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Affected sibling pairs with juvenile idiopathic arthritis

An immunogenetic study of the disease in multicase families

Hanna Säilä

ACADEMIC DISSERTATION

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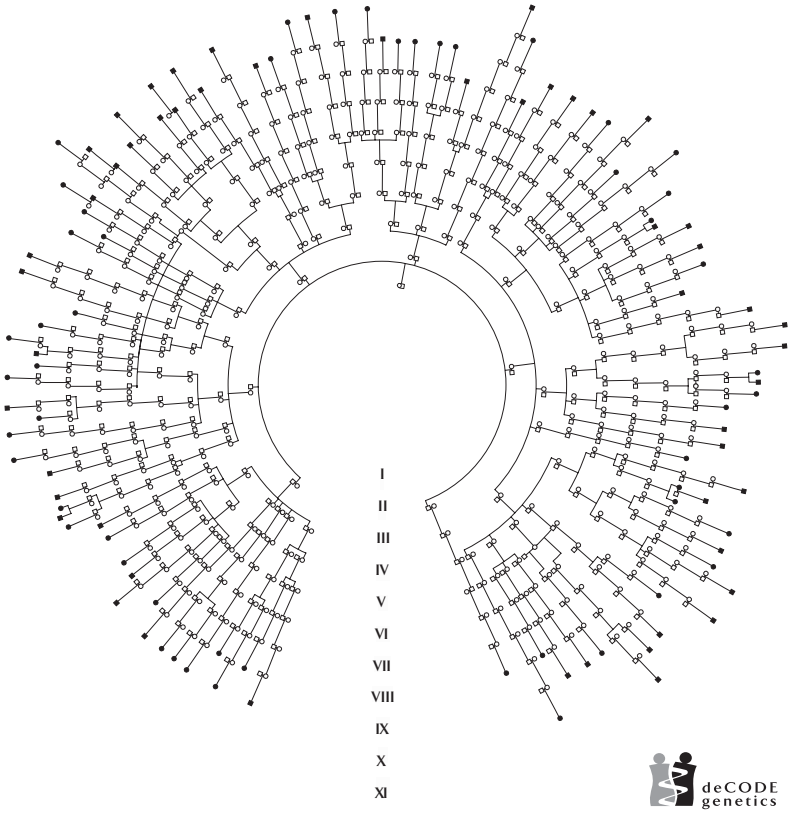
To the families that participated in this study



Contents

Suomenkielinen tiivistelmä

Lastenreuman perimmäiset syyt ovat yhä tuntemattomat. Se on monitekijäinen sairaus ja nykykäsityksen mukaan sen syntyyn vaikuttavat sekä perintö- että ympäristötekijät. Sekä geneettiset että ympäristötekijät vaikuttavat taudin ja sen alatyyppien esiintyvyyden eroihin eri väestöissä ja roduilla. Perintötekijöiden osuutta sairastavuuteen vahvistaa taudin suvuittainen kertyminen sekä kaksosaineistossa todettu konkordanssi. Lisäksi lastenreuman assosiotuminen tiettyihin kromosomi kuuden HLA-polymorfismeihin vahvistaa geneettisen komponentin osuutta.

Perintötekijöiden etsinnässä lastenreumaa sairastavien potilaiden verinäytteistä määritellyistä markkereiden genotyyppitiedoista pyritään selvittämään mahdollista kytkeytymistä tautia aiheuttavaan geeniin. Aikaisemmissa tutkimuksissa vahvin lastenreumalle altistava geenialue näyttää koko genomin kattavan analyysin perusteella sijoittuvan HLA-alueelle, mutta muillakin genomin alueiden geeneillä on ilmeisesti vaikutusta.

Suomalaisten geneettisiltä taustoiltaan homogeenisten potilaiden tutkiminen mahdollistaa monitekijäisten tautien, kuten lastenreuman tautikytkentöjen selvittämisen pienemmästäkin potilasmäärästä, koska (1) kytkentäepätasapainon uskotaan käsittävän suurempia kromosomipalasia ja (2) on todennäköistä, että suppeassa ns. perustajaväestössä on altistavia geenejä rajoitetusti.

Tässä väitöskirjatutkimuksessa tutkittiin lastenreuman suvuittaista kertymistä ja HLA alleeli assosiaatioita kytkentä- ja assosiaatioanalyyseilla Reumasäätiön sairaalan sisaruspari-aineistossa.Väitöstutkimuksen tulokset osoittavat, että lastenreuman periytyvyys on selvästi voimakkaampaa kuin aikuisten nivelreumassa. Monotsygoottisten, perimältään identtisten kaksosten aineistossa todettiin, että 25 %:ssa kumpikin kaksosparin jäsen sairasti lastenreumaa. Tätä verrattaessa lastenreuman esiintyvyyteen väestössä (1/1000) identtisen kaksosen riski sairastua, jos toisella on lastenreuma, on 250-kertainen. Muiden sisrusten sairastumisriski on arviolta n. 15–20 kertainen.

Lastenreumaan usein liittyvän silmän värikalvotulehduksen esiintyvyys molemmilla sisaruspareilla ei poikennut odotetusta eikä selvää geneettistä tekijää tälle silmäkomplikaatiolle voitu osoittaa.

Lastenreumaa sairastavien sisarusten vanhemmilla todettiin odotettua enemmän kroonisia tulehduksellisia reumasairauksia, jotka joko olivat alkaneet vanhemmillakin jo lapsuudessa tai sitten ne kliiniseltä kuvaltaan sekä HLA-alleeliesiintyvyyden perusteella olivat lastenreuman kaltaisia.

Väitöstutkimuksessa määritettiin HLA-haplotyyppejä suomalaisilta lastenreumaa sairastavilta potilailta. Assosiaatioanalyysien tulokset vahvistivat taudille ainakin osittain altistavien geenien periytyvän yhdessä DRB1*08, DQB1*04 sekä Cw*04 alleelin kanssa.

Abstract

Genetic susceptibility to juvenile idiopathic arthritis (JIA) was studied in the genetically homogeneous Finnish population by collecting families with two or three patients affected by this disease from cases seen in the Rheumatism Foundation Hospital. The number of families ranged in different studies from 37 to 45 and the total number of patients with JIA, from among whom these cases were derived, was 2 000 to 2 300. Characteristics of the disease in affected siblings in Finland were compared with a population-based series and with a sibling series from the United States. A thorough clinical and ophthalmological examination was made of all affected patients belonging to sibpair series. Information on the occurrence of chronic rheumatic diseases in parents was collected by questionnaire and diagnoses were confirmed from hospital records.

All patients, their parents and most of the healthy sibs were typed for human leukocyte antigen (HLA) alleles in loci A, C, B, DR and DQ. The HLA allele distribution of the cases was compared with corresponding data from Finnish bone marrow donors.

The genetic component in JIA was found to be more significant than previously believed. A concordance rate of 25% for a disease with a population prevalence of 1 per 1000 implied a relative risk of 250 for a monozygotic (MZ) twin. An estimate for the sibling risk of an affected individual was about 15- to 20-fold.

The disease was basically similar in familial and sporadic cases; the mean age at disease onset was however lower in familial cases, (4.8 years vs 7.4 years). Three sibpairs (3.4 expected) were concordant for the presence of asymptomatic uveitis. Uveitis would thus not appear to have any genetic component of its own, separate from the genetic basis of JIA. Four of the parents had JIA (0.2 cases expected), four had a type of rheumatoid factor-negative arthritis similar to that seen in juvenile patients but commencing in adulthood, and one had spondyloarthropathy (SPA). These findings provide additional support for the conception of a genetic predisposition to JIA and suggest the existence of a new disease entity, JIA of adult onset.

Both the linkage analysis of the affected sibpairs and the association analysis of nuclear families provided overwhelming evidence of a major contribution of HLA to the genetic susceptibility to JIA. The association analysis in the Finnish population confirmed that the most significant associations prevailed for DRB1*0801, DQB1*0402, as expected from previous observations, and indicated the independent role of Cw*0401.

List of original publications

This thesis was based on the following original publications, referred to in the text by their Roman numerals. In addition some unpublished results are presented.

I Säilä H, Savolainen A, Tuomilehto-Wolf E, Tuomilehto J, Leirisalo-Repo M: Type of onset of juvenile idiopathic arthritis in monozygotic twins can be phenotypically different. J Rheumatol 2000; 27: 2289.

II Savolainen A, Säilä H, Kotaniemi K, Kaipiainen-Seppänen O, Leirisalo-Repo M, Aho K: Magnitude of the genetic component in juvenile idiopathic arthritis. Ann Rheum Dis 2000; 59: 1001.

III Säilä H, Savolainen A, Kotaniemi K, Kaipiainen-Seppänen O, Leirisalo-Repo M, Aho K: Juvenile idiopathic arthritis in multicase families: comparison with a population-based series and a United States sibling series. Clin Exp Rheumatol 2001; 19: 218–20.

IV Säilä H, Kotaniemi K, Savolainen A, Kautiainen H, Leirisalo-Repo M, Aho K: Uveitis in sibling pairs with juvenile idiopathic arthritis. Rheumatology 2001; 40: 221–4.

V Säilä H, Savolainen A, Kauppi M, Alakulppi N, Kinnunen L, Tuomilehto-Wolf E, Tuomilehto J, Leirisalo-Repo M, Aho K. Occurrence of chronic inflammatory rheumatic diseases among parents of multiple offspring affected by juvenile idiopathic arthritis. Clin Exp Rheumatol 2003; 21: 263–5.

VI Säilä H, Pitkäniemi J, Tuomilehto J, Savolainen A, Alakulppi N, Tuomilehto-Wolf E, Leirisalo-Repo M, Aho K. HLA and susceptibility to juvenile idiopathic arthritis: a study of affected sibpairs in an isolated Finnish population. J Rheumatol 2004; 31: 2281–5.

 Publication IV is to be found in the thesis by Kotaniemi K (2001).

Abbreviations

ACR	American College of Rheumatology
AG	Antigen
ANA	Antinuclear antibodies
ARA	American Rheumatism Association
AS	Ankylosing spondylitis
ASP	Affected sibpair
β2-m	β2-microglobulin
CI	Confidence interval
DZ	Dizygotic
ESR	Erythrocyte sedimentation rate
EULAR	European League of Associations of Rheumatology
HLA	Human leukocyte antigen
ILAR	International League of Associations of Rheumatology
JCA	Juvenile chronic arthritis
JIA	Juvenile idiopathic arthritis
JRA	Juvenile rheumatoid arthritis
λs	sibling recurrence ratio
LOD	logarithm of odds
MHC	Major histocompatibility complex
MZ	Monozygotic
RA	Rheumatoid arthritis
RF	Rheumatoid factor
SBT	Sequence-based typing
SD	Standard deviation
SNP	Single nucleotide polymorphism
SPA	Spondyloarthropathy
SSP	Sequence-specific primer
TCR	T cell receptor
TDT	Transmission disequilibrium test
TNF	Tumor necrosis factor

Introduction

Chronic arthritis of childhood comprises heterogeneous group of disease conditions of unknown cause. Characteristic for them is inflammation in one or more joints, together with occasional manifestations in other organ systems. Some disease types such as oligoarthritis with associated chronic uveitis are seen mainly in children, whereas certain other types are seen both in children and in adults, although their respective clinical phenotypes may differ to some degree.

The overall incidence of chronic arthritides of childhood is only one-tenth of that of rheumatoid arthritis (RA) in adults. Nevertheless juvenile patients frequently live some 60–70 years with their disease. The life-long burden caused by the disease is thus considerable both for the individual and for society.

George Fredrich Still described chronic arthritis in childhood in his publication 'On a form of chronic joint disease in children' (Still 1897). After recognising childhood arthritis as distinct from chronic arthritis in adults, Still was the first author to draw attention to the heterogeneity of childhood chronic arthritis, and juvenile-onset chronic arthritis has since been thought to be more than one disease. Still also described a specific subgroup of childhood arthritis characterized by an acute onset of disease, where the patient in addition to arthritis had lymphadenopathy, splenomegaly, fever and pericarditis. In early days all chronic arthritides in children were called Still's disease, wherwas nowadays this term is reserved for the systemic form of the disease.

Much work has been done to determine the genetic contribution to childhood chronic arthritis. The relevant evidence comes mainly from observations of associations of human leukocyte antigen (HLA) with certain forms of arthritis. The genetic associations with HLA seem to vary according to disease subtypes. Few family or sibling series have hitherto been published, but as the basic populations from which these cases were derived are only poorly known, it is difficult to draw any conclusion from these studies as to the overall magnitude of the genetic component.

Review of the literature

1 Definition and classification

In the 1970s two sets of criteria were introduced for the diagnosis and classification of childhood chronic arthritis, those proposed by a committee of *The American Rheumatism Association (ARA*), and those by a committee of *The European League of Associations of Rheumatology (EULAR*). The main differences between these two sets of criteria are the requirement concerning the duration of arthritis necessary for diagnosis and the subsets of the disease included.

1.1 Juvenile rheumatoid arthritis

ARA, subsequently *The American College of Rheumatology (ACR) Criteria Subcommittee* published in 1972 the initial criteria for the classification of children with *juvenile rheumatoid arthritis (JRA*). The minimum duration of arthritis necessary for diagnosis was set at 6 weeks (Brewer et al.1972). Modifications of the original criteria were developed in 1976 to place greater emphasis on subgroups according to the onset type of the disease: systemic, polyarticular and pauciarticular (Brewer et al. 1977). A further subdivision was made according to the course of the disease after the onset period of six months (Cassidy et al. 1986).

1.2 Juvenile chronic arthritis

At a workshop arranged by *EULAR* in 1977 the term *juvenile chronic arthritis (JCA*) was proposed (Wood 1978). JCA was defined as an arthritis persisting for at least 3 months. The following subgroups were distinguished according to the EULAR criteria: rheumatoid factor (RF)-negative chronic arthritis, which includes systemic, polyarticular and pauciarticular disease, and RF-positive polyarticular disease. JCA also includes children with juvenile ankylosing spondylitis (AS), psoriatic arthropathy and the arthropathies associated with inflammatory bowel disease, whereas the ACR criteria do not cover these subsets.

1.3 Juvenile arthritis

The term *juvenile arthritis* has sometimes been used to encompass a wider range of arthritic conditions than those covered by the ACR and EULAR criteria, but the term has not been generally accepted and remains controversial (Cassidy and Nelson 1988; Cassidy et al. 1989).

1.4 Juvenile idiopathic arthritis

The ACR criteria widely used in North and South America and the EULAR criteria primarily used by European investigators are not wholly interchangeable. This lack of uniformity led to an attempt to standardize the diagnostic and classification criteria worldwide. Along these lines, the Classification Taskforce of the Pediatric Standing Committee of *The International League of Associations of Rheumatology (ILAR)* proposed a classification system with special emphasis on defining homogeneous subgroups (Fink 1995). The term suggested for the disease was *juvenile idiopathic arthritis (JIA)* according to the Durban criteria (Petty et al. 1998). A minor revision of the criteria was recently published (Petty et al. 2004).

