SUSCEPTIBILITY AND PROGNOSTIC FACTORS IN PORTUGUESE PATIENTS WITH JUVENILE IDIOPATHIC ARTHRITIS

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Tese para obtenção do grau de Doutor em Medicina

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Aos meus Pais

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Nenhuma aprendizagem, trabalho ou arte se desenvolve de forma isolada, sendo apenas na partilha das dúvidas, conhecimentos e ideias que pode nascer a verdadeira progressão do saber. O trabalho de investigação apresentado nesta tese resulta da conjugação de vários acontecimentos felizes e da colaboração imprescindível de várias pessoas.

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ABBREVIATIONS

ACR American college of rheumatology

AID Autoimmune diseases

COX2 Cyclo-oxygenase 2

CHAQ Childhood health assessment questionnaire

cJADAS Clinical juvenile arthritis disease activity score

CRP C- reactive protein

DAS Disease activity index

DMARD Disease modifying anti-rheumatic drug

ERA Enthesitis-related arthritis

ESR Erythrocyte sedimentation rate

GWAS Genome wide association studies

HLA Human leucocyte antigen

ILAR International league of associations for rheumatology

JADI Juvenile arthritis damage index

JADAS Juvenile arthritis disease activity score

JADAS3 Juvenile arthritis disease activity score with 3 variables (without ESR or CRP)

JIA Juvenile idiopathic arthritis

MAF Minor allele frequency

MTX Methotrexate

MHC Major histocompatibility complex

NSAIDs Non-steroidal anti-inflammatory drugs

OligoJIA Oligoarticular JIA

PolyJIA Polyarticular JIA

PsA Psoriatic arthritis

RA Rheumatoid arthritis

RCT Randomized controlled trial

Reuma.pt Portuguese register of rheumatic diseases

RF Rheumatoid factor

SNP Single nucleotide polymorphism

SoJIA Systemic onset JIA

TNF Tumor necrosis factor

VAS Visual analogue scale

SUMMARY

The aim of the present thesis was to validate genetic predictors of susceptibility to juvenile idiopathic arthritis (JIA), and to identify genetic and clinical predictors of poor prognosis in JIA. As a relevant component of long-term prognosis we also aimed at evaluating a clinical disease activity score and the clinical effectiveness, safety and retention rate of biological therapies, used in a subset of poor prognosis JIA patients.

In the first part of this thesis we investigated whether polymorphisms in the promoter area of TNF (-308 genotypes) were relevant in disease susceptibility and activity, based on previous results obtained by our group in rheumatoid arthritis patients. We observed that TNF 308 GA/AA genotypes were related to higher inflammatory and disease activity. These genotypes were not associated with susceptibility to JIA.

Later on, we have increased our sample and aimed to confirm whether 15 single nucleotide polymorphisms (SNP) of selected genes, found in previous studies to be associated with an increased risk for the development of JIA, were associated with susceptibility for JIA in the Portuguese population. Our results provide additional evidence for an association between polymorphisms in genes PTPN2, PTPN22 and ANGPT1 and the risk of RF-positive polyarticular, extended oligoarticular and systemic JIA, respectively, supporting the current concept of genetic heterogeneity of JIA categories.

Additionally, we found that polyarticular categories of JIA, longer duration of disease modifying anti-rheumatic drugs (DMARD) treatment and higher physician visual analogue scale (VAS) had a significant association with poor prognosis in JIA patients. Our study did not confirm the association between a panel of selected SNP and poor prognosis in patients with JIA, as opposed to what had been described in other studies.

Missing values in the laboratorial variables, especially erythrocyte sedimentation rate (ESR), are a common problem for national databases of rheumatic diseases and we had to face this issue during the development of this project while using the national registry, Reuma.pt. This prompted us to evaluate the correlation between the recently developed tool for evaluation

of disease activity in JIA, the Juvenile Arthritis Disease Activity Score 27-joint reduced count (JADAS27) using ESR, and JADAS27 with C-reactive protein (CRP). We found that JADAS27 based on CRP level correlated closely with JADAS27-ESR across all disease activity states and JIA categories, indicating that both measures can be used in clinical practice. Moreover, we found that the correlation of JADAS27 with and without ESR (clinical JADAS) was also high, suggesting that this tool might be useful even in the absence of any laboratorial measures.

We subsequently studied the use of biological therapy in JIA patients within the national registry, Reuma.pt, and demonstrated a sustained effectiveness and safety and high long-term retention rate for the first biological agent throughout the follow-up period.

The results presented in this thesis allowed us to identify genetic predictors of susceptibility to specific categories of JIA. On the other hand, although we did not find any consistent genetic predictor of poor prognosis, we found clinical variables that can be related with poor prognosis. Of relevance for studies on the prognosis of JIA we provided evidence for the use of clinical JADAS27, indicating that when laboratorial variables are not available, this instrument can reliably be used to monitor disease activity. Finally, with future implications for the long-term prognosis of JIA, we have demonstrated a high retention rate of biological therapies in JIA.

SUMÁRIO

A presente tese teve como objetivo validar preditores genéticos de suscetibilidade para a artrite idiopática juvenil (AIJ) e identificar preditores genéticos e clínicos de mau prognóstico na AIJ. Como componente relevante do prognóstico a longo prazo, visámos igualmente avaliar um instrumento de determinação de atividade da doença (JADAS), bem como a eficácia, segurança e a taxa de retenção das terapêuticas biológicas.

Na primeira parte desta tese, com base nos resultados anteriormente obtidos pelo nosso grupo em doentes com artrite reumatoide, investigámos se os polimorfismos na região promotora do gene do TNF (posição -308) eram relevantes na suscetibilidade e atividade da AIJ. Observámos que os genótipos 308 GA/AA do TNF estavam relacionados com o aumento dos parâmetros inflamatórios e maior atividade da doença. Estes genótipos não estavam associados a maior suscetibilidade para a AIJ.

Posteriormente, aumentámos a nossa amostra e testámos se 15 polimorfismos de nucleóticos simples (SNP) de genes selecionados previamente associados a maior risco de desenvolver AIJ em outras populações, também estavam associados a suscetibilidade para AIJ na população portuguesa. Os nossos resultados sugerem a associação entre polimorfismos nos genes PTPN2, PTPN22 e ANGPT1 e o risco de AIJ poliarticular com FR positivo, oligoarticular estendida e sistémica, respetivamente, sustentando o conceito atual de heterogeneidade genética entre as categorias de AIJ.

Observámos igualmente que as categorias poliarticulares de AIJ, a maior duração do tratamento com agentes antirreumáticos modificadores da doença e uma pontuação mais elevada na escala visual analógica de atividade da doença avaliada pelo médico, estavam associados a pior prognóstico em doentes com AIJ. O nosso estudo não confirmou a associação entre um painel de SNPs selecionados e o mau prognóstico dos doentes com AIJ, contrariamente ao que foi descrito em outros estudos.

Um dos problemas das bases de dados de registos de doentes, nomeadamente do registo nacional das doenças reumáticas (Reuma.pt) inclui a falta de dados de variáveis laboratoriais,

em concreto a velocidade de sedimentação (VS). Esta situação levou-nos a avaliar a correlação entre o instrumento recentemente desenvolvido para avaliação da atividade da doença na AIJ, o *Juvenile Arthritis Disease Activity Score* 27 (JADAS27) utilizando a VS (JADAS27-VS) e o JADAS27 utilizando a Proteína-C-Reativa (PCR). Verificámos que o JADAS27 utilizando a PCR estava significativamente correlacionado com o JADAS27-VS em todos os níveis de atividade da doença e categorias de AIJ. Tais resultados confirmam que ambos os instrumentos podem ser utilizados na prática clínica. Adicionalmente, observámos que a correlação com o JADAS27, com e sem VS (JADAS clínico), também era muito significativa, sugerindo que este instrumento pode ser útil na monitorização da atividade da doença, mesmo na ausência de parâmetros laboratoriais.

Na parte final desta tese, estudámos a utilização de terapêuticas biológicas em doentes com AIJ no âmbito do registo nacional Reuma.pt, e verificámos mantidas eficácia clínica e segurança, bem como uma elevada taxa de retenção para o primeiro agente biológico a longo prazo.

Os resultados apresentados nesta tese permitiram-nos identificar preditores genéticos de suscetibilidade para categorias específicas de AIJ. Embora não tenhamos conseguido encontrar consistentes preditores genéticos de mau prognóstico, conseguimos sugerir variáveis clínicas potencialmente relacionadas com mau prognóstico. De relevância para os estudos de prognóstico na AIJ fornecemos ainda evidência que suporta a utilização do JADAS27 clínico, reforçando o facto de que mesmo quando não estão disponíveis variáveis laboratoriais, este instrumento pode ser utilizado para monitorizar a atividade da doença. Por fim, com implicações futuras para o prognóstico a longo prazo, demonstrámos uma elevada taxa de retenção de terapêuticas biológicas na AIJ.

INTRODUCTION

1. WHAT IS JUVENILE IDIOPATHIC ARTHRITIS?

Juvenile idiopathic arthritis (JIA) is not a single disease, but a term that encompasses all forms of arthritis that begin before the age of 16 years, persist for more than 6 weeks, and are of unknown cause (1–3). The term represents, therefore, an exclusion diagnosis that includes all forms of childhood chronic arthritis of unknown cause. JIA comprises several disease categories, each of which has distinct methods of presentation, clinical signs and symptoms, and, in some cases, genetic background. JIA is the most common rheumatic disease in children (4,5) and may result in significant pain, joint deformity, and growth impairment, with persistence of active arthritis into adulthood. The cause of this disease is still poorly understood, but seems to be related to both genetic and environmental factors. The fundamental process in JIA is chronic inflammation, in which the immune system understandably plays a critical role (6). Both innate and adaptive immune systems have been implicated in the pathogenesis of the various subtypes of JIA (7). Although none of the available drugs has a curative potential, a substantial progress in disease management has occurred. The recent introduction of biological therapies is expected to have a long-term impact on prognosis, particularly if retention rates will prove to be high and associated with sustained efficacy and safety.

According to the International League of Associations for Rheumatology (ILAR) (1,2), JIA consists of seven heterogeneous subgroups, namely oligoarthritis (OligoJIA), rheumatoid factor (RF)-positive polyarthritis (PolyJIA), RF-negative PolyJIA, systemic onset JIA (SoJIA), enthesitis-related arthritis (ERA), psoriatic arthritis (PsA) and undifferentiated arthritis (Table 1).

JIA has replaced former classification nomenclature, including juvenile rheumatoid arthritis and juvenile chronic arthritis. The primary aim for the reclassification of JIA was to define more clearly distinctive clinical phenotypes, thus facilitating research into the underlying genetic background, disease processes, as well as prognosis and response to therapy in this group of conditions. The original classification of JIA has been revised several times, most

recently in 2004 resulting in further classification of the various subsets, correcting prior incongruences, and improving its clinical utility to the rheumatologist (8–10). However, the classification still needs validation and consensus; it has restrictions intrinsic to any classification founded on clinical criteria and will probably be modified as new information on pathogenesis becomes available. As with most classification criteria in rheumatology, the diagnosis of JIA is one of exclusion, forcing the clinician to rule out other causes of chronic arthritis including rheumatic, infectious and other potential causes of chronic synovitis.

Table 1. Classification of the Juvenile Idiopathic Arthritis categories

SYSTEMIC ONSET
Arthritis with or preceded by at least 2 weeks of daily fever, with at least 3 days of documented daily ("quotidian") fever. Plus one of more of the following: 1. Evanescent, non-fixed erythematous rash 2. Generalized lymphadenopathy 3. Hepatomegaly and/or splenomegaly 4. Serositis
OLIGOARTHRITIS ONSET
Arthritis affecting 1-4 joints during the first 6 months of disease Persistent oligoarthritis: Arthritis of 4 or fewer joints throughout disease course Extended oligoarthritis: Arthritis of 5 or more joints after initial 6 months of oligoarticular disease
POLYARTHRITIS ONSET
Rheumatoid Factor-negative: Arthritis of 5 or more joints during initial 6 months of disease; Rheumatoid Factor negative **Rheumatoid Factor-positive**: Arthritis of 5 or more joints during initial 6 months of disease; Rheumatoid Factor positive on two or more occasions, at least 3 months apart
PSORIATIC ARTHRITIS
Arthritis and psoriasis **or** Arthritis and at least two of the following: 1. Dactylitis 2. Nail pitting or onycholysis 3. Psoriasis in a first-degree relative
ENTHESITIS-RELATED ARTHRITIS
Arthritis and enthesitis **or** Arthritis or enthesitis with at least two of the following: 1. Sacroiliac joint tenderness and/or inflammatory lumbosacral pain 2. HLA B27 positive 3. Arthritis in a male over 6 years of age 4. Acute anterior uveitis 5. History of ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with inflammatory bowel disease, reactive arthritis (Reiter's syndrome), or acute anterior uveitis in a first-degree relative
UNDIFFERENTIATED ARTHRITIS
Fulfills none of the above subsets or fulfills more than one of the above subsets

Adapted from Ravelli A, Martini A. Juvenile Idiopathic Arthritis. Lancet. 2007;369(9563):767-8. (9)

2. EPIDEMIOLOGY OF JIA

Juvenile idiopathic arthritis is the most common chronic rheumatic disease in children and an important cause of short- and long-term disability (9). The incidence of JIA is estimated at 2 to 20 cases per 100,000 children, with a prevalence of 16 to 150 cases per 100,000 children worldwide (9,11). Unlike many other autoimmune diseases, JIA is more common in children of European ancestry and the distribution of JIA subtypes does differ significantly across ethnic groups (12,13). The prevalence of this disease is considered to be underestimated, largely due to the lack of awareness and skills for diagnosing this disease in those who may be the first point of contact in the evaluation of a child with musculoskeletal disease, such as the pediatrician, family practitioner, or emergency room physician, most of whom never had any formal training in pediatric musculoskeletal exam. Furthermore, there is a relative shortage of pediatric rheumatologists, furthering the limitations in medical education, as well as lack of adequate clinical care.

Similar to most rheumatic diseases, twice as many girls may develop JIA, mainly reflecting the female predominance of the oligoarticular subset, which is the largest subgroup. Certain subsets have an age-specific peak incidence; however, it is unusual for children to develop JIA before 6 months of age, similar to the epidemiology of most other childhood rheumatic diseases (11).

In an attempt to determine the prevalence of rheumatic diseases in Portugal, the study EpiReumaPt – epidemiological study of rheumatic diseases in Portugal (14,15) - was conducted and represented a landmark in the epidemiology of rheumatic and musculoskeletal diseases in Portugal, involving more than 10,000 participants. The study design had anticipated the inclusion of chronic arthritis in children. Unfortunately the reduced number of reported cases, directly related with the fact that JIA is a rare condition, turned not possible to determine the prevalence of JIA in Portugal.

3. SUSCEPTIBILITY TO JIA

JIA seems complex in nature, with both environmental and multiple genetic risk components (16–18). Research suggests that some individuals may have a genetic tendency to develop JIA, but develop the condition only after exposure to an infection or another unknown trigger. Multiple and overlapping environmental risk factors have been identified (16,19–24), but the role of these environmental factors in JIA risk is not well characterized and is not the focus of this thesis.

Over the last two decades our understanding of the pathophysiology of JIA has substantially improved with several new genetic associations being recognized (7,17,18,25,26). As it is true for most autoimmune diseases, in JIA there will be many genomic regions contributing with relatively small amounts to overall disease risk (27).

Compared to some adult onset disorders, genetic contribution may be higher in JIA, since children have had less time for environment and behavior to influence disease risk development (28). Family studies have provided firm evidence for genetic susceptibility in JIA and it is not uncommon to discover a family history of autoimmune diseases (8–10,29).

Genetic susceptibility to JIA has been a focus of interest because it holds the promise of two very relevant possible clinical contributions: on one hand it might provide evidence for new key physiopathology pathways that could lead to new treatment targets and on the other hand it might identify markers useful for an early diagnosis.

3.1 How can we investigate JIA susceptibility?

Given the challenges associated with JIA genetics, resulting from the relative rarity and compounded by the clinical heterogeneity of the disease, researchers used varied strategies in an attempt to uncover the genetic basis of JIA susceptibility (17). These include:

3.1.1 Gene candidate association

This approach consists in selecting genes based on expression profiling results and those previously associated with other immune mediated chronic inflammatory diseases (16,19),

since genetic studies in these diseases have revealed the presence of shared common susceptibility loci (7). This overlap of immune mediated chronic inflammatory disease susceptibility loci may occur where the same variants contribute to multiple diseases or it may be that different variants in the same gene lead to different diseases.

In the candidate gene association approach, the cases and control populations must be well genetically matched. Confirmation in a different cohort (also known as replication) is needed usually before one has some confidence that the result does not represent a false positive. It is reasonable to suggest that the small sample sizes in most of the replication studies, as well as positive association publication bias, have led to a likely scenario of over-representation of false positives reported in the literature. It is also true that in many cases, the lack of replication may merely reflect the lack of availability of sufficiently large cohorts in which to seek replication. It is also likely that in some instances, combining clinically distinct entities, such as SoJIA and OligoJIA, might confound the results.

3.1.2 Genome wide association studies (GWAS)

In recent years, the candidate-gene approach has been largely superseded by the genome-wide approach (genome-wide association study or GWAS), in which hundreds of thousands of single nucleotide polymorphisms (SNP) across the entire genome are screened in a single assay. This has led to a plethora of novel susceptibility loci being identified for a variety of complex diseases, many of which have since been confirmed in replication studies (13,28,30–35). Although expensive, the cost of genome-wide studies has considerably decreased, making it cost-effective. In addition, the genome-wide approach has the advantage of being largely “hypothesis-free”, in that it makes no assumptions about which genes may be important in the disease (33). Many of the associations identified to date in other complex diseases are not in genes that would have been selected as candidates; indeed, many are not in genes at all but lie in intergenic regions, presumed to be regulatory. A recent study representing the largest collaborative study of JIA to date (32) identified 14 new genes linked to the disease and confirmed three previously discovered genes, specifically HLA genes, PTPN22 and PTPN2. This study also suggested that another 11 genetic regions might be involved in the disease.

3.2 Overlap susceptibility loci with RA

Since the pathogenesis of JIA shares many similarities with that of adult rheumatoid arthritis (RA), RA-associated loci were re-evaluated in JIA patients (30). This strategy resulted in the identification of several new genetic variants, including the STAT4 gene (36), the TRAF1 and C5 region on chromosome 9 (37), the C1858T polymorphism of PTPN22 gene (38,39), a region on chromosome 10p15 close to the PRKCQ gene (40), CD247 (30), 6q23/TNFAIP3 (40), PTPN2, COG6 and ANGPT1 (26), IL2RA (30), CCR5 (41), AFF3 and the IL2/IL21 (42), which contribute to both RA and JIA.

The presence of TRAF1 and TNFAIP3 among possible candidate genes for association with JIA may suggest a significant role of pro-inflammatory TNF-dependent signaling in the pathogenesis of JIA (43). JIA-associated variants of TRAF1 were shown to predispose to disease (30,37,44) whereas TNFAIP3 variants associated with JIA were protective (30). Such a difference may be explained by opposite functions of these proteins in regulating TNF-dependent signaling (43). TRAF1 forms a heterodimeric complex with TRAF2 that mediates TNF-dependent activation of MAPK8/JNK and stimulates translocation of the nuclear factor (NF)-kB to the nucleus, where it induces expression of multiple pro-inflammatory and anti-apoptotic genes (45). Indeed, overactivity of TRAF1 should enhance pro-inflammatory signaling. By contrast, TNFAIP3 possess anti-inflammatory and anti-apoptotic properties, as a negative regulator of NF-kB signaling through ubiquitin modifications of adaptor proteins downstream of TNF and Toll-like receptors (46). Therefore, decreased expression levels or lowered activity of TNFAIP3 may promote TNF-mediated inflammation. In addition, the FAS gene, whose variants are associated with both RA (47) and JIA (32), belongs to the TNF-receptor superfamily and is critical for TNF-induced apoptosis (48). Thus, activation of TNF-dependent signaling observed in the synovium of JIA patients should substantially contribute to joint inflammation (43).

Notably, almost all loci shared between JIA and RA showed concordance in their effects on susceptibility to rheumatic disease. For example, IL2RA, PTPN2, and PTPN22 variants were found to predispose to both diseases while ANKRD55, IL2, and CD247 play a protective role (30).

3.3 HLA contribution

HLA variants only explain part of the genetic susceptibility to JIA. It has been estimated that HLA-DR accounts for only about 17% of the genetic burden of JIA, which suggests that other variants within and outside the Major Histocompatibility Complex (MHC) play a role in susceptibility (28,49). HLA variants differ according to the various categories of JIA: OligoJIA has been shown to be associated with HLA-A2, DR5 and DR8, whereas DRB1*04, DRB1*07 and DQA1*03 are said to be protective (7,25,50). HLA-A2, DRB1*08, DQA1*04 and DPB1*03 are associated with RF-negative PolyJIA and DRB1*04, DQA1*03 and DQB1*03 with RF-positive PolyJIA. RF-positive PolyJIA is also associated with HLA-DR4, DR1 and DR14, whereas DQA1*02 is protective (7,25,50). HLA associations for OligoJIA and RF-negative PolyJIA overlap, suggesting that these are genetically related. RF-positive PolyJIA appears to be a genetically distinct disorder and has HLA linkages similar to adult rheumatoid arthritis (7). Moreover, HLA DRB1*11/12 have been associated with PsA, whereas ERA is associated with HLAB27 (31,51).

