW OF PULMONARY FIBROSIS

The pathogenesis of fibrotic diseases is similar regardless of whether the lung or extrapulmonary tissues are involved. The disease is characterized by chronic inflammation, excessive accumulation of matrix proteins and destruction of normal tissue structure.[1,2] Although the etiology of pulmonary fibrotic diseases is diverse, the process universally involves an imbalance between matrix formation and degradation. Studies have shown that accumulation of matrix proteins, mainly produced by fibroblasts and myofibroblasts, is responsible for the alveolar wall damage and distortion of lung parenchyma. Three, sometimes overlapping, phases have been proposed in the development of pulmonary fibrosis including initial triggering events, the inflammatory response and the fibrotic response. A diverse group of insults including drugs (bleomycin, cisplatin), radiation, physical injury, or viral infections as well as collagen-vascular diseases, such as systemic sclerosis, may trigger the development of fibrosis.[1,3] Regarding environmental factors, an elevated risk for idiopathic pulmonary fibrosis (IPF) occurs in subjects exposed to mineral dusts (e.g. silica or asbestos), metal or wood dust and tobacco smoking.[4–6]

The tissue response involves sequentially alveolar epithelial cell injury or activation, inflammation, proliferation, and differentiation of interstitial cells and collagen production. Chronic inflammation occurs following the initial injury and is characterized by activation of resident cells and inflammatory cell infiltration, interstitial edema and local cell proliferation.[1,7] Cytokines, such as interleukin-1 (IL)-1, IL-4, IL-8 and tumor necrosis factor (TNF)-α, and growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β1, are involved in the recruitment of inflammatory cells into the alveolar walls and spaces. A number of these mediators also play a role in the remodeling process through fibroblast proliferation and collagen synthesis. Therefore, the early and persistent expression of pro-inflammatory cytokines and subsequent presence of cell-surface adhesion molecules and chemotactic molecules are important in mediating

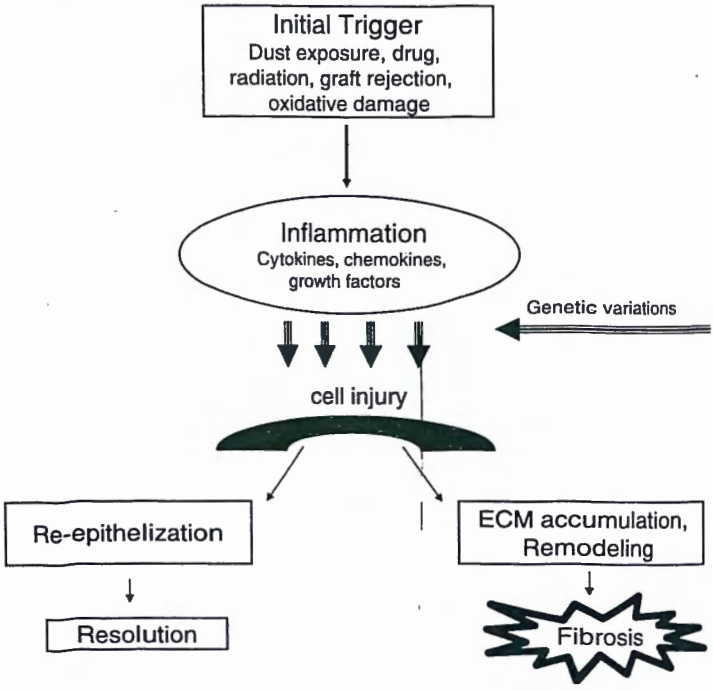

FIGURE 24.1 Simplified scheme of the phases involved in the pathogenesis of pulmonary fibrosis. A diverse group of insults may trigger the initial lung injury followed by chronic inflammation. If inflammation is not resolved, fibrotic phase emerges with the overproduction of collagen and other matrix proteins, resulting in the development of fibrosis. In this pattern, genetic variations in related genes may influence susceptibility and modify progression or severity of disease.