The basic requirements are arthritis in one or more joints, persisting for at least 6 weeks and onset before the 16th birthday. The disease is divided into seven subtypes: systemic arthritis, polyarticular form positive and negative for RF, oligoarthritis (persistent and extended oligoarthritis), enthesitis-related arthritis, and psoriatic arthritis. In addition, a category termed 'other' comprises children who do not fit into any of the above categories or who fit into more than one. A new disease group, extended oligoarthritis, covers a group of patients in whom the pattern of arthritis alters from an initial oligoarthritis to polyarthritis. In addition, certain extra-articular clinical features are used for the classification of enthesitis-related arthritis and psoriatic arthritis. The term enthesitis-related arthritis is more useful than those previously employed, for example juvenile AS, because few children in fact evince radiographic changes in the sacroiliac joints or definite signs of AS in their spine before 16 years of age. This subgroup was described as early as 1982 by Rosenberg and Petty in their work on the RF-negative enthesopathy and arthropathy syndrome (Rosenberg and Petty 1982); they recognized it as a juvenile manifestation of AS. The subgroup psoriatic arthritis is more useful as a separate entity rather than being lumped into a spondyloarthropathy group, since juvenile psoriatic arthritis may present with different joint patterns; only a minority of patients have axial involvement, and few are HLA B27-positive.

1.5 Juvenile idiopathic arthritis versus adult rheumatoid arthritis

The subtype of RF-positive polyarthritis is considered to be the pediatric equivalent of adult rheumatoid arthritis; indeed, this subtype shares genetic markers with adult RA (Moore et al. 1984; Vehe et al. 1990). Systemic onset JIA can sometimes occur in adults, and is referred to as 'adult-onset Still's disease'. A subtype which has been thought to occur exclusively in children is oligoarthritis beginning in early years, often associated with chronic anterior uveitis. This disease subtype has been the subject of the largest number of HLA studies and is genetically the best defined entitity (Fernendez-Viña et al. 1994). A proportion of RF-negative polyarthritis is possibly related to the RF-negative RA of adults, on the basis of certain genetic links which they are reported to have in common (Gao et al. 1991).

The various concepts used to classify chronic arthritis in childhood have mainly emerged through clinical features. Some serological features have been used for further subclassification without any official recommendations until those made by ILAR criteria comittee (Petty et al. 2004). A list of additional features, called descriptions, such as the presence of antinuclear antibodies (ANA), do not form a part of the classificatin as such, but may be important indicators of outcome. The different terms used for chronic arthritis in childhood and their contents are set out in Table 1.

2 Occurrence of juvenile idiopathic arthritis

Data published on the incidence and prevalence of JIA have shown marked variation, which can be accounted for by differences in the diagnostic criteria used, the nature of the population studied, the number of patients and sizes of the populations on which the figures are based, methods of patient retrieval, and the length of the period studied. The ideal approach might be prospective population-based studies of well-defined disease groups from different geographical areas and in populations with different genetic backgrounds.

The different methodologies used for case identification include population-based cohorts, practitioner surveys, clinic-based studies (in which case identification is made in pediatric rheumatology specialty clinics), and disease registers. Population surveys have yielded the highest rates, followed by practitioner surveys, whereas the lowest numbers have been obtained from clinic-based studies, where the proportion of severe cases is fairly high and the studies in question have missed a notable proportion of mild cases (Oen and Cheang 1996).

Table 1 *A comparison of the sets of criteria used for chronic arthritis in childhood.*

Definition		**Classification for chronic juvenile arthritis**		
		Juvenile chronic arthritis	**Juvenile rheumatoid arthritis**	**Juvenile idiopathic arthritis**
Criteria (term)		**EULAR (JCA)**	**ACR (JRA)**	**ILAR (JIA)**
Time	Disease duration	Three months	Six weeks	Six weeks
Clinical features	Arthritis type:	Poly	Poly	Poly
		Oligo	Oligo	Oligo:
		Systemic	Systemic	persistent extended
		Psoriatic		Systemic
		Juvenile ankylosing spondylitis		Psoriatic
		Arthritis in connection with inflammatory bowel disease		Enthesitis-related
				Other
Serological features	Rheumatoid factor	Subclassification: RF-positive: juvenile rheumatoid arthritis	Subclassification: RF-positive polyarthritis	A criterion for RF-positive polyarthritis
	HLA-B27	Not considered	Not considered	A criterion for enthesitis-related arthritis

2.1 Prevalence

Based on reliable data, the prevalence of JIA in the pediatric population has been estimated to be about 100/100 000 from sufficiently extensive studies (Manners and Bower 2002; Andersson Gäre 1999; Towner et al. 1983; Moe and Rygg 1998; Andersson Gäre and Fasth 1992). Nonetheless wide variations in prevalence have been reported from different geographical locations, ranging from 8 to 400/100 000 in the pediatric population (Table 2). The lowest estimate, 8/100 000, is from one region in France and was

probably influenced by patient selection; it is possible that a considerable proportion of mild cases were not reported (Prieur et al. 1987). The highest prevalence, 400/100 000, was reported in a study from Australia where case ascertainment was based on population questionnaires in combination with clinical examination by a pediatric rheumatologist (Manners and Diepeveen 1996).

2.2 Incidence

The worldwide annual incidence of JIA is reported as 0.8 to 22.6 per 100 000 children from areas where reliable data are available (Table 2) (Manners and Bower 2002). Lower figures may have been influenced by flaws in methodology. Thus, both the Finnish study by Laaksonen and associates (1966), which reported a very low incidence figure, 3.8/100 000, and the Japanese study by Fujikawa and Okuni (1997), reporting an annual incidence of 0.8/100 000, covered hospital cases only.

The incidence of JIA in Finland reported in recent studies by Kaipiainen Seppänen and Savolainen (1996, 2001) ranged from 13.5 to 19.5/100 000. The average age of JIA onset is around 7–8 years and if the disease is persistent, the duration (to age 16) is around 8 years. Thus according to incidence figures, the prevalence figures in Finland would be about 108–156/100 000.

Differences in the occurrence of JIA have been reported between patient groups depending on their ethnic and geographic background. These variations may result either from genetic or from environmental influences. In population-based studies with a Caucasian dominance, the incidence of JIA has ranged from 10 to 23 per 100 000 (Berntson et al. 2003; Andersson Gäre 1999). A clearly lower occurrence has been reported in a Hispanic population, an incidence of 6.8 per 100 000 (Arguedas et al. 1998) and in African American children, in whom the frequency of the oligoarticular subtype (34.3% vs 54.7% in Caucasian children) and the presence of antinuclear antibodies (ANA) in oligoarticular JIA patients (33.3% vs 70.7%) were also less common than in their Caucasian counterparts (Schwartz et al. 1997).

A tendency toward a decreasing north to south gradient in incidence has been noted in Europe. No such gradient would appear to exist in North America (Andersson Gäre 1999). The high incidence rates reported from Scandinavian countries may be a true phenomenon based on genetic or environmental factors. Interestingly, the incidence of another important autoimmune childhood disease, type 1 diabetes, is also high in Nordic countries, especially in Finland.

A cyclical pattern of incidence reported from North America and Finland suggests a role of environmental influences. Peterson and colleagues (1996) found an overall decrease in the incidence of JIA during 1960–1993 in Rochester, Minnesota, especially in the case of the oligoarticular and systemic onset types. On the other hand, Kaipiainen-Seppänen and Savolainen (1996) reported a slight increase in the incidence of oligo-

Table 2 *Incidence and prevalence of JIA, lowest and highest figures reported in different populations.*

Reference (first author)	Population	Diagnostic criteria	Incidence (100 000/year)	Prevalence (/100 000)
Laaksonen (1966)	Finland	Other*	3.8	ND†
Towner (1983)	Minnesota, USA	ARA EULAR	13.9 (1960–79) 10.8	113 84
Kunnamo (1986)	Finland	ARA	19.6 (1982–83)	ND
Prieur (1987)	France	EULAR	1.3–1.9 (1981–82)	8–10
Andersson Gäre (1992)	Sweden	EULAR	10.9 (1984–88)	86.3
Kaipiainen-Seppänen (1996)	Finland	ARA	13.8 (1980) 15.1 (1985) 13.5 (1990)	ND
Symmons (1996)	UK	EULAR	10 (1990–95)	ND
Peterson (1996)	Minnesota, USA	ARA	15.0 (1960–69) 14.1 (1970–79) 7.8 (1980-93)	94.3 (1980) 86.1 (1990)
Manners (1996)	Australia	EULAR	ND	400
Fujikawa (1997)	Japan	ARA	0.8 (1994)	ND
Moe (1998)	Norway	EULAR	22.6 (1985–94)	148
Arguedas (1998)	Costa Rica (Hispanic, American Indian)	EULAR	6.8 (1993–95)	34.9
Kaipiainen-Seppänen (2001)	Finland	ARA	19.5 (1995)	ND

* ARA or EULAR criteria not used at that time. † Not done.

and polyarticular groups of disease in 1995 compared to the years 1980, 1985 and 1990, while no such increase was observed in the systemic group. Seasonal variation reported in incidence studies concerning systemic JIA suggests the role of environmental triggers such as infections (Lindsley1987; Peterson et al. 1996).

3 Clinical features and course of juvenile idiopathic arthritis

3.1 Systemic onset juvenile arthritis

Systemic features are most prominent in the early phase of systemic onset juvenile arthritis. They include high, intermittent fever, typical papulomacular rash and visceral involvements such as hepatosplenomegaly, lymphadenopathy, pericarditis or other evidence of serositis. These features, which are typically intermittent, persisting from a minimum of 2 weeks up to 5 years at the longest, gradually vanish. Children may have significant arthralgias and myalgias or even arthritis during the febrile period, but the development of overt arthritis is necessary for a diagnosis of systemic arthritis. Arthritis may be oligoarticular or polyarticular. Typical laboratory findings are elevated white cell and platelet counts and a high erythrocyte sedimentation rate (ESR). Serum ferritin levels are of diagnostic value for the disease and reflect the response of the fever to treatment (Pelkonen et al. 1986). RF and ANA are usually absent. The peak age at onset is 2 years and the disease occurs with almost equal frequency in boys and girls (Woo and Wedderburn 1998). In some children, the systemic phase lasts from only a few months to several years, but others have recurring episodes or persistent systemic manifestations. Important for the outcome is the type of arthritis, which is more favorable in patients with oligoarthritis compared with the polyarticular form.

3.2 Polyarthritis, rheumatoid factor-positive/-negative

In polyarticular juvenile arthritis five or more joints are affected within the first 6 months after onset. Most of the children in question have symmetric arthritis of knees, ankles, wrists, elbows and shoulders, and the cervical spine is often involved in this type of disease. Temporomandibular joint arthritis is relatively common in children with polyarthritis (Cassidy and Petty 2001).

Polyarticular RF-positive JIA is predominantly a disease of teenage girls. Some of the prominent features are rapid progression and severity of the arthritis, affecting especially small joints and leading to early erosions. The severity of RF-positive polyarthritis is about the same as that of the polyarticular type of systemic onset disease (Cassidy et al. 1989).

RF-negative polyarthritis occurs in children at all ages, with two peaks, one at 2 to 3 years and another at preadolescent age. The proportion of boys is higher than in RF-positive patients, and boys are more likely to be in the younger age group. Chronic uveitis develops in approximately 5–10% of these children (Cassidy and Petty 2001; Fink et al. 1995), and such cases are frequently ANA-positive.

3.3 Oligoarthritis, persistent/extended

Oligoarticular onset JIA, involving 1 to 4 joints, is the largest patient category (50–75% of patients). Most patients are girls and the peak incidence is at the age of 3 years. These young children are frequently ANA-positive and chronic uveitis is common (10–20%) (Kotaniemi et al. 2003). The first affected joint is most typically the knee. There are two subgroups of oligoarthritis: persistent and extended, in which latter the arthritis spreads to more than 4 joints after the first 6 months of disease. Patients with extended oligoarthritis have an outcome comparable to those with initial polyarthritis. In those with persistent oligoarthritis the articular prognosis is often good; remission frequently occurs in 4 or 5 years (Woo and Wedderburn 1998). No outcome difference has been shown between patients with persistent monoarthritis and those with oligoarthritis (Stillman and Barry 1977).

3.4 Enthesitis-related arthritis

Patients with enthesitis-related arthritis are often boys of the age of six or older. They typically have asymmetrical arthritis of the lower limbs and enthesitis, the latter being an inflammation of the attachment between the bones and tendons. There is a strong association with the HLA allele B27 and such patients are at risk of developing acute anterior uveitis. Subsequently they may develop a disease resembling chronic spondyloarthropathy of adults (Woo and Wedderburn 1998).

3.5 Juvenile psoriatic arthritis

Juvenile psoriatic arthritis is defined as a chronic inflammatory arthritis occurring before the age of 16 years, preceded by, accompanied by, or followed by psoriasis. The diagnosis of psoriatic arthritis is based either on arthritis associated with a typical psoriatic rash, or arthritis associated with at least two of the following three minor criteria: nail abnormalities, dactylitis and a positive family history of psoriasis in a first-degree relative.

Patients with psoriasis may have different types of articular involvements. A large majority of these children have an asymmetric oligoarthritis affecting small as well as large joints at disease onset, although with time most of them become polyarticular. Patients are usually RF-negative, but some with psoriasis may have RF-positive polyarthritis resembling adult RA (the presence of RF is regarded as an exclusion criterion for psoriatic arthritis) (Petty et al. 1998). A few HLA B27-positive patients will develop ankylosing spondylitis with sacroilitis. Juvenile psoriatic arthritis is quite rare and there is a slight predominance of girls (Southwood et al. 1989).

3.6 Distribution of subtypes of juvenile idiopathic arthritis

Studies of the occurrence of JIA have revealed some ethnic variation in the proportion of disease subtypes. In populations of North European ancestry oligoarthritis is the most common type of JIA, comprising 50 to 75% of cases. The proportion of polyarthritis is estimated to be 20% to 40% of JIA cases and the great majority of these are RF-negative. Systemic onset JIA is diagnosed in about 3 to 10% of JIA patients. According to the Durban classification the proportion of enthesitis-related arthritis is about 7% to 10% and that of psoriatic arthritis about 4% to 5% (Cassidy and Petty 2001).

In line with the previous comment, in community-based studies from Finland, 66–76% of children have had oligoarthritis, 18–31% had polyarthritis, and only 3–6% systemic onset JIA (subclassification according to ARA criteria) (Lantto and von Wendt 1985; Kunnamo et al. 1986; Kaipiainen-Seppanen and Savolainen 1996).

The proportion of polyarticular JIA appears to be high and that of pauciarticular JIA strikingly low among African, East Indian, Japanese, and Thai children (Grahan and Glass 1997). This may be due in part to selective loss of mild oligoarthritis cases from the patient series. On the other hand, there is probably a true dearth of early-onset pauciarticular disease in non-Caucasian patients (Oen and Cheang 1996). A high frequency of seronegative spondyloarthropathies was reported among Inuit children in the Northwest territories of Canada (Oen et al. 1986). No differences have been noted between racial groups in the proportion of patients with systemic onset JIA.