Interestingly, there is an apparent lack of association between SoJIA and HLA, with the possible exception of HLADRB1* 04, which has been weakly associated with SoJIA in some studies (33,52). There has been only one reproducible association with SoJIA (rs1800795 in the promoter of IL-6), although this does not reach genome-wide significance levels (53). Other cytokine genes have also been described to be associated with SoJIA, but not yet replicated in other populations (33). These findings, in addition to the clinical features, suggest that the genetic background of SoJIA may differ substantially from OligoJIA and PolyJIA (PolyJIA). Indeed, there is a growing body of evidence to suggest that SoJIA should be considered separately from other JIA subgroups (54). Recent recognition of a group of systemic inflammatory illnesses that are largely genetically determined and characterized by unprovoked episodes of inflammation (the autoinflammatory syndromes) (33,54) has led many clinicians and researchers in JIA to reconsider whether SoJIA fits better within this group rather than JIA on clinical and genetic grounds. More recently, gene expression profiling in active JIA showed striking differences between subtypes, with SoJIA being the most distinct (55).

3.4 Non-HLA genes

Many non-HLA susceptibility genes have also now shown to be linked with subtypes of JIA and the list of putative markers has been expanding over the years. A systematic review of the literature reveals that about 100 different non-HLA candidate loci have been investigated for the association with JIA in different cohorts (56), and, overall, there are now more than 25 regions represented by SNPs that show strong genetic associations (57). The causal variants and corresponding functions have not yet been defined for the majority of these regions. Although many of such associations have been suggested, these have not been subsequently replicated in follow-up studies in different populations (26,39,41,42,58–60).

Most of the non-HLA loci belong to immune-related genes. Thompson et al (26) in a landmark study, examined a cohort of 809 JIA cases of non-Hispanic European ancestry and reported that PTPN2, COG6 and ANGPT1 were associated with OligoJIA and RF-negative PolyJIA. In a subsequent study published in 2012, Thompson et al (28) reported a new susceptibility locus at chromosome region 3q13 in their cohort of 814 JIA patients among Caucasians. This cohort consisted predominantly of OligoJIA and RF-negative PolyJIA. Novel associations were established at 3q13 within C3orf1 and near rs4688011 regions with GWAS analysis. A new locus at 10q21 near rs647989 region was reported to be associated with JIA. However, the investigators did not analyze the two subtypes (i.e., OligoJIA and RF-negative PolyJIA) separately, probably due to lack of sufficient sample size. Behrens et al (44) and Hinks et al (30) reported an association of TRAF1/C5 and VTCN1 with JIA by GWAS. However, these studies lacked power and no replication studies have been performed.

A consortium to investigate shared loci identified in GWAS across immune mediated chronic inflammatory disorders developed a custom genotyping array called the Immunochip (61). The Immunochip has almost 200 000 SNPs, including dense coverage of the MHC region, and approximately 180 loci with strong statistical evidence of association with one or more of 12 autoimmune diseases (61) and can be done at a fraction of the cost of a genome-wide SNPs array (57).

For JIA, an international consortium has collaborated to maximize sample size, which included 2,816 cases with OligoJIA or RF-negative PolyJIA and 13,056 controls (32). According to the results of this Immunochip array study, a total of 16 non-HLA immune-related loci contribute to the predisposition to Oligo and PolyJIA, the two commonest JIA subtypes. In fact, association of seven loci such as PTPN22, PTPN2, IL2RA, STAT4, IL2–IL21, ANKRD55, and SH2B3–ATXN2 was confirmed in this study (32,43). As stated, there was supporting evidence of their role in JIA susceptibility from previous studies (26,30,37–40,42,58,62). The remaining nine immune-related loci need to be confirmed in independent JIA cohorts.

The presence of IL2-IL21, IL2RA, and IL2RB loci encoding IL-2 itself and two subunits of the IL-2 receptor among JIA susceptibility genes may indicate for a key role of disturbances in IL-2-mediated signaling for JIA pathogenesis. IL-2 is crucial for growth and function of T lymphocytes, especially CD4+CD25+ regulatory T cells (63,64).

The involvement of PTPN22, PTPN2, TYK2, and SH2B3 variants in conferring susceptibility to JIA underlines significance of intracellular protein phosphatases and protein kinases, which suppress T cell receptor and pro-inflammatory cytokine signaling in JIA etiology (43). Importantly, functionally relevant non-synonymous amino acid changes such as R620W (rs2467701) in PTPN22, W262R (rs3184504) in SH2B3, and P1104A (rs34536443) in TYK2 may represent a likely etiological variant responsible for susceptibility to JIA in a corresponding genomic region (43). The functional significance of PTPN22 R620W and SH2B3 W262R is well characterized in a number of organ-specific immune mediated chronic inflammatory conditions (65–69). The role of the rare P1104A variant of TYK2 has been recently revealed, with a hypomorphic state of mutated enzyme (e.g. having one normal and one mutant subunit) capable of impairing (overactivating) the downstream signaling in a cytokine-dependent manner (43,70).

As previously mentioned, the JIA Immunochip consortium has demonstrated the benefits of an international collaboration by successfully identifying a large number of variants predisposing to the most common forms of JIA (61).

In conclusion, genetic research has given an unprecedented contribution to better understanding the physiopathology of JIA. Nevertheless, it has been hypothesized that these genetic findings only explain a portion of disease susceptibility. Using a variance component liability model, Thompson et al (28) estimated that common SNPs variation accounts for approximately one third of JIA susceptibility. The genetic associations of disease subsets in JIA still needs to be clearly defined by meta-analysis of comprehensive genome-wide association studies involving all ethnicities across the globe. In fact, isolated smaller studies in different populations bring out confirmatory results that are of outmost relevance for validating initial observations and reinforce the hypothetical physiopathology key role of that particular gene.

4. PROGNOSIS

4.1 Disease activity in JIA: the Juvenile Arthritis Disease Activity Score (JADAS)

As stated in 1883 by Lord Kelvin, "when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot, your knowledge is of a meagre and unsatisfactory kind" (Thompson W. electrical units of measurement. Popular lectures, vol 1,1883). The evaluation of disease activity is a crucial component of the clinical assessment of children with JIA because persistently active disease plays a major role in causing joint damage and physical disability, consisting in the major driver of long-term prognosis (71).

A variety of clinical measures are available for the assessment of disease status of children with JIA in clinical trials, clinical care and observational studies (72). The primary outcome measure for the assessment of response to therapy in JIA clinical trials is represented by the so-called American College of Rheumatology (ACR) Pediatric 30 criteria (73). The ACR Pedi response criteria are defined relative to each patient's baseline parameters but do not enable the quantification of absolute disease activity or comparison of absolute responses

amongst patients. For instance, these response criteria cannot distinguish between a patient who has improved by 30% starting with 30 active joints (still has 21 active joints) and one who started with 3 active joints (still has 2 active joints)(74). This limitation is relevant in the light of the recent advances in the management of JIA, which have moved the therapeutic goals increasingly towards the attainment of a state of inactive disease or, at least, of low disease activity (75–79). In recent years, several measures of disease activity state in JIA have been developed, with special emphasis on the criteria of inactive disease and clinical remission for JIA (75,78).

In 2009, the first composite disease activity score for JIA, named Juvenile Arthritis Disease Activity Score (JADAS), was published (71). This tool includes the following 4 variables: 1) physician global assessment of disease activity; 2) parent/patient global assessment of well-being; 3) count of joints with active arthritis, assessed in 71 (JADAS71), 27 (JADAS27), or 10 (JADAS10) joints; and 4) erythrocyte sedimentation rate (ESR), normalized to a 0–10 scale. The JADAS is calculated as the arithmetic sum of the scores of its 4 components, which yields a global score of 0–101, 0–57, and 0–40 for JADAS71, JADAS27, and JADAS10, respectively. The clinical measures included in JADAS are part of the ACR pediatric core set of outcome variables (73). A major advantage of JADAS when compared to ACR pediatric measures of improvement criteria is the ability to assess disease activity at a single visit and also to compare disease activity between individuals or groups; in other words, to provide clinicians and researchers with status and change scores. Nevertheless there are no perfect instruments and the major caveat of JADAS is that systemic features are not contemplated, limiting its use in systemic JIA.

The clinical JADAS (cJADAS) refers to the JADAS (71/27/10) without the fourth variable (ESR or C-reactive protein (CRP)). It is a relevant concept as frequently patients are not being evaluated with a recent ESR or CRP and in retrospective data retrieval from clinical records or in databases these are frequent missing values. However, full validation of the correlations between the cJADAS and JADAS is still lacking.

Due to the need for identifying different states of JIA activity and to provide simple and intuitive reference values that could be used to monitor the disease course over time in an

individual patient or to compare disease status across individual patients or patient groups (80), JADAS criteria (i.e., cutoff values) for JIA disease states have been recently developed (81) (**Table 2**). These criteria are ideally suited to implement a treat-to-target strategy and aim at achieving and maintaining tight disease control, with treatment escalation if a target score was not reached or is lost (81).

Table 2. JADAS and cJADAS cut-off values for JIA disease states

DISEASE STATE	OLIGOARTHRITIS	POLYARTHRITIS
JADAS		
Inactive disease	≤ 1	≤ 1
Physician-assessed remission	≤ 2	≤2
Parent-assessed remission	≤2.3	≤2.3
Child-assessed remission	≤2.2	≤2.2
Minimal disease activity	≤2	≤3.8
Parent acceptable symptom state	≤ 3.2/3.5*	≤ 5.2/5.4*
Child acceptable symptom state	≤ 3	≤ 4.3/4.5*
High disease activity	> 4.2	> 8.5/10.5*
cJADAS**		
Low disease activity	≤ 1.5	≤2.5
Moderate disease activity	1.51-4	2.51-8.5
High disease activity	>4	>8.5

Cut-off values apply to all versions of the Juvenile Arthritis Disease Activity Score (JADAS) versions, unless otherwise indicated. *Cut-off value for JADAS27/cut-off value for JADAS10 and JADAS71; ** Cut-off values for non-systemic JIA using the clinical Juvenile Arthritis Disease Activity Score (cJADAS).

4.2 Damage in JIA: the Juvenile Arthritis Damage Index (JADI)

Damage in JIA may be related to prolonged synovial inflammation, which may lead to permanent alterations in joint structures. Permanent changes may also develop in extra-articular organ/systems (*e.g.* the eye, as a result of uncontrolled iridocyclitis) or result from adverse effects of medications (72).

In the majority of the studies published in the last decade, the long-term morbidity in JIA has been most frequently evaluated in terms of functional disability. The most widely used tool

for assessment of functional status is the Childhood Health Assessment Questionnaire (CHAQ) (82,83). However, despite its advantages and widespread use, the CHAQ has been shown to have specific limitations in research and clinical settings. First, it has been demonstrated to have a ceiling effect, with a tendency for scores to cluster at the normal end of the scale, particularly in patients with fewer joints involved (84–86). Second, its estimation of physical disability in patients with active disease can be inflated by symptoms of inflammation, particularly joint pain (87,88). Third, the parent's observation of the child's physical function has been found to be frequently inaccurate, being affected by both the severity of arthritis and the level of pain (89). Finally, the CHAQ may not capture information on several possible forms of damage that may develop in JIA patients over time, such as micrognathia, height retardation, localized growth disturbances, pubertal delay, or visceral organ failure (84).

The lack of a clinical instrument that encompasses all forms of damage that may accumulate in patients with JIA over time prompted the development of the Juvenile Arthritis Damage Index (JADI) (84). This instrument comprises two parts: one devoted to the assessment of articular damage (JADI-A) and the other devoted to the assessment of extra-articular damage (JADI-E). In the JADI-A, 36 joints or joint groups are assessed for the presence of damage and the damage observed in each joint is scored on a three-point scale (0 = no damage; 1 = partial damage; 2 = severe damage, ankylosis, or prosthesis). The maximum total score is 72. The JADI-E includes 13 items in five different organs/systems. Each item is scored as 0 or 1 if damage is absent or present, respectively. Due to the relevant impact of ocular damage on the child's health, in each eye a score of 2 is given in case the patient has had ocular surgery and a score of 3 in case the patient has developed legal blindness. The maximum total score is 17.

Another important method for the assessment of disease severity and course is represented by the evaluation of radiographic joint damage and its progression. In recent years, there has been a great deal of effort to devise new radiographic scoring systems or validate existing methods for use in JIA. Some of these measures have undergone a thorough validation process and have proved to be valid and reliable for the assessment of radiographic

progression in children with chronic arthritis (90). However, given the nature of the disease full radiological characterization of a patient in a prospective follow up raises safety issues in children limiting the practical use of these scores.

4.3 Predictors of prognosis

Despite significant improvements in the management of children with JIA, for many, the likelihood of long-term disease activity remains high (91). Therefore it is essential to know the prognosis for the individual patient early in the course of the disease and preferentially at the time of diagnosis in order to immediately start the most appropriate treatment. Furthermore, patients and their parents not only want to know what kind of disease JIA is in general, but especially how it will affect their personal lives and prospects, for which, too, it is crucial to know the individual prognosis.

Much effort has already been done to elucidate clinical predictors of prognosis. Published evidence demonstrates that clinical subtype, disease activity and duration, and response to treatment, all of them influence the prognosis (92–98). In outcome studies using a variety of criteria for remission, an overall remission rate of 40% has been reported (99). The highest remission rate was consistently observed in persistent OligoJIA compared to extended OligoJIA and RF-negative PolyJIA (100–102). The percentage of active disease in the first years is not only predictive over the course of disease in the following years (100,103) but a prolonged disease activity is also related to joint damage or functional impairment (104). Therefore, the aim of the treatment is to control disease activity and prevent damage.

In a recent review by Dijkhuizen EHP et al (97), the authors conclude that there is considerable variability among prognostic studies, partly as a result of a lack of standardized criteria, making it harder to draw consistent conclusions. Overall, demographic, clinical and laboratory values are insufficient as early predictors for long-term outcome.

In addition to clinical factors, it is clinically important to understand the genetic determinants of disease severity and long-term outcomes. Genetic markers would be ideal as predictive factors, already present at disease onset and not influenced by disease activity or

medication. However, the vast majority of genetic research in JIA to date has aimed to identify variants that affect the risk of developing JIA (susceptibility) or pathways modulating drug response (pharmacogenetics); studies that evaluate prognosis hardly exist and the few published studies of genetic predictors of outcome were performed with small sample sizes and have yet to be replicated independently. A GWAS in a large cohort would be an ideal approach to look for genetic associations with various key long-term outcomes such as pain and disability.

In 2005, Oen K et al (105) had advanced the correlation of the IL6 genotype with pain and the possible association of the TGF-b1 codon 25 genotype with short-term radiographic damage (G/C with greater risk and G/G with decreased risk), suggesting that both these polymorphisms might be useful early prognostic indicators.

Some other examples of genetic research into JIA outcomes include a study in the ERA subtype that found that the presence of HLA-DRB1*08 predicts failure to attain disease remission (106), a study of 272 children with JIA that found that VTCN1 SNP rs10923223 and JIA subtype were the strongest independent predictors of disease course (99), and a recent work by Scardapane A et al (107), which showed that in a sample of 74 patients including all JIA subtypes, those carrying the TNF-α -308 GA/AA and -238 GA genotypes were associated with a worse prognosis and a lower response to anti-TNF drugs.

Genetic polymorphisms also appear to influence the outcome in SoJIA, as illustrated by the work done by Benedetti et al (108) who showed that a polymorphism in the macrophage migration inhibitory factor gene (i.e. MIF 173*C allele) was a poor prognostic marker in SoJIA.

Currently, international JIA outcome studies (*CAPS* in UK (109), *ReACCh-Out* in Canada (110), *CLARITY* in Australia (111)) are ongoing and will be vital resources enabling us to answer some very interesting questions, not only from a genetic perspective, but also incorporating general epidemiological data on disease presentation and course, treatment patterns and psychological aspects of the disease.

5. MANAGEMENT

5.1 General Treatment Aspects

Management of JIA is based on a combination of pharmacological interventions, physical and occupational therapy, and psychosocial support (9,112–114).

Although there are still no drugs that are able to cure the disease, prognosis has greatly improved, when compared to even a decade ago, because of substantial advance in disease management. The aim of treatment is to reach complete control of the disease, to preserve the physical and psychological integrity of the child and to prevent any long-term consequence related to the disease or its therapy. These aims need a careful long-term follow-up, in which monitoring treatment, disease activity, and disease damage is crucial. Since JIA is not a single disease, treatment approach varies across subtypes. However, as previously mentioned, a rational therapeutic approach is often not clear. Which children will enter remission and which children will go on having unremitting disease with substantial risk of joint destruction and permanent disability is unknown at disease onset (9).

Non-steroidal anti-inflammatory drugs (NSAIDs) have been the mainstay treatment for JIA for decades. Their role remains important and most children with juvenile idiopathic arthritis are started on an NSAID. Just a few NSAIDs are approved for use in children—the most commonly used include naproxen, ibuprofen, and indometacin. They are usually quite well tolerated and side-effects are less common than in adults. Experience with cyclo-oxygenase 2 (COX2) inhibitors in children is scarce (115). Meloxicam, an inhibitor of both COX1 and COX2, has proven to be effective and safe in children (116).

Intra-articular steroid injections with triamcinolone hexacetonide are frequently needed at disease onset or during disease course. In monoarticular or oligoarticular arthritis they could be used with, or substituted for, NSAIDs. They are rapidly effective and, most importantly, they break the vicious circle that leads to deformities secondary to contractures. Ultrasound guided injections are more accurate and can be very useful for joints with difficult access.

Patients whose disease is not well controlled by these approaches and an appropriate program of physical therapy are candidates to receive more aggressive interventions. Moderate or high-dose systemic corticosteroid therapy should be reserved for patients with SoJIA whose disease is not controlled by NSAIDs. In subtypes of JIA other than the systemic subtype, corticosteroids should be used very selectively because their potential toxic effects, including growth arrest or retardation, might outweigh any benefits to articular disease.

Methotrexate (MTX) has become the second-line agent of choice for persistent, active arthritis because of its effectiveness and acceptable toxic effects (117,118). In an attempt to determine in which subtype of JIA MTX is more effective, investigators from UK undertook a randomized controlled trial (RCT) and concluded that MTX produced significant improvement in patients with extended OligoJIA, but was much less effective in patients with SoJIA (119). MTX is sometimes used in ERA, but there are no consistent reports of its efficacy in this group of children (120). Moreover, anecdotal reports suggest that MTX is less effective in ERA than in other types of JIA (121). No RCTs have been conducted in PsA. In inflammatory bowel disease arthropathies MTX results in improvement of both GI and joint symptoms (122–124). Studies on the management of uveitis in children with JIA concluded that MTX was very effective (125,126).

Treatment with other DMARDs is not as well established as with MTX. Some studies have shown that sulfasalazine is able to improve arthritis in the late-onset OligoJIA and in patients with ERA (127,128). It is also commonly used in arthritis associated with inflammatory bowel disease. A trial has shown the efficacy of leflunomide in PolyJIA (129,130) but experience with this drug in children is still scarce.

In approximately 40% of the patients, a complete treatment efficiency cannot be provided with long-acting drugs (131). At this point, biological drugs which have been used widely in the last 10 years and shown to be efficient come into question.

In 2007 were published the Portuguese recommendations for the use of biologics in JIA aiming to improve the medical practice and guarantee their safest and most effective use in children and adolescents (132). In 2011 they were revised and updated (133) and, at this

time, they are being updated again. Patients are eligible for biological agents if presents 5 or more active joints on two separate occasions at least 3 months apart, despite standard treatment with synthetic DMARDs. The decision to initiate a biologic earlier or in patients with fewer active joints, enthesitis or systemic manifestations should be made on an individual basis and taking into account prognostic features, functional status and drug side effects (133).

5.2 Biologics in JIA

The introduction of biological medications has provided a crucial therapeutic option for the treatment of patients with JIA who are resistant to conventional anti-rheumatic agents. Its use has been expanded from patients with moderate-to-severe PolyJIA and SoJIA to further JIA categories including extended OligoJIA, PsA, as well as ERA.

Biological treatments have transformed the outcome of JIA from severe joint damage with disability and prolonged active disease to normal joint function with early and sustained remission (134). It is expected that the timely introduction of biologics in the treatment of JIA will change dramatically the long-term prognosis of these patients. However, biological treatments are not devoid of adverse effects and thus identifying the appropriate subset of patients for early initiation of biological treatment is an important objective in the clinical care of these children.

TNF inhibitors clearly have the widest application in most categories, except for systemic JIA. TNF antagonists are less effective in children with SoJIA than in those with other categories (135,136) probably due to the unique pattern of cytokine abnormalities that characterizes systemic arthritis. Anti-IL1 and anti-IL6 have proven efficacy in the treatment of SoJIA (137,138).

In all JIA categories early initiation of aggressive treatment may take advantage of the window of opportunity, allowing for a rapid remission and thus altering the course of the disease.

There are currently five biologics licensed for the treatment of JIA: etanercept (after 2 years-old), abatacept (after 6 years-old), adalimumab (after 2 years-old), tocilizumab (after 2 years-old) and canakinumab (after 2 years-old). **Table 3** shows the current biologicals used in the treatment of JIA, including off label drugs.

Given the potential change of long-term prognosis that the judicious use of biological treatments might have on JIA patients, an adequate evaluation of the long-term clinical effectiveness and safety and retention rate of biological therapies is of outmost relevance. This can be achieved using national patient registries, such as the Portuguese registry of rheumatic diseases (Reuma.pt)(139).

Table 3. Biologicals currently used in the treatment of JIA.

