Possible environmental determinants of juvenile idiopathic arthritis

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Abstract

Like other autoimmune diseases, including adult RA, risk of developing juvenile idiopathic arthritis (JIA) is thought to be determined by a complex combination of genetic and environmental factors. Although some predisposing JIA genes are now being identified, research aimed at identifying environmental influences lags behind most other autoimmune conditions. Here we review research to date, from which some evidence has been generated to support a role for breastfeeding, infection and maternal smoking in determining JIA risk. We also propose further hypotheses worthy of testing, based on knowledge acquired for other autoimmune diseases. These include the role of vitamin D and sun exposure, and the role of early-life infection (‘the hygiene hypothesis’) in determining risk. Finally, we discuss future directions including practical study designs to more comprehensively test hypotheses and provide new insight into this important area of research.

Key words: Breastfeeding, Hygiene hypothesis, Infection, Maternal smoking, Sun exposure, Vitamin D.

Introduction

The epidemiology of autoimmune disease is a topic of growing focus. The majority of autoimmune diseases in adults and children are considered complex in aetiology, with risk conferred by both genes and the environment. Some autoimmune diseases, such as type 1 diabetes (T1D), have markedly grown in incidence during the past few decades, more swiftly than would be expected by genetic mechanisms alone. This suggests that changes to the environment that have occurred over the same period have likely contributed to the increased incidence. Environmental risk factors may also be more amenable to population-level intervention than genetic risk factors. Hence, there has been much interest in uncovering the environmental risk factors.

One of the less studied of the autoimmune diseases is juvenile idiopathic arthritis (JIA). JIA constitutes a heterogeneous group of conditions that have chronic inflammation in one or more joints in common, in children aged ≤16 years at onset. Over the past few decades, the classifications used for JIA have changed and, along with it, the commonly used term to describe this disease group. Previously, JIA had been referred to as juvenile arthritis (JA), JCA and JRA. These descriptors have been altered as the classification system has become more clinically refined. JIA is the term now recommended by the ILAR, and coincides with the ILAR’s classification of JIA into seven subtypes based on the number of affected joints, biochemistry and associated comorbidities [1]. Oligoarticular JIA refers to four or fewer affected joints, and may be further subcategorized into persistent (stable after 6 months of disease) or extended (increasing to more than four affected joints after 6 months of disease). Polyarticular JIA refers to the involvement of more than four joints, and is further subcategorized by the presence or absence of the RF. The remaining categories are systemic JIA, enthesitis-related JIA and psoriatic JIA. These changing classifications, along with the fact that paediatric rheumatology is a relatively new subspecialty, probably account for the fact that research aimed at risk-factor identification has been lacking in comparison with many other autoimmune diseases such as adult RA, T1D and multiple sclerosis (MS).

In this review, we consider what is currently known regarding risk factors for the development of JIA, with a particular focus on environmental risk factors. We propose some testable hypotheses for additional...
risk factors based on knowledge generated for other autoimmune diseases, and present a study currently underway, which has developed a strong focus on the identification of environmental, in addition to genetic, risk factors for JIA.

**Variation in place, time and person**

There is a reasonable body of work, produced over several decades, describing estimates of incidence and prevalence of JIA. The studies conducted have used a variety of diagnostic criteria, sampling approaches (clinic- and community-based), sample sizes and ethnic groupings. A review by Manners and Bower in 2002 [2] presented the results of 34 studies conducted since 1966. Across these studies, prevalence ranged from 0.07 to 4.01/1000 children, and annual incidence ranged from 0.008 to 0.226/1000 children. The highest rates were derived by studies using a community-based sampling approach; for example, clinical assessment of children in classrooms or homes. This is likely to be due to the presence of undiagnosed children in the community with mild illnesses that have never presented to clinics. Other factors contributing to the large variation may include a lack of consistency in case definition across the time period covered by the review, and the predominance of small studies that may have introduced random fluctuations [2].

Another important consideration in comparing regional prevalence rates is population ethnicity. With some exceptions, the majority of the studies reviewed by Manners and Bower were conducted in Caucasian populations from Europe, the USA and Canada. However, in the non-Caucasian populations the incidence and prevalence rates were among the lowest reported. For example, the annual incidence of JIA was found to be 0.0083/1000 children, and annual incidence ranged from 0.008 to 0.226/1000 children. The highest rates were derived by studies using a community-based sampling approach; for example, clinical assessment of children in classrooms or homes. This is likely to be due to the presence of undiagnosed children in the community with mild illnesses that have never presented to clinics. Other factors contributing to the large variation may include a lack of consistency in case definition across the time period covered by the review, and the predominance of small studies that may have introduced random fluctuations [2].

