UPDATE ON GENETIC SUSCEPTIBILITY AND PATHOGENESIS IN JUVENILE IDIOPATHIC ARTHRITIS

Morten Herlin,[1,2] Michael B. Petersen,[1,2] *Troels Herlin[3]

1. Department of Clinical Genetics, Aalborg University Hospital, Aalborg, Denmark
2. Department of Clinical Medicine, Aalborg University, Aalborg, Denmark
3. Pediatric Rheumatology Clinic, Department of Pediatrics, Aarhus University Hospital, Aarhus, Denmark
**Correspondence to troeherl@rm.dk*

Disclosure: No potential conflict of interest.
Received: 04.04.14 **Accepted:** 26.05.14
Citation: EMJ Rheumatol. 2014;1:73-83.

ABSTRACT

Juvenile idiopathic arthritis (JIA) is a multifactorial disease with a pathogenesis which remains inexplicable. However, genome-wide association studies brought forward within recent years have discovered several new susceptibility genes, and accumulating evidence supports genetic variability as playing a key role in JIA development. This review summarises the present knowledge of human leukocyte antigen (HLA) and non-HLA polymorphisms conferring disease susceptibility, and discusses the areas in JIA genetics, which are still to be investigated in order to apply JIA genetics in a clinical setting.

Keywords: Juvenile idiopathic arthritis, arthritis, juvenile, genetics, genetic predisposition to disease, individualised medicine.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in children, with a reported prevalence of around 16-150 per 100,000.[1] The term JIA encompasses a diverse group of arthritides characterised by onset of disease before the age of 16, with arthritis lasting >6 weeks, and with an unknown cause. JIA is divided into seven subgroups according to the classification provided by a task force within the International League of Associations for Rheumatology:[2] systemic arthritis (sJIA), oligoarthritis (oJIA; further divided into persistent oJIA [per-oJIA] and extended oJIA [ext-oJIA]), rheumatoid factor-negative polyarthritis (RFneg-pJIA), rheumatoid factor-positive polyarthritis (RFpos-pJIA), enthesitis-related arthritis (ERA), psoriatic arthritis (PsA), and undifferentiated arthritis. Characteristically, an uneven distribution of female and male cases is seen in the different subgroups of JIA. A female majority is seen in oJIA, pJIA, and PsA, in contrast to ERA where male cases predominate.[1]

The aetiology of JIA is believed to be multifactorial with both genetic and environmental factors[3] - such as infections, breastfeeding, and maternal smoking during pregnancy - although knowledge of the latter remains sparse. For many years, genetic studies were limited to a candidate gene approach with both productive and unproductive outcomes.[4] During the last decade, the introduction of genome-wide association studies (GWAS) with a hypothesis-free approach has proven to be a valuable tool for investigating the genetics of JIA, and has discovered several new loci conferring JIA susceptibility. However, despite this new knowledge, a complete understanding of JIA pathogenesis still remains elusive.

JIA AS A GENETIC DISEASE

JIA is considered a complex genetic disease[5] with a non-Mendelian inheritance pattern. In addition to the knowledge we have today of JIA pathogenesis and multiple genetic loci conferring susceptibility to JIA, the idea of a genetic aetiology has been supported by reports of affected twins and sib pairs with phenotypic concordance in each family

regarding JIA subtypes.[6-9] A study using the Utah Population Database sought to quantify the familial contribution to JIA and found a significant risk in relatives of JIA patients compared to the background population; 11.6 in siblings ($p<2.59x10^{-8}$) and 5.82 in first-degree cousins ($p<6.07x10^{-5}$), respectively. Additionally, the contribution of familial factors in JIA risk was estimated to be 13%.[9]

It has also become evident that other autoimmune disorders, such as Type 1 diabetes, coeliac disease, autoimmune thyroiditis, and other chronic arthritides, are clustering in relatives of JIA patients and in patients as well.[10-14] In a US cohort, the odds ratio (OR) of a JIA relative having at least one autoimmune disorder compared to controls was 3.4 ($p<1x10^{-6}$).[10] This suggests a shared genetic aetiology in an otherwise heterogeneous group of diseases and has to be considered when investigating the pathogenesis and genetic susceptibility of JIA.

HLA GENES AND JIA SUSCEPTIBILITY

Ever since the discovery of the human leukocyte antigen (HLA) B27 association in ankylosing spondylitis,[15,16] and subsequently juvenile rheumatoid arthritis (JRA),[17] the HLA gene complex has been subject to vast investigations concerning its role in arthritis pathogenesis. The HLA gene complex, located at 6p21.3, remains the single most significant gene region in conferring susceptibility to JIA with particular importance of the HLA-DRB and HLA-DQA/B genes; this was recently confirmed in the largest genetic study on JIA patients (oJIA and RFneg-pJIA) done so far.[18] A single nucleotide polymorphism (SNP) rs7775055 (G>A), tagging the HLA-DRB1*0801-HLA-DQA1*0401-HLA-DQB1*0402 haplotype, showed definite association with oJIA and RFneg-JIA (OR=6.01, $p<3.14x10^{-174}$), and in particular, oJIA (OR=6.78, $p<2.24x10^{-162}$).[18] An important feature regarding HLA genes is the high degree of linkage disequilibrium (LD); thus, it has been a challenge to determine which of the multiple adjacent loci is truly associated with disease risk. This always has to be taken into account when interpreting genotyping results in the HLA complex.

Table 1 summarises the results (only significant results shown) from two of the largest studies[19,20] investigating associations between HLA alleles/haplotypes and JIA in UK/US Caucasians. The best documented allele is the HLA-DRB1*08 which confers susceptibility to JIA (driven by per-oJIA, ext-oJIA, and RFneg-pJIA). Similar results were found with DQA1*04 and DQB1*04, but these alleles are in LD with the DRB1*08 allele and evidence has been reported indicating that DRB1*08 is the true risk allele.[20,21] Other DRB1-alleles associated with disease predisposition are: DRB1*01 (only ERA and PsA; LD with B27 and DQA1*0101), DRB1*11, and DRB1*13 (only per-oJIA). Protective alleles are: DQA1*0102, DQA1*02, DQA1*03, DRB1*04, and DRB1*07, of which DQA1*03 and DRB1*04 are in LD. It should be noted that DRB1*04, which is a rheumatoid arthritis (RA) susceptibility allele, is associated with risk of the juvenile analogue to RA, RFpos-pJIA. Other alleles outside the DRB1 and DRQ genes, such as A*0201, C*0202, and DPB1*0201, are also associated with oJIA risk, whereas A*0101 is protective.