CLASS	GENERIC NAME	MECHANISM
TNF inhibitor	Adalimumab	Full human monoclonal antibody against TNF
	Certolizumab pegol	Pegylated Fab' fragment of a humanized TNF monoclonal antibody
	Etanercept	Fusion protein of human TNF receptor to human IgG
	Golimumab*	Fully human monoclonal antibody against TNF
	Infliximab*	Chimeric monoclonal antibody against TNF
IL1-Blockade	Anakinra	Fully human recombinant IL-1 receptor antagonist
	Rilonacept*	IL-1 trap
	Canakinumab	Fully humanized anti-IL-1β monoclonal antibody
IL-6 Blockade	Tocilizumab	Humanized monoclonal IL-6 receptor antibody
CTLA-4	Abatacept	Costimulation blocker binding to CD80 and/or CD86
CD20	Rituximab*	Chimeric monoclonal antibody to CD20

TNF: tumor necrosis factor; IL-1: interleukine 1; IL-6: interleukine 6; CTLA-4: cytotoxic T lymphocyte antigen 4; CD: cluster of differentiation; * off label; # not available in Europe.

Adapted from Zhao Y and Wallace C. Judicious Use of Biologicals in Juvenile Idiopathic Arthritis. Curr Rheumatol Rep (2014) 16:454(140).

AIMS

The aims of this thesis were:

1. To validate the association between selected SNPs and susceptibility to JIA.
2. To identify genetic and clinical predictors of poor prognosis.
3. To analyse the long-term effectiveness, safety and retention rate of biologic therapies in a daily-life clinical setting of JIA.

RESULTS

The results presented and discussed in this thesis were published in the following scientific peer-reviewed journals:

PART I. Mourão AF, Caetano-Lopes J, Costa P, Canhão H, Santos MJ, Pinto P, Brito I, Nicola P, Cavaleiro J, Teles J, Sousa A, Melo Gomes JA, Branco JC, Teixeira da Costa J, Gome Pedro J, Viana de Queiroz M, Fonseca. Tumor necrosis factor-alpha -308 genotypes influence inflammatory activity and TNF-alpha serum levels in children with Juvenile Idiopathic Arthritis. *J* Rheumatol 2009; 36(4):837-42.

PART II. Mourão AF, Santos MJ, Mendonça S, Oliveira-Ramos F, Salgado M, Estanqueiro P, Melo-Gomes JA, Martins F, Lopes A, Bettencourt BF, Bruges-Armas J, Costa J, Furtado C, Figueira R, Brito I, Branco JC, Fonseca JE, Canhão H. Single nucleotide polymorphism in *PTPN2*, *PTPN22* and *ANGPT1* are associated with susceptibility to Juvenile Idiopathic Arthritis specific categories. (*Submitted*).

PART III. Mourão AF, Santos MJ, Mendonça S, Oliveira-Ramos F, Salgado M, Estanqueiro P, Melo-Gomes JA, Martins F, Bettencourt BF, Bruges-Armas J, Cost a J, Furtado C, Figueira R, Brito I, Branco JC, Fonseca JE, Canhão H. Genetic predictors of poor prognosis in Portuguese patients with juvenile idiopathic arthritis: data from Reuma.pt. J Immunol Res 2015;2015:706515.

PART IV. Mourão AF, Santos MJ, Melo-Gomes J, Martins FM, Costa JA, Ramos F, Brito I, Duarte C, Figueira R, Figueiredo G, Furtado C, Lopes A, Oliveira M, Rodrigues A, Salgado M, Sousa M, Branco JC, Fonseca JE, Canhão H. Using the Juvenile Arthritis Disease Activity Score based on erythrocyte sedimentation rate or C-reactive protein level: results from the Portuguese register. Arthritis Care Res (Hoboken) 2014 Apr; 66(4):585-91.

PART V. Mourão AF, Santos MJ, Melo Gomes JA, Martins FM, Mendonça SC, Oliveira Ramos F, Fernandes S, Salgado M, Guedes M, Carvalho S, Costa JA, Brito I, Duarte C, Furtado C, Lopes A, Rodrigues A, Sequeira G, Branco JC, Fonseca JE, Canhão H. Effectiveness and long-

term retention of anti-TNF treatment in juvenile and adult patients with Juvenile Idiopathic Arthritis: data from Reuma.pt. (*Accepted for publication in August 2015: Rheumatology*).

PART I

Tumour necrosis factor-alpha -308 genotypes influence inflammatory activity and TNF-alpha serum levels in children with Juvenile Idiopathic Arthritis

Tumor Necrosis Factor-α –308 Genotypes Influence Inflammatory Activity and TNF-α Serum Concentrations in Children with Juvenile Idiopathic Arthritis

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ABSTRACT. ***Objective.*** Considering the relevance of tumor necrosis factor-α (TNF-α) in the pathophysiology of juvenile idiopathic arthritis (JIA), it is likely that polymorphisms in its promoter area may be relevant in disease susceptibility and activity. We investigated if clinical measures of JIA activity and TNF-α serum concentrations were associated with TNF-α –308 genotypes.

Methods. Portuguese patients with JIA in 5 pediatric rheumatology centers were recruited consecutively, along with a control group of healthy subjects. Demographic and clinical data and blood samples were collected from each patient. DNA was extracted for analysis of TNF-α gene promoter polymorphisms at position –308 by restriction fragment-length polymorphism.

Results. One hundred fourteen patients and 117 controls were evaluated; 57% of patients presented the oligoarticular subtype, 25% the polyarticular subtype, 8% the systemic subtype, and 9% had enthesitis-related arthritis and 5% psoriatic arthritis. Twenty-four percent of the patients presented the –308 GA/AA genotypes and 76% the –308 GG genotype, similar to findings in controls. Patients with the –308 GA/AA genotype had higher degree of functional impairment, erythrocyte sedimentation rate, 100-mm visual analog scale score for disease activity, and TNF-α levels compared to those with the –308 GG genotype.

Conclusion. TNF-α –308 GA/AA genotypes were found to be related to higher inflammatory activity and worse measures of disease activity in Portuguese patients with JIA. They were not associated with susceptibility to JIA. (First Release Jan 15 2009; J Rheumatol 2009;36:837–42; doi:10.3899/jrheum.080615)

Key Indexing Terms:
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–308 GENOTYPES
PROGNOSIS

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Juvenile idiopathic arthritis (JIA) is the most common chronic arthritis in childhood; it comprises a heterogeneous group of syndromes, of which onset occurs before the age of 16 years, with a disease duration greater than 6 weeks[1]. The etiology of JIA remains unclear, but some genetic factors acting in concert are believed to predispose to development of the disease[2,3]. The best-defined genetic associations have been made with human leukocyte antigen (HLA) system genes[4-7]. Other molecules, such as interleukin 1 (IL-1), IL-6, IL-17, and tumor necrosis factor-α (TNF-α) have also been implicated in the etiopathogenesis of JIA[8-12].

TNF-α is a cytokine that plays an important role in inflammation, stimulating the production of many other proinflammatory cytokines. TNF-α is involved in the pathogenesis of JIA[11,13], and the level of this cytokine in the serum and synovial fluid of patients with JIA has been shown to vary with disease activity[14,15]. Moreover, some studies have shown that the production of TNF-α is influenced by polymorphisms in its gene promoter region. In particular, the G/A transition at position –308 appears to be crucial for the regulation of TNF-α translation[16-19]. Ozen, *et al*[20] assessed the TNF-α –308 and –238 polymorphisms in Czech and Turkish patients with JIA and suggested an association between the –308A allele and poor disease outcome in the Turkish group. Zeggini, *et al*[21] also found a positive association with TNF-α polymorphisms (positions –308A, –238G, +489A, +851A) in a large panel of UK Caucasian patients with oligoarticular JIA. In contrast, Modesto, *et al*[22] found no association of 4 TNF-α gene promoter polymorphisms (at positions –376, –308, –238, and –163) with oligoarticular and systemic JIA, in a group of Spanish Caucasian children.

JIA and rheumatoid arthritis (RA) are 2 distinct disease entities, although they share some clinical and pathogenetic factors as well as genetic background[23]. Of interest, the –308 TNF-α GA/AA genotypes have been shown to be correlated with disease activity and response to treatment in Portuguese patients with RA[24,25]. Therefore, our aim was to examine whether clinical, functional, and laboratory measures of JIA activity and serum levels of TNF-α were associated with TNF-α gene promoter polymorphisms at position –308.

MATERIALS AND METHODS

Patients. Patients with the diagnosis of JIA according to the Durban criteria[1] followed in 4 hospitals (Santa Maria Hospital, Egas Moniz Hospital, Garcia de Orta Hospital, and São João Hospital) and one author's private clinic in Portugal were recruited consecutively from March 2005 to May 2007. Patients who did not fulfil all the criteria were excluded. Written informed consent was obtained from all parents and also from patients who were older than 12 years of age. Research was carried out in compliance with the Declaration of Helsinki, and all the ethics committees of the participating hospitals and clinic approved the study.

For each patient, a data collection protocol was applied to evaluate age, sex, weight, height, ethnic origin, disease and followup duration, history of uveitis, family history of rheumatic diseases, number of joints with active disease and/or limited range of motion, visual analog scale (VAS; 100 mm) score for disease activity, patient's functional status, as assessed by the Portuguese version of the Childhood Health Assessment Questionnaire (CHAQ)[26], use of steroids and antiinflammatory drugs, and presence of serum rheumatoid factor (RF) and antinuclear antibodies (ANA). Patients were classified, at 6 months after diagnosis, into one of 7 disease categories, each with its own specific characteristics, as follows: oligoarticular (affecting up to 4 joints during the first 6 months of disease), which can be subdivided into persistent oligoarthritis (up to 4 joints even after the first 6 months of disease) or extended oligoarthritis (affecting ≥ 5 joints after the first 6 months); polyarticular (affecting ≥ 5 joints during the first 6 months of disease), which can be subdivided as RF-positive or RF-negative polyarthritis; and systemic arthritis was defined as arthritis coincident with or preceded by daily fever for at least 2 weeks of duration, accompanied by one or more manifestations (including transient evanescent rash, lymphadenopathy, hepatomegaly or splenomegaly, and serositis). The 2 other JIA categories considered were enthesitis-related arthritis and psoriatic arthritis. Patients whose diagnosis was established in the previous 6 months of protocol application were considered as presenting zero years of disease duration. We also included young adults with active JIA (whose disease was diagnosed before age 16 years).

A blood sample was collected from each patient for determination of erythrocyte sedimentation rate (ESR) and serum TNF-α levels. In patients receiving TNF-α antagonists (etanercept) the blood sample was collected under this therapy. DNA was extracted from blood to determine the genotype at position –308 of the TNF-α gene by restriction fragment-length polymorphism (RFLP). The same polymorphism was also assessed in a sample of healthy controls from the same geographic and ethnic origin as the patients.

DNA analysis and TNF-α assessment. Blood was collected in EDTA-containing tubes and DNA extraction was performed using the QIAmp DNA Blood Mini kit (Qiagen, Hilden, Germany), according to the manufacturer's recommendations.

TNF-α gene –308 polymorphisms (G/A) were analyzed by RFLP using the forward primer 5'-AAT AGG TTT TGA GGG CCA TG-3' and the reverse primer 5'-ATC TGG AGG AAG CGG TAG TG-3'. The forward primer contained one nucleotide mismatch (underlined above), which allowed use of the restriction enzyme *NcoI* (New England Biolabs, Hitchin, UK) for detection of –308G/A polymorphisms. Polymerase chain reaction was performed in a 50 µl reaction mixture containing 100 ng genomic DNA, 40 nM of each primer, 0.2 mM of each dNTP, 15 mM of $MgCl_2$, and 0.4 U of *Taq* DNA polymerase (ABgene, Epson, UK). The reaction mixture was incubated 3 min at 95°C, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 58°C for 45 s, and extension at 72°C for 60 s. Digestion with *NcoI* was performed at 37°C, as described by the manufacturer.

The TNF-α concentration was determined in duplicate in serum samples from patients using the DuoSet ELISA kit (R&D Systems, Minneapolis, MN, USA), as recommended by the manufacturer.

Statistical analysis. Continuous variables were described as mean ± standard deviation with a normal distribution, or median (interquartile range) if otherwise. The Wilcoxon rank-sum test and Pearson's chi-square test were used to compare continuous or categorical variables, respectively, among genotype groups. As only one subject was found with the AA genotype, this was grouped with the GA genotype (GA/AA) for statistical analysis. Excluding the subject with AA genotype did not change the conclusions. Statistical tests were considered significant when p values were ≤ 0.05. Statistical analysis was performed using R software[27].

RESULTS

One hundred fourteen Portuguese Caucasian patients with JIA were evaluated, 68% were girls, with a mean age of 12.7 ± 6.5 years. The mean body mass index was 19.0 ± 3.2 kg/m^2. The median disease duration was 4 years (range

0–28; zero years in 4 patients whose diagnosis was established in the 6 months before the protocol and 28 years in a 42-year-old patient with symptoms since age 14 years) and the median followup period was 3 years (range 0–28). One hundred seventeen healthy subjects were used as controls. The frequency of –308 TNF-α genotype was similar between patients with JIA and controls (patients 24% GA/AA and 76% GG vs controls 22% GA/AA and 78% GG; p = 0.876; Table 1).

Thirty-one patients (27%) had a family member with a rheumatic disease (RA, rheumatic fever, psoriasis, JIA, or ankylosing spondylitis). Forty-one patients (36%) had been treated with oral steroids, 89 (78%) with nonsteroidal anti-inflammatory drugs, 65 (59%) with methotrexate, and 15 (13%) with etanercept.

Sixty-five (57%) patients presented the oligoarticular disease subtype [48 (42%) had the oligoarticular persistent pattern and 17 (15%) developed the oligoarticular extended form], 24 (21%) the polyarticular subtype, 10 (9%) enthesitis-related arthritis, 9 (8%) systemic arthritis subtype, and 6 (5%) psoriatic arthritis subtype.

ANA were detected in 39% of patients with JIA (11 missing results). The –308 genotype frequencies were similar between patients with ANA-positive JIA and ANA-negative JIA (16.0% GA/AA and 84.0% GG vs 24.5% GA/AA and 75.5% GG, respectively). The frequency of ANA was particularly high in the group of patients with the oligoarticular subtype: 49% of tested individuals had this autoantibody in the serum. A lower frequency was observed in the other subtypes: 26.3% in the polyarticular, 20.0% in enthesitis-related arthritis, 14.3% in the systemic, and 25.0% in the psoriatic.

In addition, among patients with the oligoarticular subtype, uveitis occurred in 12 (19%) patients and ANA were detected in 5 (42%) of these patients.

Forty-five percent of patients with polyarticular JIA and 9% of patients with the oligoarticular subtype were RF-positive (5 missing results). No RF was detected in serum of patients with systemic, psoriatic, or enthesitis-related arthritis.

Patients with the oligoarticular persistent subtype presented a trend for a higher frequency of –308 GG genotype (90%) as compared to other JIA subtypes and controls (p = 0.080). In contrast, a trend for a higher frequency of the –308 GA/AA genotype was present in patients with the polyarticular (38%; p = 0.123) and the psoriatic (50%) subtypes (p = 0.141). We observed that the distribution of genotype frequencies of oligoarticular subtype patients (oligoarticular persistent and extended) was strongly associated with the GG genotype, compared with "non-oligoarticular" subtypes (polyarticular, enthesitis-related arthritis, systemic, and psoriatic arthritis; p = 0.007; Figure 1).

We also found that patients with the –308 GA/AA polymorphism had significantly higher ESR (28.4 ± 24.2 vs 15.0 ± 11.7 mm/h, respectively; p = 0.007) and TNF-α levels (215.1 ± 176.0 vs 96.2 ± 124.2 pg/ml; p = 0.003), and a trend to a higher degree of functional impairment, as evaluated by CHAQ (0.46 ± 0.66 vs 0.27 ± 0.52; p = 0.243) and disease activity VAS (22.3 ± 26.0 vs 13.7 ± 20.3 mm; p = 0.166; Figure 2).

Among the 17 patients treated with etanercept, 87% had –308 GG genotype and 13% were –308 GA/AA (not statistically different from the genotype frequency in other patients and controls). Serum TNF-α levels in this group

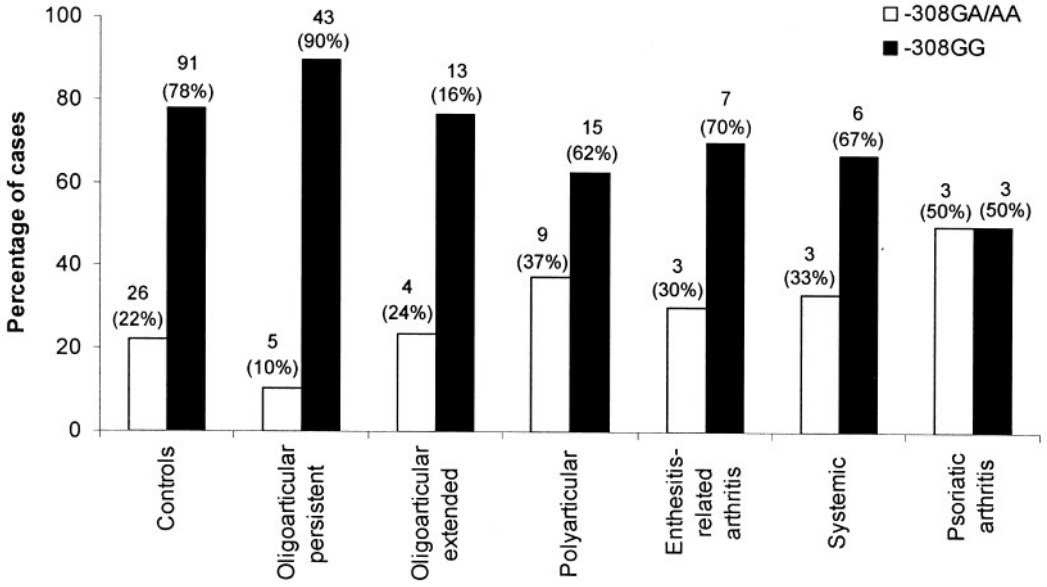

Figure 1. TNF-α promoter –308 genotype frequency in JIA subgroups and controls. No statistically significant differences between patients and controls were observed for –308 genotype (p = 0.876). The oligoarticular persistent subtype presented the highest proportion of GG (90%), while GA/AA was significantly increased in the polyarticular (38%) and psoriatic arthritis (50%) groups.

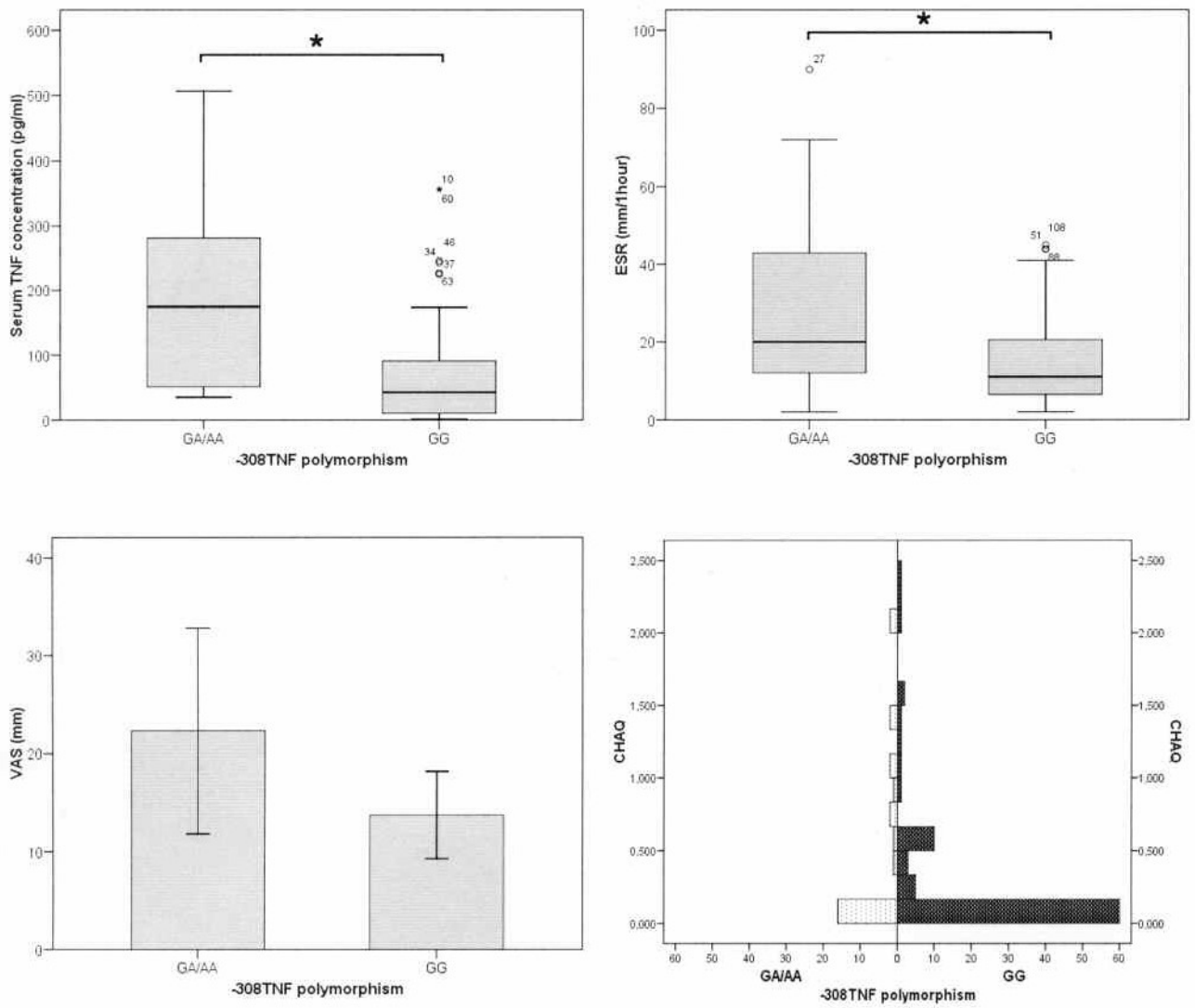

Figure 2. –308 GA/AA was shown to be strongly associated with inflammatory and disease activity indicators. We observed that erythrocyte sedimentation rate (ESR; p = 0.007) and serum TNF-α levels (p = 0.003) were significantly higher in subjects with –308 GA/AA genotype. CHAQ (p = 0.243) and visual analog scale (VAS) scores for disease activity (p = 0.166) were higher among subjects with –308 GA/AA (not statistically significant).

were not significantly different from the rest of the study population (114.1 ± 138.5 vs 139.0 ± 200.4 pg/ml; p = 0.487).