fibrosis.[2,3] Although this pathway is common for most interstitial lung diseases, IPF may develop in the absence of an inflammatory process.[8,9] If inflammation is not resolved, fibroblasts migrate and proliferate in areas of acute injury and secrete an excessive amount of collagen and other matrix proteins. These proteins are deposited in the interstitial space and gradually cause irreversible pulmonary fibrosis.[10–12] Fibroblasts also release proteases that degrade and remodel the matrix proteins.[2] The aberrant tissue remodeling involves the families of matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs). The changes in the levels, activities and balance between MMPs and TIMPs play a significant role in the altered extracellular matrix (ECM) metabolism due to their capacity to cleave structural proteins such as collagens and elastin.[13,14] Figure 24.1 shows the hypothetical scheme of the events in the pathogenesis of pulmonary fibrosis.

24.2 CYTOKINES, METALLOPROTEINASES AND FIBROGENIC MEDIATORS IN FIBROTIC DISEASES

24.2.1 Inflammatory Cytokines

The pathogenesis of pulmonary fibrosis appears to be driven by persistent inflammation where pro-inflammatory cytokines, such as TNFα, IL-1 and IL-6 play a central role. TNFα is one of the earliest cytokines implicated as its over-expression promotes fibroblast proliferation and collagen deposition.[15] Administration of neutralizing antibodies to TNFα or soluble TNFα receptors prevent the development of silica or bleomycin-induced

pulmonary fibrosis in mice.[15,16] Furthermore, mice that over-express TNFα develop IPF-like fibrosis, while TNFα deficient mice resist bleomycin-induced fibrosis.[17,18] TNFα also promotes matrix-degrading gelatinases and fibroblast migration which result in matrix-remodeling.[19] Increased amounts of TNFα can be found in BALF of patients with IPF or asbestosis.[20] Furthermore, polymorphisms at position −308 and −238 in the promoter region of the TNFα gene confer increased risk of developing pulmonary fibrosis.[21–23] These genetic variants result in increased production of TNFα protein.[24]

The IL-1 family that includes IL-1α, IL-1β, and IL-1 receptor antagonist (RA), like TNFα, has pro-inflammatory and fibrogenic properties that contribute to the initial fibrotic process. Both IL-1α and IL-1β induce fibroblasts to produce additional cytokines such as IL-6, and collagens.[25] Recently, it has been reported that transient expression of IL-1β can lead to progressive fibrosis, even after IL-1β levels have declined.[26] IL-1Ra, a naturally occurring antagonist of the IL-1 receptor, can attenuate IL-1 signaling and help resolve inflammation after injury. Administration of IL-1Ra decreases lung fibrosis in mice exposed to bleomycin and silica.[27] Significantly increased levels of IL-1Ra can be found in the BALF of patients with IPF and sarcoidosis.[28,29] Two genetic variations of the IL-1Ra gene (IL-1RN) at position +2018 and intron 2, which express variable numbers of tandem repeat [VNTR], have been associated with increased production of IL-1Ra and accelerated fibrosis.[21,30]

IL-6 has been shown to help mediate interstitial lung diseases either alone or in concert with TNFα.[31] Although the contribution of IL-6 to fibrosis is not fully understood, it may stimulate fibroblast proliferation and ECM deposition.[32] In animal models, over-expression of IL-6 showed a marked inflammatory response, but only weak signs of fibrosis.[33,34] However, IL-6, together with TNFα, participates in fibrosis induced by bleomycin by stimulating expression of the pro-fibrotic chemokine macrophage inflammatory protein-1 alpha (MIP-1α).[35] Furthermore, increased levels of IL-6 are found in BALF from patients with sarcoidosis.[36] A polymorphism at position −174 in the promoter region of the IL-6 gene leads to reduced transcription of the gene and its presence is associated with IPF progression.[22,37]