3.7 Complications of juvenile idiopathic arthritis

Ocular complications are common in children with JIA and visual impairment may be a consequence of chronic silent uveitis. Two types of uveitis are linked to JIA. Chronic uveitis is usually asymptomatic and often bilateral. It is most commonly seen in ANA-positive girls with early-onset oligoarthritis. The same type of uveitis occurs less frequently in polyarticular disease and very rarely in the systemic form of JIA. In most instances, uveitis manifests within 7 years from the onset of arthritis, but may in fact precede arthritis by years (Kanski 1990). The other type of uveitis, acute anterior uveitis, is more acute and clearly symptomatic. Patients are generally preteen and teenage boys and they are usually ANA-negative. Acute anterior uveitis is often associated with enthesitis-related arthritis (Woo and Wedderburn 1998).

Abnormalities of growth and development sometimes complicate severe JIA. Both the chronic inflammatory state and anti-inflammatory treatment with glucocorticosteroids may impede linear growth. Inflammation of joints can result in local growth disturbances, causing micrognathia, bradydactyly and unequal length of long bones. Long

duration of active disease can lead to shortening of muscles and tendons, which may give rise to flexion contractures. Some serious extra-articular manifestations associated mainly with systemic onset JIA are heart involvements such as pericarditis, myocarditis and endocarditis, severe anemia or, more rarely, disseminated intravascular coagulation (Schwartz et al. 1992).

Secondary amyloidosis is one of the most serious long-term complications of JIA. According to some studies the majority of amyloidosis patients have the systemic disease subtype (Stoeber 1981). The incidence of secondary amyloidosis as a complication of JIA has been reported to be rare in North America, but is found more commonly in European children with JIA. There is evidence that the incidence of amyloidosis in JIA is declining (Savolainen and Isomäki 1993).

3.8 Autoantibodies in juvenile idiopathic arthritis

Many immunological abnormalities, including the production of autoantibodies, have been observed in children with various forms of chronic arthritis, but few findings are clinically useful or well understood (Leak 1988; Lawrence et al. 1993). The most common immunological abnormality in JIA is a positive ANA, which usually exhibits a homogeneous or speckled pattern in the indicator cell. The frequency of positive reaction depends on the techniques applied. On average, ANA has been detected by immunofluorescence in roughly 30% of children with JIA, most frequently in patients with oligoarthritis, less so in those with RF-negative polyarthritis and only seldom in the other subtypes of JIA. ANA-positive JIA patients run a greatly increased risk of developing uveitis. The underlying reason for this association is not known.

Specific components of nuclei have been identified as the antigens for the ANA reaction in patients with systemic lupus erythematosus and certain other systemic rheumatic diseases such as double-stranded DNA, Sjögren syndroma antigens (SS-A, SS-B) and Sm (Smith) antibodies. Antibodies against these components do not occur in patients with JIA; the nature of the antigen(s) responsible for positive ANA in JIA patients has not yet been settled with certainty.

Antibodies against soluble bovine retinal S protein may be linked to ocular damage in patients with JIA, since children with JIA-associated uveitis have been reported to have higher antibody levels compared to those without eye disease and to normal controls (Petty et al. 1987). Other investigators, however, have not been able to confirm this finding (Edelsten et al. 1996). Type II collagen induces arthritis, and occasionally uveitis in animals. In children with arthritis and uveitis, antibodies and cell-mediated immunity to native or denaturated human or bovine collagen are not more frequent than in children with arthritis alone (Petty and Hunt 1989).

RF as determined by conventional test techniques occurs only seldom in patients

with JIA. Those children who do have a persistent high-titred RF quite obviously have a genuine RA which has its onset in childhood. Some attention has been paid to the occurrence of so-called hidden RF, i.e. RF blocked by autologous IgG, in other forms of JIA (Lawrence et al. 1993), but there seems to be no unanimity as to the validity of these findings.

4 Genetic epidemiology of juvenile idiopathic arthritis

Three lines of evidence support the conception of a role of genetic predisposition to JIA: racial differences in risk and the predominance of different JIA subtypes in different ethnic groups, the occurrence of multiple cases of JIA in families, and associations between particular HLA alleles and certain forms of JIA.

4.1 Familial aggregation of juvenile idiopathic arthritis

The starting-point in determining the genetic component involved in the etiology of the disease is to observe the clustering of disease in certain families. However, familial clustering can be caused by genetic factors, shared environment, or both. Reports of families with multiple affected members are rare in JIA.

Ansell and associates (1969) made detailed family studies examining clinical, serological and radiological variables among 228 first-degree relatives of 92 probands meeting the criteria for a JCA-resembling disease, and found some excess of inflammatory rheumatic diseases compared to the adult population as a whole. Most of the cases among relatives were of adult onset; no data were given on diseases already commencing in childhood. In a study from the Netherlands a higher than expected prevalence of spondyloarthropathy (4% vs 0.25%) was noted among parents of 70 children with oligoarticular arthritis (Hertzberger-ten and Dijkmans 1993). In a Swedish population-based cohort study 5.6% of patients with JIA had mothers who had been diagnosed with RA. Of these patients 9.7% had a first-degree relative with psoriasis and 2.4% a father with AS (Andersson Gäre and Fasth 1995). Prahalad and colleagues (2002) demonstrated that relatives of patients with JIA evinced an increased frequency of autoimmune disorders (12.6% vs 4.0% in control individuals) and that the prevalence of inflammatory arthritis was higher among relatives of JIA-affected sibpairs (ASP) than in simplex families. No details were given concerning the type of arthritis.

Typical for diseases with a genetic predisposition is that first-degree relatives of an affected individual are at increased risk of disease (Risch 1990). The most commonly used indicator of the magnitude of the genetic component is the sibling recurrence ratio (λs), i.e. the prevalence of the disease in sibs of affected probands divided by the preva-

lence of the disease in the general population (Glass and Giannini 1999). The familial aggregation of JIA was recently demonstrated by identifying multiplex JIA pedigrees in a study by Prahalad and associates (2004), who provided a population-based estimate of the recurrence risk for first-degree relatives of about ~ 30.

4.2 Affected sibling pairs with juvenile idiopathic arthritis

Other reports of families with multiple affected members involve affected sibpairs. Yodfat and colleagues (1971) described a family in which five out of seven siblings had JIA with an oligoarticular course. The fifth had an initial history of arthralgia but it remained unclear whether true arthritis was ever documented. Rosenberg and Petty (1980) reported two ASPs in whom the clinical course was concordant in both members of the pair. One pair with pauciarticular JIA were also concordant for the presence of chronic uveitis. Ansell and Albert (1984) published data on 19 families with ASPs collected in Taplow and Northwick Park, England.

Two small sibling series and a larger one with more detailed data on HLA haplotype sharing have been published. Clemens and associates (1985) reported data on 12 families with sibling pairs affected by RF-negative JIA. The ASPs were gathered from among patients admitted to Juvenile Rheumatism Units at Taplow, England and Garmish-Partenkirchen, Germany. The study focused on HLA haplotype sharing and provided rather little clinical information on the patients. Perhaps the most striking clinical finding was that 11 pairs were concordant for the onset type. Likewise, in 11 pairs the disease began at about the same age. Suciu-Foca and associates (1980) evaluated 14 ASPs with pauciarticular JIA, but mainly from a genetic (HLA) point of view.

An ASP series from the United States was collected by means of a multiple advertising campaign directed to United States physicians likely to care for patients with JIA. Moroldo and associates (1997) reported 71 ASPs. A higher than expected degree of concordance for onset type suggested a role of genetic influences. Prahalad and colleagues (2000/a) continued the study and described altogether 118 pairs of siblings registered in the above National Institute of Arthritis and Musculoskeletal and Skin Diseases–sponsored Research Registry for JRA ASPs. Recently, complete clinical information has become available on 183 ASPs (Moroldo et al. 2004).

The prevalence figure for JIA in the general population is based on fairly reliable data, but that for JIA in sibs of those affected is only an estimate, since there are no satisfactory data on the population from which the ASP series were derived. In a study described by Clemens and colleagues (1985) six of the 12 ASPs were reported to be derived from 2 000 patients in a clinic population. On the basis of the above figures it is difficult to compute the risk to siblings of affected family members. Glass and Giannini (1999) suggested a λs of 15 from figures for the estimated total number of 300 JIA ASPs in the US population, in which there are about 71 000 patients with JIA.

4.3 Twin pairs with juvenile idiopathic arthritis

A classic twin study compares concordance of monozygotic (MZ) and dizygotic (DZ) twins for disease, higher concordance rates observed in MZ than in DZ twins providing evidence for the involvement of genetic factors in the disease. However, the MZ/DZ ratio is dependent on the number of genes involved, not only on the magnitude of the genetic component. Likewise, in diseases of multifactorial etiology the twin concordance rate is largely a measure of disease prevalence (Järvinen and Aho 1994).

Comparison of MZ and DZ twins, assuming that they share disease-relevant environmental factors to a similar degree but differ in their genetic similarity, allows assessment of heritability, which estimates the extent to which variation in the liability to disease in a population can be explained by genetic variations. It is independent of disease prevalence, and tells of prevailing individual genetic differences.

Baum and Fink (1968) reported an MZ twin pair with identical disease onset and course; both patients were diagnosed as having pauciarticular JIA. Kapusta and associates (1969) described one pair of identical twin boys and their mother who all had JIA. In all three, the disease progressed to chronic deforming polyarthritis, the onset of the disease was at an early age and the disease differed only in that the sons were ANA-positive and the mother ANA-negative. Husby and associates (1988) published a report on immunological studies of an MZ twin pair concordant for severe polyarticular JIA but discordant for monoclonal gammopathy and amyloidosis. Ansell, in her review of chronic arthritis in childhood, (1978) reported altogether six concordant MZ pairs. Two pairs had juvenile AS; no details were given on the phenotype in the other pairs. Meyerowitz and colleagues (1968) reported eight sets of MZ twins discordant for arthritis. Three of these sets had juvenile onset disease, one-RF positive and the other two pauciarticular.

From the ASP series reported by Moroldo and colleagues (1997) and by Prahalad and colleagues (2000/a) altogether 14 pairs of twins concordant for JIA were reported. Eleven pairs were MZ, one pair was opposite-sex DZ, and the zygosity of two pairs is yet to be determined (Prahalad et al. 2000/b). The most recent clinical information includes 19 pairs of twins (Moroldo et al. 2004). No conclusions were drawn as to the frequency of JIA among MZ or DZ twins, since the information was derived from a registry to which ASPs were selectively referred.

To judge from reported twins affected by JIA concordance for onset and course type has been fairly high in MZ twins. In addition, the onset of disease in twins has been reported to be much closer chronologically compared with that in non-twin ASPs. However, it is likely that MZ twin pairs concordant for a number of features are reported more frequently than pairs discordant for these features. To avoid selection bias, patients should ideally be identified from a population-based source.

5 Associations with genetic markers

5.1 The whole-genome scan

Genome-wide screening, in which all chromosomes are systematically screened in search of novel suscebtibility loci, can be used as an initial step in identifying genes predisposing to disease. The markers traditionally used are short tandem repeats, e.g. microsatellites, where DNA sequences show considerable variation among individuals (polymorphism). Recently, single nucleotide polymorphisms (SNP), single base pair variations, have been increasingly used for more detailed genotyping in chromosomal regions where a potential linkage may exist.

Data have been published from the first genome-wide screen in JIA populations, carried out in 121 pedigrees involving 130 JRA-affected sibpairs from the National Institute of Arthritis and Musculoskeletal and Skin Diseases sibpair registry (Thompson et al. 2004). Six chromosomal regions showed some evidence of linkage with JIA, logarithm of odds (LOD) score > 1 (p-value = 0.014), the most promising at chromosome 6. However, the thresholds for significant linkage (LOD > 3.3) and for suggestive linkage (LOD > 1.9) were not reached (Lander and Kruglyak 1995).

5.2 Candidate gene approach

A chromosomal region, once having indicated an area of interest for further analyses, can be used in the evaluation of candidate loci. Several aspects may be considered when selecting genes for investigation in JIA. The nature of the histopathology of the inflamed synovium is one starting-point. There is thus evidence that the underlying driving force for the chronic synovitis of JIA is antigen-driven and T-cell-mediated. Another important starting-point for genetic investigation is the expression of inflammation-associated proteins in affected children (Thomson and Donn 2002). However, there is hardly anything specific for JIA; the same arguments can be used for selecting candidate genes for most inflammatory joint diseases.

HLA genes probably account only for a certain part of total susceptibility, leaving the rest still to be identified. Non-HLA gene associations with JIA have recently been reviewed by Forre and Smerdel (2002), Thomson and Donn (2002/b) and Rosen and colleagues (2003). With one exception, the work done so far comprises only candidate gene studies, being therefore limited in respect of an overall assessment of genetic susceptibility to JIA. Of the non-HLA genes, attention has been drawn to T cell receptor genes, genes encoding different cytokines, natural resistant-associated macrophage protein 1 gene (regulating the expression of chemokine/cytokine genes), interferon regulatory factor and macrophage migration inhibitory factor. Taken as a whole, only few of

the observations have been confirmed, the odds ratios in most instances have been low and inconclusive, and many initially promising findings of candidate genes have suffered from lack of replication.

5.3 Analytical methods

The methods available for genetic investigation of complex traits are linkage analysis, which uses one of two basic techniques: lod score methods or allele-sharing methods, and association studies.

5.3.1 Linkage analysis

Linkage studies are designed to map previously unknown genes of etiologic relevance. Parametric linkage analysis follows co-segregation of two genetic factors at specific loci in pedigrees, using the frequency of meiotic recombination as an estimate of genetic distance. The establishment of linkage through conventional linkage analyses (logarithm of odds score) has been difficult in JIA due to lack of families with multiple cases affected. These linkage studies are likely to require several hundreds, possibly even thousands, of affected sibling pairs to provide sufficient power to detect linkage (Risch and Merikangas 1996).

One means of measuring linkage in complex diseases where the mode of inheritance is unknown is to use non-parametric linkage analysis. Allele-sharing methods and the simplest form of this method, affected sibling pair analysis, is a test for an excess of disease-associated allele-sharing in ASPs (Risch 1990). The method does not depend on the existence of linkage disequilibrium between the markers being tested and the disease genes, since the analysis is performed within families. The method can be divided into modifications which depend on marker alleles shared identical-by-descent and those which rely on alleles shared identical-by-state. Two alleles are said to be identical-by-state if they are the same variant of some polymorphic system, whereas identical-by-descent depends in addition on whether these alleles are inherited from a common ancestor. Each ASP is expected to share identical-by-descent 0, 1 and 2 alleles with respective probablities of 0.25, 0.5 and 0.25 according to random Mendelian segregation. When the parental genotypes are unknown, the probabilities of sibs sharing alleles identical-by-descent can be estimated depending on the frequency of the observed alleles at the marker locus in question (Lange 1986).

Two small sibling series have been published detailing haplotype sharing (Suciu-Foca et al. 1980; Clemens et al. 1985) and a larger one on allele sharing (Prahalad et al. 2000/a) in JIA patients. A marked excess of shared haplotypes was recorded in the very small series reviewed by Clemens and associates (1985) ($p = 0.006$). Prahalad and colleagues (2000/a) focused on the DR locus, as this is believed to be the major HLA locus

determining susceptibility to JIA. Based on statistics designed for identity-by-state, the authors showed a significant excess of sharing 0, 1 or 2 DR alleles: 8 : 40 : 32, instead of the expected ratio of 20 : 40 : 20 ($p < 0.001$).