A complex genetic disease is one which has been shown to have a heritable risk component, but that does not demonstrate a single gene-based Mendelian inheritance pattern. The risk of developing the majority of complex diseases is thought to be influenced by a combination of multiple genes and environmental factors.

The evidence for a heritable component to JIA risk comes mainly from twin and family studies, which demonstrate an increased prevalence of JIA in twins and siblings of JIA probands [11, 12]. In monzygotic twins, concordance rates have been estimated in the range of 25–40% [13], and among twins who are both affected with JIA, a much higher than expected proportion are monzygotic [14], suggesting an important role for shared genetic composition. In affected non-twin sibling pairs, evidence suggests that there is significant concordance between the siblings in terms of age at onset, and disease course, with a prevalence 15–30 times that of the general population [11, 15].

These twin studies also indicate the important contribution of the environment. Monozygotic twins share an identical underlying genetic make-up at a DNA sequence level. If the risk of JIA were determined solely by genetics, it would then be expected that monozygotic twins would also share the identical risk of developing JIA. The concordance rates quoted above are far from 100%, indicating that certain environmental influences that the twins are individually exposed to must also impact on the JIA risk. Thus, there appears to be a complex interplay between the genetic pre-disposition and environmental influence at work, and it is the varied combination of these factors from individual to individual that determines disease risk within families and across populations.

**Genetic risk factors**

A thorough review of the current knowledge of genetic risk factors for JIA has recently been published elsewhere [12], and does not fall within the main focus of this review. However, in summary, efforts to identify genes contributing to the JIA disease risk are gaining pace, with reports describing good sample sizes, reasonable levels of statistical power and replication of findings in independent populations now appearing in the literature. While many
candidate genes have been associated with JIA over the past two decades, the findings of older studies have often failed to replicate, in larger, more well-designed studies appropriate for the identification of genes, those that confer modest risk. However, a few genes have now been identified with considerable certainty. The genes of the HLA region of the MHC on chromosome 6 appear to confer the largest contribution to this risk [12], although the exact HLA genes and risk alleles remain to be precisely defined. Strong HLA associations are common to many autoimmune diseases, including T1D and RA [16]. Outside the HLA region, the gene with the most robust evidence for association is PTPN22 (encoding a lymphoid tyrosine phosphatase) [17]. Again, this gene has been associated with multiple autoimmune diseases including T1D and RA [16]. Very recently, a replicated association with a novel gene, VTCN1, was reported [18], along with evidence to support the involvement of the IL-2RA/CD25 (encoding IL-2 receptor α) [19], and the TRAF1-C5 locus (encoding TNF receptor-associated factor 1 and complement component 5) in the pre-disposition to JIA [20].

Environmental risk factors

Genetic risk factor identification in JIA, when compared with many other autoimmune diseases, has received modest research attention. However, the recent establishment of sufficiently large cohorts capable of robustly identifying genes through genome-wide association studies or candidate gene approaches bodes well for significant advancement in this area in the next few years. In contrast, research to identify environmental risk factors has been strikingly sparse. Over the past 15 years there have been only a handful of studies, and the outcomes of such studies have not generally been replicated in independent populations.

Barriers to environmental risk factor research

There have been a number of barriers to advancing research in this area. Most of these barriers are common to many autoimmune diseases, but are heightened in JIA due to the fact that the subspecialty of paediatric rheumatology is relatively new and remains poorly resourced internationally.

Barriers include, but are not limited to:

- the changing subtype classification system, preventing comparison of studies over time;
- low disease incidence precluding prospective collection of pre-disease environmental data and limiting sufficient case sampling at diagnosis in a timely fashion;
- the need to separately consider subtypes of JIA, which necessitates the consideration of even smaller subgroups of cases within already small studies; and
- the selection of appropriate paediatric controls that represent the population from which they are drawn, but which also allows for collection of biospecimens for direct measures of exposure.

Here we review the limited past studies in approximately sequential order (summarized in Table 1). To gather these data, we performed searches of the NCBI PubMed database using the search term ‘juvenile arthritis’ (to encompass the various terminology over time) with terms including ‘environment’, ‘epidemiology’, ‘aetiology’, ‘cause’, ‘determinant’ and ‘risk’. We also scrutinized the bibliographical lists of identified papers and searched for ‘juvenile arthritis’ with specific environmental factors known to be associated with other autoimmune diseases. We acknowledge that studies captured by this approach may not be entirely exhaustive. Note that we have retained the disease terminology used in the original papers. We then turn to knowledge generated through research into other autoimmune diseases, which might assist in formulating hypotheses testable in JIA studies designed to capture relevant data. The further identification of environmental risk factors, and indeed examination of how they interact with genetic factors, would significantly enhance our understanding of the JIA aetiology.