DISCUSSION

We found that TNF-α –308 genotype frequencies were similar between patients with JIA and controls. Compared to patients with the other JIA subtypes, the oligoarticular subtype (including persistent and extended forms) presented a higher frequency of the GG genotype. Patients with the polyarticular and psoriatic JIA subtypes presented a trend for a higher frequency of –308 GA/AA genotype compared to other subtypes and controls. The presence of the –308A allele was associated with higher level of inflammatory activity, revealed by higher ESR values and serum TNF-α levels ($p < 0.05$), and also with a trend for a lower functional capacity and higher disease activity VAS values, although these were not statistically significant. In a previous study our group focused on Portuguese patients with RA[25], and we found a positive association between position –308 of the TNF-α gene promoter and work disability and radiologic progression. Our current results are coherent with the data we obtained in that study and reinforce the relevance of –308 polymorphisms in arthritis activity and severity in the Portuguese population.

Fifteen percent of the patients (17 patients) were treated with the TNF-α blocker etanercept. Among these patients, we found no difference in genotype frequency compared to other patients and controls, suggesting that –308 polymorphisms of TNF-α gene do not influence the selection for treatment with biological agents. Serum samples were collected in patients undergoing this therapy, which might have

caused underestimation of the total serum TNF-α concentration. Data on the influence of the polymorphism at position –308 on clinical response to TNF-α antagonists are controversial. In a study from a French group[28] performed in 59 patients with RA, those carrying the rare A allele were twice as likely to have no response to infliximab compared to those with the GG genotype of the –308 polymorphism. In another study[24], Portuguese RA patients with the –308 GA/AA genotypes presented a worse clinical response to anti-TNF-α therapies and a trend for worse HAQ result. In contrast, Marotte, *et al*[29] did not observe any link between the TNF –238 and –308 polymorphisms and joint destruction or selection for infliximab treatment. Similarly, TNF genotypes had no effect on the clinical response to infliximab. Miceli-Richard, *et al*[30] also found no association between clinical response to another TNF-α blocker (adalimumab) and any of 3 TNF-α gene promoter polymorphisms (–238, –308, –857) tested individually.

We found an association between the –308 GA/AA polymorphisms and total serum TNF-α concentration. However, in a study from Marotte, *et al*[31] the –308A allele was associated with higher level of circulating TNF-α bioactivity, but not with protein levels, indicating that endogenous inhibitors must be taken into account. To date, there is no consensus regarding the functional significance of TNF-α gene polymorphisms, and there is no evidence that simple determination of plasma TNF levels allows such prediction[32].

In a study involving simplex families consisting of a parent and a child with JIA, as well as healthy individuals, Zeggini, *et al* reported that the –308A allele was associated with oligoarthritis in the whole group and the persistent and extended disease subsets separately[21]. In contrast, in a recent study with a cohort of 107 patients with JIA, Cimaz, *et al*[33] studied TNF-α and IL1 gene polymorphisms, including the TNF-α –308 polymorphism, and no relationship was detected between these polymorphisms and the disease phenotype or response to TNF inhibitors. In accord with that report, we found no association between TNF-α –308 G/A promoter polymorphisms and susceptibility to JIA. Nevertheless, the frequency of the –308 GG genotype was higher in the oligoarticular JIA subtype, compared to the other subtypes, and the –308A allele was associated with higher level of inflammatory activity. In agreement with our observation, in a study performed in Turkish and Czech patients, the –308A allele was significantly associated with a poor outcome in the Turkish group ($p = 0.005$), but there was no association in the Czech patients[20].

The relatively small number of patients in some of the disease subgroups may have significantly reduced the power of our study to detect potential differences in allele or genotype frequency between JIA subtypes. Our populations of patients and controls were both from Portugal, with a similar genetic background. However, we acknowledge the limitations of direct comparisons of existing studies due to different ethnic populations and systems of nomenclature and classification. The genetic component of JIA is complex, involving the effects of multiple genes at various points in the disease pathology[3]. TNF-α is a proinflammatory cytokine implicated in the etiopathogenesis of a broad range of diseases, including JIA. In our study, nearly one-third of the patients had a relative with a rheumatic disease, which reinforces the role of genetic factors in these diseases.

In conclusion, in our study population –308 TNF-α GA/AA genotypes were found to be associated with higher level of inflammatory activity and higher serum concentrations of TNF-α, and the –308 GG genotype was associated with the oligoarticular subtype of the disease. However, polymorphisms in the TNF-α –308 position do not appear to have a relevant role in susceptibility to JIA.

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PART II

Single nucleotide polymorphism in *PTPN2*, *PTPN22* and *ANGPT1* are associated with susceptibility to Juvenile Idiopathic Arthritis specific categories

TITLE

Single nucleotide polymorphism in *PTPN2*, *PTPN22* and *ANGPT1* are associated with susceptibility to Juvenile Idiopathic Arthritis specific categories.

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Each author listed on the manuscript has seen and approved the submission of this version of the manuscript and takes full responsibility for the manuscript.

Abbreviations: JIA: juvenile idiopathic arthritis; SNP: Single nucleotide polymorphism; DMARD: disease modifying anti-rheumatic drug; RF: rheumatoid factor; GWAS: genome wide association studies; HW: Hardy-Weinberg; OR: odds ratio.

Key-words: Susceptibility gene, juvenile idiopathic arthritis, single nucleotide polymorphisms.

Word count: 1504

ABSTRACT

Objectives: This study aimed to confirm whether 15 single nucleotide polymorphisms (SNPs) of selected genes are also associated with susceptibility for Juvenile idiopathic Arthritis (JIA) in the Portuguese population.

Methods: Our study was conducted on Reuma.pt, the Rheumatic Diseases Portuguese Register, which includes patients with JIA receiving biological therapies and synthetic Disease Modifying Anti Rheumatic Drugs (DMARDs) since June 2001. Fifteen SNPs were investigated using Taqman® SNP genotyping assays in 291 Portuguese patients with JIA and 300 ethnically matched healthy controls.

Results: Prior to Bonferroni correction for multiple testing, significant genotype association between one SNP and overall group of JIA was observed (*PTPN22* rs2476601). In subgroup analysis, associations between six SNPs and the subgroup of patients with rheumatoid factor (RF)-positive Polyarticular (*PTPN2* rs7234029), Extended oligoarticular (*PTPN22* rs2476601), Systemic (*PTPRC* rs10919563, *ANGPT1* rs7151781 and *TNF* rs361525) and Psoriatic JIA (*IL2RA/CD25* rs2104286) were found. After Bonferroni correction for multiple testing, 3 genotype associations remained significant in the subgroup of patients with RF-positive polyarticular JIA (*PTPN2* rs7234029 [corrected *P* 0.026]), extended oligoarticular (*PTPN22* rs2476601 [corrected *P* 0.026]) and systemic JIA (*ANGPT1* rs7151781 [corrected *P* 0.039]).

Conclusion: Our results provide additional evidence for an association between polymorphisms in genes *PTPN2*, *PTPN22* and *ANGPT1* and the risk of RF-positive polyarticular, extended oligoarticular and systemic JIA, respectively, in a Portuguese population.

INTRODUCTION

Although relatively rare, JIA is the most common of the childhood rheumatic diseases.[1] JIA has autoimmune and inflammatory features and appears complex in nature, with both environmental and multiple genetic risk components.[2,3] JIA may have a stronger genetic contribution compared to some adult onset disorders since children with JIA have had less time for environment and behavior to influence disease risk relative to adults.[4] Given the challenges associated with JIA genetics resulting from the relative rarity and clinical heterogeneity of the disease (there are currently seven ILAR subtypes),[5] researchers used varied strategies in an attempt to uncover the genetic basis of JIA susceptibility.[6] These include selecting genes based on expression profiling results,[7] those previously associated with other autoimmune diseases [8,9] and genome wide association studies (GWAS).[10,4] Using a variance component liability model, *Thompson et al* estimated that common SNP variation accounts for approximately one-third of JIA susceptibility.[4]

Polymorphisms in various genes associated with rheumatic diseases have been reported to vary substantially according to allele frequency in different ethnic groups [11,12] and thus, further ethnicity-specific association studies are required to confirm genetic associations with disease susceptibility in different populations.

This study aimed to confirm whether 15 single nucleotide polymorphisms (SNP) of selected genes, found in previous studies to be associated with an increased risk for the development of JIA, are also associated with susceptibility for JIA in the Portuguese population.

METHODS

Patient population

Our study was based on Reuma.pt, the Rheumatic Diseases Portuguese Register, which includes patients with JIA receiving biological therapies and synthetic Disease Modifying Anti Rheumatic Drugs (DMARDs) since June 2001. Patients registered up to December 2013 were included. The parent's consent and patient's assent (as appropriate) were obtained according to the declaration of Helsinki. The study was approved by local Ethics Committee. All patients fulfilled the ILAR criteria for the classification of JIA.[5] This study did not have any interference with the standard of care of JIA patients.

We analyzed the patients registered in Reuma.pt with the diagnosis of JIA, who had collected a blood sample for DNA analysis. For each patient, the disease characteristics protocol included gender, age at disease onset, disease duration, age at study visit and the category of JIA.

Three hundred unrelated healthy individuals, sex and ethnic-matched, selected from the Biobanco-IMM, Lisbon Academic Medical Center, Lisbon, Portugal, served as controls for the genomic typing. Genetic single nucleotide polymorphisms (SNP) were studied to verify if there was any association with the risk of JIA.

Genetic analysis

The choice of single nucleotide polymorphisms (SNP) variants was based in previous studies of susceptibility factors in JIA and included the following 15 SNPs of genes with a known function in the immune system: *PTPN22* rs2476601 A/G, *PTPRC* rs10919563 A/G, *TRAF1/C5* rs3761847 A/G, *ANGPT1* rs1010824 A/G, *ANGPT1* rs7151781 C/T, *AFF3* rs1160542 A/G,

AFF3 rs10865035 A/G, *CTLA4* rs3087243 A/G, *IL2-IL21* rs6822844 G/T, IL2RA/CD25 rs2104286 C/T, *PTPN2* rs1893217 A/G, *PTPN2* rs7234029 A/G, *STAT4* rs3821236 A/G, *STAT4* rs7574865 G/T, *TNF* rs361525 A/G.

All samples were genotyped using Taqman® SNP genotyping assays (Applied Biosystems, Foster City, USA) performed as described in the manufacturers' protocol. Genotyping reactions were carried out with an ABI 7500-fast thermocycler. The allele call was obtained by the AB software v2.0.5, by the analysis of allelic discrimination plots. SNPs with deviation from Hardy-Weinberg equilibrium ($p<0.05$) or minor allele frequency (MAF) <1% were excluded from further analysis.

Statistical analysis

First we assessed the Hardy-Weinberg (HW) equilibrium and identified the minor allele for each SNP in the control population.

Genotype and allele frequencies were calculated separately for cases and controls. Initially we compared the JIA group, as a whole, with the control group. Secondly, each JIA category was compared with controls. We used the seven ILAR disease categories [5]: polyarticular rheumatoid factor (RF) positive (n=26), polyarticular RF negative (n=49), persistent oligoarticular (n=96), extended oligoarticular (n=47), enthesis-related arthritis (ERA) (n=34), systemic (n=23) and psoriatic arthritis (n=16).

We used the additive model to study the association between SNPs and JIA susceptibility. Homozygotes for the major allele were classified as zero, heterozygotes as 1 and homozygotes

for the minor allele as 2. We report odds ratio (OR) as the association measure obtained from univariate logistic regression.

Statistical significance was considered at the 5% level. Bonferroni correction was applied based on 13 SNPs that passed the inclusion criteria.

Statistical analysis was made in R version 3.0.2.

RESULTS

Twenty-one centers and 77 rheumatologists and pediatricians contributed with data to Reuma.pt. Of the 812 patients children and adults with JIA registered in Reuma.pt (mean age 19.9 ±11.3 years, 65% females, mean age at JIA onset 6.9 ±4.7 years), 291 caucasian patients had a blood sample to perform the genetic analysis. The cohort characteristics are presented in **Table 1**.

Unrelated healthy controls (n = 300) consisted of 192 caucasian female and 109 caucasian male of similar age.

Table 1. Demographics and disease characteristics of JIA patients.

Characteristics	
Total JIA patients	291
Age, mean ± SD years	15.2±9.2
Gender (F(%))	187(64.3)
Age at disease onset, mean ± SD years	6.6±4.6
Disease duration, mean ± SD years	11.0±8.2
JIA category (%)	
RF-negative Polyarticular	49 (16.8)
RF-positive Polyarticular	26 (8.9)
Extended oligoarticular	47 (16.2)
Persistent oligoarticular	96 (33)
Systemic	23 (7.9)
Enthesitis-related arthritis	34 (11.7)
Psoriatic arthritis	16 (5.5)

Legend: JIA: Juvenile idiopathic arthritis; SD: standard deviation; RF: rheumatoid factor

Two SNPs exhibiting significant deviation from HW equilibrium in the control population were excluded (*AFF3* rs1160542 A/G and *AFF3* rs10865035 A/G) leaving 13 SNPs for further analysis.

The genotype and allele frequencies were analyzed in the overall group of controls and patients with JIA, as shown in **Table 2**.

Table 2. Characteristics of the SNP analysed.

Gene	SNP	Controls				Cases	
		N	Minor allele	MAF	HW p-value	N	MAF
PTPN22	rs2476601 A/G	300	A	6.3	0.8431	291	9.8
PTPRC	rs10919563 A/G	299	A	9	0.0713	291	10.3
TRAF1/C5	rs3761847 A/G	300	G	38.2	0.2501	291	40.5
ANGPT1	rs1010824 A/G	300	A	16.3	0.3989	291	14.3
ANGPT1	rs7151781 C/T	299	C	38.3	0.4859	291	39.5
IL2-IL21	rs6822844 G/T	300	T	11.3	0.2174	291	8.6
CTLA4	rs3087243 A/G	300	G	46.7	0.5362	291	52.4
IL2RA/CD25	rs2104286 C/T	300	C	19	0.4156	291	18.4
PTPN2	rs1893217 A/G	300	G	14.8	0.8552	291	16.3
PTPN2	rs7234029 A/G	299	G	13.5	0.2144	291	17.7
STAT4	rs3821236 A/G	299	A	18.4	0.2298	291	21.6
STAT4	rs7574865 G/T	300	T	22.2	0.8042	291	23
TNF	rs361525 A/G	298	A	2.7	0.6339	291	3.8

Legend: SNP: single nucleotide polymorphisms. MAF: minor allele frequency; HW: Hardy-Weinberg.

Because of the importance of investigating genetic risk factors in homogeneous, well-defined phenotypic groups, we also analysed genetic association within each category of JIA. The significant genetic associations of the selected SNP with JIA are shown in **Table 3**.

Table 3. Genetic associations of the SNP with JIA and JIA categories.

JIA categories	Gene	SNP	OR (95% CI)	p-value Bonferroni
Overall JIA patients	PTPN22	rs2476601 A/G	1.59 (1.04-2.43)	0.416
RF-positive Polyarticular	PTPN2	rs7234029 A/G	2.67 (1.43-4.99)	0.026
Extended oligoarticular	PTPN22	rs2476601 A/G	2.73 (1.43-5.21)	0.026
Systemic	PTPRC	rs10919563 A/G	2.45 (1.21-4.96)	0.169
	ANGPT1	rs7151781 C/T	2.62 (1.38-4.97)	0.039
	TNF	rs361525 A/G	3.71 (1.13-12.2)	0.403
Psoriatic arthritis	IL2RA/CD25	rs2104286 C/T	2.14 (1.02-4.5)	0.572

Legend: SNP: single nucleotide polymorphisms; JIA: juvenile idiopathic arthritis; OR: odds ratio; CI: confidence interval. Corrected *p-values* < 0.05 were considered statistically significant.

DISCUSSION

This study aimed to confirm whether 15 single nucleotide polymorphisms (SNP) of selected genes, found in previous studies to be associated with an increased risk for the development of JIA, are also associated with susceptibility for JIA in the Portuguese population. Our results reinforce the relationship between *PTPN2*, *PTPN22* and *ANGTP1* polymorphisms and specific JIA sub types in the Portuguese population.

JIA is phenotypically heterogeneous and the 7 categories may represent distinct clinical entities. Limited genetic research has been performed on the individual categories of JIA due to the inevitable reductions in sample size and the corresponding decrease in power to detect weaker genetic effects and issues regarding multiple testing. Our study shows an association between polymorphisms in *PTPN2* with RF-positive polyarticular JIA, polymorphisms in gene *PTPN22* with extended oligoarticular JIA and polymorphisms in gene *ANGPT1* with Systemic JIA susceptibility. These results support the genetic heterogeneity of JIA categories.

Thompson et al in a landmark study, examined a cohort of 809 JIA cases of non-Hispanic European ancestry and reported that 'PTPN2', 'COG6' and 'ANGPT1' were associated with oligoarticular and RF-negative polyarticular JIA.[13] In our study the same SNPs analyzed in *PTPN2* and *ANGPT1* genes were associated with RF-positive polyarticular and systemic JIA, respectively. Still in that study,[13] in accordance to other susceptibility studies on JIA,[14-16] the SNP in *PTPN22* gene (rs2476601 A/G) that we have found associated with the risk of extended oligoarticular JIA, was associated with oligoarticular and RF-negative polyarticular

JIA. Similarly, *Dimopoulou DG et al*,[12] demonstrated that the same *PTPN22* polymorphism was associated with JIA in a Greek population. These discrepancies of genetic associations across different racial or ethnic groups underlines the importance of assessing genetic variants in different populations, even within Europe, to conclusively define the genetic architecture of JIA and the magnitude of the effects of specific risk alleles in different populations.[12]

In our study, a SNP in *ANGPT1* gene was associated with systemic JIA. It is thought that systemic JIA is particularly different from the other subtypes, because of its clinical features typical of an autoinflammatory disease. In addition, it lacks a strong MHC association, shows cytokine disregulation and various innate immune system abnormalities.[17] It has already been seen that systemic JIA is associated with SNPs within genes such as *IL10*,[18] IL6 [6,19] and *SLC26A2*.[20] While our understanding of the genetic susceptibility to oligoarticular and RF-negative polyarticular JIA is rapidly improving due to recent focus and large, well-powered studies,[16] systemic JIA remains a relatively poorly understood subtype. However, this is being addressed in a large multi-national GWAS of systemic JIA.

A limitation of this study is the small sample size, which increases the possibility of type 2 statistical error. In studies like the one we herein present, where a search for genetic factors having a small effect on the risk is being made, the sample size is instrumental.[12] Thus, the failure to replicate the findings may reflect insufficient power. In addition, clinical heterogeneity of JIA may also contribute to this problem.[12]

There is still much work to be done until we have a comprehensive understanding of the genetic architecture of JIA. The coming years will hopefully provide insight into important pathways involved in disease, will identify genes implicated in outcomes such as disability and pain and also genetic predictors of response to treatments such as MTX.[3]

In conclusion, our results provide additional evidence for an association between polymorphisms in genes *PTPN2*, *PTPN22* and *ANGTP1* and the risk of RF-positive polyarticular, extended oligoarticular and systemic JIA, respectively, in a Portuguese population.

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PART III

Genetic predictors of poor prognosis in Portuguese patients with juvenile idiopathic arthritis: data from Reuma.pt

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Research Article

Genetic Predictors of Poor Prognosis in Portuguese Patients with Juvenile Idiopathic Arthritis: Data from Reuma.pt

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Introduction. This study aimed to assess the genetic determinants of poor outcome in Portuguese patients with juvenile idiopathic arthritis (JIA). *Methods.* Our study was conducted in Reuma.pt, the Rheumatic Diseases Portuguese Register, which includes patients with JIA. We collected prospectively patient and disease characteristics and a blood sample for DNA analysis. Poor prognosis was defined as CHAQ/HAQ >0.75 at the last visit and/or the treatment with biological therapy. A selected panel of single nucleotide polymorphisms (SNPs) associated with susceptibility was studied to verify if there was association with poor prognosis. *Results.* Of the 812 patients with JIA registered in Reuma.pt, 267 had a blood sample and registered information used to define "poor prognosis." In univariate analysis, we found significant associations with poor prognosis for allele A of *TNFA1P3/20* rs6920220, allele G of *TRAF1/C5* rs3761847, and allele G of *PTPN2* rs7234029. In multivariate models, the associations with *TRAF1/C5* (1.96 [1.17–3.3]) remained significant at the 5% level, while *TNFA1P3/20* and *PTPN2* were no longer significant. Nevertheless, none of associations found was significant after the Bonferroni correction was applied. *Conclusion.* Our study does not confirm the association between a panel of selected SNP and poor prognosis in Portuguese patients with JIA.