5.3.2 Association-based methods

Association studies are non-parametric tests which have been used to determine whether marker allele frequencies at a given locus differ between affected and unaffected individuals. An allele is said to be associated with a trait if it occurs at a significantly higher frequency among affected compared with control individuals. Association studies are either case-control or family-based. In case-control studies the frequencies of genetic markers in patients are compared with those in control populations. Nearly 100 studies comprising different population groups have shown some consistent associations of HLA loci with specific subtypes of disease (for details see section 5.4.)

In family-based association studies the affected proband and members of the family are typed and assessed for evidence of disease. The tests require the presence of both association and linkage between the locus tested and the disease gene. Genetic linkage is assumed when two gene loci are physically close together on the chromosome. A disease gene will be linked to a particular allele within a given affected family but will not necessarily be associated with the same allele in unrelated affected persons.

The most popular extension of this approach is the transmission disequilibrium test (TDT), which focuses solely on heterozygous parental genotypes. TDT determines how many times potential disease alleles are transmitted from heterozygous parents to affected offspring and compares this number to the number of times transmission does not occur (Spielman and Ewens 1996). This method is an oversimplification for a situation where the affection status among other relatives is completely ignored.

In an American study by Moroldo and associates (1998) the TDT results established family-based associations of HLA alleles with pauciarticular onset JRA (HLA-A2, B27, B35, DR5 and DR8). Two other marker association studies have been conducted examining multiple markers in the major histocompatibility complex (MHC) region in addition to specific HLA genes (Zeggini et al. 2002; Runstadler et al. 2003).

5.4 Associations with HLA alleles

5.4.1 HLA system

Until recently, most of the genetic work on JIA has centred on HLA genes within the MHC located on the short arm of chromosome 6 (6p21.3). This is the most polymorphic system known in the human genome, consisting of several closely linked loci. Many of the genes within the MHC complex are associated with immune functions, and associa-

tions of specific HLA polymorphisms with several rheumatic diseases have been recognized (Nepom and Nepom 2003; Klein and Sato 2000).

The HLA region has been divided into three different subregions, class I, class II and class III (Figure 1). Class I antigens: HLA A, B and C consist of a polymorphic α chain encoded by HLA genes, and a β chain, in which beta-2 microglobulin is coded by a gene on chromosome 15. Class II genes (three major loci: HLA DR, HLA DQ and HLA DP) are heterodimeric glycoproteins. Most of the class II antigens consist of two different polypeptide chains, α and β, both controlled by genes in the HLA region. The role of these HLA molecules includes presenting foreign and self antigens to T-cells. T cell interacts with T cell receptor (TCR), CD4 and CD8 coreceptors to HLA molecules (Figure 2).

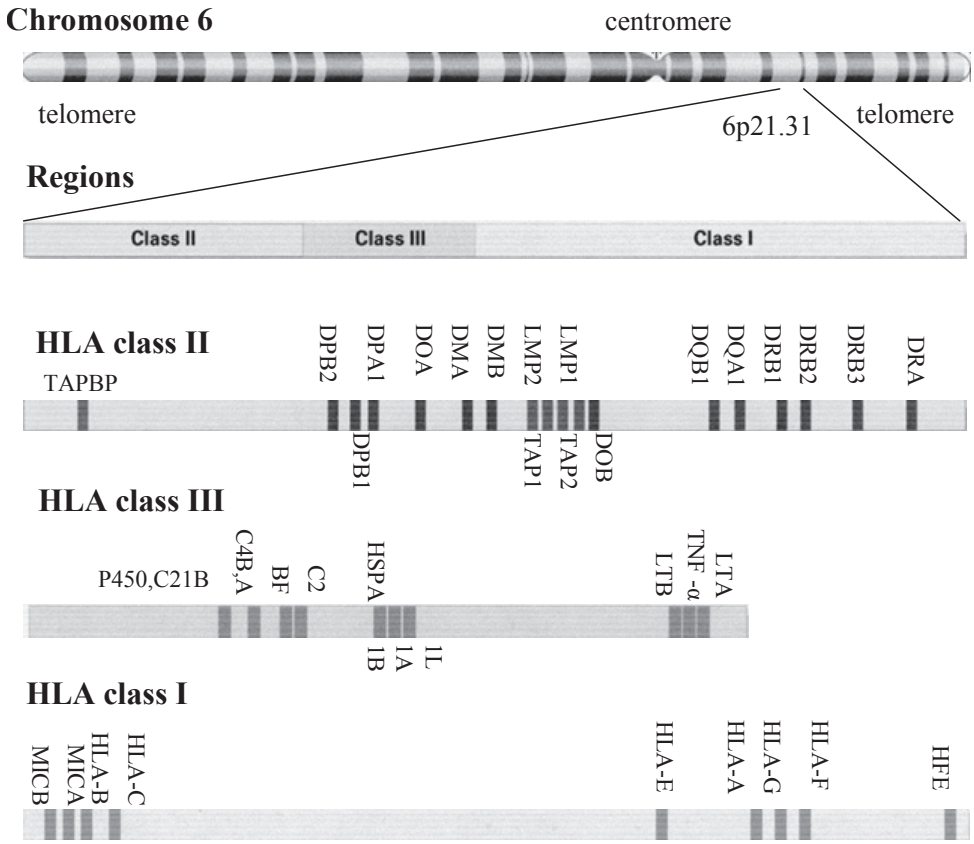


Figure 1 *Location and organization of the MHC complex on chromosome 6 (the figure is modified from Klein and Sato 2000). The complex is conventionally divided into three regions: I, II and III. Each region contains numerous loci (genes), only some of which are shown. Of the class I and II genes, only the expressed genes are depicted. Class III genes are not related to class I and class II genes structurally or functionally.*

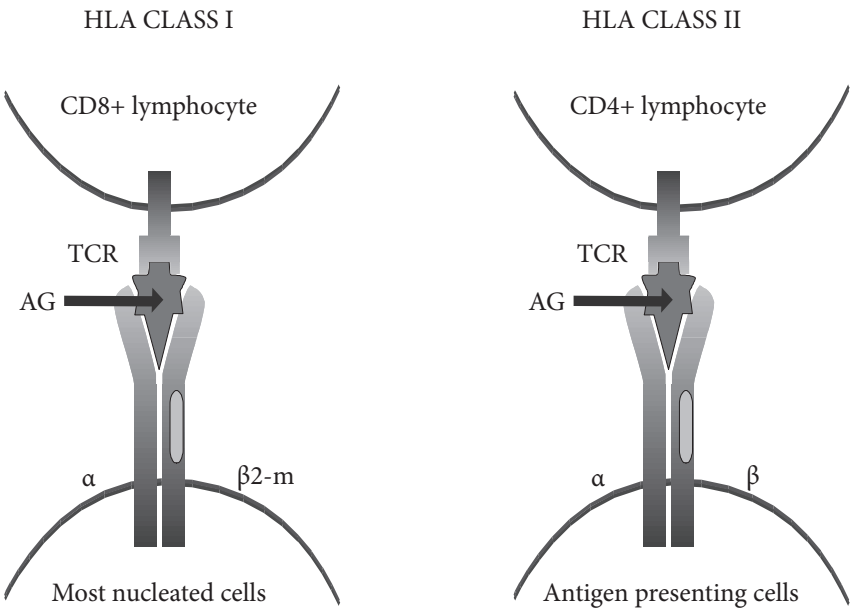


Figure 2 *Interaction between HLA and T-cell receptor. AG = antigen; TCR = T cell receptor; β2-m = β2-microglobulin*

HLA class I antigens are expressed in most nucleated cells and present cytosolic proteins (both self and non-self e.g., viral polypeptides, genetic origin) to CD8+ 'cytotoxic' lymphocytes. HLA class II antigens are expressed only in antigen-presenting cells (activated macrophages, B-lymphocytes, dendritic cells and thymic epithelial cells) and present degraded exogenous antigen fragments to CD4+ 'helper-inducer' lymphocytes.

The class III genes are situated between the class I and II genes. They encode for instance the complement factors C2 and C4 of the classical complement pathway and the properdin factor of the alternative complement pathway, the tumor necrosis factors (TNF) alpha and beta, the heat shock proteins and the enzyme 21-hydroxylase. In addition, genes for large multifunctional proteases involved in antigen processing in the cytosol (LMP2 and LMP7) and genes for transporters of antigen peptides (TAP1 and TAP2) have been mapped within the HLA region.

5.4.2 Difficulties in determining significant HLA associations

Progress in characterizing the genetic components of JIA and interpreting the significance of its HLA associations has proved difficult, for several different reasons. First, JIA is not a single disease but a group of clinical syndromes and HLA associations may vary between the disease subtypes. Secondly, HLA associations may appear differently

depending on age at onset of the disease. Murray and colleagues (1999) have shown HLA-specific windows of susceptibility which are, at least in part, related to JIA subgroups. Thirdly, irrespective of JIA the frequency of HLA alleles varies widely among different ethnic groups, being in fact one of the best anthropologic markers. Differences in HLA typing methodologies have resulted in difficulties in comparing allele frequencies. There are, moreover, differences in the occurrence of different subtypes of the disease among populations.

Finally, the strong linkage disequilibrium within the HLA region will render it difficult to identify the actual predisposing genes. The term linkage disequilibrium means that certain alleles at neighbouring loci occur together more frequently than expected by chance (Jorde 1995). In principle, the HLA region on chromosome 6 is inherited *en bloc,* suggesting that these genes have strong linkage disequilibrium and that jointly they have a certain biological meaning.

5.4.3 HLA-gene associations in juvenile idiopathic arthritis

HLA B27 was the first HLA allele found to be associated with JIA, reported in 1974 (Rachelefsky et al. 1974). Since 1979/80 other HLA associations with JIA as distinct from adult RA have been reported (Glass et al. 1980; Stastny and Fink, 1979). Subsequently, a replete of nearly 100 studies involving different population groups have dealt with HLA associations of JIA. Most have shown virtually the same allelic associations within the same subtype and marked differences among the subtypes (Scholz et al. 1993; Donn and Ollier, 1996; Glass and Giannini, 1999; Thomson and Silman, 2001; Forre and Smerdel, 2002). From Finland there is only one earlier study from 1978 (Mäkelä and Tiilikainen, 1978) covering the HLA A and B loci with a limited number of alleles known at that time.

The HLA B27 allele, which is strongly related to ankylosing spondylitis (Brewerton et al. 1973; Schlosstein et al., 1973), also contributes to the risk of late onset pauciarticular disease with a strong male predominance (Rachelefsky et al. 1974). Patients typically meet the current criteria for enthesitis-related arthritis. The prevalence of HLA B27 is also marginally increased in some other subtypes of JIA and may be associated with poor prognosis. In a Finnish study by Savolainen and associates. (1998) HLA-B27-positive cases were clearly accummulated among the most severe cases, i.e. among patients needing treatment with cytostatics and patients with amyloidosis or arthroplasty.

An increased frequency of the HLA A2 (HLA A*0201) allele has been demonstrated in children with JIA, particularly in girls with pauciarticular arthritis of early onset (Oen et al. 1982). Few studies have dealt with Cw locus alleles. There is some evidence that the prevalence of Cw4 is increased in pauciarticular disease (Howard et al. 1985).

RF-positive polyarthritis in children represents the counterpart of RF-positive RA in adults. The HLA associations are the same in these two patient groups; in particular, certain subtypes of HLA DR4 are involved (Vehe et al. 1990).

Early onset oligoarthritis occurs predominantly in girls and is not infrequently (in about 20% of cases) complicated by chronic iridocyclitis. In addition to HLA A2, a number of HLA class II alleles have been associated with this subtype (DRB1*08, DR5 (DRB1*11 and DR12), DR6 (DRB1*1301) as well as DQA1 *0401, *0501, *0601 and finally DP2 (DPB1*0201) (Albert and Scholz 1998; Nepom and Glass 1992; Paul et al. 1995). According to some studies HLA allele DRB1*1104 (a split of HLA DR5) is associated with the occurrence of chronic iridocyclitis (Malagon et al. 1992; Melin-Aldana et al. 1992). Recently, some evidence has been presented that persistent oligoarthritis and extended oligoarthritis are immunogenetically distinct (Zeggini et al 2002). Thomson and colleagues (2002) demonstrated a possible increase in the frequency of one haplotype (DRB1*13-DQA1*01-DQB1*06) in persistent oligoarthritis.

The HLA DRB1*0801 allele has the strongest association with RF-negative JIA, in both oligoarthritis and polyarthritis. The HLA DR8/DQ4 haplotype has been reported to represent a marker of seronegative arthritis in children in many populations (Nepom et al. 1992; Smerdel et al. 2002/b). The relative importance of the closely linked DR and DQ loci is difficult to establish.

In contrast to HLA DR/DQ alleles, reported disease associations with the DP locus are few. A number of studies have shown an association between oligoarthritis and HLA DPB1*0201 (Albert and Scholz 1998). RF-negative polyarthritis has been reported to be associated with HLA DPB1*0301 (Fernandez-Vina et al. 1990; Barron et al. 1992). This allele has been suggested to be associated with some subsets of RF-negative RA of adults (Gao et al. 1991).

The HLA associations in systemic JIA appear to be relatively weak and inconsistent. Some studies have shown an increase in DR4 (especially with severe arthritis in children with systemic onset JIA), although the association appears to be limited to Northern European populations (Miller et al. 1985; Nepom and Glass 1992; Paul et al. 1995), and may be a reflection of a higher frequency of DR4 in northern Europe.

Linkage disequilibrium across different HLA regions is strong. However, Zeggini and colleagues (2002) showed that at least the loci HLA-A and HLA DRB1 contribute independently to oligoarhritis and that these associations seem not to be a result of linkage disequilibrium between the markers involved, suggesting that in some patients several different HLA alleles (i.e. several HLA genes) are involved in the pathogenesis of JIA (Forre and Smerdel 2002). The available information on HLA associations is summarized in Table 3 (modified from Forre and Smerdel 2002).

In addition to the much studied HLA class II loci, the MHC contains many other genes which could be directly involved in JIA risk, or might interact with HLA II alleles. Of these, special emphasis has been laid on TNF protein and its receptors. The TNF region is highly polymorphic. Many of the alleles in question are in linkage disequilibrium with DR/DQ alleles, rendering it difficult to assess the independent role of genes in this region.

Recently, a positive association was described for a marker by the microsatellite

Table 3 *HLA association with juvenile idiopathic arthritis.*

JIA subgroups	HLA genes
Oligoarticular	Class I: A*0201, Cw4?, B27?
	Class II: DRB1*08, DR5 (DRB1*11, DRB1*12), DR6 (DRB1*1301) DQA1*0401, DQA1*0501, DQA1*0601 DPB1*0201
Polyarticular RF-negative JIA	Class II: DRB1*0801, DQB1*0402 DPB1*0301?
Polyarticular RF-positive JIA	Class II: DRB1*04
Systemic JIA	Class II: DRB1*04
Enthesitis-related arthritis	Class I: B27

D6S265 located in the vicinity of HLA-A, (Smerdel et al. 2002/a). This novel susceptibility gene in linkage disequilibrium with D6S265 will influence the risk of JIA independently of the DR-DQ haplotype. This provides additional evidence for the JIA susceptibility genes in the HLA class I region.