Breastfeeding

In response to reports in the 1980s of a relationship between breastfeeding and the development of T1D [33] and Crohn’s disease [34], three studies aimed at determining the role of breastfeeding in the JIA disease risk were published between 1995 and 1998 [21–23]. Mason et al. [21] conducted a study to analyse the relationship between breastfeeding and the development of JIA. Published in 1995, the study involved 54 US children with JRA diagnosed by a paediatric rheumatologist and defined by criteria developed in 1977. Case participants were asked to nominate two playmates of similar age and ethnicity to serve as controls, and 79 were recruited into the study. A phone survey was conducted with each case and control participant to retrospectively collect data on breastfeeding, including duration. Statistically, cases were less likely to have been breastfed than playmate controls [odds ratio (OR) 0.40; 95% CI 0.20, 0.81], but particularly for those with pauciarticular (now referred to as oligoarticular) JRA (OR 0.31; 95% CI 0.20, 0.93), indicating a possible protective effect of breastfeeding on the risk of JRA development. The OR for all cases vs controls decreased with the longer duration of breastfeeding (0–3 months; OR 0.56; 95% CI 0.23, 1.14; >3 months; OR 0.28; 95% CI 0.10, 0.67) [21].

Potential problems with the study by Mason et al. included low statistical power, selection bias (particularly the degree to which the controls accurately reflected the pattern of breastfeeding in the general population) and possible measurement bias pertaining to recall. However, overall, the study suggested that children with JRA were less likely to have been breastfed as an infant [21]. In a subsequent study by Rosenberg [22], 137 children with JRA drawn from a population survey of childhood rheumatic disease in Canada were compared with 331 healthy control children who were nominated by case parents. Data on breastfeeding occurrence and duration were collected via a self-administered
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<td>Mason et al. [21] NC, USA PRD cases from single centre, N=54 (n=28 pauci, n=24 poly, n=2 systemic)</td>
<td>Playmate controls (up to two per case), n=79</td>
<td>PRD JRA (1977 ARA criteria): - Pauciarticular - Polyarticular - Systemic</td>
<td>Retrospective phone survey: Ever breastfed Duration of breastfeeding (a) None (b) 0–3 months (c) &gt;3 months</td>
<td>Nil</td>
<td>(i) 0.40 (all) 0.20, 0.81 (ii) 0.31 (pauci) 0.20, 0.93 (iii) 0.60 (poly) 0.21, 1.70 (iv) 0.06 (pauci) 0.21, 1.70 (v) 0.56 0.23,1.14 (vi) 0.28 0.10, 0.67</td>
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<td>Rosenberg [22] Canada PRD cases from single centre, N=137 (n=88 pauci, n=49 poly)</td>
<td>Unmatched controls (selection undefined), n=331 Parent-selected age and sex-matched controls, N=77 (n=54 matched to pauci cases, n=23 matched to poly cases)</td>
<td>PRD JRA (1977 ARA criteria): - Pauciarticular - Polyarticular</td>
<td>Parent-administered questionnaire: Ever breastfed Duration of breastfeeding Introduction of cow’s milk before 1 year</td>
<td>Nil</td>
<td>Unmatched: (i) P (x²) = 0.20 (all) P (x²) = 0.01 (pauci) P (x²) = 0.006 (poly) (ii) P (t) = 0.98 (pauci) P (t) = 0.23 (poly) (iii) P (x²) = 0.15 (pauci) P (x²) = 0.99 (poly) Matched: (i) 2.17 (pauci) 1.17 (poly) 0.33, 4.2 (ii) 0.24 (pauci) 0.06, 0.99* 0.33, 3.55 (iii) 0.79 (poly) 0.16, 3.75 0.19, 13.5 (iv) 0.59 (pauci) 1.82 (oligo vs healthy controls) 0.16, 47.6</td>
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<td>Kasapcopur et al. [23] Turkey PRD cases from single centre, N=53 (n=26 oligo, n=18 poly, n=9 systemic)</td>
<td>Disease controls (primary nephritic syndrome), n=32 healthy controls not further defined n=54</td>
<td>PRD JRA (1986 ARA criteria): - Oligoarticular - Polyarticular - Systemic</td>
<td>Investigator-administered interview: breastfeeding duration, months</td>
<td>Nil</td>
<td>1.5 (oligo vs disease controls) 0.19, 13.5</td>
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<td>Soderlund et al. [24] Finland Cases referred for JCA treatment, n=37</td>
<td>Joint trauma controls, n=27</td>
<td>Chronic arthritis in at least one joint (criteria not further defined)</td>
<td>(i) Parvovirus B19 DNA in SM (ii) Parvovirus B19 IgG in serum</td>
<td>NA</td>
<td>(i) P (x²) = 0.11 (ii) P (x²) = 0.44</td>
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<td>Oguz et al. [25] Turkey Cases with acute arthritis, n=75</td>
<td>Outpatient clinic healthy controls, n=75</td>
<td>(i) Acute arthritis (ii) Progression to chronic arthritis (&gt;6 weeks) (iii) Progression to JRA (&gt;6 months) (ARA criteria, year undefined)</td>
<td>Parvovirus B19 IgM in serum</td>
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<td>(i) P (x²) = 0.003 vs controls (ii) P (x²) = 3.7 x 10⁻⁵ vs acute (iii) P (x²) = 0.03 vs acute</td>
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<td>Gonzalez et al. [26] Chile JIA cases drawn from single paediatric immunology clinic, 1993–96, n=50</td>
<td>Otolaryngology ward patient controls from same site, free of acute and chronic illness related to parovirus, n=39</td>
<td>Diagnosis defined by ARA 1997/EULAR 1978 criteria: - Pauciarticular - Polyarticular - Systemic - ARA 1987 criteria for active disease ILAR 1997 definitions: - Early onset pauciarticular - Non-early onset pauciarticular - Polyarticular - Psoriatic - Enthesitis associated - Systemic</td>