24.2.2 Th Cytokines

The imbalance between T helper 1 (Th1) and T helper 2 (Th2) cytokine responses is also important in the pathogenesis of fibrosis.[7,8,38–40] Th1 and Th2 cytokines have the ability to influence either resolution or progression to end-stage fibrosis. While Th1 cytokines [interfeon-γ (IFN-γ), IL-2, IL-12, IL-18, TNFβ] appear to be involved in the restoration of normal tissue structure by inhibiting fibrosis, Th2 cytokine response (IL-4, IL-5, IL-10, and IL-13) stimulate fibroblast activation, proliferation, and ultimately the deposition of ECM protein.[38,41,42] In particular, IL-4 and IL-13 have been implicated in fibroblast proliferation and increased production of ECM, including type-I and type-III procollagens and fibronectin.[43–45] IL-4 mRNA levels are increased in bleomycin-induced pulmonary fibrosis in mice while IL-13 expressing transgenic mice demonstrates airway epithelial cell hypertrophy and subepithelial airway fibrosis.[42,45] IL-5 is also up-regulated at sites of active pulmonary fibrosis in mice treated with bleomycin.[46]

In addition, increased levels of IL-4, 5 and 13 are found in BAL fluid from patients with IPF.[47] With regards to Th1 response, Th1 cytokines have a profound antifibrotic effect mediated primarily by IFN-γ. The administration of IFN-γ suppresses the proliferation of fibroblasts, production of ECM proteins, such as collagen and fibronectin, and downregulates TGF-β production.[44,48,49] Clinical studies indicate that IFN-γ is one of the more encouraging drugs for fibrosis treatment and patients with higher serum IFN-γ levels respond better to corticosteroids.[50] Treatment of IPF patients with IFN-γ for

a one-year period can provide substantial improvement in lung function.[51] Furthermore, a variable length CA repeat polymorphism in the first intron of the IFN-γ gene has been associated with pulmonary fibrosis.[52,53] The other Th1 cytokines, IL-12 and IL-18, act synergistically to stimulate IFN-γ induction in T cells.[54]

24.2.3 METALLOPROTEINASES

Degradation of the ECM is mediated primarily by MMPs which are grouped based on their structure and activity into different subfamilies such as collagenases, gelatinases, and stromelysins.[3,8] The TIMPs control ECM turnover with their MMP inhibitory actions. Disruption of the regulated balance between MMPs and TIMPs during normal tissue metabolism plays a crucial role in the formation of the ECM and remodeling.[14,55–57] Increased expression of MMP-1, -2, -8 and -9 are found in experimental models of pulmonary fibrosis[58,59] and in patients with IPF and sarcoidosis.[60]

24.2.4 FIBROGENIC MEDIATORS

TGF-β is the most widely-studied cytokine in the context of fibrosis due to its pleiotropic activity in inflammatory/immune and structural cells, wound healing, and tissue remodeling.[61,62] Both laboratory animal and human studies support a role for TGF-β in fibrosis. For example, TGF-β gene expression and protein production are increased in bleomycin, silica, asbestos, and radiation-induced pulmonary fibrosis in experimental animals.[63–66] Increased TGFβ1 production has also been demonstrated in patients with IPF and asbestosis.[67,68] Among the three TGFβ isoforms identified, TGFβ1, -2 and -3, TGFβ1 is the most potent promoter of ECM production, being involved in wound healing and tissue remodeling. Also, TGFβ1 is a potent chemotactic and activating factor for monocytes and macrophages. Once activated, these cells, in turn, release additional cytokines, such as IL-1β, PDGF, fibroblast growth factor (FGF), TNFα and TGFβ1.[62] Other potentially fibrogenic molecules, such as TNFα and granulocyte-macrophage colony-stimulating factor (GM-CSF), may mediate their fibrogenic effect through up-regulation of TGF-β expression.[69,70] Furthermore, gene polymorphisms influencing TGFβ1 production at positions +915, +29, and −509 are associated with pulmonary fibrosis.[71,72] Other profibrotic factors, such as PDGF, macrophage chemotactic protein (MCP-1), insulin-like growth factor (IGF-1), endothelin-1 (ET-1), MIP-1α and IL-8 also have been implicated in different stages of human and experimental pulmonary fibrosis. Table 24.1 provided a list of these mediators and their roles in fibrosis. Functional polymorphisms in genes which control these mediators have been associated with a variety of lung diseases. However, contributions of these genetic variations in pulmonary fibrosis are yet to be identified.