1. Introduction

Juvenile idiopathic arthritis (JIA) is the most common childhood rheumatic disease [1]. Despite significant improvements in the management of children with JIA, the likelihood of long-term persistent disease activity remains high [2]. Published evidence demonstrates that clinical subtype, disease activity and duration, and response to treatment all influence the prognosis [3, 4]. In addition, diagnostic delay, severity and extension of arthritis at onset, symmetric disease, early hip or wrist involvement, involvement of cervical spine, the presence of rheumatoid factor (RF) or anticyclic citrullinated peptide, early age at onset, female gender, and family history of rheumatic disease were the best predictors of a poor outcome [3, 5–11]. However, in most studies of prognostic predictors in JIA, the authors are unanimous in concluding that there is considerable variability in results, making it harder to draw consistent conclusions [8].

Identifying earlier JIA worse prognosis cases is crucial to start appropriate treatment and to correctly inform patients and their parents. Much effort has already been done to elucidate prognosis predictors. Besides clinical factors, identification of genetic predictors of poor prognosis would be a significant contribution to the development of optimal treatment strategies for JIA.

Studies that evaluate nonclinical predictors, such as genetic or immunological parameters, hardly exist. Most of the genetic research aimed to identify variants that affect the risk of developing JIA or pathways modulating drug response in this disease. On the contrary, the goal of this study was to assess the genetic determinants of poor outcome in Portuguese patients with JIA. Our secondary objective was to find potential clinical predictors of poor prognosis.

2. Methods

2.1. Patient Population. Our study was conducted based on Reuma.pt, the Rheumatic Diseases Portuguese Register, which includes JIA patients treated with synthetic and biological Disease Modifying Antirheumatic Drugs (DMARDs) since June 2001. Patients registered up to December 2013 were included. The parent's consent and patient's assent (as appropriate) were obtained according to the declaration of Helsinki. The study was approved by local Ethics Committee. All patients fulfilled the ILAR criteria for the classification of JIA [12]. This study did not have any interference with patients' standard of care.

We analyzed the patients registered in Reuma.pt with the diagnosis of JIA, who had collected a blood sample for DNA analysis. The following data were collected at the time of the last visit to rheumatology clinics: gender, age, JIA subtype, disease duration, time until diagnosis (time since the beginning of the symptoms until the diagnosis of JIA), extra-articular manifestations of the disease, duration of therapy with DMARDs, corticosteroids and biological therapies, Childhood Health Assessment Questionnaire (CHAQ)/Health Assessment Questionnaire (HAQ) [13], patient's/parent's pain visual analogue scale (VAS), patient's/parent's disease global activity VAS, and physician's global disease activity VAS.

One of the barriers found in prognostic studies of JIA is that there is no universal definition of "poor prognosis." We have chosen to integrate in our definition of "poor prognosis" two variables: one instrument that combines disease activity and damage (CHAQ), dichotomized in accordance with other studies [14–19], using 0.75 as the cut-off point, combined with "the need for biological therapy," as a surrogate marker of disease severity and higher likelihood of a worse outcome. We have classified as patients in "need for biological therapy" all patients that were ever treated with biological agents for more than 3 months, due to articular or extra-articular manifestations of the disease. Thus, for the purpose of this study a patient was classified as having poor prognosis if CHAQ >0.75 and/or if the patient was ever treated with biological therapy for more than 3 months.

Genetic single nucleotide polymorphisms (SNPs) were studied to verify if there was any association with poor prognosis.

2.2. Genetic Analysis. The choice of SNP variants was based on information from previous studies of susceptibility and prognosis factors in JIA and included the following 32 SNPs of genes with a known function in the immune system: *PTPN22* rs2476601, *PTPRC* rs10919563, *TNFAIP3/A20* rs10499194, *TNFAIP3/A20* rs6920220, *TRAF1/C5* rs3761847, *ANGPT1* rs1010824, *ANGPT1* rs7151781, *AFF3* rs1160542, *AFF3* rs10865035, *CTLA4* rs3087243, *ERAP1/ARTS1* rs30187, *IL1* rs6712572, *IL1* rs2071374, *IL1* rs1688075, *IL10-1080GA* rs1800896, *IL10-819CT* rs1800871, *IL1R* rs12712122, *IL23R* rs11209026, *IL2-IL21* rs6822844, *IL2RA/CD25* rs2104286, *MIF-173CG* rs755622, *PTPN2* rs1893217, *PTPN2* rs7234029, *SLC26A2* rs1541915, *STAT4* rs3821236, *STAT4* rs7574865, *TNF-238* rs361525, *TNF-308* rs1800629, *VTCN1* rs10923223, *VTCN1* rs12046117, *WISP3* rs2280153, and *EYA4* rs17301249.

All samples were genotyped using Taqman SNP genotyping assays (Applied Biosystems, Foster City, USA) performed as described in the manufacturers' protocol. Genotyping reactions were carried out with an ABI 7500-fast thermocycler. The allele call was obtained by the AB software v2.0.5, by the analysis of allelic discrimination plots. SNPs with deviation from Hardy-Weinberg equilibrium ($P < 0.05$) or minor allele frequency (MAF) <1% were excluded from further analysis.

2.3. Statistical Analysis. We used the additive model to study the association between SNPs and poor prognosis, where homozygotes for the major allele were classified as zero, heterozygotes as 1, and homozygotes for the minor allele as 2. We report crude odds ratio (OR) based on a univariate logistic regression and adjusted OR from a multivariate model including significant clinical predictors. The following clinical variables were characterized: gender, disease category (classified into five groups including polyarticular JIA (RF negative, RF positive, and extended oligoarticular), persistent oligoarticular JIA, systemic arthritis, enthesitis-related arthritis (ERA), and psoriatic arthritis), time until diagnosis

Table 1: Distribution of the clinical characteristics of patients with and without poor prognosis.

Variable	Total	Patients with poor prognosis	Patients without poor prognosis	*P* value
Number	267	85	182	
Female gender *n* (%)	171 (64)	60 (22.59)	111 (41.6)	0.166
JIA categories (%):				
Polyarticular RF negative	48 (18)	19 (39.6)	29 (60.4)	
Polyarticular RF positive	25 (9.4)	19 (76)	6 (24)	
Extended oligoarticular	43 (16.1)	17 (39.5)	26 (60.5)	
Persistent oligoarticular	89 (33.3)	7 (7.9)	82 (92.1)	<0.001
Systemic	22 (8.2)	11 (50)	11 (50)	
Enthesitis-related arthritis	28 (10.5)	8 (28.6)	20 (71.4)	
Psoriatic arthritis	12 (4.5)	4 (33.3)	8 (66.7)	
Age at disease onset (median (IQR))	5.3 (2.2–9.7)	6.6 (3.1–11.6)	4.8 (2.1–8.8)	0.056
Age at diagnosis (median (IQR))	6.6 (2.8–11.6)	8.7 (3.4–13.7)	5.7 (2.5–10.6)	0.013
Time until diagnosis (median (IQR))	0.33 (1.14–1.00)	0.50 (0.17–1.0)	0.26 (0.14–0.88)	0.130
Age at last visit (median (IQR))	14.3 (8.9–18.3)	16.9 (13.1–24.1)	12.7 (6.9–13.3)	<0.001
Disease duration (median (IQR))	6.4 (3.1–12.0)	10.4 (5.2–16.0)	4.9 (2.3–10.4)	<0.001
CHAQ/HAQ (median (IQR))	0 (0–0.25)	0.25 (0-1)	0 (0–0.13)	<0.001
Patient's/parent's VAS (median (IQR))	5 (0–30)	10 (0–50)	0 (0–30)	0.017
Physician VAS (median (IQR))	0 (0–20)	10 (0–35)	0 (0–11.3)	<0.001
Extra-articular manifestations	98	41	57	0.011
Duration of DMARD use (median (IQR))	2.37 (0–5.8)	5.46 (3.02–9.54)	1.43 (0–4.02)	<0.001
Corticosteroid use (Y/N)	124	52	68	<0.001

F: female; M: male; JIA: juvenile idiopathic arthritis; RF: rheumatoid factor; IQR: interquartile range; CHAQ: Childhood Health Assessment Questionnaire; HAQ: Health Assessment Questionnaire; SD: standard deviation; VAS: visual analogue scale; DMARD: Disease Modifying Antirheumatic Drug; Y: yes; N: no. *P* values < 0.05 were considered statistically significant. Note: *P* values are from Pearson's chi-squared or Mann-Whitney tests, as appropriate.

(years), age at disease onset, disease duration (years), duration of DMARD treatment (years), corticosteroid treatment (ever or never), patient's/parent's disease global activity VAS, physician's global disease activity VAS, and extra-articular manifestations (yes or no). Continuous variables were modelled as linear. All clinical variables crudely associated with poor prognosis ($P < 0.20$) were included in a multivariate model. Then backward selection was applied to retain the clinical variables most associated with the outcome, using a significance level of 5%. Due to small sample size for most of the disease categories we carried out the analysis using all JIA categories combined. The stratified analysis was only possible for the polyarticular categories (polyarticular RF positive, polyarticular RF negative, and extended oligoarticular JIA) with 116 patients.

There was missing data for some of the variables, as follows: age at disease onset (1.5%), age at diagnosis (1.9%), patient's/parent's VAS (4.9%), and physician VAS (9.7%).

Statistical significance was considered at the 5% level. After Bonferroni correction for the 32 SNPs analyzed, results were considered significant for $P < 0.0016$.

Statistical analysis was made in R version 2.15.3 [20].

3. Results

Twenty-one centers and 77 rheumatologists and pediatricians contributed with data to Reuma.pt. Of the 812 patients with JIA registered in Reuma.pt (mean age 19.9 ± 11.3 years old, 65% females, and mean age at JIA onset 6.9 ± 4.7 years old), 291 had a blood sample to perform the genetic analysis and, from those, 267 had registered information about CHAQ/HAQ and/or the need for biological therapy used to define "poor prognosis." Of the 267 patients included, 85 had a poor prognosis, according to the definition: CHAQ/HAQ >0.75 and/or the treatment with biological therapy for more than 3 months. Nineteen patients had a CHAQ/HAQ >0.75 at the last appointment, 58 were treated with biological therapy, and 8 fulfilled both criteria.

Table 1 shows the distribution of the clinical characteristics of patients with and without poor prognosis.

3.1. Clinical Predictors of Poor Prognosis. Almost all the clinical variables, except gender, age at disease onset, and delay in diagnosis, were significantly different between the group of JIA patients with poor prognosis and the group who did not had poor prognosis (Table 1).

Clinical variables significantly associated with poor prognosis and included in the multivariate models were DMARD treatment (OR 1.17 [95% confidence interval 1.07–1.27]), higher physician VAS (1.03 [1.01–1.04]), and disease category. In particular, the persistent oligoarticular category had a much lower chance of worse prognosis (0.09 [0.04–0.22]) compared to the polyarticular category; ERA (0.44 [0.18–1.09]), systemic arthritis (1.11 [0.45–2.76]), and psoriatic arthritis (0.55 [0.16–1.94]) categories were not significantly different to the polyarticular group of JIA.

Table 2: Crude and adjusted *odds ratio* for the association between single nucleotide polymorphisms and poor prognosis.

	Minor allele	Crude		Adjusted[†]	
		OR (95% CI)	*P* value	OR (95% CI)	*P* value
All categories					
TNFA1P3/20 rs6920220 A/G	A	1.53 (1.01–2.33)	0.0436	1.67 (0.98–2.83)	0.0579
TRAF1/C5 rs3761847 A/G	G	1.49 (1.00–2.21)	0.0491	1.96 (1.17–3.3)	0.0110
PTPN2 rs7234029 A/G	G	1.86 (1.17–2.95)	0.0085	1.75 (0.99–3.1)	0.0540
Polyarticular categories					
CTLA4 rs3087243 A/G	A	1.98 (1.14–3.45)	0.0153	2.9 (1.39–6.08)	0.0047
PTPN2 rs7234029 A/G	G	3.08 (1.53–6.19)	0.0016	3.3 (1.48–7.37)	0.0035

[†]Clinical covariates included disease category, DMARD treatment, and physician VAS. Disease category was omitted from the model for the polyarticular categories of JIA.
OR: odds ratio; SNPs: single nucleotide polymorphisms. *P* values < 0.05 were considered statistically significant.

3.2. Genetic Predictors of Poor Prognosis. Crude and adjusted odds ratios for the association between studied SNPs and poor prognosis are shown in Table 2. In univariate analysis including all disease categories we found significant associations with poor prognosis for allele A of *TNFA1P3/20* rs6920220, allele G of *TRAF1/C5* rs3761847, and allele G of *PTPN2* rs7234029. In multivariate models adjusted for relevant clinical predictors (disease category, DMARD treatment, and physician VAS) the association for *TRAF1/C5* rs3761847 (1.96 [1.17–3.30]) remained significant at the 5% level while *TNFA1P3/20* rs6920220 (1.67 [0.98–2.83]) and *PTPN2* rs7234029 (1.75 [0.99–3.10]) were no longer significant.

In the univariate analysis for the polyarticular categories we found associations for allele A of *CTLA4* rs3087243 and allele G of *PTPN2* rs7234029. After adjusting for clinical factors the associations for *CTLA4* rs3087243 (2.90 [1.39–6.08]) and *PTPN2* rs7234029 (3.30 [1.48–7.37]) were still significant at the 5% level.

Nevertheless, none of associations found was significant after the Bonferroni correction ($P < 0.0016$).

4. Discussion

The aim of our study was to identify genetic and clinical predictors of poor outcome in JIA. In a Portuguese sample of patients with JIA, we have not found genetic associations with a poor outcome. Longer duration of DMARD treatment, higher physician VAS, and polyarticular categories of JIA had a significant association with poor prognosis.

A growing number of studies have been focused on susceptibility to JIA, including genome wide association studies [21]. However, studies on genetics of JIA outcomes are still scarce. In a recent systematic literature review of early predictors of prognosis in JIA [8], the authors concluded that demographic, clinical, and laboratory values were insufficient to predict the individual prognosis. The authors also pointed out that hardly any other potential predictors were evaluated, such as cytokine levels, cell characteristics, results of imaging obtained early in the disease course, or genetic evaluations, such as HLA and SNPs in genes with a known function in the immune system.

There are some examples of genetic research on JIA outcomes, including a study that suggests that the *MIF-173* polymorphism (*MIF-173*∗C allele) is a predictor of poor outcome in systemic-onset JIA [22], another study that found SNPs in the *IL6* gene associated with pain [14] and a correlation between *TGF-b1* gene codon 25 genotypes and early radiological damage [14], and, in the ERA subtype, a publication suggesting that the presence of *HLA-DRB1∗08* predicts failure to attain disease remission [23].

RA shares several clinical and pathological features with JIA and previous studies reported considerable overlap in genetic susceptibility loci for the two diseases [24–26]. JIA is a heterogeneous disease and genetic differences across the JIA categories and some category-specific effects have been identified [27, 28]. However, stratified analysis leads to small sample sizes for many of the categories. Larger cohorts of the ILAR categories are required to improve the power to detect any category-specific effects. We have stratified our analysis to investigate the polyarticular categories (polyarticular RF positive, polyarticular RF negative, and extended oligoarticular) which are the largest category in our sample.

We have found an association between a variant in the *TRAF1/C5* locus and poor prognosis in Portuguese with JIA regardless of the disease category. Only in the polyarticular category of JIA did we find an association between 2 variants in the *CTLA4* and *PTPN2* loci and a poor outcome. Nevertheless, none of the associations found was significant after the Bonferroni correction was applied ($P < 0.0016$).

The analysis of the clinical variables identified a number of parameters associated with poor outcome. Patients with a poor prognosis were more likely to have polyarticular categories of JIA (polyarticular RF negative, polyarticular RF positive, and extended oligoarticular), to be on treatment with DMARDs for a longer period, and to have higher values of physician VAS at the last visit. Additionally, patients with a poor prognosis were less likely to have persistent oligoarticular JIA. Our results are in accordance with other studies that revealed that children with persistent oligoarticular JIA have a substantially better outcome than those with either systemic or polyarticular JIA, as measured by attaining remission, degree of disability, and structural damage [8, 10, 11].

There are some limitations in our study, namely, the definition used to determine poor prognosis. There is no universal definition of "poor prognosis" in patients with JIA. We have chosen to integrate in our definition of "poor

prognosis" two variables: (1) an instrument that combines disease activity and damage (CHAQ/HAQ); (2) the need for biological treatment, because patients that do not respond to conventional DMARDs, namely, methotrexate, have a higher chance of a poor outcome. Regarding this last point, our study included patients at different phases of their disease and we are aware that the access to biological therapy could not have been the same for all patients, leading to a selection bias. In addition, some patients could have started biological therapy mainly for extra-articular manifestations of the disease (e.g., uveitis) and not due to joint disease. This could also have potentially confounded our results.

Another limitation of our study is the problem of multiple comparisons: our results may simply be attributable to chance. The sample size in our cohort was too small to adequately test replication and a further study in a larger cohort is still required in order to confirm or refute our findings.

5. Conclusions

In summary, our study does not confirm the association between a panel of selected SNPs and poor prognosis in Portuguese patients with JIA. A search for additional genetic variants is required. Moreover, combination of genetic factors together with environmental exposures should also be considered. Further studies, in different populations of JIA patients, should be performed to replicate these findings.

Abbreviations

JIA:	Juvenile idiopathic arthritis
DMARD:	Disease Modifying Antirheumatic Drug
CHAQ:	Childhood Health Assessment Questionnaire
SNP:	Single nucleotide polymorphism
VAS:	Visual analogue scale
MAF:	Minor allele frequency
OR:	Odds ratio
RF:	Rheumatoid factor
ERA:	Enthesitis-related arthritis.

Disclosure

There are no financial relationships relevant to the work.

Conflict of Interests

The authors have declared no conflict of interests.

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PART IV

Using the Juvenile Arthritis Disease Activity Score based on erythrocyte sedimentation rate or C-reactive protein level: results from the Portuguese register

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ORIGINAL ARTICLE

Using the Juvenile Arthritis Disease Activity Score Based on Erythrocyte Sedimentation Rate or C-Reactive Protein Level: Results From the Portuguese Register

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Objective. **Our aims were to evaluate the correlation between Juvenile Arthritis Disease Activity Score 27-joint reduced count (JADAS27) with erythrocyte sedimentation rate (ESR) and JADAS27 with C-reactive protein (CRP) scores and to test the agreement of both scores on classifying each disease activity state. We also aimed at verifying the correlation of the 2 scores across juvenile idiopathic arthritis (JIA) categories and to check the correlation between JADAS27-ESR and clinical JADAS27 (JADAS27 without ESR).**

Methods. **A nationwide cohort of patients with JIA registered in the Portuguese Register, Reuma.pt, was studied. JADAS27-CRP was adapted by replacing ESR with CRP level as the inflammatory marker. JADAS27-CRP was calculated similarly to JADAS27-ESR as the simple linear sum of its 4 components. Pearson's correlations and K statistics were used in the analyses.**

Results. **A total of 358 children had full data to calculate JADAS27; 65.4% were female and the mean ± SD disease duration was 11.8 ± 9.1 years. The correlation coefficient between JADAS27-ESR and JADAS27-CRP was 0.967 ($P < 0.0001$), although the correlation coefficient between ESR and CRP level was 0.335 ($P < 0.0001$). The strong correlation between JADAS27-ESR and JADAS27-CRP was maintained when compared within each JIA category. The agreement between JADAS27-ESR and JADAS27-CRP across the 4 activity states was very good, showing 91.1% of the observations in agreement; K = 0.867 (95% confidence interval 0.824–0.91). The correlation between JADAS27 with ESR and JADAS27 without ESR was high (r = 0.97, $P < 0.0001$).**

Conclusion. **JADAS27 based on CRP level correlated closely with JADAS27-ESR across all disease activity states and JIA categories, indicating that both measures can be used in clinical practice. Moreover, the correlation of JADAS27 with and without ESR was also high, suggesting that this tool might be useful even in the absence of laboratorial measures.**

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most common arthritis of childhood. It is a heterogeneous disease group of unknown etiology with distinct presentation, clinical features, immunopathogenesis, and genetic background (1). In fact, some of the categories of JIA may represent different diseases. Evaluation of disease activity is a crucial

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Significance & Innovations

- Juvenile Arthritis Disease Activity Score (JADAS) is a valid instrument for assessment of disease activity in juvenile idiopathic arthritis.
- JADAS based on erythrocyte sedimentation rate (ESR) correlated closely with JADAS based on C-reactive protein level.
- The correlation of JADAS with and without ESR is also high, suggesting that this tool might be useful even in the absence of laboratory measures.

component of the clinical assessment of children with JIA because persistently active disease plays a major role in causing joint damage and physical disability (2).