<td>Parvovirus B19: (i) Serum IgG (ii) Serum IgM (iii) Serum DNA</td>
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<td>(i) P (x²) = NS (ii) P (x²) = 0.01 (iii) P (x²) = 0.01</td>
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<td>Weisbrich et al. [27] Germany PRD cases from single centre, 1986–2001, N=172 (n=67 early onset pauci, n=13 non-early onset pauci, n=19 poly, n=54 enthesitis associated, n=11 psoriatic, n=8 systemic)</td>
<td>Children referred to endocrinology or paediatric rheumatology hospital unit presenting without complaint associated with arthropathy or infection, n=146</td>
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<td>Serum B19 IgG</td>
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<td>(i) P (x²) = 0.05 (ii) P (x²) = NS (iii) P (x²) = NS (iv) P (x²) = NS (v) P (x²) = NS (vi) P (x²) = NS</td>
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<td>Massa et al. [28]</td>
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<td>Cases aged 3–24 years with active oligoarticular JIA and serological evidence of past EBV infection, drawn from undefined source, n = 17</td>
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<td>ILAR 1997 criteria for oligoarticular JIA</td>
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<td>(i) P (U) = NS (ii) P (U) &lt; 0.05</td>
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<td>Feldman et al. [29]</td>
<td>Canada</td>
<td>PRD systemic JRA cases drawn from 11 of 15 tertiary care paediatric rheumatology clinics across Canada, 1980–88, n = 221</td>
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<td>Onset (date, season, region) of systemic JRA (pre-1995 definitions)</td>
<td>(i) Season (ii) Region-specific viral incidence data</td>
<td>(i) NS (ii) NS</td>
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<td>Nielsen et al. [30]</td>
<td>Denmark</td>
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<td>Controls (four per case) matched for sex, age, county of residence, n = 880</td>
<td>Diagnosis based on EULAR criteria</td>
<td>(i) Only child (ii) High income (iii) Urban living</td>
<td>(i) 1.6 (ii) 1.9 (iii) 2.7</td>
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<td>Carlens et al. [31]</td>
<td>Sweden</td>
<td>Nationwide register-identified JIA cases, 1973–2002, n = 3334</td>
<td>Birth register identified controls (four per case) matched by sex, year, delivery unit, n = 13 336</td>
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<td>Prospective register-recorded data on: (i) Maternal age (ii) No. of older sibs (iii) Season of birth (iv) Caesarean delivery (v) Birth weight (vi) Gestational age (vii) Maternal smoking (viii) Apgar 5 mins (ix) Hospitalized for infection before 1 year: (a) Any (b) Respiratory (c) Gastrointestinal (d) Skin</td>
<td>Multivariate analyses</td>
<td>(i) 1.0 (ii) 0.9 (≥ 3 vs 1) (iii) 1.1 Oct-Dec vs Apr–Jun (iv) 1.1 (v) 0.9 (vi) 1.0, 1.3 (vii) 0.8, 1.1 (viii) 0.7 (≤ 6 vs 9–10) 0.5, 1.0 (ix) 1.9 (a) 1.7, 2.1 (b) 2.0, 2.4 (c) 2.3 (d) 1.5 (i) 1.00 (ii) 0.87, 4.66 (iii) 0.89 (iv) 0.90 (v) 0.11, 7.21 (vi) 1.1, 2.2</td>
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<td>Jaakkola and Gissler [32]</td>
<td>Finland</td>
<td>Nationwide register-identified JRA cases, singleton births born 1987, followed 1987–94, n = 31</td>
<td>Individuals from nationwide register, singleton births born 1987, followed 1987–94, no diagnoses of arthropathy, n = 47 845</td>
<td>International Statistical Classification of Diseases: ICD-9 code 714.3</td>
<td>Maternal smoking during pregnancy Birthweight, pre-term delivery, birth</td>
<td>(i) Boy, none (ii) Girl, none (iii) Boy, less than 10/day (iv) Girl, less than 10/day (v) Boy, 10 or more/day (vi) Girl, 10 or more/day</td>
<td>(i) Ref (ii) 2.02 (iii) 0.87, 4.66 (iv) 0.89 (v) 0.90 (vi) Maternal age, (vii) 0.11, 7.90 (viii) 0.11, 7.90 (ix) marital status, (x) socio-economic status</td>
<td>6.79, 22.9</td>
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Bold type is used to indicate statistically significant associations. PRD: Paediatric Rheumatologist diagnosed; mo: months; JIA: juvenile idiopathic arthritis; NS: non-significant; P(t): P-value from t-test; P (χ²): P-value from chi-square analysis; P (U): P-value from Mann–Whitney U test. *As stated in reference, however, CIs do not encompass OR value.
parent questionnaire. In relation to breastfeeding occurrence, no significant differences were observed between total JRA cases and healthy controls (68 vs 62%), or between polyarticular arthritis cases (n = 49) and healthy controls (53 vs 62%). An excess of breastfeeding was observed among cases with pauciarticular arthritis (n = 88; 76% pauciarticular cases vs 62% controls; P = 0.01). When pauciarticular arthritis cases were compared with polyarticular arthritis cases, a higher proportion were breastfed (76 vs 53%; P = 0.006). The authors state that, after adjustment for multiple testing, no significant differences between any of the groups were identified, and they note that had such adjustments been applied to the results of Mason et al. no significant differences would have been apparent [22]. However, in contrast to the previous study, there appeared to be a tendency towards more frequent breastfeeding in JRA cases, suggesting an increased risk of breastfeeding on disease development. Again, this study was small.