Additionally, Hollenbach et al.[20] showed age-at-onset effects of different haplotypes; 0801-0400-0402 and 1103/04-0500-0301 conferring risk of an early onset of disease (<6 years). Still, the exact mechanisms of how the different alleles are involved in JIA pathogenesis are poorly understood. More recently, studies have tried to identify the actual causal variants in the different DRB1 alleles by focusing on particular amino acid residues in the antigen binding cleft of the DR1 protein.[22,23] Thomson et al.[22] studied the amino acid residues (9-86) encoded by exon 2 in the HLA-DRB1 gene and found that variations in the amino acids 13, 37, 57, 67, 74, and 86 are important risk factors in JIA susceptibility. Prahalad et al.[23] investigated the frequency of RA-associated amino acid sequences in residues 70-74 (the so-called shared epitope [SE]) in RFpos-pJIA patients and found a significantly higher frequency of SE alleles in cases compared to healthy controls. Further investigations on the role of key amino acids are important to elucidate the mechanisms of HLA in JIA pathogenesis and autoimmunity in general.

NON-HLA GENES AND JIA SUSCEPTIBILITY

Prahalad et al.[24] estimated the HLA-DR region to account for 17% of JIA risk, and more recent estimates of the whole HLA complex have been as low as 8-13%.[18,25] This supports the increasing amount of research focusing on finding susceptibility loci outside the HLA gene complex. As mentioned earlier, studies have - for many years - been limited to a candidate gene approach often based on findings from gene expression levels.

But in the past 5 years, with the use of genome-wide genotyping methods and analyses, many new susceptibility loci have been identified.[25-27]

Additionally, the idea of a closely related genetic pathogenesis among several different autoimmune diseases has also proved to be a successful method in finding new JIA susceptibility loci.[18,28-32]

Table 1: JIA subtypes and associated HLA allele/haplotype variants in US/UK Caucasians.

Allele/haplotype	Ref.	JIA	Per-oJIA	Ext-oJIA	RF-pJIA	RF+pJIA	sJIA	ERA	PsA
A*0101	20*			0.46					
A*0201	20			1.96					
B*27								Criteria	
C*0202	20		[a]2.05						
DPB1*0201	19		2.1	2				0.1	
DQA1*0101	19							2.8	4.2
DQA1*0102	19	0.6	0.6	0.4					
DQA1*0103	19	2.7	5.7						
DQA1*02	19	0.6	0.4			0.1			
DQA1*03	19	0.6	0.4	0.2		4			0.3
DQA1*04	19	4.4	5.1	10	4.2				
DQA1*05	19	1.7	2.5				2.6		
DQB1*03	19				0.7	6.1			0.5
DQB1*04	19	3.5	5	7.4					
DQB1*05	19			1.8				3.5	4.4
DRB1*01	19			2				3.6	2.7
DRB1*04	19	0.6	0.3	0.1	0.7	3.2			0.3
DRB1*07	19	0.6	0.3		0.5	0.1		0.3	
DRB1*08	19	3.0	3.9	6.3	3.1				
DRB1*11	19	2.0	2.5	2.5			2.8		
DRB1*13	19		1.8						
[b]01-0101-0501	19			1.9				4.9	3.8
0401-03-03	19	0.6	0.4	0.1		3.9			
0401-0300-0301	20	[c]0.34/NS	0.21/NS						
0701-0201-0201	19,20	0.42/NS[20]	0.2[19,20]						
0801-0400-0402	20	7.14/4.08	8.70/5.35	5.36	5.52/3.31				
0801-0401-0402	19	4.1	6.1	10.3					
11-05-03	19	2.1	2.2	3			4.3		
1103/04-0500-0301	20	5.99/3.55	5.43/NS	4.65	6.90/NS				
13-01-06	19	3.0	6.4						4.5
1301-0103-0603	20	2.04/NS	2.3						
1501-0102-0602	20	0.28/0.61	0.21/0.54	0.48	0.28/NS				

JIA: juvenile idiopathic arthritis; HLA: human leukocyte antigen; Per-oJIA: persistent oligoarticular juvenile idiopathic arthritis; Ext-oJIA: extended oligoarticular juvenile idiopathic arthritis; RF-pJIA: rheumatoid factor-negative polyarticular juvenile idiopathic arthritis; RF+pJIA: rheumatoid arthritis-positive polyarticular juvenile idiopathic arthritis; sJIA: systemic juvenile idiopathic arthritis; ERA: enthesitis-related arthritis; PsA: psoriatic arthritis; NS: not significant.

*Hollenbach et al.[20] investigated a cohort limited to per-oJIA, ext-oJIA, and RF-pJIA cases; [a]OR: odds ratio; [b]Haplotype DRB1-DQA1-DQB1; [c]<6 years/≥6 years.

In 2013, the results from the largest genetic JIA study so far, utilising both of these two approaches, were published.[18] A study which included 2,816 patients (oJIA and RFneg-pJIA) and 13,056 healthy controls using the Immunochip array,[33] analysed a total of 123,003 SNPs. 17 loci reached genome-wide significance ($p<5x10^{-8}$), of which 3 loci (HLA, PTPN22, and PTPN2) previously have shown genome-wide significant associations with JIA; 5 loci have supporting evidence from previous studies (STAT4, ANKRD55, IL2-IL21, IL-2RA, and SH2B3-ATXN2). Furthermore, an additional 11 loci almost reached genome-wide significance ($5x10^{-8}$, $p<1x10^{-6}$).