Due to a higher than expected prevalence of certain HLA alleles, other alleles may be compensatorily decreased. Claims that there may be alleles which seem to be truly protective are thus not easily confirmed.

Aims of this study

This study was undertaken to investigate in the genetically homogeneous Finnish population the genetic factors predisposing to JIA and to determine the clinical characteristics of the disease in families with multiple cases derived from a defined basic JIA population. Special emphasis was laid on HLA alleles and haplotypes.

The specific aims were:

1 To obtain information on the magnitude of the genetic component in JIA.

2 To compare the disease type in familial and sporadic JIA patients to obtain information on whether these represent basically the same disease.

3 To obtain information on the occurrence and characteristics of uveitis in sibling pairs affected with JIA.

4 To study the prevalence of chronic inflammatory rheumatic diseases among parents who have two or more offspring affected by JIA.

5 To determine the effects of class I (A, C, and B) and II (DRB1 and DQB1) HLA loci alleles on genetic susceptibility to JIA in families with two or more affected siblings.

Patients and methods

1 Patients and families

Multicase JIA families have been traced systematically at the Rheumatism Foundation Hospital in Heinola over a period of about 15 years. The hospital is a semi-private institution which receives patients from university and other central hospitals and directly from primary care, especially if the patient lives in an area near the hospital. It provides in- and outpatient care in conservative rheumatology, orthopedics and rehabilitation for children and adults. According to the patient register it has been estimated that about two-thirds of all Finnish JIA patients are seen at the Rheumatism Foundation Hospital, some regularly and others only occasionally.

1.1 Number of affected sibling pair families

During the period 1985 to 2000 41 families with 88 siblings affected by JIA satisfying the Durban criteria for JIA were found among the 2 312 patients treated at the pediatric department of the Rheumatism Foundation Hospital. Of these, about 90% had JIA. It can thus be estimated that the population of JIA cases from which the multicase families were derived amounted to some 2000 patients. This corresponds to about 60% of JIA cases in the country as estimated on the basis of the incidence of JIA in Finland (Kaipiainen-Seppänen and Savolainen 1996). In addition, four new families were later ascertained (study V).

The number of ASPs varied to some degree in different studies; the precise figures are shown in Table 4. Families having three affected siblings were counted as having three ASPs. No family had more than three affected siblings.

Of the 41 identified ASP families, HLA genotyping was undertaken in 38 nuclear families (study VI), as one family refused DNA analysis and one sibling pair was excluded due to technical reasons. One family was excluded because the pair was an MZ twin pair. The genetic analysis of an MZ twin pair cannot be regarded as a full sibling case, since identical twins would be expected to share all of their genetic information.

1.2 Diagnostic issues

All JIA patients and their parents were asked for a family history of rheumatic diseases. Those providing a positive answer filled a questionnaire requesting more details of the

Table 4 *Number of families, patients and ASPs available in different studies.*

	Study		
	II	**III, IV**	**VI**
No. of families	41	37	38
No. of patients	88	80	83
boys	*34*	*31*	*31*
girls	*54*	*49*	*52*
No. of ASPs	52	49	50

medical history of rheumatic diseases in the family. The diagnoses were confirmed from patient records.

Information on the parents' chronic inflammatory rheumatic diseases in multiple JIA families were collected from the parents themselves and the diagnoses were checked from the hospital records; the parents were not clinically studied. A total of nine parents from eight families were found to have some chronic rheumatic disease entitling to medication specially reimbursed by the National Social Insurance Institution. Eligibility for such approval in Finland requires a comprehensive medical certificate written by the attending specialist physician and approved by an expert adviser on behalf of the sickness insurance scheme. The certificates are not keyed to any specific criteria but are written to provide evidence that a subject has a chronic rheumatic disease and needs drug treatment for it (Myllykangas-Luosujärvi et al. 1995).

On the basis of information collected the diagnoses in children with chronic inflammatory joint disease were classified using the Durban criteria for JIA (Petty et al. 1998). The diagnoses of RA in parents were classified using the ACR classification criteria (Arnett et al. 1988) and for the diagnosis of spondyloarthropathy the preliminary criteria of the European Spondylarthropathy Study Group were used (Dougados et al. 1991).

In patients with JIA, the relevant clinical data and laboratory test results (ANA, RF) were recorded during the first six months from diagnosis (onset type) and during the subsequent follow-up (course type). The characteristics of the disease were evaluated during the follow-up.

1.3 Clinical findings in affected sibling pairs

The 50 ASPs in Study VI were derived from 83 patients, comprising 31 affected ASPs and seven sets of triples (three affected siblings in a family) with JIA (62 + 21 patients). As

noted above, the genetic analysis of an MZ twin pair cannot be regarded as full-sibling, as identical twins would be expected to share all of their genetic information. Thus a triple set including MZ twins was considered as one sibling pair. Each other triple set was counted as three sibling pairs; the series thus comprised a total of 50 ASPs (32 + 18 ASPs). Of the affected index cases, 31 were boys and 52 girls. The difference in distribution of diagnosis between boys and girls was not statistically significant ($p = 0.32$). Of the 50 ASPs, 8 were boy–boy, 17 girl–girl and 25 boy–girl pairs. Of the six trios, four were boy–boy–girl and two boy–girl–girl.

The onset type was oligoarticular in 55 patients (66%). The disease progressed into extended oligoarthritis in 16% of the patients. The disease was persistent oligoarticular in 42 (51%) of the patients and in most instances ran a mild course. The distribution of the subtypes is depicted in greater detail in Table 5.

Table 5 *Disease subtypes among the 83 patients classified according to the Durban criteria.*

Disease subtypes	**Boys (N = 31)**	**Girls (N = 52)**	**ALL**
	N (%)	**N (%)**	**N = 83**
Systemic	1 (3)	2 (4)	3 (4)
Oligo:			
persistent	17 (55)	25 (48)	42 (51)
extended	3 (10)	10 (19)	13 (16)
Poly			
RF+	0 (0)	1 (2)	1 (1)
RF–	7 (23)	14 (27)	21 (25)
Enthesitis-related	2 (6)	0 (0)	2 (2)
Psoriatic	1 (3)	0 (0)	1 (1)

The mean age at diagnosis of JIA was 4.5 years, range 1.0–15.7 years, median 3.2 years. In girls the mean age was 4.2 (median 3.0) and in boys 5.1 years (median 4.1).

To detect insidious uveitis in study IV, the patients were examined by an ophthalmologist at least once, usually two to four times a year. The examination involved detection of best corrected visual acuity, ocular pressure (when possible), biomicroscopy and examination of the fundus of the eye. The type of uveitis was determined and complications of uveitis were sought. Patients with active uveitis were checked at intervals of 1–3 months.

1.4 Population-based controls (study III and VI)

For the comparison of patients with JIA in the sibling series and in the population-based series in Finland, those 114 JIA patients in whom the diagnosis had been made in 1980, 1985 and 1990 in five of the 21 central hospitals districts of Finland (Keski-Suomi, Tampere, Päijät-Häme, Kymenlaakso, Kuopio) were used. Hospital records of the patients in a population-based series published by Kaipiainen-Seppänen and Savolainen (1996) were reanalysed to obtain additional information on certain characteristics of disease, for example the number of joints involved, and on certain laboratory measures at diagnosis.

Data on HLA allele frequencies among Finnish bone marrow donors (14 752 persons) were used to represent frequencies in the Finnish background population, obtained through the courtesy of Dr Jukka Partanen, PhD, from the Finnish Red Cross Blood Transfusion Service.

2 DNA extraction and HLA genotyping

Samples from JIA patients and their relatives have been collected from 1997 on. Most of the samples were fresh 10 ml whole blood samples delivered in EDTA tubes to the National Public Health Institute, Helsinki, where DNA isolations were carried out by PUREGENE DNA isolation protocol (Web site: http://www.gentra.com/protocols/00190.pdf). There were also 6 samples of saliva, for which DNA isolation was carried out (DNA isolation protocol for 3 ml saliva, Web site: http://www.gentra.com/protocols/00882.pdf).

HLA A, C, B, DRB1 and DQB1 typing was performed in 83 JIA patients, their parents and 45 healthy sibs. This family-based approach enabled unambiguous determination of haplotypes for each sibling. HLA polymerase chain reactions were performed according to the appropriate Dynal® Allset+™ SSP (sequence specific primers) kit protocol (Dynal, Oslo, Norway) and serologic typing or sequence-based typing (SBT) by Visible Genetics® GeneKit™ (Visible Genetics, Toronto, Ontario, Canada).

3 Statistical methods

3.1 Statistical comparisons between series

Statistical comparisons between sibling and population series were made using the chi-squared test, Fisher's exact test (Freeman-Halton), Student's t-test and the Mann–Whitney test. In genetic studies variables observed in individuals in pedigrees are not fully independent observations. Statistical comparisons between sibling and population series in original publications III, IV and VI were made using the data for all individuals, ignoring their relationships. For a summary we computed the figures using a single individual from each family. The single case used was a proband who was the individual causing a family to be identified, and in the current study he/she was the first affected member in the family.

3.2 HLA data on patients versus control cases

The frequencies of HLA-A, -C, -B, -DRB1 alleles in the patients with JIA were compared with unaffected controls. Finnish bone marrow donors (14 752 cases) from the Finnish Red Cross Blood Transfusion Service were used to represent frequencies in the Finnish background population. HLA results on bone marrow donors were available only at serological level. Odds ratios and their 95% confidence intervals (CI) were applied to compare the HLA allele frequencies.

3.3 Affected sibling pair analysis method

ASPs are defined as full-biologic siblings who both (or all) have JIA. HLA genotyping was performed in 38 nuclear families (study VI). The series consisted of 32 families with an affected sibpair and 6 families with an affected sibling trio. Each affected trio was counted as comprising three ASPs (sib1–sib2, sib1–sib3, and sib2–sib3), the total number of ASPs being thus $32 + 6 \times 3 = 50$. However, trio sets are not independent and various means to correct the resulting error have been suggested (e.g. Holmans 1998). In this study none of these counting methods was used.

3.3.1 Linkage analysis

The ASP analysis is based on the mendelian paradigm of random segregation of alleles; each sibpair is expected to share identical-by-descent 0, 1 and 2 alleles with respective

probabilities of 0.25, 0.5, and 0.25. For deviations of observed allele-sharing frequencies from the expected, chi-square tests with 2 degrees of freedom were used.

3.3.2 Association methods

Two association-based methods were used: the independent TDT association and the family-based association test. The classical TDT test compares the transmission of alleles from heterozygous parents to the affected offspring with the number of times transmission does not occur. The JIA-affected sibpairs were treated as independent and the *p*-value then adapted using a within-family Monte Carlo permutation z'-score procedure with continuity correction (Spielman and Ewens 1998). The *p*-value for the z'-score was obtained using standard normal distribution. Multiple comparison corrections to *p*-values were made by the Bonferroni method according to the number of alleles at a given marker locus.

Family-based association analysis of the JIA and HLA alleles uses diseased vs not diseased phenotype. The test uses all collected pedigree data and no assumptions of independence of familial cases are made in the same way as in the classical TDT. In this study family-based association tests using the FBAT program were applied (Laird et al. 2000). (http://www.biostat.harvard.edu/~fbat/default.html). Each HLA locus was analysed by unvariate analysis (allelic effects and overall test) and also joint-allelic main effects were analysed to assess independent allele effects.

4 Ethics

The parents had given written informed consent to their family participation in the study. The study was approved by the Ethics Committee of the Rheumatism Foundation Hospital.

Results

1 Twins (I, II)

1.1 Concordance rate in monozygotic twins

Over the study period of 15 years, eight sets of MZ twins were found; two twin pairs were concordant for JIA. There are about four MZ births per 1 000 in the Finnish population. Thus our eight MZ pairs indicated that most, if not all, such pairs had been traced among the approximately 2 000 JIA patients. A pairwise concordance rate of 25% for a disease with a population prevalence of 1 per 1 000 implies a relative risk of 250 for an MZ twin, while the probandwise concordance of 40% (4/10) implies an even higher relative risk.

1.2 Disease phenotypes of monozygotic twins

The disease subtypes of MZ twin pairs concordant for JIA were further studied. In study I a phenotypically different MZ twin pair was described; the onset type was systemic in one, pauciarticular in the other. The subsequent course was similar in both, polyarticular. In another MZ twin pair the patients were concordant for the disease phenotypes; both had oligoarticular onset and polyarticular course. The age difference at onset of the disease was six months in the first twin pair and only two months in the other pair.

2 Affected sibpair studies (II, III, IV)

2.1 Sibling recurrence risk

The computed sibling recurrence risk in this study is only an estimate, for the following reasons. First, the number of affected sibs among the 41 probands was 47, which may not be the final figure, because an unaffected family member may carry susceptibility genes to JIA and a proportion of healthy sibs may later develop disease. Second, our 41 multicase families were found in the basic population of 2 000 patients with JIA. Among these families, the exact number of healthy sibs was not known; an estimate was obtained from the average number of children, 1.8, in Finnish families; 45% of families have only one child, 38% have two children, 13% have three children and 4% ≥ four

children. Finally, the prevalence figure of 1 per 1 000 in the Finnish population ought to be replaced with an estimate of cumulative incidence, because the sibpairs were collected over a study period of 15 years. The best estimate for λ_s from the above data is of the order of 15–20.

2.2 Comparison of sibling series and population-based series

A population-based series of patients with JIA previously published in Finland (Kaipiainen-Seppänen 1996) was compared with our ASP series, consisting of 80 patients with JIA from 37 families. Comparisons of sibling series and population-based series were re-calculated using only one individual (37 probands) from each sibship (Table 6). Distributions of cases according to disease subtype in ASP series/ population-based series were similar in both series: pauciarticular onset 68% vs 70%, polyarticular onset 27% vs 18%, enthesitis-related onset 0% vs 5%, systemic onset 5% vs 6%. The proportion of boys was slightly higher in the sibling vs the population series (41% vs 30%). Uveitis and ANA-positivity were significantly more frequent in the ASP series, when only probands were compared (32% vs 16%, $p = 0.027$ and 70% vs 43%, $p = 0.004$ respectively). A further significant difference detected was the lower age at diagnosis in the series of affected siblings (4.0 years vs 7.4 years, $p < 0.001$).