A third study by Kasacopur et al. [23] was subsequently conducted in Turkey, a developing country in which breastfeeding rates are generally higher than in developed countries. In 32 children with JRA, they found no differences in occurrence or duration of breastfeeding when compared with 32 disease control children with primary nephritic syndrome, or to 54 healthy control children. However, the data indicate that there may be a difference in the duration of breastfeeding between JRA subtypes, with the mean duration of breastfeeding being 10 months less in oligoarticular JRA cases than in polyarticular JIA cases (9.0 ± 10.1 vs 19.7 ± 10.9 months) [23]. Of potential interest, this finding is consistent with the study of Rosenberg that identified a lower breastfeeding duration in pauciarticular JRA cases (5.9 months) when compared with polyarticular cases (7.3 months).

The only other study to have addressed the relationship between breastfeeding and JIA, albeit indirectly, was published by Young et al. [35] in 2007. In this study, interactions between the RF positivity (RF+), HLA-DR4 genotype (associated with increased risk of RA and polyarticular JIA) and early childhood factors, including breastfeeding duration, were considered. In individuals who were HLA-DR4 negative (i.e. those that did not carry this aspect of genetic predisposition to polyarticular JIA), breastfeeding for >3 months was more frequent in RF− children than RF+ children (OR 0.18; 95% CI 0.04, 0.99; P = 0.049), suggesting a protective effect of breastfeeding on the development of RF positivity [35]. These findings are relevant only to the small proportion of JIA cases who are of the RF+ polyarticular subtype. Additionally, it should be noted that the comparison was made between 180 RF−, HLA-DR4 participants and only 9 RF+, HLA-DR4 participants. Therefore, the small number of RF+ children in particular may, by chance, not reflect the true rate of breastfeeding RF+ children in the broader population.