The PTPN22 Gene

Table 2 lists the genetic polymorphisms that have shown an association with JIA susceptibility and have been confirmed in ≥2 cohorts (a comprehensive review of both productive and unproductive candidate gene studies has previously been done by Sampath and Glass[4]). The best verified non-HLA gene involved in JIA susceptibility and several other autoimmune diseases, such as RA, Type 1 diabetes, systemic lupus erythematosus, and Graves' disease,[34,35] is PTPN22 (OR ~1.55).[18,28,35-40] PTPN22 encodes the lymphoid protein tyrosine phosphatase (Lyp) involved in modulation of T cell receptor signalling. Of particular interest is the non-synonymous SNP rs2476601 (G>A, also known as c.1858C>T or R620W), a missense mutation proposed to be functional by compromising the ability of Lyp to bind to the C-terminal Src kinase (Csk) and create the Lyp/Csk complex. This complex is responsible for the inactivation of lymphocyte-specific protein tyrosine kinase (Lck, a key mediator of T cell receptor signalling).[34]

Other Potential Disease-Causing Variants

Many of the SNPs associated with JIA risk are intergenic or intronic, and therefore these SNPs are less likely to be the actual disease-causing variants. Instead, these SNPs suggest an involvement of the adjacent genes in disease pathogenesis. Still, it is important to note that the role of the non-coding SNPs should not be neglected entirely, since a study on the genetic variation in different common diseases (JIA not included) found many of the non-coding SNPs to be located in regulatory DNA.[64] The current knowledge of the mechanistic effects of associated SNPs, however, is generally limited to non-synonymous SNPs located in exons or SNPs in the promoter regions of genes. rs3184504 (also known as c.784T/C or R262W), which is associated with JIA susceptibility (OR 1.20),[18] is located in exon 3 of the SH2B3 gene and is therefore a possible causal variant. This polymorphism has been reported to influence the level of T cell proliferation.[65] The 32bp deletion (32) of CCR5, associated with a lowered JIA risk (OR ~0.80), causes a frame-shift leading to a non-functional chemokine receptor. This is thought to impair the recruitment of T cells in the autoimmune reaction.[55]

Other polymorphisms such as rs755622 (-173G>C, macrophage migration inhibitory factor [MIF]), rs1800629 (-308G>A, tumour necrosis factor [TNF]), and allele 3 of the $(GT)_n$ microsatellite repeat (SLC11A1) are located in the promoter regions of their respective genes. These variants have been associated with a higher expression of their gene products and are therefore likely contributors to JIA pathogenesis.[43,45,66] Conversely, the protective SNP rs1800795 (-174G>C, Interleukin 6 [IL-6]) is associated with low IL-6 levels.[58] The functions of other genes, listed in Table 2, support their relevance as JIA susceptibility genes, but how polymorphisms/mutations in these regions affect gene expression or function is still to be discovered.

Of the loci, whose associations are still to be replicated, those reported in the Immunochip study,[18] of course, also need to be mentioned (TYK2, ERAP2-LNPEP, UBE2L3, C5orf56-IRF1, RUNX1, IL-2R, ATP8B2-IL6R, FAS, and ZFP36L1). Of particular interest is the rs34536443 SNP, located in a coding region (exon 23) of TYK2; thus, it is likely to be a causal variant.

SYSTEMIC JIA – A SEPARATE ENTITY

Systemic JIA (formerly known as Still's disease) is defined by the presence of arthritis accompanied or preceded by a quotidian fever lasting >2 weeks and including at least one of the following features: evanescent erythematous rash, generalised lymphadenopathy, hepatosplenomegaly, or serositis.[2] The pathogenesis of sJIA is characterised by an activation of the innate immune system leading to an unbalanced secretion of inflammatory cytokines. Due to the distinct clinical features along with a characteristic pathogenesis of autoinflammation rather than T cell-driven autoimmunity, sJIA is regarded a separate disease entity.[67,68]

This idea is also supported by the findings of genes conferring susceptibility to sJIA. First of all, variation in the HLA genes is generally not associated with risk of sJIA, although Thomson et al.[19] did find the HLA-DRB1*11-allele to be associated with sJIA (Table 1). Instead, several genes encoding cytokines have been reported to be associated with sJIA susceptibility. The synonymous SNP, rs1800795 (G>C), in the promoter region of IL-6, has been reported to confer protection in Caucasians in two studies,[57,58] and rs1800896 (G>A) near IL-10 has been associated with sJIA risk in both UK and German cohorts.[51,52] Additionally, studies have found an association between sJIA susceptibility and variants in the IL-1A[69,70] and IL-20[52,71] genes. But due to overlapping cohorts, these findings still need to be replicated. So far, no GWAS has been published investigating patients with sJIA. However, a study is currently underway.[72]

The distinct characteristics of sJIA pathogenesis also seem to influence the efficacy of different disease-modifying anti-rheumatic drugs (DMARDs). Methotrexate (MTX) and corticosteroids (the side-effects considered), as well as biological anti-TNF agents, have been rather unsuccessful. The knowledge of sJIA pathogenesis has instead proven valuable in generating new treatments, and today, anakinra (IL-1 receptor antagonist) and tocilizumab (humanised antibody against the IL-6 receptor) are the most efficacious options in the treatment of sJIA patients.[73]

Table 2: Non-HLA polymorphisms conferring JIA susceptibility*.