Table 6 *Comparison of patients with juvenile idiopathic arthritis in the sibling and population-based series in Finland (corrected comparisons using only probands).*

Variables	**Sibling series**	**Population series**	*P* **value**
Number of patients	37 (probands)	114	
Number of boys (%)	15 (41)	34 (30)	0.23
Onset type, number (%)			0.46
Pauciarticular	25 (68)	80 (70)	
Polyarticular	10 (27)	21† (18)	
Enthesitis-related	0 (0)	6 (5)	
Systemic	2 (5)	7 (6)	
Number of uveitis detected, n (%)	12 (32)	18 (16)	0.027
Number positive for ANA, (%)	26 (70)	49‡ (43)	0.004
ESR at diagnosis, (mean, SD)	40 (30)	32 (25)	0.11
Age at diagnosis, years (mean, SD)	4.0 (3.0)	7.4 (4.6)	< 0.001

† 3 patients seropositive ‡ information on one patient lacking

2.3 Comparison of the Finnish versus the United States affected sibling pair series

The Finnish JIA-affected sibling series and the series from the United States published by Moroldo and colleagues (1997) were compared. The disease subtypes were re-classified using the same classification of disease types as used in the American study. For comparative purposes, two enthesitis-related arthritis cases were included in our series in the oligoarthritis group, and one psoriatic arthritis case and one RF-positive polyarthritis case were included in the common polyarthritis group.

The Finnish series consisted of 49 ASPs and the number of American ASPs recruited through the JRA-ASP registry was 71. Some differences between these series emerged. First, there were somewhat more cases with oligoarthritis (71% vs 61%) and fewer with polyarthritis (25% vs 34%) in our series. The mean age at onset of the disease in the United States series was 5.3 years; that at diagnosis in our series was 4.8 years. The proportion of girls in the United States series was significantly higher (84% vs 61%, $p < 0.001$) than in ours.

2.4 Phenotype concordance rates

2.4.1 Finnish affected sibling pairs

In the Finnish sibling series 49% of the pairs were concordant for sex; most (71%) were female pairs; 57% were concordant for onset type and 89% of these were pauciarticular pairs. Onset type differed from course type in 20% of patients. Concordance by course type was 61%; 53% of the pairs were pauciarticular and 47% polyarticular. Concordances for individual sibling pairs are presented in Table 7. Among the 6 families with

Table 7 *Comparison of similarity of 49 affected sibpairs according to certain characteritics.*

Characteristic	Concordant for presence of characteristic N (%)	Discordant N (%)	Concordant for absence N (%)
Sex (male)	7 (14)	25 (51)	17 (35)
Uveitis	3 (6)	17 (35)	29 (59)
ANA positivity	21 (43)	15 (31)	13 (27)
HLA B27 positivity	14 (29)	7 (14)	28 (57)

3 affected sibs, 2 of the trios were concordant for onset type and 3 were concordant for course type.

2.4.2 Concordance rate variations in the Finnish series

In the Finnish series there was no difference with regard to onset type and a small difference with regard to course type. Concordance for ANA positivity was somewhat higher than expected; among the ANA-positive cases (56%) in the series, one would expect about 15 to have an ANA-positive sib. We observed 21 pairs concordant for ANA positivity. The concordance for the presence of uveitis was 3, not different from the expected figure of 3.4.

4 Occurrence of uveitis in sibpairs (IV)

This study examined the prevalence of uveitis in familial JIA and found no increase in concordance. Uveitis was diagnosed in 21 (26%) of the 80 patients. The corresponding figure among the girls was 10/49 (20%) and 11/31 (35%) for boys. Oligoarthritis was the most frequent type of JIA (67%), and ANA positivity was common (67%) among the patients with uveitis derived from the whole sibling series. Three pairs were concordant for the presence of asymptomatic uveitis, the expected number being 3,4.

The comparisons of patients with and without uveitis were re-computed using only probands from sibling series (shown in Table 8). There were more boys in the group with uveitis, and frequencies of ANA-positive and HLA B27-positive cases were also higher in this group. The mean age at diagnosis of JIA was lower in patients with uveitis. However, the differences were not statistically significant.

In four patients (19%) uveitis was found before the diagnosis of JIA, two of these having enthesitis-related arthritis with episodes of acute unilateral uveitis, preceding the onset of joint symptoms. In two patients also asymptomatic uveitis preceded arthritis. In all the other patients with uveitis the eye disease appeared within five years from the onset of arthritis.

During the routine diagnostic procedure at the hospital the patients had been typed by serological methods for HLA B27. The occurrence of this allele in familiality-corrected results (only one proband per family used in comparisons) was somewhat more frequent in patients with uveitis than in those without (42% vs 32% $p = 0.72$) compared to 14.5% in the Finnish population. Interestingly, all siblings concordant for the presence of asymptomatic uveitis were HLA B27-positive. None of the 16 typed patients with uveitis carried the allele DRB1*1104 (a split of HLA DR5), which in some studies has been reported to occur more frequently in patients with chronic uveitis. Six of the 16 typed patients with uveitis, however, carried the DR1 allele (DRB1*0101), a gene

Table 8 *Comparisons of JIA patients from sibling series based on probands with and without uveitis.*

	JIA patients		
Variables	**With uveitis**	**Without uveitis**	***P* value**
Number of patients, n (%)	12 (32%)	25 (68%)	
Boys/girls, n	7/5	8/17	0.16
Onset type of arthritis			
oligoarthritis, n (%)	8 (67%)	17 (68%)	0.99
polyarthritis, n	*3*	*7*	
systemic, n	*1*	*1*	
enthesitis-related, n	*0*	*0*	
Positive for ANA, n (%)	11 (92%)	15 (60%)	0.064
Positive for HLA B27, n (%)	5 (42%)	8 (32%)	0.72
Age at diagnosis of arthritis, yrs, mean (SD)	3.6 (2.0)	4.2 (3.4)	0.55

which in some studies has been claimed to be 'protective' against eye disease. Obviously, the number of patients with uveitis in our series was too small to draw conclusions regarding HLA associations. Patients with uveitis were somewhat younger at the onset of JIA than those without (3.6 yr vs 4.2 yr).

5 Parents of the affected sibpairs (V)

Among the collected 'multicase families', 9 parents from 8 families also had a diagnosis of chronic inflammatory rheumatic disease. Their disease phenotypes (first symptoms and age at onset of the disease) and HLA haplotypes, which are series of alleles at closely linked marker loci residing on the same chromosomal copy and tend to be transmitted intact, are shown in Table 9.

Both cases with a spondyloarthropathy diagnosis were positive for the HLA allele B27. Two of the three cases with juvenile oligoarthritis or polyarthritis were positive for HLA A2 and one was positive for HLA DR8 (alleles with an increased prevalence in JIA). All four cases with chronic peripheral arthritis with onset in adulthood were positive for HLA A2 and two were positive for HLA DR8. One case (that with uveitis commencing in childhood) possessed the allele HLA DR4.

Table 9 *The disease phenotypes of 9 parents with chronic arthritis.*

Family	Parent	Disease	Haplotype:	A	B	C	DR	DQ
1	Father	RF-neg JIA (oligo)	a:	0201	3901	1203	1201	0301
		Both knees (10 yr)	b:	0101	0801	0701	0301	0201
2	Mother	RF-neg JIA (poly)	c:	0201	4002	0202	1101	0301
		Both knees and left ankle (5 yr)	d:	0301	4001	0304	0801	0402
		Progression to chronic polyarthritis						
3	Mother	RF-neg JIA (poly)	c:	0301	0801	0701	0401	0302
		Knees and ankles (9 yr)	d:	2402	4001	0304	1302	0604
		Progression to asymmetric polyarthritis, secondary amyloidosis						
4	Father	RF-neg JIA/SPA	a:	0201	2705	0202	0801	0402
		Monoarthritis of the left knee (3 yr)	b:	2402	4402	0701	1101	0301
		In adulthood: back pains						
		X-ray: bilateral sacroilitis						
4	Mother	RF-neg RA	c:	0201	4001	0304	1302	0602
		Fingers and toes, left wrist (35 yr)	d:	0301	0702	0702	1501	0604
		Symmetric, erosive polyarthritis						
5	Mother	RF-neg oligoarthritis	c:	0301	1501	0303	0401	0302
		Knees (27 yr)	d:	0201	4001	0304	0801	0402
		Chronic uveitis since the age of 10						
6	Mother	RF-neg oligoarthritis	c:	0201	1302	0602	0901	0303
		Knees (25 yr)	d:	0301	3501	0401	0101	0501
7	Father	RF-neg oligoarthritis	a:	2402	3901	0702	0801	0402
		Knees (54 yr)	b:	0201	5601	0102	1301	0603
		Positive for ANA						
8	Mother	SPA	c:	2501	1801	1202	1101	0301
		X-rays verified bilateral sacroilitis (35 yr), fibromyalgia	d:	0201	2705	0202	0801	0402

The pedigrees of the families are shown in Figures 3 and 4, with the index case indicated by an arrow. In these eight families 19 children were affected by JIA; the relationships in these families are shown in Figure 3–4.

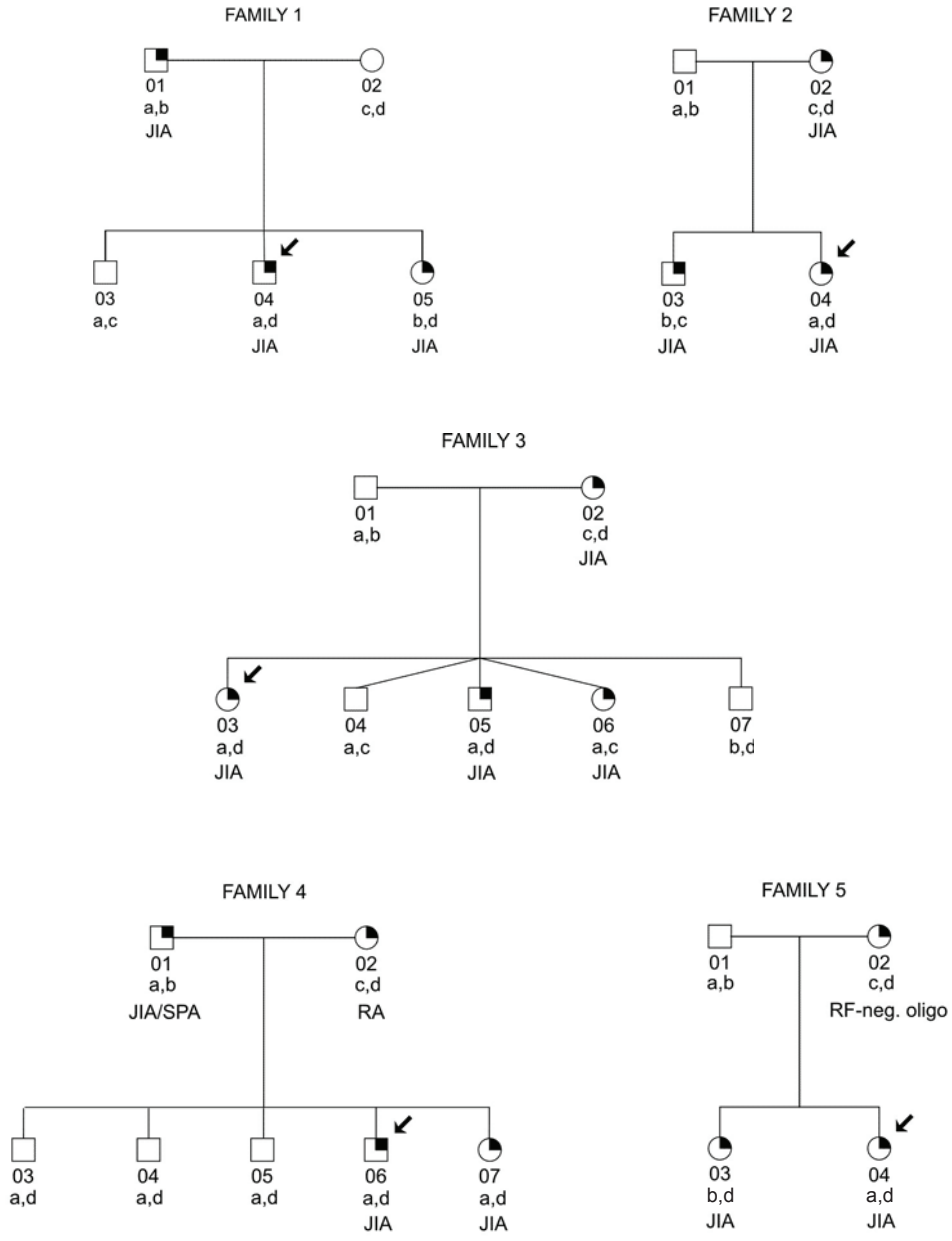


Figure 3 *The pedigrees of families 1–5 with inflammatory arthritis among the parents of JIA-affected sibpairs. Chromosome 6 haplotypes (a,b,c,d) as described in Table 9.*

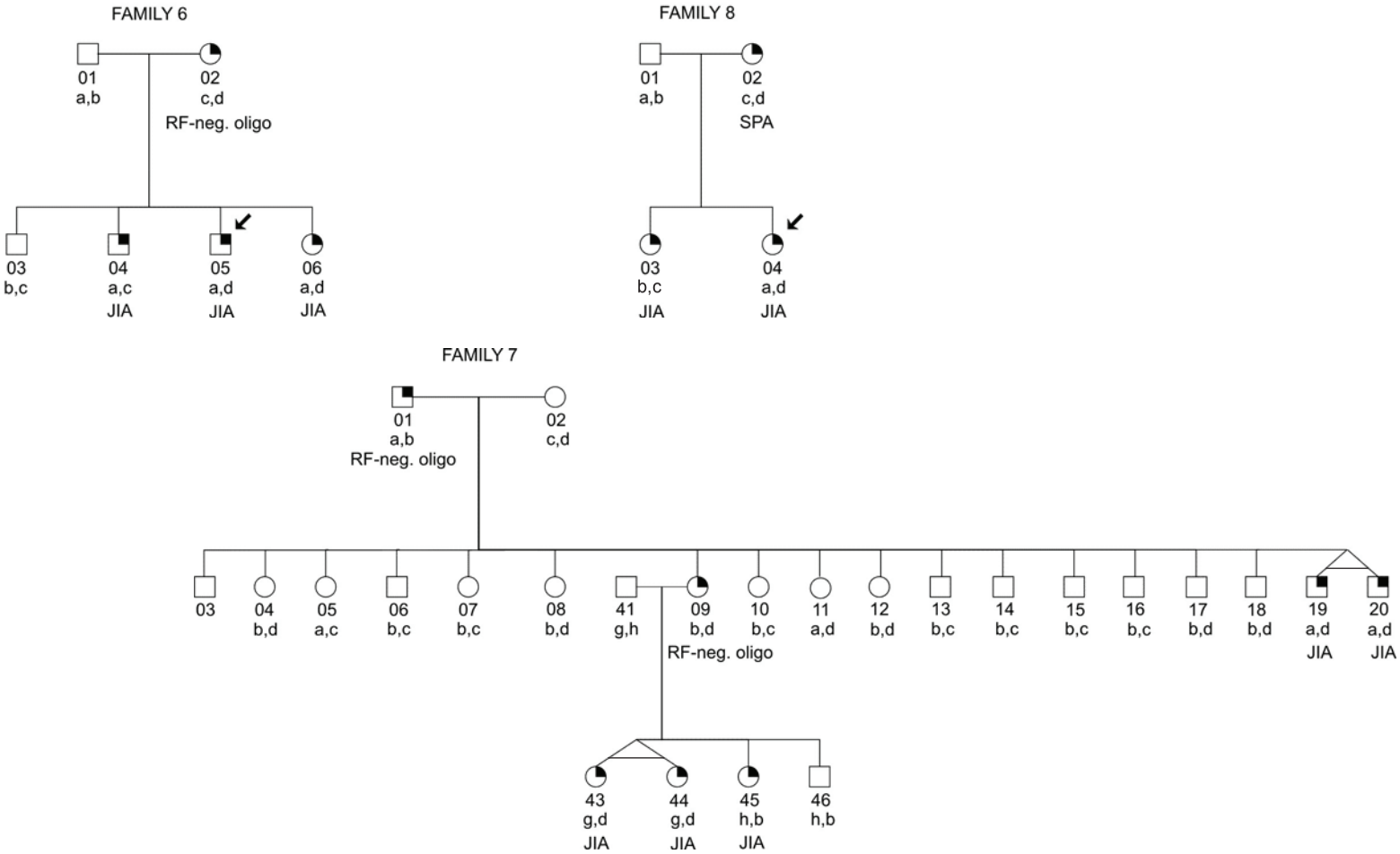


Figure 4 *The pedigrees of families 6–8.*

In family seven the affected father had three offspring affected by chronic arthritis and three second-degree relatives affected by JIA. In this family the haplotype distribution among the father and his 18 offsspring was not as expected (the paternal a and b haplotypes should have an equal (50 : 50) change to be transmitted to the children). The same phenomenom is noted in family four, where in addition all five offspring are haploidentical, but only two of them are affected.