Recently, a composite score named Juvenile Arthritis Disease Activity Score (JADAS) was found to be a valid instrument for assessment of disease activity (2). JADAS consists of 4 components: physician global assessment of disease activity, parent/patient global assessment of well-being, number of joints with active disease, and an inflammatory marker (2). The clinical measures included in JADAS are part of the American College of Rheumatology (ACR) pediatric core set of outcome variables (3). A major advantage of JADAS when compared to ACR pediatric measures of improvement criteria is the ability to assess disease activity at a single visit and also to compare disease activity between individuals or groups. There are no perfect instruments and the major caveat of JADAS is that systemic features are not contemplated, limiting its use in systemic JIA. According to the authors who validated JADAS (2), the statistical performance of the JADAS 27-joint reduced count (JADAS27) was comparable with that provided by the JADAS71. However, assessment of 27 joints is more feasible and less tedious than evaluation of 71 joints. The simplest, 10-joint reduced count revealed the best discriminating validity, responsiveness (although not in nonresponder patients), and distribution, but had a somewhat poorer construct validity. The greater responsiveness of this joint count may be explained by most JIA patients having few joints involved. Use of this reduced count, which does not enable a precise assessment of joint disease and may limit the ability to detect new joint involvement over time, is advised only for use in retrospective studies, when the total number of involved joints is known, but no information on the individual affected joint is available (2).

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JADAS was developed using the erythrocyte sedimentation rate (ESR) because C-reactive protein (CRP) values were not available in all databases used to validate the tool (2). However, as the authors of the JADAS highlighted, CRP level is a direct measure of the acute-phase response and is less confounded by other factors when compared to ESR. In rheumatoid arthritis (RA) the performance of the Disease Activity Index of 28 joints (DAS28) based on CRP level has been shown to have a similar profile to DAS28 based on ESR (4,5). Similarly, Nordal et al recently compared in a Nordic population the JADAS based on CRP level with the JADAS based on ESR and concluded that these instruments correlated closely, indicating that both scores can be recommended for assessing disease activity in JIA (6). Recently, cutoff values for classifying distinct disease activity states were proposed for the JADAS-ESR (7).

The aim of our work was to evaluate the correlation between the JADAS27-ESR and the JADAS27-CRP and to test the agreement of both scores for classifying each disease activity state. We also aimed at verifying the correlation of the 2 scores across all JIA categories and to check the correlation between the JADAS27-ESR and the JADAS27 without ESR.

PATIENTS AND METHODS

Population and ethical considerations. A nationwide cohort of patients with JIA according to International League of Associations for Rheumatology (ILAR) criteria, registered in the Rheumatic Diseases Portuguese Register, Reuma.pt (8), was studied. Patients with a diagnosis of JIA were consecutively included in the study during the visit in which they completed all study protocol and all disease activity measures were available for JADAS27-ESR and JADAS27-CRP calculation. Clinical information, inflammation markers, and physician and parent/patient visual analog scales (VAS) on global health were collected according to the study protocol and inserted by physicians. Questionnaires on self-reported physical disability were also assessed, i.e., the Childhood Health Assessment Questionnaire (C-HAQ; where 0 = best and 3 = worst) for children age <18 years (9), and the Health Assessment Questionnaire (HAQ; where 0 = best and 3 = worst) (10) for participants age >18 years. JIA categories were classified according to the ILAR criteria (11).

Parents and children between ages 12 and 18 years gave informed consent, as well as patients age ≥18 years. Reuma.pt was approved by the National Board of Data Protection and local ethics committees. Research was carried out in compliance with the Declaration of Helsinki, and all the ethics committees of the participating hospitals and clinics approved the study.

JADAS calculation. JADAS consists of 4 components: physician global assessment of disease activity on a 10-cm VAS (where 0 = no activity and 10 = maximum activity), parent/patient global assessment of well-being on a 10-cm VAS (where 0 = very well and 10 = very poor), number of joints with active disease, and an inflammatory marker

(ESR) (2). We decided to use the 27-joint reduced count (JADAS27) due to its greater feasibility. This count has been found to be a valid surrogate for the whole joint count in JIA (12). The JADAS27 includes the following joints: cervical spine, elbows, wrists, metacarpophalangeal joints (from the first to third), proximal interphalangeal joints, hips, knees, and ankles. The ESR value was normalized to a 0–10 scale according to the following formula:

$$\frac{\text{ESR mm/hour} - 20}{10}$$

Before making the calculation, ESR values <20 mm/hour were converted to 0 and ESR values >120 were converted to 10 (2).

Similarly to Nordal et al, JADAS27-CRP was adapted by replacing ESR with CRP level as the inflammatory marker (6). CRP level was truncated to a 0–10 scale according to the following formula:

$$\frac{\text{CRP mg/liter} - 10}{10}$$

This is similar to the truncated ESR used in JADAS (2). Before calculation, CRP values <10 mg/liter were converted to 0 and CRP values >110 mg/liter were converted to 10. JADAS27-CRP was calculated similarly to JADAS27-ESR, as the simple linear sum of its 4 components, yielding a global score of 0–57, which is also similar to JADAS27-ESR.

Cutoff values for inactive disease, minimal disease activity, acceptable symptom state, and active disease. Recently, Consolaro et al (7) defined, for all versions of JADAS-ESR (JADAS10, JADAS27, and JADAS71), the cutoff score for classifying a patient as having "inactive disease" as ≤1 for all JIA categories. The cutoff for classification of "minimal disease activity" was >1 and ≤2 for oligoarticular JIA and >1 and ≤3.8 for polyarticular JIA. Children with systemic arthritis, rheumatoid factor (RF)–positive polyarthritis, RF-negative polyarthritis, or extended oligoarthritis were included in the polyarthritis group. The oligoarthritis group included patients with persistent oligoarthritis. Patients with JIA classified in the remaining ILAR categories were assigned to the polyarthritis or oligoarthritis group based on the number of joints affected during disease course (>4 or ≤4, respectively). Cutoff values for JADAS for "parent's acceptable symptom state" was >2 and ≤3.2 for oligoarticular JIA and >3.8 and ≤5.2 for polyarticular JIA (7). Values above the cutoffs for "parent's acceptable symptom state" were considered as "active disease" state (JADAS >3.2 and JADAS >5.2 for oligoarticular and polyarticular JIA, respectively).

We analyzed the agreement of the classification of patients with the cutoffs of JADAS27 (using ESR) with the JADAS27-CRP classification using the categories of "inactive disease," "minimal disease activity," "parent's acceptable symptom state," and "active disease" to verify whether these 2 JADAS versions were classifying the patients similarly.

Statistical analysis. Descriptive statistics were used to summarize population characteristics. Correlations between the continuous variables were calculated and expressed as Pearson's coefficient correlation. Correlations were considered high, moderate, or weak at coefficients ≥0.7, 0.4–0.7, or ≤0.4, respectively. The Student's *t*-test and analysis of variance (ANOVA) were used to compare means between groups. ANOVA followed by Bonferroni analysis was used to compare the JADAS27-ESR and JADAS27-CRP across all JIA categories. Pearson's correlations and K statistics were used to assess the agreement between disease states set by JADAS27-ESR and JADAS27-CRP. Statistical analysis was performed using SPSS statistical software, version 20. Two-sided *P* values less than 0.05 were considered statistically significant.

RESULTS

From the 729 patients with JIA included in the Reuma.pt database, 358 children had full data to calculate JADAS27-ESR and JADAS27-CRP. Of these 358 patients, 65.4% were female. Mean ± SD disease duration was 11.8 ± 9.1 years and the mean ± SD age at the last visit was 18.5 ± 9.9 years. A total of 134 patients (37.5%) were classified as persistent oligoarticular, 53 patients (14.8%) as extended oligoarticular, 51 patients (14.2%) were polyarticular RF negative, 30 patients (8.4%) were polyarticular RF positive, 39 patients (10.9%) were systemic, 35 patients (9.8%) had enthesitis-related arthritis, 11 patients (3.1%) had psoriatic arthritis, and in 5 patients (1.4%) information was lacking on the category of JIA. The age, sex, disease duration, and JIA categories distribution of the selected patients (358 patients) was similar to the patients that were excluded due to insufficient data (371 patients).

The correlation coefficient at the last visit with all JADAS items available between JADAS27-ESR and JADAS27-CRP was 0.967 ($P < 0.0001$) (Figure 1), although the correlation coefficient between ESR and CRP level was 0.335 ($P < 0.0001$) (this correlation refers to the raw values of ESR and CRP level, not the truncated values used to calculate JADAS27). When comparing the JADAS27-ESR and JADAS27-CRP within each category of JIA, the strong

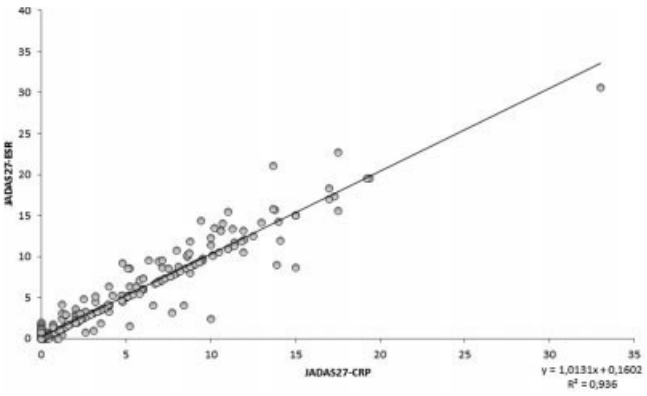

Figure 1. Correlation between Juvenile Arthritis Disease Activity Score 27-joint reduced count (JADAS27) based on erythrocyte sedimentation rate (ESR) and corresponding JADAS27 based on C-reactive protein (CRP) level in the Portuguese Reuma.pt cohort, at the same study visit (r = 0.967, $P < 0.0001$).

Table 1. Pearson's correlation coefficients of JADAS27-ESR and JADAS27-CRP across the JIA categories*

	JADAS27-ESR, mean ± SD	JADAS27-CRP, mean ± SD	Correlation coefficient, r†	*P*
PsA	5.218 ± 4.61	4.855 ± 4.68	0.883	0.0003
ERA	3.731 ± 4.29	3.514 ± 3.87	0.973	< 0.0001
OligoE	3.128 ± 4.11	3.002 ± 3.93	0.976	< 0.0001
OligoP	2.569 ± 4.05	2.309 ± 3.66	0.970	< 0.0001
PolyRFneg	5.304 ± 6.88	4.878 ± 6.54	0.964	< 0.0001
PolyRFpos	6.217 ± 5.42	6.263 ± 5.46	0.964	< 0.0001
SoJIA	3.523 ± 3.68	3.464 ± 3.63	0.967	< 0.0001
All categories	3.661 ± 4.82	3.447 ± 4.58	0.967	< 0.0001

* JADAS27 = Juvenile Arthritis Disease Activity Score 27-joint reduced count; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; JIA = juvenile idiopathic arthritis; PsA = psoriatic arthritis; ERA = enthesitis-related arthritis; OligoE = oligoarticular extended; OligoP = oligoarticular persistent; PolyRFneg = polyarticular rheumatoid factor negative; PolyRFpos = polyarticular rheumatoid factor positive; SoJIA = systemic-onset JIA.
† Correlations were considered high when $r \geq 0.7$, moderate if $0.4 > r > 0.7$, and low if $r \leq 0.4$.

correlation was maintained (all correlation coefficients >0.8 and *P* values < 0.001) (Table 1).

JADAS27-ESR and JADAS27-CRP according to JIA categories are shown in Table 1. The mean JADAS27-CRP of the oligoarticular categories differed significantly from the mean JADAS27-CRP score of the polyarticular categories (persistent oligoarticular versus polyarticular RF positive [$P < 0.0001$], persistent oligoarticular versus polyarticular RF negative [$P = 0.010$], and extended oligoarticular versus polyarticular RF positive [$P = 0.03$]). The JADAS27-ESR also differed significantly between the oligoarticular and polyarticular categories of JIA (persistent oligoarticular versus polyarticular RF positive [$P = 0.003$] and persistent oligoarticular versus polyarticular RF negative [$P = 0.01$]).

From the 358 patients included in this study using the JADAS27-ESR, 160 (44.7%) patients were classified as having inactive disease, 42 (11.7%) had minimal disease activity, 45 (12.6%) had acceptable symptom state, and 111 (31%) patients had active disease. The classification was similar using the JADAS27-CRP: 166 (46.4%) patients were classified as having inactive disease, 41 (11.4%) had minimal disease activity, 39 (10.9%) had acceptable symptom state, and 112 (31.3%) patients

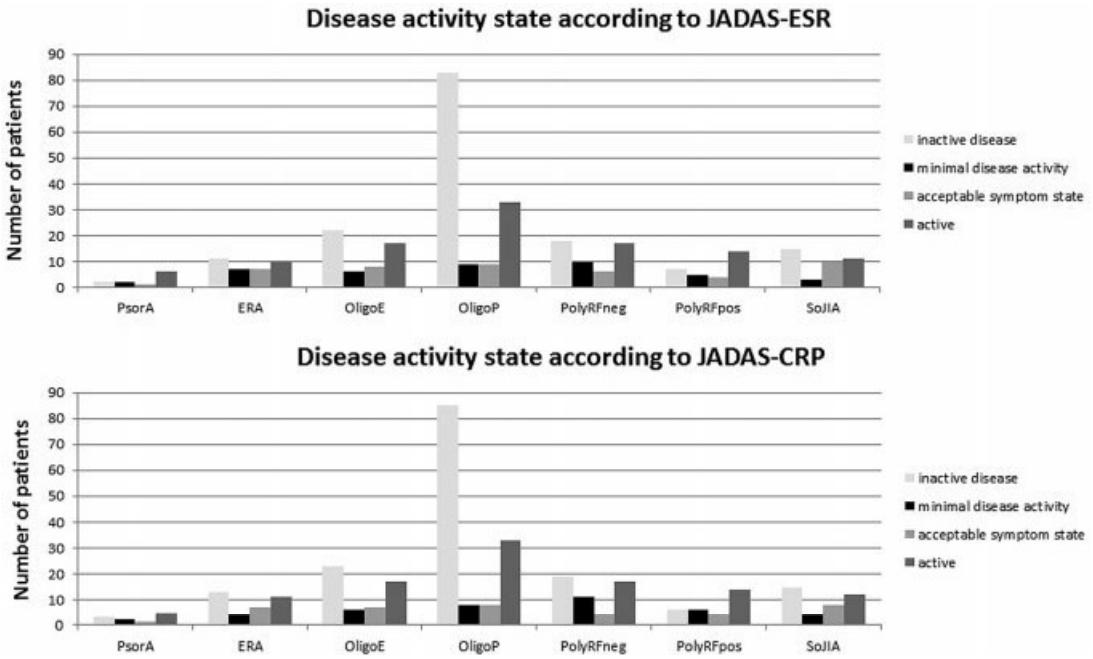

Figure 2. Disease activity state according to Juvenile Arthritis Disease Activity Score (JADAS) based on erythrocyte sedimentation rate (ESR) and JADAS based on C-reactive protein (CRP) level for the different juvenile idiopathic arthritis (JIA) categories at the study visit, in the Portuguese Reuma.pt JIA cohort. PsorA = psoriatic arthritis; ERA = enthesitis-related arthritis; OligoE = oligoarticular extended; OligoP = oligoarticular persistent; PolyRFneg = polyarticular rheumatoid factor negative; PolyRFpos = polyarticular rheumatoid factor positive; SoJIA = systemic-onset JIA.

Table 2. JADAS27-ESR and JADAS-CRP across JIA disease activity states and descriptive statistics of the components of JADAS27 (number of joints with active disease, parent/patient global assessment of well-being, physician global assessment of disease activity, and the inflammatory marker)*

	All activity states	Inactive disease	Minimal disease activity	Acceptable symptom state	Active disease
Patients, no. (%)	358 (100)	160 (44.69)	42 (11.73)	45 (12.57)	111 (31.01)
JADAS27-ESR					
Mean ± SD	3.71 ± 4.86	0.18 ± 0.31	1.78 ± 0.66	3.36 ± 0.97	9.67 ± 4.52
Median (minimum, maximum)	1.5 (0, 30.6)	0 (0, 1.1)	1.55 (1.1, 3.8)	3.3 (2, 5.4)	9 (3.6, 30.6)
JADAS-CRP					
Mean ± SD	3.50 ± 4.64	0.19 ± 0.42	1.76 ± 1.72	3.32 ± 1.56	9.01 ± 4.45
Median (minimum, maximum)	1.35 (0, 33)	0 (0, 3.1)	1.5 (0, 10)	3 (1.2, 8.4)	8.5 (1.2, 33)
JADAS-ESR vs. JADAS-CRP					
r	0.967	0.697	0.496	0.593	0.925
P	< 0.0001	< 0.0001	0.0008	< 0.0001	< 0.0001
ESR, mm/hour					
Mean ± SD	17.12 ± 17.16	9.74 ± 6.83	16.36 ± 12.7	19.49 ± 16.8	27.09 ± 23.11
Median (minimum, maximum)	12 (1, 120)	8 (1, 29)	14 (1, 44)	14 (1, 71)	20 (1, 120)
<20, %	74.02	91.88	66.67	68.89	53.15
CRP, mg/liter					
Mean ± SD	7.01 ± 14.24	3.04 ± 4.88	5.89 ± 11.43	10.78 ± 17.0	11.63 ± 20.2
Median (minimum, maximum)	2 (0, 156)	1 (0, 31.3)	2 (0, 51.9)	4 (0, 73.9)	4 (0, 156)
<10, %	81.84	91.88	92.86	73.33	66.67
Active joints					
Mean ± SD	0.39 ± 1.03	0 ± 0	0.12 ± 0.4	0.22 ± 0.56	1.13 ± 1.57
Median (minimum, maximum)	0 (0, 7)	0 (0, 0)	0 (0, 2)	0 (0, 3)	1 (0, 7)
PGA					
Mean ± SD	1.63 ± 2.32	0.10 ± 0.24	0.66 ± 0.61	1.53 ± 1.29	4.25 ± 2.42
Median (minimum, maximum)	0.4 (0, 10)	0 (0, 1)	0.7 (0, 2)	1.2 (0, 5.2)	4 (0, 10)
PhGA					
Mean ± SD	1.15 ± 1.7	0.04 ± 0.16	0.59 ± 0.56	0.97 ± 0.74	3.05 ± 1.86
Median (minimum, maximum)	0 (0, 9.3)	0 (0, 1.1)	0.5 (0, 2)	1 (0, 2.5)	2.9 (0, 9.3)

* JADAS27 = Juvenile Arthritis Disease Activity Score 27-joint reduced count; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; JIA = juvenile idiopathic arthritis; PGA = parent/patient global assessment of well-being (on a 10-cm visual analog scale [VAS], where 0 = very well and 10 = very poor); PhGA = physician global assessment of disease activity (on a 10-cm VAS, where 0 = no activity and 10 = maximum activity).

had active disease. Figure 2 shows the disease activity states according to the different JIA categories based on JADAS27-ESR and JADAS27-CRP. The agreement between JADAS27-ESR and JADAS27-CRP across the 4 activity states assessed by K statistics was very good, showing 91.1% of the observations in agreement: K = 0.867 (95% confidence interval 0.824−0.91).

The correlation between JADAS27 with ESR and JADAS27 without ESR (clinical JADAS27) was high (r = 0.97, $P < 0.0001$), as well as the correlation between JADAS27-CRP and JADAS27 without CRP (r = 0.97, $P < 0.0001$). We analyzed separately the patients with higher values of ESR (≥20 mm/hour, n = 109) and increased CRP level (≥10 mg/liter, n = 70), and verified that the correlation between JADAS27-ESR and clinical JADAS27 was still high (r = 0.96, $P < 0.0001$), as well as between JADAS27-CRP and clinical JADAS27 (r = 0.93, $P < 0.0001$). We also assessed whether the correlation between clinical JADAS27 and the JADAS27 that included an inflammatory marker still remained high in the systemic subtype. The coefficient correlation with clinical JADAS27 was 0.96 and 0.94, respectively, for JADAS27-ESR and JADAS27-CRP. Table 2 shows the correlation between JADAS-ESR and JADAS-CRP across all JIA disease activity states.

Correlation between JADAS27-ESR and parent/patient global assessment of well-being was strong (r = 0.84, $P < 0.0001$) as well as the correlation between JADAS27-ESR and physician global assessment of disease activity (r = 0.88, $P < 0.0001$). We also tested the correlation between physician global assessment of disease activity (VAS) and parent/patient global assessment of well-being on a 10-cm VAS; the correlation coefficient was 0.64 ($P < 0.0001$). A moderate correlation (r = 0.49) was found between JADAS27-ESR and C-HAQ/HAQ ($P < 0.0001$).

DISCUSSION

Composite indices or pooled indices are useful tools for the evaluation of disease activity in patients with JIA. They allow the integration of various aspects of the disease into a single numerical value and may improve patient care. The JADAS is a new tool for the evaluation of disease activity in JIA that has been developed to provide physicians with a simple and useful instrument.

Similar to the work of Nordal et al (6), in our study the JADAS27 based on ESR and on CRP level correlated closely, indicating that both measures can be used. In addition, we have tested the recently published cutoff

criteria for classification of inactive disease, minimal disease activity, parent's acceptable symptom state (7), and active disease to analyze whether patients were classified in the same state using either JADAS27-ESR or CRP. The criteria (i.e., cutoff values) were developed due to the need for identifying different states of JIA activity and may provide simple and intuitive reference values that can be used to monitor the disease course over time in an individual patient or to compare disease status across individual patients or patient groups (7). The agreement between JADAS27-ESR and JADAS27-CRP across the 4 activity states was very good, showing agreement in 91.1% of the observations, reinforcing that clinicians can use both measures to calculate the JADAS without changing the categories in which the patients are classified.

JADAS calculation may have had some limitations in our study population. Most of our JIA patients had inactive disease, which might have enhanced the results. However, when we performed the correlation of JADAS-ESR with JADAS-CRP according to the disease activity states (Table 2), the patients with active disease showed a high correlation ($r = 0.925$, $P < 0.0001$). Additionally, in patients with values of ESR ≥20 or CRP level ≥10 (i.e., converted values different than zero), the high correlation between the 2 scores was maintained ($r = 0.94$, $P < 0.0001$) (data not shown).