Association	Gene region	Chr.	SNP/allele	Gene function	Ref.
Risk	PTPN22	1	rs2476601 (G>A) rs6679677 (C>A)	Encodes the Lyp – a key modulator of TCR signalling.[34]	18, 28, 35-40
	STAT4	2	rs7574865 (G>T) rs10174238 (A>G) rs3821236 (G>A)	Stat4 is a transcription factor, particularly involved in IL-12-receptor signalling leading to Th1 cell differentiation and IFN- production.	18, 28,29, 31, 37, 39, 41
	PTPN2	18	rs7234029 (A>G) rs2847293 (T>A)	Modulating several cytokine-signalling pathways such as IL-2, IL-4, IL-6, and IFN-.	18, 28, 32
	SLC11A1	2	$(GT)_n$ allele 3 (MS)	Nramp1 is a membrane protein in macrophages, modulating their activation. Conversely, allele 2 is associated with infections.	42, 43
	SH2B3-ATXN2	12	rs3184504 (G>A) rs17696736 (A>G)	SH2B3 is a part of the TCR signalling pathway.	18, 28-30, 37
	TIMMDC1-CD80	3	rs4688011 (C>T) rs4688013 (G>A)	TIMMDC1 is a complex 1 assembly factor in the mitochondria. CD80 is placed on the surface of B cells, providing co-stimulatory signals for T cell activation.	18, 25, 37
	AFF3-LONFR2	2	rs6740838 (C>A) rs1160542 (A>G)	AFF3 is expressed in lymphoid tissue and is believed to regulate lymphoid development.	18, 44
	IL2-IL21	4	rs17388568 (G>A)	See below.	28
	MIF	22	rs755622 (G>C)	MIF binds to CD74 and induces secretion of TNF-, IFN-, IL-1, IL-6, and IL-8 by Th1 cells.	45, 46
	TRAF1/C5	9	rs2900180 (C>T) rs10818488 (G>A)	TNF-receptor associated factor 1 is involved in T cell survival through 4-1BB (CD137). C5 encodes complement component 5.	31, 37, 47, 48
	TNF†	6	rs1800629(G>A)	TNF- mediates systemic inflammation and acuse phase response.	49, 50
	IL10‡	1	rs1800896 (G>A)	Anti-inflammatory cytokine secreted by monocytes and Th2 cells.	51, 52
	TNFAIP3	6	rs6920220 (G>A)	See below.	29, 31
	WISP3	6	rs2280153 (G>A)	Regulates cellular functions (adhesion, migration, and differentiation).	53

Table 2 continued.

Association	Gene region	Chr.	SNP/allele	Gene function	Ref.
Protective	ANKRD55	5	rs71624119 (G>A) rs10040327 (C>A)	Ankyrin repeat domain containing protein 55. Involved in N-glycosylation of immunoglobulin G.	18, 32
	IL2-IL21	4	rs1479924 (A>G) rs13143866 (G>T) rs6822844 (G>T)	IL-2 is important for clonal expansion of lymphocytes. IL-21 is involved in activation and differentiation of several immune cells (macrophages, NK cells, and B cells).	18, 28, 32, 44, 54
	IL2RA	10	rs7909519 (A>C) rs2104286 (A>C)	IL-2 receptor subunit, mediates IL-2 signalling.	18, 27, 28
	COG6	13	rs7993214 (G>A)	Subunit in the oligomeric Golgi complex which ensures the integrity of the Golgi apparatus.	18, 28
	ANGPT1	8	rs1010824 (C>T)	Angiopoietin-1 activates the TIE2 receptor leading to angiogenesis, neutrophil chemotaxis, and secretion of MMPs.	28
	VTCN1	1	rs2358820 (G>A)	A B7 costimulatory protein (B7-H4) on the surface of antigen presenting cells interacting with T cells.	27
	CCR5	3	32 allele	Expressed on T cells and macrophages and serves as receptor for MCP-2, MIP1/, and CCL5.	55, 56
	TNFAIP3	6	rs10499194 (C>T) rs13207033 (G>A)	TNF-induced protein 3 modulates NF-B signalling downstream of TNF and TLRs.	29, 31, 39
	IL6‡	7	rs1800795 (G>C)	IL-6 is a proinflammatory cytokine secreted by macrophages, mediating fever and acute phase response.	57, 58

*The table shows only susceptibility loci with significant association in ≥2 cohorts. † Studies regarding polymorphisms in the TNF gene region have been rather inconclusive and unreplicated associations of several different polymorphisms have been reported[59-63]; ‡ only associated with sJIA susceptibility. Other loci are associated with JIA (often attributable to oJIA and pJIA).

HLA: human leukocyte antigen; JIA: juvenile idiopathic arthritis; MS: microsatellite; SNP: single nucleotide polymorphism; TCR: T cell receptor; Lyp: lymphoid protein tyrosine phosphatase; IL: interleukin; MIF: macrophage migration inhibitory factor; Th1: Type 1 T helper (Th1); IFN-: interferon-gamma; TNF: tumour necrosis factor; NK cells: natural killer cells; MMP: matrix metalloproteinase; TIE2: tyrosine kinase with immunoglobulin-like and EGF-like domains 2; NF-B: nuclear factor kappa-light-chain-enhancer of activated B cells; CCL5: chemokine ligand 5; TLRs: toll-like receptors.

FUTURE PERSPECTIVES IN JIA GENETICS

What is Next in Future JIA GWASs?

The vast majority of genetic studies done so far have focused on finding loci conferring susceptibility to JIA. These studies have been crucial for acquiring an understanding of the pathogenesis of JIA but also in terms of developing new treatment options. However, an increasing number of studies have been focusing on more specific outcomes, often related to either disease course or treatment responses (pharmacogenomics). A list of such findings is shown in Table 3.

As mentioned earlier, the big advantage of a genome-wide approach is that it is hypothesis-free. However, due to the many SNPs genotyped, it is challenging to reach significant results after correction for multiple testing (e.g. genome-wide significance, typically $p<5x10^{-8}$). It requires high demands on the size of the cohorts investigated and, as such, international collaborations such as the International Childhood Arthritis Genetics Consortium are essential to acquire enough patient material. The Immunochip study[18] with 2,816 cases (oJIA and RFneg-JIA), including US, UK, and German samples, is the only study to have reached genome-wide significance ($p<5x10^{-8}$) in multiple loci.

Table 3: Genetic variations associated with specific outcome in JIA patients.