6 Family-based HLA-association study of affected sibpairs (VI)

Significantly increased HLA allele frequencies among the affected siblings were observed for HLA alleles Cw4 and DR8 (Table 10). In original publication VI the estimation of allele frequencies without taking account of the relationships of affected siblings may involve a risk of biased results. The correction of dependence of family members by removal of other than propands from cases gave an even more significant increased DR 8 allele frequency among affected probands compared to controls. The frequency of the allele DR4 in JIA probands was significantly lower than that in bone marrow donors. The prevalence of DR5 did not differ from that in controls. DQ allele frequencies could not be compared because frequencies in controls were not available.

Table 10 *Odds ratios and 95% CI's of HLA C, B and DR alleles with significant difference in allele frequencies between the Finnish JIA patients and Finnish bone marrow donors (comparison of frequencies using only one proband/family (37 probands, 74 alleles).*

HLA Allele	JIA cases, %	Finnish population, %	Odds ratio (95% CI)	Odds ratio (95% CI)†
Cw4	29.7	18.6	1.85 (1.1 to 3.1)	1.7 (1.2–2.4)
Cw6	2.7	9.4	0.27 (0.03–1.0)	0.3 (0.1–0.7)
B27	13.5	8.0	1.80 (0.8–3.5)	1.8 (1.1–2.8)
B35	23.0	14.2	1.80 (0.98–3.2)	1.7 (1.1–2.4)
DR2	6.8	15.1	0.41 (0.13–1.0)	0.4 (0.2–0.7)
DR4	1.4	14.1	0.08 (0.0–0.48)	0.3(0.1–0.6)
DR8	32.4	10.4	4.14 (2.4–6.9)	3.7 (2.6–5.3)

† The odds ratios from original study VI with all affected cases/family (83 patient, 166 allele)

Table 11 *Haplotype sharing (identical-by-descent) in patients with JIA stratified by onset and course type, and by age difference at onset of disease.*

Group	No of pairs	Haplotype sharing observed			*P* value
		0	1	2	
		N (%)	N (%)	N (%)	
Total observed	50	10 (20)	23 (46)	17 (34)	0.33
By onset type					
Concordant	30	4 (13)	14 (47)	12 (40)	0.12
Discordant	20	6 (30)	9 (45)	5 (25)	0.96
By course type					
Concordant	32	8 (25)	13 (41)	11 (34)	0.47
Discordant	18	2 (11)	10 (56)	6 (33)	0.42
By age difference					
< 2 years	25	5 (20)	9 (36)	11 (44)	0.10
≥ 2 years	25	5 (20)	14 (56)	6 (24)	0.81

Abbreviations: Each ASP is expected to share identical-by-descent 0, 1and 2 alleles with respective probabilities of 25%, 50% and 25%.

The sharing of parental haplotypes was studied among the affected sibpairs (Table 11). The observed ratio of sharing 0, 1 or 2 HLA haplotypes was 10 : 23 : 17, which did not differ significantly from that expected. When stratified by onset type, course type and age difference at onset of disease, the sharing of HLA haplotypes identical-by-descent was somewhat greater in concordant compared to discordant pairs, but none of the differences was statistically significant.

Of the 57 (23 + 17 + 17) shared haplotypes the most common (16%) was A*0301, Cw4*0401, B*3501, DRB1*0101, DQB1*0501. In 12% the shared haplotype was A*0201, Cw2*0202, B*2705, DRB1*0801, DQB1*0402. Of the 57 shared haplotypes 24 (42%) contained the DRB1*0801/DQB1*0402 combination. Twenty-three of the ASPs shared one common haplotype; 15 of these were maternal and in eight cases the haplotype was of paternal origin.

The Transmission Disequilibrium Test was first used and showed significantly increased transmissions for HLA DRB1*0801, DQB1*0402 and Cw4*0401 after Bonferroni correction. Transmissions of Cw7*0701 and DQB1*0302 alleles were significantly decreased. Family-based multi-allelic tests for association of locus showed a statistically highly significant association with the DR and DQ locus (p = 0.001 and p = 0.001). The association of the C locus was of borderline significance (p = 0.057). Results of the

Table 12 *Locus-specific univariate allelic associations using the family-based association test in Finnish families with JIA.*

HLA allele	Allele frequency	Informative families	Z	*P* from FBAT
C*0401	0.210	13	2.62	0.009
C*0701	0.133	14	–2.27	0.024
B*3501	0.176	13	1.98	0.047
DRB1*0101	0.202	12	1.95	0.052
DRB1*0801	0.196	16	3.84	< 0.001
DQB1*0201	0.078	8	–2.08	0.037
DQB1*0302	0.098	9	–2.89	0.004
DQB1*0402	0.195	16	3.84	< 0.001
DQB1*0501	0.201	11	2.10	0.035

multiallelic test for allelic associations were comparable to those obtained with the independent-TDT (Table 12).

The joint allelic main effects of the HLA A, C, B, DR and DQ loci using the family-based association test were analysed to provide support for the hypothesis that the allelic effects of the HLA A, C, B, DR and DQ loci are independent of each other. No significant positive effects were detected at the A and B loci. At the C locus Cw4*0401 evinced a positive association ($p = 0.001$) and Cw7*0701 a negative association ($p = 0.003$). At the DRB1 locus, DRB1*0801 ($p < 0.001$) and DRB1*0101($p = 0.021$) were positively associated with JIA, while DRB1*0301($p = 0.011$) and DRB1*0401 ($p = 0.022$) showed a negative association with JIA. At the DQB1 locus positive associations were observed for DQB1*0402 ($p < 0.001$) and for DQB1*0501 ($p = 0.015$) and negative associations for DQB1*0302 ($p < 0.001$) and for DQB1*0201 ($p = 0.012$) when looking for independent effects.

Discussion

1 The Finnish population structure

The current Finnish population represents an isolated population descending from small founder populations. The vast majority of Finns descend from two imigration waves, the earlier wave involved eastern Uralic speakers about 4000 years ago, subsequently followed by the migration of Indo-European speakers from the south about 2000 years ago (Nevanlinna 1972, Norio 1981). Although the Finnish language belongs to the Finno-Ugric group, the Finnish gene pool is mainly European, and recent studies on mitochondrial DNA sequences have revealed that European populations, including Finns, are part of one homogeneous mitochondrial gene pool (Lahermo et al. 1996). However, Y-chromosome studies have revealed the contribution of two distinct male lineages to the present Finnish population, a paternal genetic contribution of Europeans and also of Asians (Kittles et al. 1998).

The initial founding population has undergone several bottlenecks, the most recent being a fall to 250 000 at the beginning of the 1700s (de la Chapelle 1993). For linguistic, cultural and geographical reasons the Finnish population expanded without mixing with other populations. Also migration from one rural community to another has until recently been rare in Finland. Due to this isolation, some disease genes carried by the founders remained enriched and some others were lost.

A population with founder effects is better suited for genetic studies than an ethnically mixed population. Because the genetic material is derived from a small number of founders, individuals in isolated populations (founder populations) are more likely to carry fewer susceptibility genes than individuals in mixed populations. If the population is relatively young, linkage disequilibrium, i.e. non-random association of alleles at closely linked loci, can extend for larger chromosomal intervals than in heterogeneous populations (Varilo et al. 1996).

2 Variable disease expression and allele frequency variations in the Finnish population

Differences in the occurrence of the diseases between regions and countries generate a hypothesis regarding environmental and genetic factors which may influence the disease. Some regional differences and changes in certain common diseases, for example prevalence variations of coronary heart disease (Näyhä 1989), type I diabetes (Karvo-

nen et al. 1997) and schizophrenia (Hovatta et al. 1997) have been reported in Finland. Most convincingly there exists a high-risk area of multiple sclerosis with exceptional familial clustering of cases in Western Finland (Kinnunen et al. 1983).

Allele frequencies are known to vary widely within and between populations, irrespective of disease status (The International HapMap Consortium 2005). HLA studies among healthy voluntary donors registered with the Finnish Bone Marrow Donor Registry indicate the existence of specific Finnish frequencies and some variations in the frequencies of the HLA antigen have also been reported, e.g., concerning antigens A9, B12, B35, Cw4 and DR3 (Siren et al.1996). HLA-B27 occurs in the Finnish population more frequently (14.5%) than in most other European populations, but no regional differences have been reported. In unselected Finnish patients with JIA, the prevalence of HLA-B27 has been 27–36% (Kunnamo et al. 1986; Savolainen et al. 1998) and its occurrence is of the same order of magnitude among patients with RA in Finland (Isomäki et al. 1975; Paimela et al. 1993)

When cases and contols have different allele frequencies attributable to diversity in the background population, it emphasizes the potential for false positive results in case-control studies. The positive associations observed may be a result of population stratification (Cardon and Palmer 2003). Association studies for candidate susceptibility genes in JIA have revealed significant variations between different ethnic groups in the disease association of specific alleles. Specially non-HLA polymorphisms associations with JIA have not been as reproducible in different populations as studies of HLA associations (Rosen et al. 2003) In order to limit this source of error, family-based studies and tests which control for population structure are essential and have been used widely. However, the availability of patients for family-based studies may lead to a risk of bias due to smaller materials.

3 Selection of the patient material in the current study

3.1 Selection of controls for case-control association studies

The possibility of false-positive allele associations in our study is small. Allele frequencies in the controls were derived from the genetically relatively homogeneous Finnish population and the number of healthy bone marrow donors used was large, 14 752, and derived from all parts of the country, thus representing frequencies in the Finnish background population without any significant regional accumulations.

3.2 Patients in family-based studies

Family-based methods such as TDT are useful particularly when working with a pediatric population where parents are easily available. TDT depends on both linkage and association and differs from association tests in that the frequency of the marker alleles is well controlled for ethnic ancestry and is not subject to the bias introduced in conventional association studies. The weakness of TDT and other affected family-based methods is that these lose power rapidly when the test locus is not very tightly linked with the true susceptibility locus, i.e. it relies heavily on linkage disequilibrium. This problem may be largely overcome if the study is based on a founder population. TDT does not take into account the affection status of the parents, which may limit its usefulness.

3.3 Independency of family members

Comparisons of sibling series and population-based series were first examined with cases identified by complete ascertainment. The use of all siblings may have a burden of family ascertainment and thus the result was also computed where familiality was taken into account using only the first ascertained case in the family. In comparison of sibling and population-based series the detected statistical significant difference in respect of age at diagnosis remained and correction of dependence of family members in comparison of HLA allele frequencies between Finnish JIA patients and Finnish bone marrow donors gave an even more significant increased DR8 allele frequency among affected probands compared to controls. An important improvement in allele frequency estimates may be obtained by the appropriate use of data on all individuals, but the ideal method would be a combination of family-specific estimates, using weights inversely proportional to the variances (Broman 2001).

3.4 Possible selection of the sibling series from the patients treated in the Rheumatism Foundation Hospital

The care of patients with JIA in Finland is markedly centralized to the Rheumatism Foundation Hospital, which means that almost all severe cases of JIA in the country attend the hospital and are also constantly monitored there. Milder cases visit the hospital less frequently. According to the statistics of the Finnish Social Insurance Institution (estimation on the basis of the drug reimbursement register), the total number of children with JIA is approximately 1 200 in the population of about one million children in Finland. According to the patient register of the Rheumatism Foundation Hospital it

could be estimated that about 60% of JIA patients are seen at the hospital. Our sibling series was probably selected but only slightly so; some ASPs with a mild disease treated in other hospitals might be missing from our series.

3.5 Possible selection of patients in other published affected sibling pair series

Three studies have been published from other populations concerning JIA ASPs: two are from the United States: those by Suciu-Foca et al. (1980) and by Moroldo et al. (1997), and one is from Germany and England (Clemens et al. 1985). Suciu-Foca and Clemens did not provide information on selection criteria. ASPs in the study by Moroldo were collected by means of a multiple advertising campaign directed to United States physicians likely to care for patients with Juvenile arthriris. Some differences emerged between the US and the Finnish series. First, the frequency of oligoarthritis tended to be higher and that of polyarthritis lower in our series. The reason for this is either that the US series was biased toward clinical cases with more severe disease, or that the patient clientele in Finland differs to some degree from that in the US. Second, there was more intra-pair similarity in the onset and course types among the US patients than among Finnish patients. A possible explanation is a selection bias toward cases resembling each other in the US series.

4 Magnitude of the genetic component in juvenile idiopathic arthritis

4.1 Sibling recurrence risk

The degree of familial clustering is one measure of the genetic component in disease. It is estimated from the risk of disease between two comparison populations; the prevalence of disease in first-degree relatives of the patients compared to the prevalence in the population (Risch, 1990). The genetic contribution to RA is quite small according to the sibling recurrence risk (λ_s) estimate in RA, which is about 4 (range 2–5) (Silman 2001, del Junco et al. 1984). The magnitude of the contribution of HLA to overall genetic risk in RA is probabbly around one third of the total (Deighton et al. 1989; Seldin et al. 1999), with a $\lambda_{s\,HLA}$ estimate about 1.8 (Jawaheer et al. 2001).

It has proved problematic to estimate the genetic contribution to JIA, as the number of families with multiple cases has been limited. In addition, the prevalence of the disease depends on its clinical definition. JIA is not one distinct disease but a group of clinical syndromes. Glass and Giannini (1999) suggested a λ_s for JIA of 15, although

their basic figures were not in accordance with this (estimated number of ASPs 300; prevalence of JIA in pediatric population about 1 per 1 000, U.S. population 250 million). The $\lambda_{s\ HLA\ DR}$ accounted for about 17% of the risk of JRA (Prahalad et al. 2000/a). Our estimate of λ_s from the collected study data is of the order of about 15–20, which has also been estimated for type I diabetes and multiple sclerosis. (Harjutsalo et al 2005, Lindsey 2005).