24.3 CYTOKINE GENE POLYMORPHISMS AND INTERSTITIAL LUNG DISEASES

The genes that control expression of mediators of interstitial lung diseases are highly polymorphic and the role of these polymorphisms in other common diseases is receiving considerable attention. In this respect, epidemiological studies have identified associations between specific cytokine polymorphisms and common complex human diseases such as cardiovascular diseases, cancer, neurodegenerative diseases, periodontal disease and immune-mediated diseases including allergic asthma and autoimmunity.[73–78] Most genetic polymorphisms in genes for common diseases are not directly responsible for disease causation, rather they act as disease modifiers by influencing the severity or response to specific treatment regimens. This is particularly true for polymorphisms in cytokine genes. As has

TABLE 24.1
Examples of Cytokines, Chemokines and Growth Factors That Play a Role in the Pathogenesis of Pulmonary Fibrosis

Cytokines	Their Role in the Pathogenesis of Pulmonary Fibrosis	Reference
TNFα	Chronic inflammation, mitogenic and chemotactic for fibroblasts	15,17
IL-1α and β	Chronic inflammation, fibroblast proliferation	25
IL-6	Regulates inflammatory response, stimulates fibroblast proliferation and ECM deposition	31–33
TGF-β	Fibroblast proliferation, ECM production	68,88
IL-4	Fibroblast proliferation, collagen production, suppression of Th1 cytokines	42,89
IL-10	Suppresses pro-inflammatory cytokines and chemokines	90,91
IL-12	Skewing cytokine production toward Th1	92
IL-13	Fibroblast activation, ECM production	45
IFN-γ	Inhibits fibroblasts proliferation and collagen synthesis	48,49
IL-8	Chemoattractant for neutrophils	93
MCP-1 and MIP-1	Mononuclear cell recruitment	94,95
RANTES	Eosinophil and lymphocyte chemoattractant	96
PDGF	Fibroblast proliferation, ECM synthesis	97,98
IGF-1	Fibroblast proliferation	99
MMPs	ECM destruction	13,14
ET-1, CTGF	Cell proliferation and matrix production	100,101

been suggested in the course of epidemiological studies with other chronic inflammatory diseases, genetic factors may also determine susceptibility or influence the clinical expression of pulmonary fibrosis. Table 24.2 provides a summary indicating where genetic variations have been implicated in the development or course of pulmonary fibrosis.

The association between the TGFβ1 +915 (codon 25) single nucleotide polymorphism (SNP) and pre-transplant lung fibrosis was investigated in transplant patients.[71] Stimulated lymphocytes containing the homozygous genotype (Arg/Arg) from control individuals displayed a higher production of the protein. The frequency of the minor variant was increased in patients displaying fibrosis, when compared to normal controls or patients with pre-transplant non-fibrotic pathology. The authors also found that this variation can predict the development of post-transplant lung fibrosis. Patients that developed allograft-mediated fibrosis expressed the homozygous Arg/Arg, reflecting the high TGFβ1 producer genotype. The presence of the Pro allele in codon 10 of the TGF-β1 gene has been associated with rapid deterioration in gas exchange in patients with IPF.[72]