Category	Gene region	Chr.	SNP/allele/GT	Association	Ref.
Disease course	HLA	6	B27	HLA-B27-pos. children had higher odds of not being in remission after 8 years of disease than HLA-B27-neg. children	74
	MIF	22	rs755622 (G>C)	Higher number of affected joints and higher C-HAQ scores	75
	NLRP3	1	rs4353135 (T>G)	Increased need for etanercept (anti-TNF) treatment	76
	IL6	7	rs1800795 (G)	Pain scores (VAS)	77
	TNF	6	rs1800629(G>A)	Higher disease activity, C-HAQ scores (trend), and TNF-α levels	66
			rs1800629(G>A)	Poor outcome	78
	VTCN1	1	rs10923223 (T>C)	Remitting disease course	79
	CDK6	7	rs42041 (C>G)	Remitting disease course	79
	MBL2	10	XA/O or O/O genotype	Remitting disease course	80
	TGFB1	19	rs1800471 (C>G, GG)	Protective effect against joint space narrowing	77
MAS susceptibility	IRF5	7	rs2004640 (G>T)	Higher susceptibility to MAS	81
	PRF1	10	rs35947132 (G>A)	Higher susceptibility to MAS (did not reach statistical significance)	82
	UNC13D	17	12-SNP haplotype	Higher susceptibility to MAS (9 of 16 sJIA patients with MAS)	83
MTX response	ABCB1	7	rs1045642(G>A)	Good response to MTX	84
	ABCC3	17	rs4793665 (C>T)	Good response to MTX	84
	SLC16A7	12	rs10877333 (T>G)	Good response to MTX	85
			rs3763980 (T>A)	Increased risk of non-response to MTX (validated)	85
	ATIC	2	rs12995526 (T>C)	Increased risk of non-response to MTX	86
			rs4673990 (T>C)	Increased risk of non-response to MTX	86
	ITPA	20	rs2295553 (T>C)	Increased risk of non-response to MTX	86
	SLC19A1	21	rs1051266 (C>T)	Increased risk of non-response to MTX	84
MTX toxicity	MTHFR	1	rs1801133 (C>T, TT)	High incidence of any adverse effects	87
	GGH	8	rs1800909 (T>C, CC)	Risk factor for liver dysfunction	88
Glucorticoid response	MIF	22	rs755622 (G>C)	Shorter clinical remission after intra-articular glucocorticoid injection (46% reduced time in remission)	89
			rs755622 (G>C)	Longer duration of glucocorticoid treatment (daily regimen) needed	75
Anti-TNF response	TNF	6	rs1800629(G>A, AA)	Low response to etanercept treatment and lower effect in reducing MMP-9 levels	50
Biologic effects	COL1A1	17	rs1800012 (G>T, GG)	Increased risk of LBMD in pubertal JIA children (Tanner II-III)	90
			rs1107946 (G>T, GG)	Increased risk of LBMD in pubertal JIA children (Tanner IV-V)	90

JIA: juvenile idiopathic arthritis; SNP: single nucleotide polymorphism; HLA: human leukocyte antigen; C-HAQ: childhood health assessment questionnaire; VAS: visual analogue scale; TNF: tumour necrosis factor; MAS: macrophage activation syndrome (complication in systemic JIA patients); MTX: methotrexate; MMP: matrix metalloproteinase; LBMD: low bone mineral density.

To utilise future GWASs, investigating more specific outcome measures as well as other JIA subtypes, and the necessity of international collaborations will be even more essential, both in terms of collecting large homogeneous cohorts and acquiring comparable clinical data, e.g. remission rate or treatment response, for association analysis.

Utilising Novel Sequencing Techniques in JIA Research and Personalised Medicine

As mentioned earlier, most of the common genetic polymorphisms known to confer risk or protection from JIA development are non-coding, and their particular role in the pathogenesis is unknown. Some SNPs might have a regulatory role, but it is also likely that some SNPs tag other more rare and functional variants nearby due to LD. Novel high-throughput sequencing methods (also known as next-generation sequencing [NGS]) have enabled numerous genes (targeted sequencing) or even the entire exome/genome to be sequenced in a single run. The utilisation of such approaches would be interesting to JIA research in order to detect these rare variants with a high impact on JIA pathogenesis, both in genes with known association to JIA susceptibility and new genes not detected in GWASs. This would help researchers to acquire more detailed knowledge on how genetic variation is involved in JIA.

In addition, NGS offers several other applications, such as sequencing of the methylome (bisulfate sequencing), transcriptome (RNA sequencing), transcription-factor binding sites and their interactions with proteins (chromatin immunoprecipitation [ChIP]-sequencing), nucleosome positioning (ChIP-sequencing), and chromatin interaction analysis by paired-end tag sequencing (ChIA-PET). The utilisation of these applications on disease-relevant cell types will enable researchers to investigate the role of the epigenome in JIA pathogenesis. In 2012, Ellis et al.[91] did the first genome-wide methylation study in JIA, finding a lowered methylation level at the gene encoding the proinflammatory cytokine IL-32 in CD4+ T cells. Still, the field of epigenomics in JIA remains undiscovered and it will be interesting to follow the findings of future studies on this matter. Finally, NGS also enables metagenomic analyses of the human microbiota, which is thought to contribute to the development of autoimmune diseases.[92]

In the years to come, genetics are predicted to play an ever-increasing role in the care of JIA. Knowledge of individual genetic variability can support the paediatrician's assessment in determining the right diagnosis, prognosis, and treatment in order to limit patient symptoms, treatment side-effects, and long-term disability. Bulatovic et al.[93] constructed a prediction model for MTX response including information on erythrocyte sedimentation rate and genotyping of four SNPs; it showed a 72% power to predict MTX non-responders. This confirmed the relevance of including genetic variability in determining the right treatment for JIA patients. However, much more knowledge is needed on how results from genome-wide genotyping or sequencing are to be interpreted and used in patient care.

CONCLUSION

Our knowledge of how genetic variability influences JIA susceptibility has evolved rapidly in recent years, and several new susceptibility loci outside the HLA gene complex have been discovered. These associated polymorphisms, however, are often non-coding and much investigation is still to be done on finding the functional genetic variants and pathways directly involved in the pathogenesis. Genetic variability has also been shown to influence more specific clinical outcomes, such as disease course and treatment response. Expectedly in the years to come, this accumulating knowledge - along with the increasing availability of genome-wide genotyping and sequencing analyses - will assist in diagnosis, prognosis, and more personalised medicine.