4.2 Concordance rate in twins

The comparison of MZ and DZ twins provides an extension to separate the influence of genetic and environmental risk factors in disease. A disease with a higher concordance rate in MZ compared to DZ twins is assumed to have a genetic component. The concordance rate in MZ twins observed in two population-based surveys of RA twins varied from 12% (Aho et al. 1986) to15% (Silman et al. 1993), around four times greater than seen in DZ twins.

Under the assumption that MZ and DZ twins share environmental factors equally, the comparison of concordance rates in MZ and DZ twins can be used to derive an estimate of heritability. Heritability is the proportion of the variance in the disease liability which can be explained by genetic variance (Wright et al. 2003). From the aforementioned twin studies (Aho et al. 1986, Silman et al. 1993) the heritability of RA has been estimated to be about 60% (MacGregor et al. 2000). The discordance (i.e. phenotypic differences) among genetically identical MZ twins has usually been attributed to the effects of non-shared environment, but may also be an example of epigenetic regulation during cell differentation and embryonic morphogenesis (Wong et al. 2005)

The concordance rate cannot be computed from the American JIA twin study figures. In a study by Prahalad and associates (2000/b), 14 pairs of twins were identified in which both twins have arthritis. Thirteen pairs (11 MZ twin pairs) were concordant for disease type (Prahalad et al. 2000/b).

The concordance rate can be expressed as either a pairwise or a probandwise rate, depending on the concordant pairs ascertained. In probandwise estimation both members of the twin pairs are ascertained independently and twin pairs are counted twice, while pairwise concordance rate estimation uses concordant twinships identified by complete ascertainment, in which every pair is counted only once. Within our study series of 2 000 JIA patients there were eight sets of MZ twins, two of which were concordant for JIA. Both sets of twins were concordant for course type but different for onset type (Study 1). A pairwise concordance rate of 25% for a disease with a population prevalence of 1 per 1000 implies a relative risk of 250 for a MZ twin, while probandwise concordance 4/10 implies an even higher relative risk. This concordance rate is very similar to that found for type 1 diabetes in a nationwide series of Finnish twins (Hyttinen et al. 2003).

5 Familial disease, evidence for a stronger genetic background?

In certain common diseases there may be small genetically determined subsets. In some instances, but not always, they differ clinically from sporadic disease. A proportion of breast cancers develop as a result of inherited predisposition genes, with BRCA1 and BRCA2 accounting for the majority of these genes (Lux et al. 2005). Other diseases such as familial adenomatous polyposis colorectal cancer and a subset of non-polyposis colorectal cancer are dominantly inherited and in both of these gene mutations have been already identified (Aaltonen 2000). These cancers differ from sporadic forms by an earlier age of onset, by some clinical manisfestations and by having a family history of colorectal and/or other extra-intestinal cancers.

Additionally, familial forms of Alzheimer's disease, affecting approximately 10% of patients (Haass and Baumeister 1998), and Parkinson´s disease (Gasser 2001) have been identified. Patients are younger at disease onset, and have more affected first-degree relatives.

Differences between familial and sporadic RA have been looked for in many previous studies. Basically the disease is clinically the same and associated with the same genetic risk factors. The different relative risks are explained by comparison of HLA DR4 frequency between familial and non-familial RA patients; the effect of DR4 seems to be larger in multicase families than in sporadic cases (Laivoranta-Nyman 2000). The only clinical difference is a tendency to earlier age at disease onset in familial compared to non-familial RA (Kwoh et al. 1996; Laivoranta-Nyman 2000).

Differences between sporadic and familial JIA were for the first time compared by Moroldo and colleagues (1997). Clinical features of the disease within the various subtypes appeared to be similar in both populations. Comparisons of Finnish sibling series with a Finnish population-based series (Study III) were corrected by taking into account the dependence of family members. Comparison of only the propands to population-based series revealed that uveitis and ANA positivity were significantly more frequent in ASP series and a tendency to earlier age at the disease onset in familial JIA was noted. The HLA allele distributions between familial and sporadic JIA patients could not be compared as there were no HLA data available on Finnish sporadic JIA patients.

6 Genetic component in uveitis

Chronic uveitis occurs most commonly in patients with persistent oligoarthritis, especially in young girls with early onset disease. This extra-articular manifestation is also frequently seen in patients with RF-negative polyarthritis, whereas it only seldom oc-

curs in systemic disease. The position of chronic uveitis in the classification scheme of JIA is not clear; it may represent a separate entity, irrespective of the type of arthritis accompanying its course.

Histocompatibility allele profiles have been studied in uveitis associated with early-onset oligoarthritis. In two American series, the HLA allele DRB1*1104 (a split of HLA DR5) occurred significantly more frequently in patients with chronic uveitis than in those not suffering from the disorder (Malagon et al. 1992; Melin-Aldana et al. 1992). No corresponding difference emerged in a patient series collected in Central Europe (Haas et al. 1994). In all three series, the frequency of the allele DRB1*01 was reduced. Data on HLA allele distributions in chronic uveitis associated with other forms of JIA have not been published, probably due to the small number of cases available.

The main purpose of this present study was to assess the concordance rate of uveitis in ASPs. Hereby a higher than expected concordance rate would point to the existence of a genetic component of its own for uveitis. However, our data combined with figures from two earlier series (Clemens et al. 1985; Moroldo et al. 1997) did not provide support for this contention.

Nonetheless, the findings do not rule out a modest association with some genetic marker (genotype relative risk), provided that its frequency is fairly high. This is because the frequency of a common genetic marker is not appreciably higher among siblings of patients with uveitis than in the general population and, accordingly, a modest genotype relative risk adds little to the sibling recurrence ratio (del Junco et al. 1984).

7 Increase in occurrence of chronic inflammatory rheumatic disease among the parents of affected sibling pairs

The different subtypes of JIA are based primarily on clinical characteristics and most of the disease subtypes in children are considered as being different from adult cases. There are nonetheless subtypes which on the basis of clinical characteristics and immunogenetic profile may be related to adult rheumatic diseases. Systemic JIA and adult onset Still's disease represent the same disease entity on the basis of clinical features of the diseases. Enthesitis-related arthritis and RF-positive polyarthritis appear to be childhood counterparts of spondyloarthropathy and RA, respectively. A proportion of RF-negative polyarthritis is possibly related to RF-negative RA in adults, on the basis of a proposed genetic link (Gao et al. 1991). A subtype of oligoarthritis often associated with chronic anterior uveitis and ANA positivity is considered to be a subtype existing exclusively in children.

Previous family studies of JIA patients and first-degree relatives have demonstrated a higher than expected prevalence of spondyloarthropathy (Hertzberger-ten Cate and

Dijkmans 1993) and psoriasis (Andersson Gäre and Fasth 1995). Our aim was to determine the frequency of chronic rheumatic inflammatory diseases among the parents in multicase families, where the disease genes are assumed to be accumulated.

The prevalence of JIA in children of North European ancestry is about 1 per 1 000 (Andersson Gäre 1999). Since the mean age at falling ill is 7–8 years, the cumulative incidence of JIA in adults is about 2 per 1 000. In the present study, one could expect about 0.2 cases among the 90 parents of the children. However, a marked increase in the JIA cases among the parents of JIA ASPs was noted; four cases were found. In addition there were four cases of RF-negative chronic peripheral arthritis with onset of disease in adulthood. One of these satisfied the ACR criteria for RA (Arnett et al. 1988), and three had RF-negative oligoarthritis, i.e. resembling oligoarticular JIA. These findings provided evidence for the existence of a JIA of adult onset. HLA findings on adult cases of chronic peripheral arthritis supported this contention.

HLA findings in adult onset chronic peripheral arthritis cases were compatible with earlier reported HLA associations with oligoarticular JIA. All cases were positive for HLA A2 and two were also positive for HLA DR8. One case (the patient with uveitis commencing in childhood) possessed the allele HLA DR4 (expected prevalence). In this series we did not study the HLA DP locus, but in earlier studies, HLA DPB1*0301 has been found to be a risk factor for both polyarticular JIA and for RF-negative adult RA (Gao et al.1991).

Accumulation of JIA-type diseases in two generations supports our earlier contention that the genetic component in JIA is higher than previously believed (Study II).

8 HLA association and linkage studies in juvenile idiopathic arthritis

JIA is not one distinct disease but a group of syndromes including several diseases with heterogeneous etiologies and with different reported genetic associations. RF-positive polyarthritis, psoriatic arthritis and enthesitis-related arthritis have their counterparts in adults and available evidence is in accordance with the view that these subsets have the same genetic basis as the diseases commencing in adulthood. Oligoarticular disease and RF-negative polyarthritis are the most common subsets of JIA. They have been reported to be associated with certain alleles at the HLA A, C, B, DR/DQ and DP loci. A characteristic feature of JIA is the interaction of these HLA genes within the same locus or between different loci, each having a small and additive effect (Forre and Smerdel, 2002).

8.1 Population-based association study

Previously published studies of sporadic JIA have mainly relied on association methods in non-founder populations, which are problematic due to the uncertain contribution of population admixture. The reported associations carry the risk of generating biased results for many reasons, e.g., population stratification (Cardon and Palmer 2003). To date, only one study from 1978 has been published dealing with the HLA allele distribution in Finnish JIA patients (this includes only HLA A and B alleles) (Mäkelä and Tiilikainen 1978). The present is the first study describing whole HLA haplotypes in Finnish JIA patients. We looked at the HLA allele distribution in our sibling series at HLA loci A, C, B and DR. The study sample was too small to carry out separate analyses by JIA subgroups. Based on the entire study material, significantly increased HLA allele frequencies among the affected siblings compared to control cases were found for the HLA-Cw4, -B27, -B35 and -DR8 alleles; that with DR8 was the strongest. These HLA alleles also have been found to be increased in some earlier studies in other populations (Howard et al. 1985; Murray et al. 1999; Forre and Smerdel 2002).

8.2 Linkage to HLA detected by family-based HLA haplotype sharing

Nonparametric linkage studies such as haplotype sharing among affected siblings are the traditional means of assessing linkage. They have, however, a limited statistical power to detect linkage if genetic associations are weak and each susceptibility locus in the haplotype provides only a limited relative risk.

Two small sibling series detailing haplotype sharing (Suciu-Foca et al. 1980; Clemens et al. 1985) and a larger one on allele sharing (Prahalad et al. 2000/a) in JIA have been published. A marked excess of shared haplotypes was recorded in the very small series of Clemens and associates (1985). Prahalad and group (2000/a) focused their study on the DR locus, this being believed to be the major HLA locus determining susceptibility to JIA. According to their paper affected siblings only had been tested, no other family members. Thus, information on identity by descent could not be obtained. Based on statistics designed for identity by state, the authors showed a significant excess of sharing 0, 1 or 2 DR alleles: 8 : 40 : 32, instead of the expected ratio of 20 : 40 : 20 ($p < 0.001$). The authors computed a sibling recurrence risk of 2.5 for the DR locus.

In addition to affected siblings, we here typed all the parents and most of the unaffected sibs. This family-based approach enabled an unambiguous definition of haplotype and allele sharing by descent in each instance. The observed ratio of sharing 0, 1 and 2 HLA haplotypes (A, C, B, DR and DQ) was 10 : 23 : 17, statistically not significantly different from that expected, although there was a tendency to an increase in HLA-identical siblings. The relatively high number of HLA non-identical siblings

among these ASPs may suggest that one parent carried not only one but both HLA haplotypes which conferred the susceptibility to JIA, or that both parents carried susceptibility haplotypes, although different ones.

8.3 Linkage in the presence of association

The TDT test confirmed linkage in the presence of association. Aside from the current study, linkage to HLA has been confirmed in TDT studies of JIA nuclear families with only one affected (single cases) in three populations, in American pauciarticular onset JRA patients, according to ARA criteria (Moroldo et al. 1998), in UK Caucasian JIA patients, according to ILAR classification (Zeggini et al. 2002) and in Finnish JIA patients with oligoarticular and RF-negative polyarticular subtypes (Runstadler et al. 2003).

The family-based association test was used to test association in the presence of linkage between HLA markers and any gene influencing JIA. The association analysis of HLA A, C, B, DR and DQ by independent TDT test and by family-based association tests now confirmed in the Finnish population that the most significant associations prevailed for DRB1*0801, DQB1*0402, as expected from previous observations, and also supported an independent role of Cw*0401.

8.4 HLA associations in juvenile idiopathic arthritis

Unlike adult RA, which has a strong association with HLA DRB1*0401, JIA shows a pattern of more polygenic inheritance. A characteristic feature of JIA is the interaction of HLA genes, which increases the risk of the disease (Forre and Smerdel 2002). Some HLA associations are due to linkage disequilibrium between markers involved, but accumulating evidence indicates that several different genes in the HLA region are implicated in the pathogenesis of JIA (Zeggini et al. 2002). It will thus be difficult to assess the independent role of different HLA loci, alleles and haplotypes contributing to the genetic component associated with the HLA region, although this would be important for understanding the etiology of the disease.

9 What do we know, what we need to know

The HLA region probably represents only a modest fraction in the overall genetic component of JIA. Most of the findings of non-HLA gene associations are unconfirmed. It is reasonable to assume that new information will accumulate, in part based on progress in genome-wide screening techniques or on findings achieved by in vitro stimulation of

cytokine production, which, however, do not always correspond to the situation in vivo. Accordingly, it would be important to extend patient series and in the case of JIA ASP series international co-operation will be needed.

The aim of genetic studies is to make the determination of genetic markers available for clinical practice and useful for tailoring treatment schedules. Only little is known about what factors are predictive of outcome of JIA. The phenotype, e.g. the disease subtype, certainly plays a role. At the moment there are no good means to differentiate in the early phase between children with a progressive course of their disease and children who have the genetic make-up associated with mild disease and should not be exposed to possible toxic side effects of medical treatment.

Conclusions

1 The genetic component in JIA seems to be greater than previously estimated, and greater than that in adult-onset RA. The best estimate for the sibling recurrence risk was 15–20.

2 The comparison of population-based JIA series and ASP series and the HLA associations among sibling series suggested that genetically familial and sporadic JIA patients represent the same disease.

3 The concordance rate for uveitis in sibling pairs with JIA was of the same order of magnitude as expected by chance. Thus, chronic uveitis in JIA patients has no specific genetic component of its own to separate these from other JIA patients.

4 Parents in families of multiple offspring affected with JIA had a markedly increased frequency of chronic inflammatory arthritis, either JIA or a similar type of disease (in most instances RF-negative oligoarthritis) commencing in adulthood. This can be taken as evidence for the existence of adult-onset JIA.

5 Haplotype sharing analysis showed a tendency towards increased sharing of common haplotypes, but this did not reach the level of statistical significance. The TDT and FBAT analyses provided evidence for genetic linkage between the HLA region and JIA and further evidence for a major contribution of HLA genes to genetic susceptibility to JIA. The association analysis of HLA A, C, B, DR and DQ alleles by both TDT and FBAT tests now confirmed in the Finnish population that the most significant associations prevailed for DRB1*0801, DQB1*0402, as expected from previous observations, and also supported the independent role of Cw*0401.

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