Silicosis, an interstitial lung disease resulting from inhalation of crystalline silica, is characterized by chronic inflammation leading to severe pulmonary fibrotic changes. Laboratory animal and clinical studies have indicated that TNFα and IL-1 are important in regulating the development of silicotic lesions. In this respect, an association was found between disease severity and the functional polymorphism at position −238 of the TNFα gene (OR = 4, CI; 2.4–6.8). Irrespective of disease severity, the other functional variants TNFα −308 and IL-1RA +2018 conferred an increased risk for the presence of disease (OR = 2.2, 95% CI; 1.4–3.6 and OR = 2.1, 95% CI;1.3–3.5, respectively).[79,80] In studies of South African miners, TNFα polymorphisms in positions −238, −308, −376 of the promoter region were associated with severe silicosis, providing confirmation to these associations ($p = 0.022$, 0.034, and 0.016, respectively).[81]

Coal worker's pneumoconiosis (CWP) is characterized by chronic inflammation that usually leads to fibrosis. In a study investigating associations between TNFα gene

TABLE 24.2
Examples of Associations between Cytokine Polymorphisms and Pulmonary Fibrotic Diseases

Disease	Gene/Polymorphism	Sample Size (Case/Control)	Ethnicity	Associated Variant	*P* Value	Reference
Coal workers' pneumoconiosis	TNFα −308	78/56	Caucasian	A-308 allele	$P = 0.04$	82
		253	Caucasian	A-308 allele	$P = 0.003$	87
Cystic fibrosis	TGFβ1 codon 10	171/107	Caucasian	TT genotype	$P < 0.02$	102
	TNFα +691g	180/85	Caucasian	+691 ins	$P = 0.008$	103
Fibrosing alveolitis	IL-1RN +2018	88/88	Caucasian	IL-1RN*2	$P = 0.03$	21
	TNFα −308	61/103	Caucasian	A-308 allele	$P = 0.022$	
Idiopathic pulmonary fibrosis	Co-carriage of the IL-6 intron 4 and the TNF-RII 1690	74/100 (IL-6) 74/192 (TNF-RII)	Caucasian	4 G and 1690 C alleles	$P < 0.00093$	22
Lung allograft fibrosis	IFNγ/CA repeat TGFβ1 codon 25	82/164	Caucasian	12 CA repeats	$P < 0.005$	52
		45/107	Caucasian	+915 Arg allele	$P < 0.03$	71
Sarcoidosis	TNFα −308	101/216	Caucasian	A-308 allele	$P < 0.0078$	104
Silicosis	TNFα −308, TNFα −238 (severe)	325/164	Caucasian	A-308 allele A-238 allele	$P < 0.01$ $P = 0.04$	79
	IL-1RN +2018	325/164	Caucasian	IL-1RN*2	$P < 0.01$	80

polymorphisms and development of CWP, the frequency of the TNFα −308 variant was significantly increased in miners with CWP (50%), as compared with miners without lung disease (25%) or non-miners (29%) (OR = 3.0, 95% CI; 1.0–9.0).[82] The TNFα −308 and lymphotoxin-α (LTA) *NcoI* polymorphisms were investigated in 253 coal miners exposed to low and high levels of coal dust and cigarette smoke. TNFα can alter the oxidant/antioxidant balance in the lung by generating reactive oxygen species and modulating glutathione levels.[83] The LTA gene is located in tandem with the TNFα gene on chromosome 6p21.31 and a functional polymorphism in LTA gene (*NcoI*) was found to be associated with pulmonary fibrosis.[84] Reactive oxygen species derived from coal dust and activated inflammatory cells are known to be important in CWP pathogenesis.[85] In a study investigating the relationship between coal mine dust exposure and antioxidant enzyme activities, a strong association was found between catalase activity, cumulative dust exposure, and CWP severity.[86] While the LTA *NcoI* polymorphism was associated with CWP prevalence in miners with low blood catalase activity ($p = 0.05$), the TNFα −308 SNP showed an interaction with erythrocyte GSH-Px activity in individuals with high occupational exposure ($p = 0.003$).[87] This study showed that the TNFα −308 and the LTA *NcoI* polymorphisms, along with occupational exposure and intermediate response phenotypes, may play a role in the pathogenesis of CWP.