Another limitation of JADAS is that the conversion of the higher values of ESR and CRP level to 10 can mislead the results: a JIA patient with CRP level of 200 mg/liter obtains the same JADAS score as a patient with a CRP level of 110 mg/liter (both values of CRP are converted to 10).

Finally, as the authors of JADAS point out, although the score was designed to be robust enough to cover all categories of JIA, a thorough assessment of disease activity in children with systemic JIA requires quantification of extraarticular manifestations, particularly fever and rash (2).

Measurement of CRP level presents some advantages compared to ESR: CRP level is a direct measure of the acute-phase response and is less confounded by other factors, including comorbidities. Also, CRP level assessment is more rapid and the cost is comparable to ESR (4). Still, although the inclusion of CRP level and ESR is fully justified by their face and content validity, the delay associated with their assessment might be one reason why many physicians do not apply composite scores to guide their clinical decisions. In a study of RA, Aletaha et al concluded that acute-phase reactants add little to composite disease activity indices (13). These inflammatory parameters did not seem to contribute with sufficiently important information to composite scores to change judgment of disease activity, in addition to merely using clinical measures. Because laboratory tests are frequently missing at patient visits, we have also tested JADAS27 with and without ESR. The correlation between JADAS27 with ESR and JADAS27 without ESR (clinical JADAS) was high ($r = 0.97$, $P < 0.0001$), indicating that when ESR is not available JADAS27 can be calculated without this variable. The clinical JADAS27 can therefore be used to conduct a disease activity evaluation anytime and anywhere. Recently, McErlane et al concluded that for the majority of JIA categories, clinical applicability of JADAS would be improved by exclusion of ESR and that the amended score (JADAS3-71), which omits the ESR, correlates well with JADAS71 (14).

We have also tested the correlation between physician global assessment of disease activity (VAS) and parent/patient global assessment of well-being on a 10-cm VAS in order to see whether it would be possible to cut one of these components of JADAS27, similar to the DAS28 of 4 and 3 variables in RA (15). The correlation was moderate and insufficient to exclude a component from the tool. In fact, it is crucial to include these 2 scales in JADAS. Parent/patient global assessment is important because it is the only parameter that incorporates the parent's/patient's perception of disease activity. The physician global assessment is also relevant because it represents the most responsive measure in JIA (2).

As with Consolaro et al (2), in our study the correlation between JADAS27-ESR and functional impairment according to C-HAQ/HAQ was only moderate ($r = 0.499$, $P < 0.0001$). This moderate correlation was expected because C-HAQ/HAQ scores combine the effect of both disease activity and damage. The authors of JADAS decided not to include functional status assessment because it has been shown to be relatively insensitive to change in JIA (2).

In conclusion, in our study the JADAS27 based on CRP level and ESR correlated closely, and both classify patients similarly regarding disease activity state, indicating that both measures can be used interchangeably in clinical practice. In addition, clinical JADAS, a score that does not include laboratory measures, was also well correlated with JADAS-ESR and JADAS-CRP.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. Mourão had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Mourão, Santos, Martins, Branco, Fonseca, Canhão.

Acquisition of data. Mourão, Santos, Melo-Gomes, Martins, Costa, Ramos, Brito, Duarte, Figueira, Figueiredo, Furtado, Lopes, Oliveira, Rodrigues, Salgado, Sousa, Fonseca, Canhão.

Analysis and interpretation of data. Mourão, Santos, Melo-Gomes, Martins, Branco, Fonseca, Canhão.

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PART V

Effectiveness and long-term retention of anti-TNF treatment in juvenile and adult patients with Juvenile Idiopathic Arthritis: data from Reuma.pt

Original article

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Effectiveness and long-term retention of anti-tumour necrosis factor treatment in juvenile and adult patients with juvenile idiopathic arthritis: data from Reuma.pt

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Abstract

Objectives. Assess the effectiveness and safety of biologic therapy as well as predictors of response at 1 year of therapy, retention rate in biologic treatment and predictors of drug discontinuation in JIA patients in the Portuguese register of rheumatic diseases.

Methods. We prospectively collected patient and disease characteristics from patients with JIA who started biological therapy. Adverse events were collected during the follow-up period. Predictors of response at 1 year and drug retention rates were assessed at 4 years of treatment for the first biologic agent.

Results. A total of 812 JIA patients [65% females, mean age at JIA onset 6.9 years (S.D. 4.7)], 227 received biologic therapy; 205 patients (90.3%) were treated with an anti-TNF as the first biologic. All the parameters used to evaluate disease activity, namely number of active joints, ESR and Childhood HAQ/HAQ, decreased significantly at 6 months and 1 year of treatment. The mean reduction in Juvenile Disease Activity Score 10 (JADAS10) after 1 year of treatment was 10.4 (S.D. 7.4). According to the definition of improvement using the JADAS10 score, 83.3% respond to biologic therapy after 1 year. Fourteen patients discontinued biologic therapies due to adverse events. Retention rates were 92.9% at 1 year, 85.5% at 2 years, 78.4% at 3 years and 68.1% at 4 years of treatment. Among all JIA subtypes, only concomitant therapy with corticosteroids was found to be univariately associated with withdrawal of biologic treatment ($P = 0.016$).

Conclusion. Biologic therapies seem effective and safe in patients with JIA. In addition, the retention rates for the first biologic agent are high throughout 4 years.

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Key words: juvenile idiopathic arthritis, efficacy, safety, biological treatment.

Key Messages

- Our data from Reuma.pt suggest that biologic therapies seem effective and safe in patients with JIA.
- The majority of patients with JIA respond to therapy after 1 year of biologic treatment.
- Long-term retention in biologic treatment is high in JIA patients.

Introduction

JIA is the most common rheumatic disease of childhood, affecting 1/1000 children, and includes a heterogeneous group of chronic arthritis of unknown aetiology that begins before 16 years of age. According to disease onset, seven categories can be identified [1].

The management of JIA has traditionally been modelled on the RA treatment strategy, with MTX and, more recently, biologic medications forming the mainstay of therapy [2–6]. When inflammatory activity is not controlled with MTX or if MTX is not tolerated, the next therapeutic step is usually the addition of a TNF inhibitor (with the exception of systemic JIA), either alone or in combination with MTX [7]. However, JIA is an umbrella term for a group of childhood-onset arthritides, many of which are quite different from RA. Knowing that the JIA ILAR categories represent, in fact, different diseases, the response to anti-TNF therapies may also differ according to the subtype of the disease. To date, there are currently five biologics licensed for the treatment of JIA: etanercept, abatacept, adalimumab, tocilizumab and canakinumab. Other biologic options are under evaluation and some are often prescribed off-label.

Large registries played a crucial role in analysing the effectiveness and long-term safety of biologic treatments in JIA [8–11]. However, little information is available from registries that include JIA patients starting biologics at any age, including adulthood. Moreover, data on predictors of response to biologic therapy and long-term retention rates are scarce.

In 2008, the Portuguese Society of Rheumatology developed an observational registry of patients with rheumatic diseases, including JIA, the Portuguese Register of Rheumatic Diseases (Reuma.pt) [12]. In 2012, national recommendations for the use of biologic therapies in children and adolescents with JIA were updated, supporting physicians in their therapeutic decisions [13]. The use of biologic therapy in Portugal is limited to those patients who are either intolerant to MTX and/or have arthritis activity that is not controlled by MTX [13].

This study aims to assess the effectiveness of the first biologic therapy at 6 months and 1 year of treatment as well as safety during the overall follow-up period in JIA patients registered in Reuma.pt. Our secondary objectives were to study the predictive factors of response to treatment at 1 year, the retention rate at 4 years of biologic treatment and the factors associated with biologic drug withdrawal in the treatment of JIA.

Methods

Our study was based on Reuma.pt, which includes JIA patients receiving biologic therapies and synthetic DMARDs. Our study was approved by the scientific committee of Reuma.pt. Reuma.pt is approved by the National Commission for Data Protection. All patients fulfilled the ILAR criteria for the classification of JIA [1].

We analysed all patients with a diagnosis of JIA registered in Reuma.pt until September 2013, irrespective of age at entry into the cohort (patients who started biologic therapy in adulthood were also included). At the start of biologic treatment (baseline), we collected the following data: age, gender, JIA category, age at JIA onset, disease duration, number of active joints, patient's pain visual analogue scale (VAS), patient's disease global activity VAS, physician's global disease activity VAS, extra-articular manifestations, Childhood HAQ (CHAQ) or HAQ (as appropriate) [14], ESR, CRP and concomitant therapy with DMARDs and/or corticosteroids. Follow-up data were considered during the first biologic therapy.

Follow-up data for effectiveness (disease activity) were obtained at 6 months and 1 year after starting the first biologic and included the number of active joints, ESR and CHAQ/HAQ. To calculate response to treatment we used the delta Juvenile Disease Activity Score (JADAS), a recent composite score found to be a valid instrument for assessment of disease activity in JIA [15] and, in addition, the definition of improvement using the JADAS10 [16]. According to this new definition of improvement using the JADAS [16], if the JADAS10 baseline was between 5 and 15 (low disease activity), there is a response to therapy if delta JADAS is >4; if the JADAS10 baseline is between 15 and 25 (moderate disease activity), there is a response to therapy if delta JADAS is >10; and if the JADAS10 baseline is between 25 and 40 (high disease activity), there is a response to therapy if delta JADAS is >17. Safety analysis (severe adverse events) was performed with the cumulative events at the end of the follow-up. Retention rates for the first biologic were calculated yearly in the first 4 years of treatment. The reason for biologic withdrawal was also collected.

Statistical analysis

Each patient contributed data regarding the course of their first biologic treatment only.

In order to study retention rates, we included only patients with follow-up periods of at least 1 year. Drug

retention rates were calculated using the Kaplan–Meier method.

The Cox regression model was used to identify predictors of drug discontinuation until 4 years, so patients were censored at the time of last consultation or at 4 years of treatment, whichever came first. At first, crude hazard ratios were obtained using all JIA categories combined. Subsequently the analysis was repeated using only patients with polyarthritis (polyarticular RF positive, polyarticular RF negative and extended oligoarticular JIA). The proportional hazards assumption was verified. As a secondary analysis, we repeated all of the main analyses using only the patients that started biologic treatment before the age of 18 years. Statistical analysis was made in R version 2.15.3 (R Project for Statistical Computing, Vienna, Austria) [17].

Results

Twenty-one centres and 77 clinicians across the country contributed data for this study. Of the 812 patients with JIA registered in Reuma.pt [mean age 19.9 years (S.D. 11.3), 65% females, mean age at JIA onset 6.9 years (S.D. 4.7)], 227 received biologic therapy and the median duration of the first biologic agent treatment was 4.5 years [interquartile range (IQR) 2.2–5.9] (the characteristics of the patients treated with biologic agents are presented in Table 1). The mean age at disease onset of JIA patients ever treated with biologic DMARDs was 7.5 years (S.D. 4.9; IQR 0.8–11.6) and the mean age for starting biologic therapy was 16.2 years (S.D. 9.4; IQR 1.8–20.4). Sixty-nine (30.4%) patients started biologic therapy in adulthood.

Most patients (90.3%) were treated with anti-TNF as a first line treatment: etanercept 69.2% (157 patients), adalimumab 12.8% (29 patients) and infliximab 8.4% (19 patients). All patients taking anakinra (4.8%) had systemic JIA (Table 2). During the follow-up, 32 (14.1%) patients switched biologic treatment once, 13 (5.7%) patients switched twice, 2 (0.9%) switched three times, 3 (1.3%) patients switched four times and 1 (0.4%) patient switched five times.

Twenty-eight patients had uveitis and 10 of them were treated with infliximab.

In the subgroup of patients who started biologic therapy as adults (30.4%), there was a greater proportion of female patients (76.8% *vs* 59.5%, $P = 0.02$), older age at JIA onset [9.5 years (S.D. 5.3) *vs* 6.6 (S.D. 4.5), $P < 0.0001$] and longer overall disease duration [24.4 years (S.D. 11.3) *vs* 9.5 (S.D. 5.3), $P < 0.0001$] compared with those who started in childhood. The proportion of each category of JIA in the two subgroups was similar, as well as the distribution of the first biologic agent used.

Effectiveness of biologic treatment

All the parameters used to evaluate disease activity, namely the number of active joints, ESR and CHAQ/HAQ, decreased significantly at 6 months and 1 year of treatment with biologic agents. The mean active joint count reported at the beginning of biologic therapies was 5.1 (S.D. 5.8) and decreased to 1.2 (S.D. 2.4; $P < 0.0001$) and 1.0 (S.D. 3.1; $P < 0.0001$) after 6 months and 1 year of therapy, respectively (Fig. 1A). Mean ESR was 33.9 mm/first hour (S.D. 25.3) at biologic treatment start and was of 22.0 (S.D. 24.0; $P < 0.001$) and 19.1 (S.D. 18.0; $P < 0.0001$) after 6 months and 1 year of treatment, respectively (Fig. 1B). The mean CHAQ/HAQ decreased from 0.8 (S.D. 0.7) at baseline to 0.4 (S.D. 0.5; $P < 0.0001$) at 6 months and 0.4 (S.D. 0.5; $P < 0.0001$) at 1 year (Fig. 1C). In accordance, the mean CRP was 2.4 mg/dl (S.D. 3.7) at biologic treatment start and of 1.2 (S.D. 3.3) at 6 months ($P = 0.043$) and 0.6 (S.D. 1.1) at 1 year ($P < 0.0001$). Patient global disease activity, evaluated by VAS, was 43.5 mm (S.D. 26.5) at baseline, 18.2 (S.D. 19.6) at 6 months ($P < 0.0001$) and 16.3 (S.D. 17.7) at 1 year ($P < 0.0001$).

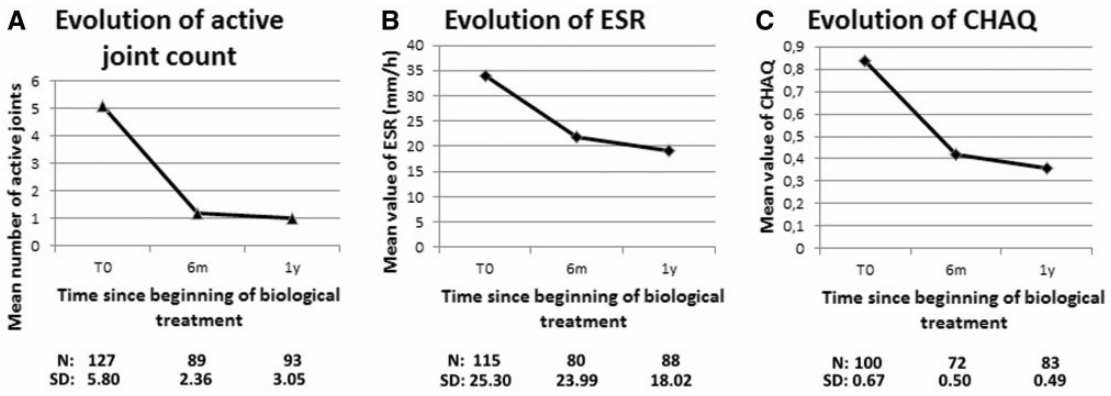
Table 1 Characteristics of JIA patients treated with biologic agents

	Patients ever treated with biologic agents
Total number of patients	227
Gender, female/male, *n* (%)/n (%)	147 (64.8)/80(35.2)
Age at disease onset, mean (S.D.), years	7.5 (4.9)
Disease duration, mean (S.D.), years	13.7 (10.1)
JIA categories fulfilled, *n*	206
JIA category not stated, *n*	21
Persistent oligoarticular, *n* (%)	20 (9.7)
Extended oligoarticular, *n* (%)	33 (16)
Polyarticular RF positive, *n* (%)	36 (17.5)
Polyarticular RF negative, *n* (%)	48 (23.3)
Systemic, n (%)	28 (13.6)
Enthesitis-related arthritis, *n* (%)	31 (15.1)
Psoriatic arthritis, *n* (%)	10 (4.8)
Unclassified, *n*	0

Table 2 Patient ($N = 227$) and disease characteristics treated with biologic agents

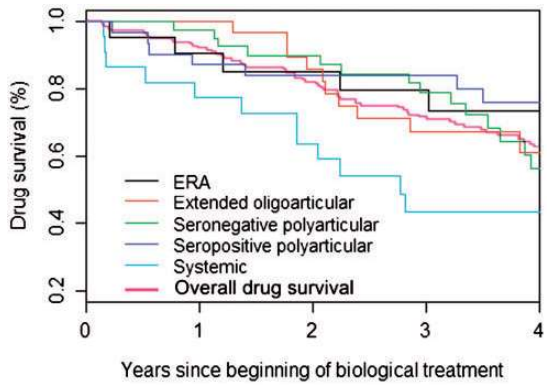
Characteristic	Value
Age, mean (S.D.), years	16.2 (9.4)
Total amount of biologic treatment exposure, mean (S.D.), years	4.5 (3.1)
Concomitant DMARD therapy, *n* (%)	181 (79.7)
Methotrexate	170 (93.9)
Sulfasalazine	16 (8.8)
Other DMARDs	11 (6.1)
First biologic treatment, *n* (%)	227 (100)
Etanercept	157 (69.2)
Adalimumab	29 (12.8)
Abatacept	8 (3.5)
Tocilizumab	2 (0.9)
Anakinra	11 (4.8)
Infliximab	19 (8.4)
Rituximab	1 (0.4)

FIG. 1 Evolution of the disease parameters in the first year of biologic treatment



Evolution of the (A) active joint count, (B) ESR and (C) CHAQ/HAQ in the first year after the beginning of biologic treatment. Act JC: active joint count; CHAQ: Childhood Health Assessment Questionnaire.

FIG. 2 Drug survival on the first biologic agent (Kaplan–Meier plot)



Safety analysis

The total length of exposure to the first biologic agent was 706.92 patient-years and, during the follow-up period, there were 1.98 events/100 patient-years. A total of 14 clinically significant adverse events (defined by the need for biologic treatment discontinuation) were reported, including infusion reaction (one patient), respiratory and urinary infections (six patients), inflammatory bowel disease (four patients), diarrhoea (one patient), tuberculin skin test conversion (one patient) and active tuberculosis (one patient).

There were no reported deaths or malignancies during the overall follow-up period of biologic treatment, 134 treatments were stopped for the following reasons: 14 (10.5%) due to an adverse event, 60 (44.8%) due to a lack/loss of efficacy (primary or secondary failure), 13 (9.7%) due to disease remission, 2 (1.49%) were lost to follow-up, 4 (3%) refused to continue treatment and 40 (29.8%) for other reasons (not specified).

Predictors of treatment response at 1 year

The mean reduction in JADAS10 after 1 year of treatment was 10.4 (S.D. 7.4) [median 9.9 (IQR 4.8–13.7)]. According to the definition of improvement using the JADAS10 score [16], 58 individuals had registered information of the variables that allowed determining JADAS10 response at 1 year of biologic therapy: 83.7% responded to therapy at 6 months of biologic treatment and 83.3% responded to biologic therapy after 1 year. These 58 individuals were comparable to the ones excluded regarding disease category ($P = 0.397$), however, they were younger (mean age 13.5 *vs* 17.3 years, $P = 0.013$) and more likely to be male (53% *vs* 30%, $P = 0.002$). Due to the small number of patients and high proportion of responders, there was no possibility of calculating the predictors of response to biologic therapy using a binary outcome.

Retention rate and predictors of drug discontinuation

A total of 179 patients were followed up for >1 year after the beginning of the first biologic therapy, and the median treatment duration was 5.8 years (IQR 4.8–8.3). The retention rates with the first biologic were 92.9% (CI 88.5, 97.5) in the first year, 85.5% (CI 79.5, 91.9) in the second year, 78.4% (CI 71.4, 86.1) in the third year and 68.1% (CI 59.7, 77.7) in the fourth year of treatment (Fig. 2).

Taking all JIA categories into consideration, only concomitant therapy with systemic corticosteroids at baseline was found to be crudely associated with withdrawal of biologic treatment [hazard ratio (HR) 1.93 (95% CI 1.13, 3.29), $P = 0.016$]. However, this association showed low statistical significance when adjusting for the other clinically relevant covariates [HR 1.47 (95% CI 0.64, 3.38), $P = 0.362$]. We found a higher risk of biologic drug withdrawal among systemic JIA patients [HR 2.32 (95% CI 1.19, 4.52), $P = 0.014$] compared with the polyarticular categories of disease. In addition, we failed to identify any predictors of drug discontinuation in the stratified analysis using the polyarticular categories of disease.

We analysed separately the subgroup of patients that started biologic therapies in childhood. In these patients, the retention rates were similar to those of patients who started biologic treatment in adulthood.

Discussion

This article presents the results from the Portuguese national register in which we consecutively included all patients with JIA treated with biologic therapies. The distribution of JIA categories in our registry is similar to those found in recently published inception cohorts [18–20–6]. These data prove, in a real-life setting with an unselected population, the sustained effectiveness and safety of biologic treatments in all JIA categories as highlighted by a high retention rate after 4 years of treatment. Etanercept was the most frequently used TNF inhibitor, most likely because it was the first biologic agent approved for JIA treatment [2].

There were few cases (20 patients) of persistent oligoarticular JIA treated with biologics, in agreement with the Portuguese guidelines for prescribing biologic therapy in JIA [13]. Paediatric rheumatologists often need to prescribe TNF blocking agents in oligoarticular JIA due to disabling active oligoarthritis or related to the higher prevalence of uveitis in this category. In multivariable models, uveitis was strongly and independently associated with non-biologic and biologic DMARD use. This implies that uveitis may frequently be the determining factor in the systemic treatment of children with oligoarticular JIA [18–21]. Nevertheless, there are no published sizable randomized studies of the systemic treatment of uveitis in children [24]. More research in this area is needed.