REFERENCES

1. Ravelli A, Martini A. Juvenile idiopathic arthritis. Lancet. 2007;369:767-78.

2. Petty RE et al. International League of Associations for Rheumatology Classification of Juvenile Idiopathic Arthritis: Second Revision, Edmonton, 2001. J Rheumatol. 2004;31(2):390-2.

3. Ellis JA et al. Possible environmental determinants of juvenile idiopathic arthritis. Rheumatology. 2010;49(3): 411-25.

4. Prahalad S, Glass DN. A comprehensive review of the genetics of juvenile idiopathic arthritis. Pediatr Rheumatol Online J. 2008;6:11.

5. Glass DN, Giannini EH. Juvenile rheumatoid arthritis as a complex genetic

trait. Arthritis Rheum. 1999;42(11):2261-8.

6. Rosenberg AM, Petty RE. Similar patterns of juvenile rheumatoid arthritis within families. Arthritis Rheum. 1980;23(8):951-3.

7. Clemens LE et al. Sibling pairs affected by chronic arthritis of childhood: evidence for a genetic predisposition. J Rheumatol. 1985;12(1):108-13.

8. Prahalad S. Genetic analysis of juvenile rheumatoid arthritis: approaches to complex traits. Curr Probl Pediatr Adolesc Health Care. 2006;36(3):83-90.

9. Prahalad S et al. Quantification of the familial contribution to juvenile idiopathic arthritis. Arthritis Rheum. 2010;62(8):2525-9.

10. Prahalad S et al. Increased prevalence of familial autoimmunity in simplex and multiplex families with juvenile rheumatoid arthritis. Arthritis Rheum. 2002;46(7):1851-6.

11. Säilä H et al. Occurrence of chronic inflammatory rheumatic diseases among parents of multiple offspring affected by juvenile idiopathic arthritis. Clin Exp Rheumatol. 2003;21(2):263-5.

12. Prahalad S et al. Familial aggregation of juvenile idiopathic arthritis. Arthritis Rheum. 2004;50(12):4022-7.

13. Stagi S et al. Thyroid function, autoimmune thyroiditis and celiac disease in juvenile idiopathic arthritis. Rheumatology. 2005;44(4):517-20.

14. Pohjankoski H et al. Diabetes, coeliac disease, multiple sclerosis and chronic arthritis in first-degree relatives of patients with juvenile idiopathic arthritis. Acta Pediatr. 2012;101(7):767-71.

15. Schlosstein L et al. High association of an HL-A antigen, W27, with ankylosing spondylitis. N Engl J Med. 1973;288(14):704-6.

16. Brewerton DA et al. Ankylosing spondylitis and HL-A 27. Lancet. 1973;301(7809):904-7.

17. Rachelefsky GS et al. Increased prevalence of W27 in juvenile rheumatoid arthritis. N Engl J Med. 1974;290(16): 892-3.

18. Hinks A et al. Dense genotyping of immune-related disease regions identifies 14 new susceptibility loci for juvenile idiopathic arthritis. Nat Genet. 2013;45(6):664-9.

19. Thompson W et al. Juvenile idiopathic arthritis classified by the ILAR criteria: HLA associations in UK patients. Rheumatology. 2002;41(10):1183-9.

20. Hollenbach JA et al. Juvenile idiopathic arthritis and HLA class I and class II interactions and age-at-onset effects. Arthritis Rheum. 2010;62(6): 1781-91.

21. Smerdel A et al. Juvenile idiopathic arthritis (JIA) is primarily associated with HLA-DR8 but not DQ4 on the DR8-DQ4 haplotype. Ann Rheum Dis. 2002;61(4):354-7.

22. Thomson G et al. Sequence feature variant type (SVFT) analysis of the HLA genetic association in juvenile idiopathic arthritis. Pac Symp Biocomput. 2010: 359-70.

23. Prahalad S et al. Hierarchy of risk of childhood-onset rheumatoid arthritis conferred by HLA-DRB1 alleles encoding the shared epitope. Arthritis Rheum. 2012;64(3):925-30.

24. Prahalad S et al. Juvenile rheumatoid arthritis: linkage to HLA demonstrated by allele sharing in affected sibpairs. Arthritis Rheum. 2000;43(10):2335-8.

25. Thompson SD et al. Genome-wide association analysis of juvenile idiopathic arthritis identifies a new susceptibility locus at chromosomal region 3q13. Arthritis Rheum. 2012;64(8):2781-91.

26. Behrens EM et al. Association of the TRAF1-C5 locus on chromosome 9 with juvenile idiopathic arthritis. Arthritis Rheum. 2008;58(7):2206-07.

27. Hinks A et al. Identification of a novel susceptibility locus for juvenile idiopathic arthritis by genome-wide association analysis. Arthritis Rheum. 2009;60(1): 258-63.

28. Thompson SD et al. The susceptibility loci juvenile idiopathic arthritis shares with other autoimmune diseases extend to PTPN2, COG6, and ANGPT1. Arthritis Rheum. 2010;62(11):3265-76.

29. Prahalad S et al. Variants in TNFAIP3, STAT4, and C12orf30 loci associated with multiple autoimmune diseases are also associated with juvenile idiopathic arthritis. Arthritis Rheum. 2009;60(7):2124-30.

30. Hinks A et al. Investigation of type 1 diabetes and coeliac disease susceptibility loci for association with juvenile idiopathic arthritis. Ann Rheum Dis. 2010;69(12):2169-72.

31. Hinks A et al. Overlap of disease susceptibility loci for rheumatoid arthritis and juvenile idiopathic arthritis. Ann Rheum Dis. 2010;69(6):1049-53.

32. Hinks A et al. Investigation of rheumatoid arthritis susceptibility loci in juvenile idiopathic arthritis confirms high degree of overlap. Ann Rheum Dis. 2012;71(7):1117-21.