Overall, the studies, while generally inconsistent, do suggest that a relationship may exist between breastfeeding and JIA risk, but the relationship has not yet been well defined in large studies. However, there is significant further impetus to clarify the role of breastfeeding in the JIA disease risk. There is a large body of evidence that shows breastfeeding assists in the development of the infant immune system through the transfer of the mother’s ‘immunological memory’, and of compounds with immune-modulating capacities such as cytokines. This process appears to assist in imprinting and programming the immune system of the infant. It is thought that improper programming of the infant immune system may lead to an increased risk of immune disorders, including autoimmune disease [36].

For T1D in particular, a number of follow-up studies have been performed to elucidate the relationship between breastfeeding and this autoimmune disease. Whereas many have provided further evidence for a relationship, or a relationship between the disease risk and early introduction of formula or cow’s milk [37], several studies, including a recent prospective cohort [38], have not confirmed the association [39]. As for JIA, these inconsistencies may be due to many study-specific factors, such as case and control selection and diagnostic criteria, sample size and method of data collection.

Infection
The role of infection in the initiation or augmentation of JIA symptomatology has long been suspected. A number of infections can lead to transient post-infectious arthritis, usually lasting only a few weeks. However, this ReA can occasionally become chronic, resembling JIA in children and RA in adults [40].

ARF often follows streptococcal infection and is characterized by the inflammation of the large joints and is more common in children. There is some evidence to suggest that streptococcal infection may trigger JIA, or cause flares in disease activity. In a study of 173 children with recent onset arthritis (ARF, known post-streptococcal ReA [PSRA], JIA, transient arthritis or other arthritides), 18% of children tested positive for recent streptococcal infection. Of the 33 children diagnosed with JIA, only 9% tested positive. Ten per cent of the PSRA patients had active joint disease at 6 months, but these cases remained quite distinct clinically from JIA, particularly in relation to hip involvement and biochemical measures, suggesting that the PSRA was unlikely to progress to JIA [41]. However, a study that examined all cases of JIA treated in a hospital paediatric rheumatology service across a period of 10 years found a high co-occurrence of disease flares with streptococcal infection. Nine of the 41 patients examined had two or more flares coinciding with acute infection. This suggests that streptococcus may impact on the disease course [42].

Viruses known to initiate ReA include the human parvovirus B19 and EBV, both of which have been examined in relation to the population-based risk of JIA development. In many cases, parvovirus B19 infection is asymptomatic, and evidence of past infection is high in the general population. In children, infection can manifest as erythema infectiosum, also known as ‘fifth disease’ or ‘slapped-cheek’ syndrome. Approximately 10% of children who...
develop erythema infectiosum also develop arthritic symptoms. These symptoms are usually symmetrical, mainly affecting small joints such as those in the hands, wrists and knees, and are more common in females [40]. In 1997, Soderlund et al. [24] presented a study in which viral B19 DNA was tested for in a variety of tissues from children with chronic arthritis (n=37) and from young adults with joint trauma (n=27). Interestingly, all SF samples from both the children with arthropathy and young adults with joint trauma were negative for B19 DNA. B19 DNA was however detected in the synovial tissue, but in a higher proportion of young adult joint trauma patients (48%) than in children with arthritis (28%). B19 IgG antibodies were detected in serum samples from all individuals who were synovial tissue B19 DNA positive, suggesting a past parvovirus infection that had persisted in the SM, but not the SF. The authors concluded that the higher proportion of evidence of B19 infection in the non-arthropathic individuals was suggestive of a lack of association between the B19 infection and JA [24]. However, reservations have been expressed regarding the fact that the 'control' group was significantly older (mean 20.2 years) than the 'cases' (mean 8.5 years). Given that the study detected past infection, as demonstrated by the presence of the IgG antibodies in the serum, the differences between the groups may be a function of the increased chance of exposure with age in the controls [43].

A different approach to the problem was taken by Oguz et al. [25]. In this study, 75 children with acute arthropathy (mean age 7.7 years) were tested for the presence of the parvovirus IgM antibodies in serum, and were followed longitudinally to assess the progress of the arthropathy to JIA. In addition, 75 healthy control children (mean age 7.6 years) were tested for B19 IgM antibodies. The presence of B19 IgM antibodies was detected in 22% of the cases, but in only 4% of the controls. The B19 seropositive cases were significantly more likely to progress to chronic arthritis (>6 weeks duration) than the B19 seronegative cases (12/16 vs 5/58; \( P = 4 \times 10^{-7} \)), and more likely to progress to a diagnosis of JIA (3/16 vs 1/58, \( P = 0.03 \)). The authors concluded that the higher rate of B19 seropositivity in the arthropathy group, and the higher number of B19-positive cases that progressed to JIA, suggests a role for the parvovirus B19 in JIA pathogenesis [25].