Biologic agents were safe during the overall follow-up period of treatment. Infections, particularly tuberculosis, are a concern for every physician prescribing biologic agents. In our study, and despite previous screening, there was one case of pulmonary tuberculosis associated with the use of a monoclonal antibody (adalimumab), and in one patient taking etanercept, we found a tuberculin skin test conversion that led to discontinuation of biologic therapy. Four patients were diagnosed with chronic inflammatory bowel disease (IBD) during biologic treatment, all with etanercept. Several other cases of new-onset IBD during etanercept use were reported [25–30]. The mechanism behind this effect is still unknown and more research is required in this field. We did not find any discontinuations associated with the development of psoriasis, lupus-like or other chronic inflammatory or autoimmune diseases. No cases of malignancy were observed.

We have observed a large gap between the mean age at disease onset of JIA patients ever treated with biologic agents [7.5 years (S.D. 4.9)] and the mean age for starting biologic therapy [16.2 years (S.D. 9.4)]: almost 10 years between disease onset and the beginning of biologic treatment. This could be related in part to the long disease duration of the adults with JIA starting biologics in adulthood and also to the retrospective insertion of these data in the registry, with a large proportion of patients being diagnosed before the approval of biologic therapies in national policies. Almost one-third of the patients included in this study started biologic treatment as an adult, a fact that is unusual in other JIA registries. Although innovative, we are aware that this could have introduced a bias in the results, since in patients with prolonged disease duration, the outcomes may be quite different from those of children starting biologics far earlier in their disease evolution. Although we believe that including adults with JIA brings an added value to our registry and data, the instruments to measure disease activity in JIA have never been validated for adults, and this should be taken into account when analysing the data. More studies including adults with JIA are necessary to validate these instruments in this population.

TNF inhibitors are not always effective or universally tolerated, which may lead to switching among biologic agents. In our study, 22.5% of the patients switched biologic treatment during their disease course. This proportion is higher than the ~10% reported from biologics registries in the UK [6] and the Netherlands [31], but is lower than the ~35% reported from Finland [32] and the 28% reported in the USA [18].

Patients with JIA had a high retention rate of biologic treatment in the first 4 years of therapy: 93% remained on treatment in the first year and 68% in the first 4 years of treatment. The retention rates found in our study were similar to the JIA British cohort [6]. The prolonged use of biologic agents suggests that for the majority of patients the drug was effective and well tolerated.

Although we found a crude association between treatment withdrawal and corticosteroid use, we found this association to be at least partly confounded by the CHAQ/HAQ score at baseline. In addition, this finding was not confirmed in the polyarticular category of JIA. Thus we cannot associate with certainty concomitant therapy with systemic corticosteroids and discontinuation of biologic treatment. We also found a higher risk of drug cessation among systemic arthritis patients, and this group has been proposed previously to be associated with a poorer response to etanercept [33, 34].

This study is purely observational and the sample might not be completely representative of the JIA population since most patients [526 (65%)] were from rheumatology centres in Lisbon. We choose the JADAS10 score as our outcome measure for treatment response, although this instrument has limitations, as pointed out by its authors. Due to the small number of patients and high proportion of responders, there was no possibility of calculating the predictors of response to biologic therapy.

The lack of follow-up information on limited joint count precluded the use of the ACR Pediatric response criteria. The JADAS calculation may have had some limitations in our study population. As the authors of the JADAS point out, although the score was designed to be robust enough to cover all categories of JIA, a thorough assessment of disease activity in children with systemic JIA requires quantification of extra-articular manifestations, particularly

fever and rash. Missing data and the small number of patients with available information for calculating response to treatment limited the use of multivariate models and stratified analyses by disease category. Because laboratory tests are frequently missing, in accordance with McErlane *et al.* [35], our group also tested the JADAS with and without ESR [36]. The correlation between the JADAS with ESR and JADAS without ESR (clinical JADAS or three-variable JADAS) was high ($r = 0.97$, $P = 0.0001$), indicating that when ESR is not available, the JADAS can be calculated without this variable, allowing the measurement of disease activity anytime and anywhere. Another limitation concerns the decision to report an adverse event, which is up to the treating physician, and physicians probably reported only the clinically relevant ones, which might have led to underreporting compared with controlled clinical trials.

Conclusion

Our data from Reuma.pt reinforce that biologic therapies seem effective and safe in all JIA categories. In addition, retention rates with the first biologic agent were high during the first 4 years of treatment.

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A.F.M.: conceptualized and designed the study; participated in the acquisition, analysis and interpretation of data; coordinated and supervised data collection; drafted the initial and final manuscript and approved the final manuscript as submitted. M.J.S., J.E.F. and H.C.: conceptualized and designed the study, carried out the initial analyses, critically reviewed and revised the manuscript and approved the final manuscript as submitted. F.M.M.' S.C.M.: carried out acquisition of the data and statistical analyses, reviewed and revised the manuscript and approved the final manuscript as submitted. J.A.M.G., F.O.R., S.F., M.S., M.G., S.C., J.A.C., I.B., C.D., C.F., A.L., A.R., G.S., J.C.B.: contributed in the acquisition of data, critically reviewed and revised the manuscript and approved the final manuscript as submitted.

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Disclosure statement

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DISCUSSION

The studies described in this thesis cover aspects of genetic susceptibility to JIA, clinical and genetic factors related to poor prognosis in JIA, and an evaluation of a clinical disease activity score and of the effectiveness, safety and retention rate of biological therapies in JIA.

In this final chapter we will summarize and discuss the main findings of the studies comprised in this thesis and also share our vision on research challenges in the field for the upcoming years.

In **Parts I and II** we have investigated genetic factors involved in susceptibility to JIA and disease activity.

In our first work (**Part I**) (35) we have studied selected polymorphisms in the TNF-308 position, in accordance with our previous observations in rheumatoid arthritis patients (141,142). We have found that TNF –308 genotype frequencies were similar between JIA patients and controls, suggesting that polymorphisms in the TNF-308 position do not appear to have a relevant role in susceptibility to Portuguese patients with JIA. In addition, the presence of the TNF-308A allele was associated with higher level of inflammatory activity, revealed by higher ESR values and serum TNF levels, and also with a trend for a lower functional capacity and higher disease activity values.

In previous studies from our group focused on Portuguese patients with RA, we have found a positive association between this same polymorphism with work disability, radiographic progression (141) and worse response to anti TNF treatments (142). Our present results were coherent with these data and reinforced the relevance of the –308 polymorphisms in arthritis activity and severity in the Portuguese population. In agreement with our observation, in a study performed in Turkish and Czech patients, the –308A allele was significantly associated with a poor outcome in the Turkish group ($p = 0.005$), but there was no association in the Czech patients (143). Similarly, a recent work by Scardapane et al (107) showed that in a sample of 74 patients including all JIA subtypes, those carrying the TNF -308

GA/AA genotypes were associated with a worse prognosis and with a lower response to anti-TNF drugs.

Interestingly, in our study (35) nearly one-third of the patients had a relative with a rheumatic disease, which reinforces the role of genetic factors in these diseases.

Subsequently, we have enlarged the patient's sample and aimed to confirm whether 15 SNPs of selected genes, found in previous studies to be associated with an increased risk for the development of JIA, were also associated with susceptibility for JIA in the Portuguese population (**Part II**).

Our results support the relationship between polymorphisms in PTPN2 gene and the risk of RF-positive PolyJIA, polymorphisms in PTPN22 gene with extended OligoJIA and polymorphisms in ANGPT1 gene with susceptibility to SoJIA. These results are concordant with the current state-of-the art underlining the genetic heterogeneity of JIA categories.

In accordance with other immune mediated inflammatory chronic diseases, there are many differences between the studies that evaluate susceptibility locus in JIA: Thompson et al (26) examined a cohort of 809 JIA cases of non-Hispanic European ancestry and reported that PTPN2, COG6 and ANGPT1 were associated with oligoarticular and RF-negative polyarticular JIA. Still in this study (26), and similarly to other susceptibility studies of JIA (32,38,144), the SNP in PTPN22 gene (rs2476601 A/G) that we have found associated with the risk of extended OligoJIA, was associated with oligoarticular and RF-negative polyarticular JIA. Dimopoulou et al (34) demonstrated that the same PTPN22 polymorphism marker was associated with JIA in a Greek population but not with our SNP in PTPN2 gene. In our study the same SNPs analyzed in PTPN2 and ANGPT1 genes were associated with RF-positive PolyJIA and SoJIA, respectively. These discrepancies of genetic associations across different racial or ethnic groups underlines the importance of assessing genetic variants in different populations, even within Europe, to conclusively define the genetic architecture of JIA and the magnitude of the effects of specific risk alleles in different populations (34).

Still in our study, a SNP in ANGPT1 gene was associated with SoJIA. As previously mentioned, it is thought that SoJIA is different from the other subtypes due to its lack of a strong MHC

association, presence of cytokine disregulation and various innate immune system abnormalities (145). It has already been reported that SoJIA is associated with SNPs within genes such as IL10 (146), IL6 (53,147) and SLC26A2 (148). While our understanding on the genetic susceptibility to oligoarticular and RF-negative polyarticular JIA is rapidly improving due to recent focus and large, well-powered studies (32), SoJIA remains a relatively poorly understood subtype. This is being addressed in a large multi-national GWAS of SoJIA that is currently under way, which may help to shed light into the complexity of this disease subtype.

In **Part III** (149) we have identified predictors of poor prognosis in patients with JIA. Identifying earlier JIA cases with a worse prognosis is crucial to start appropriate treatment and to correctly inform patients and their parents. As mentioned, much effort has already been done to elucidate clinical prognosis predictors. However, studies that evaluate other than early clinical predictors, such as genetic or immunological parameters, hardly exist. It is hoped that by studying the genetics of JIA outcomes, not only will we increase our understanding of the pathology of the disease, opening new treatment opportunities, but this will also enable us to identify earlier in the course of the disease those children likely to go on to experience more severe long-term outcomes, allowing a targeted care that will prevent the development of long-term disability (18).

Our study assessed the genetic determinants of poor outcome in Portuguese patients with JIA. As a secondary aim, we have identified clinical predictors of poor prognosis.

Using a large Portuguese sample of patients with JIA, we have not found genetic associations with a poor outcome. Polyarticular categories of JIA, longer duration of DMARD treatment and higher physician assessment of disease activity were significantly associated with poor prognosis. Additionally, patients with a poor prognosis were less likely to have persistent OligoJIA. Our work is in accordance with other studies that revealed that children with persistent OligoJIA have a substantially better outcome than those with either SoJIA or PolyJIA with regard to remission, disability and structural damage (97,98,150).

We are aware that there were some limitations of this study, namely the definition used to determine poor prognosis. There is no universal definition of "poor prognosis" in patients with JIA. So, we choose to integrate in our definition of "poor prognosis" two variables: 1. an instrument that combines disease activity and damage (CHAQ): in accordance with other studies (105,109,151–154) we have dichotomized the score, using 0.75 as the cut-off point; 2. the need of biological treatment, because patients that do not respond to conventional DMARDs, namely methotrexate, have a higher chance of a poor outcome. With respect to this last point, our study included patients at different time periods and we are aware that the access to biological therapy could have not been the same for all patients, leading to a selection bias. In addition, the indications for biological therapy could also be a confounder: patients could have been on biological therapy for extra-articular manifestations of the disease (for example uveitis) and not due to joint disease. Beyond the concerns in the definition of poor prognosis, this was the first genetic study of prognostic factors in Portuguese patients with JIA.

Another limitation of our study was the problem of multiple comparisons: we cannot completely exclude that some of the identified associations are attributable to chance. Again, the sample size in our cohort was too small to adequately test replication and a further study in a larger cohort in different populations of JIA patients is still required in order to confirm or refute our findings. Even so, our study gives a contribution to the urgent need for studies of genetic variants associated with poor prognosis.

In **Part IV** (155), we evaluated the correlation between JADAS27-ESR and JADAS27-CRP, tested the agreement between both scores for classifying each disease activity state and checked the correlation between JADAS27-ESR and JADAS27 without ESR (clinical JADAS). The evaluation of disease activity is a crucial component of the management of children with JIA because persistently active disease plays a major role in causing joint damage and physical disability, determining prognosis (71). Thus it is very important to have validated and simplified scores that can be used both in the daily clinical practice, where frequently laboratorial results are not immediately available, particularly ESR, or when assessing databases where also laboratorial variables are frequently missing.

JADAS was initially developed using the ESR because CRP values were not available in all databases used to validate the tool (71). However, as the authors of the JADAS highlighted, CRP level is a direct measure of the acute-phase response and is less confounded by other factors when compared to ESR. In RA, the performance of the Disease Activity Index of 28 joints (DAS28) based on CRP level has been shown to have a similar profile to DAS28 based on ESR (156,157). Similarly, Nordal et al (158) showed that a JADAS27 version including the CRP level instead of the ESR performed similarly to the original format (159), indicating that both scores can be recommended for assessing disease activity in JIA. In accordance with this work, we have also shown that the JADAS27 based on CRP level correlated closely with the JADAS27-ESR, indicating that both measures can be used in clinical practice. In addition, we have tested the published cutoff criteria for classification of inactive disease, minimal disease activity, parent's acceptable symptom state (80), and active disease, to analyze whether patients were classified in the same state using either JADAS27-ESR or JADAS27-CRP. The agreement between JADAS27-ESR and JADAS27-CRP across the 4 activity states was very good, showing agreement in 91.1% of the observations, reinforcing that clinicians can use both measures to calculate the JADAS without changing the categories in which the patients are classified.

Additionally, clinical JADAS27 (cJADAS or JADAS3) also correlated well with JADAS27-ESR and JADAS27-CRP ($r = 0.97$, $P < 0.0001$), indicating that when ESR is not available cJADAS27 can be calculated without any acute phase reactant (155). The clinical JADAS27 can therefore be used to conduct a disease activity evaluation anytime and anywhere. In accordance with our results, McErlane et al have recently demonstrated that for the majority of JIA categories, clinical applicability of JADAS would be improved by exclusion of ESR and that the amended score (JADAS3-71), which omits the ESR, correlates well with JADAS-71 (159).

In our last study (**Part V**) we have assessed the effectiveness of biological therapies in daily-life clinical setting of patients with JIA.

Biological treatments hold the promise of transforming the outcome of JIA from severe joint damage with disability and prolonged active disease to normal joint function with early and sustained remission (134). A good effectiveness and safety profile, combined with a high

retention rate are pivotal for achieving this promise. National registries, such as Reuma.pt, provide valuable data for these type of evaluations.

Using the database Reuma.pt (139), we have seen that overall, the distribution of JIA categories in our registry was similar to those found in other cohorts (109,160,161). Our data proved, in a real-life setting with an unselected population, the sustained effectiveness and safety of biological treatments in all JIA categories as highlighted by a high retention rate after 4 years of treatment. Noteworthy, we have observed a large gap between the mean age at disease onset of patients ever treated with biological agents (7.5 ± 4.9 years) and the mean age for starting biological therapy (16.2 ± 9.4 years): almost ten years between the disease onset and the beginning of biological treatment. This could be in part related to the long disease duration of the adults with JIA starting biologics in adulthood and also to the retrospective insertion of some data in the registry, with a large proportion of patients being diagnosed before the approval of biological therapies for JIA. Almost one third of the patients included in this study started biological treatment already in adult age, fact that is not usual in other JIA registries. Although innovative and relevant for the clinical practice, we are aware that this could have introduced a bias in the results, since in patients with prolonged disease duration the outcomes may be quite different to those of children starting biologics far earlier in the disease evolution. Despite our belief that including adults with JIA brings an added value to our registry and data, the instruments to measure disease activity in JIA have never been validated for adults and this should be taken into account when analyzing the data.

In what concerns to safety, the occurrence and exacerbation of infections are a major concern for every physician prescribing biological agents. The incidence of serious infections is low throughout all clinical trials performed in JIA patients. The rates varied from 3 to 10/100 patient-years in patients receiving etanercept, 2 to 14/100 patient-years with adalimumab, 5 to 13/100 patient-years with tocilizumab and seem to be lower in patients treated with abatacept (162). In registries, the rate of serious infection is higher in patients receiving biologics compared to patients treated with methotrexate (MTX) (162). Particularly, tuberculosis is a major concern in patients treated with TNF antagonists. While screening

strategies limit the risk for reactivation of tuberculosis primary infection can occur. A single JIA patient developed tuberculosis across a number of clinical trials. This patient was exposed to infliximab (163). Registries covering several thousands of JIA patients also very rarely report tuberculosis. A single tuberculosis case was reported in the Polish Etanercept registry (164) but no case so far was reported from Germany (165), which may simply reflect the lower incidence of tuberculosis in Western European countries compared to Eastern European countries.

The incidence rate of clinically significant adverse events (including serious infections) in our registry was 1.98/100 patient-years during the whole follow-up period, with an incidence rate of infections of 1.13/100 patient-years. The majority of adverse events did not lead to drug switching or discontinuation. There was one case of pulmonary tuberculosis associated with the use of a monoclonal antibody (adalimumab), and, in one patient taking etanercept, we found a tuberculin skin test conversion that led to discontinuation of biological therapy. The low number of clinically significant adverse effects that we have seen in our cohort was probably related to the fact that the decision to report an adverse event was left to the discretion of the treating physician and physicians probably reported only the clinically relevant ones, which might have led to underreporting compared to controlled clinical trials.

To gain further knowledge about risk profiles, national and international collaboration for the accumulation of long-term data should be encouraged. A large-scale international project to collect data about long-term safety and efficacy of biologics currently used in the treatment of JIA is ongoing (the Pharmachild registry, pharmacovigilance in JIA patients treated with biologic agents and/or methotrexate) and Reuma.pt is contributing to it. Another source of data can be the US based CARRA registry (166).

CONCLUSIONS AND FUTURE PERSPECTIVES

The results presented in this thesis allowed us to identify genetic predictors of susceptibility to specific categories of JIA that combined with clinical data might be useful for the construction of risk scores for the early diagnosis of JIA, in the context of unspecific symptoms, such as arthralgia or reported episodes of joint swelling not confirmed by physical inspection and imaging. Although we did not find any genetic predictor of poor prognosis, we found clinical variables that can be related with poor prognosis, suggesting that further efforts should be made in the elaboration of an exclusively clinical prognostic score for JIA. We also proved that the JADAS27-ESR and JADAS27-CRP correlate closely, and both classify patients similarly regarding disease activity state, indicating that both measures can be used interchangeably in clinical practice. In addition, clinical JADAS was also well correlated with JADAS-ESR and JADAS-CRP. This may have important implications for daily clinical practice, where time is limited and blood tests are not always available on time and might also save some unnecessary blood collections that often are not well tolerated by children. In addition, these also provide support for the calculation of JADAS in databases with laboratorial missing values. Lastly, in a real-life setting with an unselected population, we have demonstrated the sustained effectiveness and safety of biological treatments in all JIA categories, together with a high retention rate after 4 years of treatment.

One of the limitations common to all of our studies was the sample size of each one of the seven categories of JIA: within the limits of a reasonable recruitment phase, the sample sizes in single-center or national studies are usually too low to conduct controlled or even observational studies in different categories of JIA. Even though, our patient's sample is comparable to that of studies conducted in larger countries.

There is still much work to be done in order to have a comprehensive understanding of the genetic architecture of JIA. The coming years will hopefully provide insight into important pathways involved in the disease, will identify genes implicated in outcomes such as disability and pain and also genetic predictors of response to treatments such as methotrexate and biological therapies.

While there is clearly a need for increased focus on the genetics of the less frequent JIA subtypes, our current understanding already begins to define a picture of their distinct genetic landscapes.

Other challenges related to the prognosis of JIA include the identification of biomarkers that can predict response to specific therapies, the selection of patients who will not need biological therapy and/or are more likely to have adverse events, the detection of patients who are more likely to fail TNF blockade therapy and benefit from the earlier use of other agents. Another unmet need is the definition of criteria for choosing the best biological agent, the adequate dose and treatment duration for each patient. A search for additional genetic variants that might affect the prognosis of JIA in different populations is still of interest. Moreover, combination of genetic factors together with clinical risk factors should also be considered with the aim of creating scores that might be clinically useful.

Our group is currently undertaking a preliminary analysis of the association between 18 serum biomarkers with disease activity in JIA. Biomarkers are already used in many areas of clinical practice, but most biomarker studies focus on adults rather than children. Data from these studies are sometimes extrapolated to children without considering differences in disease pathogenesis, age-dependent changes in reference ranges for biological laboratory measures, growth and development of children over time, effect of ontogeny on disease evolution and response to treatment, and changes in phenotypic gene expression (167,168). Despite the huge potential of pediatric biomarkers, for JIA there are currently no validated pediatric biomarkers available to help in setting up a tailored or “personalized” approach on which drug choice can be based. A more tailored approach would be beneficial for patients because it could facilitate disease remission at an earlier disease stage, which would reduce burden of disease, limit side effects, and improve quality of life (169). We aim to give a contribution to increase the knowledge of this expanding field of biomarkers in JIA.

In conclusion, it is a very exciting time in JIA research, where recent improvements across the broad fields of genetics, immunology and imaging are enabling us to better understand JIA. The identification of predictors of prognosis and biomarkers will be crucial for the future

management of this disease. We hope that in the near future it will be possible to personalize and adjust the adequate treatment for each individual patient.

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