33. Cortes A, Brown MA. Promise and pitfalls of the Immunochip. Arthritis Res Ther. 2011;13(1):101.

34. Burn GL et al. Why is PTPN22 a good candidate susceptibility gene for autoimmune disease? FEBS Lett. 2011;585(23):3689-98.

35. Zheng J et al. Meta-analysis reveals an association of PTPN22 C1858T with autoimmune diseases, which depends on the localization of the affected tissue. Genes Immun. 2012;13(8):641-52.

36. Hinks A et al. Association between the PTPN22 gene and rheumatoid arthritis and juvenile idiopathic arthritis in a UK population: further support that PTPN22 is an autoimmunity gene. Arthritis Rheum. 2005;52(6):1694-9.

37. Ellis JA et al. Independent replication analysis of genetic loci with previous evidence of association with juvenile idiopathic arthritis. Pediatr Rheumatol Online J. 2013;11(1):12.

38. Cinek O et al. No independent role of the -1123 G>C and +2740 A>G variants in the association of PTPN22 with type 1 diabetes and juvenile idiopathic arthritis in two Caucasian populations. Diabetes Res Clin Pract. 2007;76(2):297-303.

39. Prahalad S et al. Susceptibility to childhood-onset rheumatoid arthritis: investigation of a weighted genetic risk score that integrates cumulative effects of variants at five genetic loci. Arthritis Rheum. 2013;65(6):1663-7.

40. Viken MK et al. Association analysis of the 1858C>T polymorphism in the PTPN22 gene in juvenile idiopathic arthritis and other autoimmune diseases. Genes Immun. 2005;6(3):271-3.

41. Liang YL et al. Association of STAT4 rs7574865 polymorphism with autoimmune diseases: a meta-analysis. Mol Biol Rep. 2012;39(9):8873-82.

42. Runstadler JA et al. Association of SLC11A1 (NRAMP1) with persistent oligoarticular and polyarticular rheumatoid factor-negative juvenile idiopathic arthritis in Finnish patients: haplotype analysis in Finnish families. Arthritis Rheum. 2005;52(1):247-56.

43. Sanjeevi CB et al. Polymorphism at NRAMP1 and D2S1471 loci associated with juvenile rheumatoid arthritis. Arthritis Rheum. 2000;43(6):1397-404.

44. Hinks A et al. Association of the AFF3 gene and IL2/IL21 gene region with juvenile idiopathic arthritis. Genes Immun. 2010;11(2):194-8.

45. Donn R et al. Mutation screening of the macrophage migration inhibitory factor gene: positive association of a functional polymorphism of macrophage migration inhibitory factor with juvenile idiopathic arthritis. Arthritis Rheum. 2002;46(9):2402-9.

46. Lee YH et al. The association between the functional PTPN22 1858 C/T and MIF -173 C/G polymorphisms and juvenile idiopathic arthritis: a meta-analysis. Inflamm Res. 2012;61(5):411-5.

47. Albers HM et al. The TRAF1/C5 region is a risk factor for polyarthritis in juvenile idiopathic arthritis. Ann Rheum Dis. 2008;67(11):1578-80.

48. Dimoupolou DG et al. Investigation of

juvenile idiopathic arthritis susceptibility loci: results from a Greek population. Hum Immunol. 2013;74(9):1194-8.

49. Jiménez-Morales S et al. Tumor necrosis factor-alpha is a common genetic risk factor for asthma, juvenile rheumatoid arthritis, and systemic lupus erythematosus in a Mexican pediatric population. Hum Immunol. 2009;70(4):251-6.

50. Basic J et al. Etanercept reduces matrix metalloproteinase-9 level in children with polyarticular juvenile idiopathic arthritis and TNF-alpha-308GG genotype. J Physiol Biochem. 2010;66(2):173-80.

51. Möller JC et al. IL10 promoter polymorphisms are associated with systemic onset juvenile idiopathic arthritis (SoJIA). Clin Exp Rheumatol. 2010;28(6):912-8.

52. Fife MS et al. Novel IL10 gene family associations with systemic juvenile idiopathic arthritis. Arthritis Res Ther. 2006;8(5):R148.

53. Lamb R et al. Wnt-1-inducible signaling pathway protein 3 and susceptibility to juvenile idiopathic arthritis. Arthritis Rheum. 2005;52(11):3548-53.

54. Albers HM et al. Association of the autoimmunity locus 4q27 with juvenile idiopathic arthritis. Arthritis Rheum. 2009;60(3):901-4.

55. Hinks A et al. Association of the CCR5 gene with juvenile idiopathic arthritis. Genes Immun. 2010;11(7):584-9.

56. Prahalad S et al. Association of two functional polymorphisms in the CCR5 gene with juvenile rheumatoid arthritis. Genes Immun. 2006;7(6):468-75.

57. Ogilvie EM et al. The -174G allele of the interleukin-6 gene confers susceptibility to systemic arthritis in children: a multicenter study using simplex and multiplex juvenile idiopathic arthritis families. Arthritis Rheum. 2003;48(11):3202-6.

58. Fishman D et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest. 1998;102(7):1369-76.

59. Schmeling H et al. Tumor necrosis factor alpha promoter polymorphisms in patients with juvenile idiopathic arthritis. Clin Exp Rheumatol. 2006;24(1):103-8.

60. Zeggini E et al. Linkage and association studies of single-nucleotide polymorphism-tagged tumor necrosis factor haplotypes in juvenile oligoarthritis. Arthritis Rheum. 2002;46(12):3304-11.

61. Nikitina Zake L et al. Major histocompatibility complex class I chain related (MIC) A gene, TNFa microsatellite alleles and TNFB alleles in juvenile idiopathic arthritis patients from Latvia. Hum Immunol. 2002;63(5):418-23.

62. Epplen C et al. Immunoprinting excludes many potential susceptibility genes as predisposing to early onset pauciarticular juvenile chronic arthritis except HLA class II and TNF. Eur J Immunogenet. 1995;22(4):311-22.

63. Date Y et al. Identification of a genetic risk factor for systemic juvenile rheumatoid arthritis in the 5'-flanking region of the TNFalpha gene and HLA genes. Arthritis Rheum. 1999;42(12): 2577-82.