More recently, Gonzalez et al. [26] examined the presence of the parvovirus B19 infection by the detection of serum IgM and IgG antibodies, and B19 DNA in 50 JIA patients (mean age 9.6 years) and 39 healthy controls (mean age 7.8 years). They found IgM antibodies in 20%, and viral DNA in 10%, of the JIA cases, but none of the controls. However, they detected IgG antibodies in 32% of the JIA cases and 44% of the controls [26]. Similar frequencies in 406 JIA cases and 146 healthy controls were detected in a study by Weissbrich et al. [27]. These results suggest that past infection in both groups is a common finding and does not indicate increased disease risk. However, the much higher prevalence of the more recent B19 infection in children with active JIA in the Gonzalez study supports previous findings indicating a pathogenic role [26].

An association of the EBV infection with adult RA and other autoimmune diseases, including SLE and MS, has long been suspected [44–46]. However, the EBV has not been as extensively examined in relation to JIA. The outcomes of three small studies seeking evidence of an increased frequency of the EBV infection in JIA have been conflicting [47–49]. In 2002, Massa et al. [28] examined the interaction between the HLA type and EBV infection in oligoarticular JIA. They hypothesized that HLA molecules expressed by HLA alleles that had been previously associated with oligoarticular JIA may share peptide similarities with the EBV protein. Therefore, T cells produced that recognize the EBV peptides may also recognize the self-HLA peptides triggering an autoimmune response; a concept referred to as molecular mimicry. The study compared 17 oligoarticular JIA patients with evidence of previous EBV infection, with 15 controls who carried both evidence of previous EBV infection, and at least one of the oligoarticular JIA-associated HLA alleles (HLA-DRB1*1101, DRB1*0801 or DPB1*0201). When cytotoxic T-cell lines from each patient were stimulated with the EBV peptides, all case and control cell lines responded with a similar cytotoxic response. However, when stimulated by the HLA DRB1*1101-, DRB1*0801- or DPB1*0201-derived peptides, it was found that only the cell lines from the oligoarticular JIA patients responded with the production of pro-inflammatory cytokines, in particular IFN-γ. This suggests that the normal ability to tolerate self-HLA epitopes and to self-limit cytotoxic T-cell responses may be hindered in oligoarticular JIA patients, and that the EBV may be one trigger of the inflammatory autoimmune response in JIA [28]. The study also serves as a good example of a possible interaction between genes and the environment in determining disease risk. Two further studies have examined the impact of infection on the JIA disease risk in a more general way. A study by Feldman et al. [29] carried out in Canada examined the relationship between the season and onset of systemic JIA. They hypothesized that seasonal differences might indicate differences in the risk of exposure to infectious agents. However, they failed to find an association with disease onset and season except in one Canadian region, and even in this region systemic JIA onset could not be related to incidence data on viral infection [29]. Lack of association of JIA onset with season was later supported by the work of Nielsen et al. [30] in an examination of children in Denmark over a 4-year period. More recently, as part of a larger study examining early-life environment and risk of adult RA and JIA, Carlens et al. [31] reported a prospective association between hospitalization for any infection in the first year of life and the risk of later JIA (OR=1.9; 95% CI 1.7, 2.1). Gastrointestinal infection appeared to confer the highest risk (OR=2.3; 95% CI 1.8, 2.8). The study utilized prospectively collected register-data in Sweden from 1973 to 2002, and
compared 3334 cases with JIA with 13336 controls. Thirteen per cent of the JIA cases had been hospitalized for infection in the first year of life, compared with 7% of the controls. Interestingly, early-life infection was also associated with a >2-fold increase in the RF+, adult RA risk. A distinction was not made within JIA cases based on the RF; however, the majority of JIA patients are also RF-.

Also worthy of mention are studies considering the impact of immunization on disease risk. There have been a number of studies examining the safety and efficacy of immunization in JIA patients [50–52]. There has also been some limited work assessing the risk of rheumatic disease onset or flare-up following immunization that suggests a slight increase in the risk of RA or other chronic arthritis following immunization against Hepatitis B, rubella and varicella (reviewed in [53]). Specifically for JIA, a recent report described a flare-up of systemic JIA in a child whose arthritis had been in remission for ≥4 years, immediately following the rubella vaccination [54]. This finding is consistent with past work indicating a possible link between rubella and JIA [55, 56].