Fibrosing alveolitis is characterized by persistent alveolar inflammation and interstitial pulmonary fibrosis. Occupations where workers are exposed to metals, wood dusts, or other chemicals can sometimes cause fibrosing alveolitis. The frequency of TNFα −308 and IL-1RN +2018 variants were investigated in patients with fibrosing alveolitis and controls from the UK and Italy.[21] The frequency of IL-1RN +2018 was increased in subjects with the homozygous minor variant (OR = 10.2, 95% CI; 1.3–81.4). Carriage of TNFα −308 variant was also associated with increased risk of fibrosing alveolitis (OR = 2.5, 95% CI; 1.1–5.5). These data suggest that IL-1RN +2018 and TNFα −308 minor variants might confer increased risk of developing fibrosing alveolitis.

24.4 GENE-GENE AND GENE-ENVIRONMENT INTERACTIONS

As fibrotic lung diseases are complex, it is not surprising that several gene–gene interactions have been observed. While no significant associations were found in an IPF population with respect to LTA, TNF-receptor 2 (TNF-R2) or IL-6 gene polymorphisms, an increased frequency of individuals possessing both the IL-6 intron 4G and the TNF-R2 1690 C alleles has been observed[22]. In silicosis, the presence of both the IL-1α +4845 and TNFα −238 variants was associated with an increased likelihood of severe disease.[79] The frequency of TNFα −238 was associated with severe silicosis, but the magnitude of this effect was greater in those subjects without the IL-1α +4845 variant. A second interaction was found between IL-1RA +2018 and TNFα −308 variants. The proportion of moderate cases increased independently with the presence of either minor variant. For severe disease, however, both IL-1RA and TNFα −308 variants were present.[79] Three-way interactions were identified between the proportion of cases in each possible gene–gene combination and two different categories of exposure (less than or equal to thirty years versus greater than thirty years). These analyses showed an increased prevalence of silicosis with increasing exposure, except in the case where both IL1α +4845 and TNFα −308 variants were present. Nadif et al. (2003) showed an interaction of the TNFα −308 genotype and high coal dust exposure and GSH-Px activity ($p = 0.003$). They postulated that chronic oxidative stress resulting from high dust exposure may down regulate GSH-Px, while the high-producer TNFα genotype modulates GSH-Px activity through regulation of GSH levels.[87]

24.5 CONCLUSIONS

With the identification of the DNA sequence of the human genome and increasing data on polymorphisms, genetic epidemiology offers a powerful approach to the identification of genetic variants that influence susceptibility to many common diseases. Although the pathogenesis of pulmonary fibrosis remains incompletely understood, identification and understanding the role of genetic risk factors helps provide novel insights into the pathophysiology of the disease and identify molecular regulators of inflammatory and fibrotic processes. The use of new high-throughput genotyping technology, statistical genetic methods and robust association study designs which considers population stratification, sample size, intermediate phenotypes, linkage disequilibrium, and gene–gene/gene environment interactions, will help lead to a better understanding of fibrotic mechanisms in the lung and identification of high-risk groups. This information may also aid in the development of novel therapeutic targets at a molecular level.