64. Maurano et al. Systemic localization of common disease-associated variants in regulatory DNA. Science. 2012;337:1190-5.

65 Lavrikova EY et al. The carriage of the type 1 diabetes-associated R262W variant of human LNK correlates with increased proliferation of peripheral blood monocytes in diabetic patients. Pediatr Diabetes. 2011;12(2):127-32.

66. Mourão AF et al. Tumor necrosis factor-alpha -308 genotypes influence inflammatory activity and TNF-alpha serum concentrations in children with juvenile idiopathic arthritis. J Rheumatol. 2009;36(4):837-42.

67. Prakken B et al. Juvenile idiopathic arthritis. Lancet. 2011;377(9783):2138-49.

68. Lin YT et al. The pathogenesis of oligoarticular/polyarticular vs systemic juvenile idiopathic arthritis. Autoimmun Rev. 2011;10(8):482-9.

69. Stock CJ et al. Comprehensive association study of genetic variants in the IL-1 gene family in systemic juvenile idiopathic arthritis. Genes Immun. 2008;9(4):349-57.

70. Hinks A et al. Autoinflammatory gene polymorphisms and susceptibility to UK juvenile idiopathic arthritis. Pediatr Rheumatol Online J. 2013;11(1):14.

71. Omoyinmi E et al. Association of the IL-10 gene family locus on chromosome 1 with juvenile idiopathic arthritis (JIA). PLoS One. 2012;7(10):e47673.

72. Cobb JE et al. The genetics of juvenile idiopathic arthritis: current understanding and future prospects. Rheumatology. 2014;53(4):592-9.

73. Herlin T. Tocilizumab for the treatment of systemic juvenile idiopathic arthritis. Expert Rev Clin Immunol. 2012;8(6): 517-25.

74. Berntson L et al. HLA-B27 predicts a more chronic disease course in an 8-year followup cohort of patients with juvenile idiopathic arthritis. J Rheumatol. 2013;40(5):725-31.

75. De Benedetti F et al. Functional and prognostic relevance of the -173 polymorphism of the macrophage migration inhibitory factor gene in systemic-onset juvenile idiopathic arthritis. Arthritis Rheum. 2003;48(5): 1398-407.

76. Yang CA et al. Association of NLRP3 and CARD8 genetic polymorphisms with juvenile idiopathic arthritis in a Taiwanese population. Scand J Rheumatol. 2014;43(2):146-52.

77. Oen K et al. Cytokine genotypes correlate with pain and radiologically defined joint damage in patients with juvenile rheumatoid arthritis. Rheumatology. 2005;44(9):1115-21.

78. Ozen S et al. Tumour necrosis factor alpha G→A -238 and G→A -308 polymorphisms in juvenile idiopathic arthritis. Rheumatology. 2002;41(2):223-7.

79. Albers HM et al. Genetic variation in VTCN1 (B7-H4) is associated with course of disease in juvenile idiopathic arthritis. Ann Rheum Dis. 2013. [Epub ahead of print].

80. Dolman KM et al. Mannose-binding lectin deficiency is associated with early onset of polyarticular juvenile rheumatoid arthritis: a cohort study. Arthritis Res Ther. 2008;10(2):R32.

81. Yanagimachi M et al. Association of IRF5 polymorphisms with susceptibility to macrophage activation syndrome in patients with juvenile idiopathic arthritis. J Rheumatol. 2011;38(4):769-74.

82. Vastert SJ et al. Mutations in the perforin gene can be linked to macrophage activation syndrome in patients with systemic onset juvenile idiopathic arthritis. Rheumatology. 2010;49(3):441-9.

83. Zhang K et al. Macrophage activation syndrome in patients with systemic juvenile idiopathic arthritis is associated with MUNC13-4 polymorphisms. Arthritis Rheum. 2008;58(9):2892-6.

84. de Rotte MC et al. ABCB1 and ABCC3 gene polymorphisms are associated with first-year response to methotrexate in juvenile idiopathic arthritis. J Rheumatol. 2012;39(10):2032-40.

85. Moncrieffe H et al. Generation of novel pharmacogenomic candidates in response to methotrexate in juvenile idiopathic arthritis: correlation between gene expression and genotype. Pharmacogenet Genomics. 2010;20(11):665-76.

86. Hinks A et al. Association of the 5-aminoimidazole-4-carboxamide ribonucleotide transformylase gene with response to methotrexate in juvenile idiopathic arthritis. Ann Rheum Dis. 2011;70(8):1395-400.

87. Tuková J et al. 677TT genotype is associated with elevated risk of methotrexate (MTX) toxicity in juvenile idiopathic arthritis: treatment outcome, erythrocyte concentrations of MTX and folates, and MTHFR polymorphisms. J Rheumatol. 2010;37(10):2180-6.

88. Yanagimachi M et al. Influence of polymorphisms within the methotrexate pathway genes on the toxicity and

efficacy of methotrexate in patients with juvenile idiopathic arthritis. Br J Clin Pharmacol. 2011;71(2):237-43.

89. Vivarelli M et al. Macrophage migration inhibitory factor (MIF) and oligoarticular juvenile idiopathic arthritis (o-JIA): association of MIF promoter polymorphisms with response to intra-articular glucocorticoids. Clin Exp Rheumatol. 2007;25(5):775-81.

90. Kostik MM et al. Genetic polymorphisms of collagen type I α1 chain (COL1A1) gene increase the frequency of low bone mineral density in the subgroup of children with juvenile idiopathic arthritis. EPMA J. 2013;4(1):15.

91. Ellis JA et al. Genome-scale case-control analysis of CD4+ T-cell DNA methylation in juvenile idiopathic arthritis reveals potential targets involved in disease. Clin Epigenetics. 2012;4(1):20.

92. Proal AD et al. Autoimmune disease in the era of the metagenome. Autoimmun Rev. 2009;8(8):677-81.

93. Bulatovic M et al. Prediction of clinical non-response to methotrexate treatment in juvenile idiopathic arthritis. Ann Rheum Dis. 2012;71(9):1484-9.