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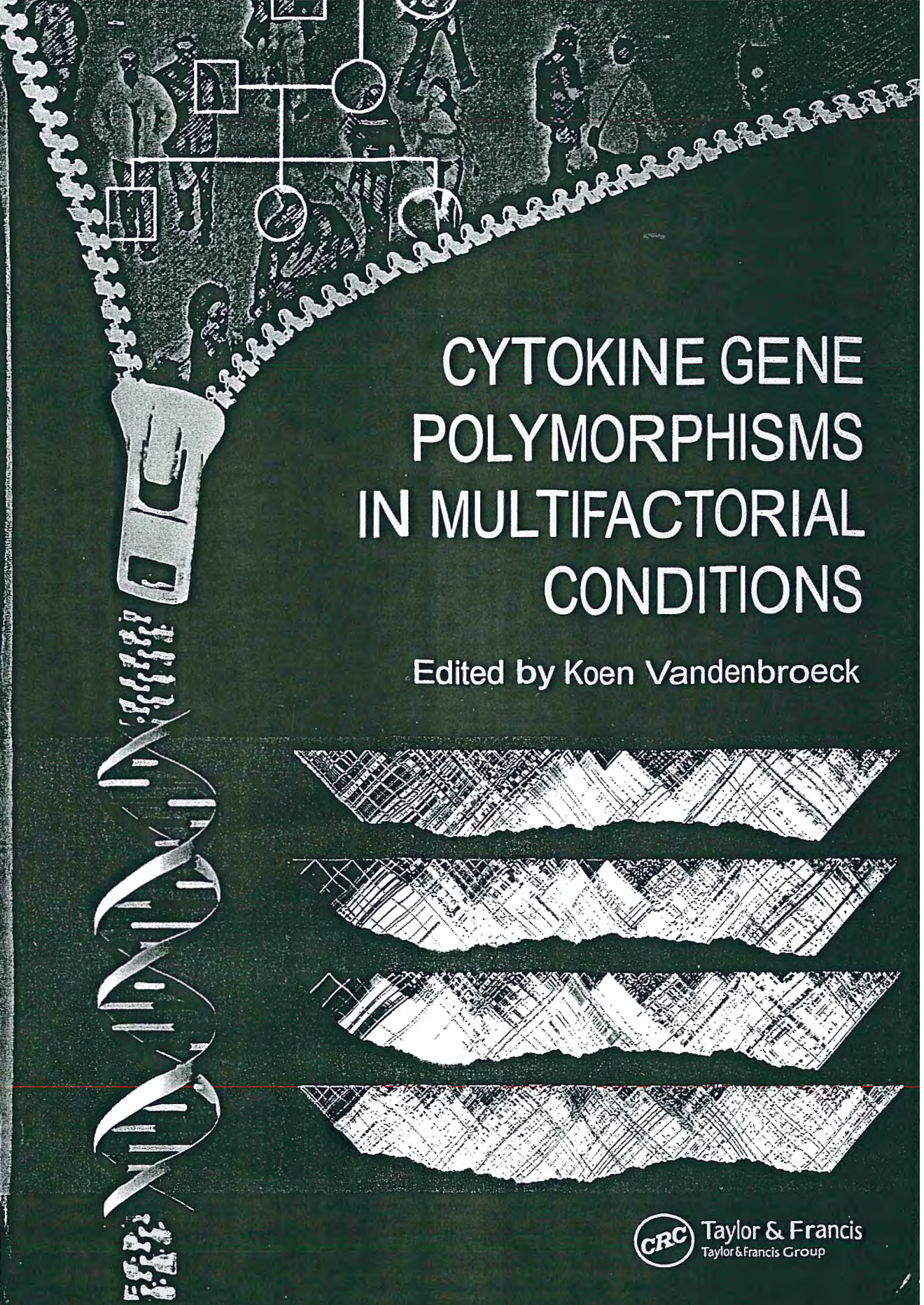

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Central front cover illustration: Pairwise linkage disequilibrium (r^2) for single nucleotide polymorphisms (SNPs) in the human cytokine cluster region (containing *IL3*, *CSF*, *IL5*, *IL13* and *IL4*) on chromosome 5q31 for the four International HapMap population panels. European American, Japanese, Han Chinese and Yoruba panels are shown in order from top to bottom. The plots include all SNPs in 2 million base pairs of chromosome 5 (5q31), from position 131090086 to 133090086 in the NCBI human genome B34. Images were downloaded from the International HapMap genome browser - http://hapmap.org on October 29, 2005, using Phase II HapMap data release #19. The illustration was prepared by Ross Lazarus and Koen Vandenbroeck.

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