On balance, the majority of these studies support the role of infection in increasing risk of JIA. However, the mechanisms through which infection affects the risk of disease in JIA and in other autoimmune diseases remains speculative, and may be distinct between the different infectious agents. Molecular mimicry, whereby self-reactivity is triggered by cross-recognition of a self-peptide and an infectious peptide due to sequence similarity, is a popular model, and one tested by the study of Massa et al. [28] in relation to the EBV as discussed above. Other models for the triggering role of infection include the polyclonal lymphocyte activation and increased immunogenicity of organs following infection-related inflammation [57]. However, there is also evidence for a protective role of infection, especially in early life, on autoimmune disease risk (the ‘hygiene hypothesis’—discussed later). A two-hit model has been proposed, whereby a hygienic early life prevents the development of adequate host responses against microbial exposure, increasing the risk of the triggering of autoimmune disease by infection later in life [57]. The protective role of early-life infections has been little examined in relation to JIA.

Maternal smoking

Finally, there is some recent evidence for an increased risk of JIA caused by maternal smoking during pregnancy. A study by Jaakkola and Gissler [32], predicated on the fact that personal smoking is a risk factor for adult RA, examined mothers’ smoking during pregnancy and the incidence of JIA across the first 7 years of life in 58841 singleton births from the Finnish birth registry. Thirty-one cases of JRA were identified. An association of JIA with smoking 10 or more cigarettes per day during pregnancy was identified for girls (adjusted OR = 6.79; 95% CI 2.00, 22.9), but not for boys. However, the authors point out that the small number of JIA cases identified is a significant study limitation. Only 21 female and 8 male cases of JIA were identified, and none of the male cases resulted from a pregnancy in which 10 or more cigarettes were smoked. Thus, while it appears that there may be a relationship between maternal smoking and JIA disease risk, the small sample size precludes definitive conclusions. The prospective study by Carlens et al. [31] introduced above was able to assess 1159 JIA cases against 4701 controls in relation to maternal smoking. They found no evidence of association (OR = 1.0; 95% CI 0.8, 1.1).

Other factors

The few remaining studies investigating the environmental determinants of JIA risk examined socio-economic variables and aspects of perinatal and early life. The Danish study by Nielsen et al. mentioned earlier, considered 220 JIA cases drawn from national registers across 1988–91, each matched to four controls. Children from urban dwellings and high-income families were found to be more likely to have JIA, as were children with no siblings [30]. These socio-economic measures may be related to a more hygienic early-life environment, and may support the ‘hygiene hypothesis’ (discussed in detail below) of the protective effect of infection on disease risk.

The study by Carlens et al. [31], along with considering the role of infection and maternal smoking, also examined the contribution of various maternal, pregnancy and infant characteristics to the risk of JIA. These included maternal age, maternal marital status, season of birth, mode of delivery, number of older siblings, birth weight, gestational age and Apgar score at 5 min following birth. Of these characteristics, borderline significant increased risk was observed for individuals born after 42 weeks gestation (OR = 1.2; 95% CI 1.0, 1.3), and by caesarean section (OR = 1.1; 95% CI 1.0, 1.3). Borderline significant reduced risk was observed for individuals with a low (<6) Apgar score 5 min post birth (OR = 0.7; 95% CI 0.5, 1.0) [31].

Summary of findings from environmental investigations

The most striking overall observation from the environmental investigations in total is the lack of large, well-designed studies ascertained to specifically test environmental hypotheses. The majority of the studies described above utilized small sample sizes, or, where larger registries were used, were capable only of analysis of limited data collected to those registries. In these cases, the data were collected for other purposes, and not always well suited to the analysis of specific aspects of the environment. For example, the study by Carlens et al. [31] utilized a robust sample size (3334 cases with JIA and 13336 controls) drawn from a prospective Canadian register. It considered the role of early-life infection in the risk of JIA. However, the analysis was restricted to infections recorded on the register i.e. infections requiring hospitalization. Children may be exposed to many infections in the first year of life, the majority of which do not require hospitalization. Therefore, restricting the analysis to more severe infections cannot capture a true spectrum
of infections that the cases and the controls, experienced prior to the first year [58].

Few conclusions can be drawn from this body of prior work. The role of breastfeeding warrants detailed follow-up in larger sample sizes, given that all studies did seem to identify relationships between breastfeeding occurrence and/or duration and JIA risk. Similarly, there does seem to be support for a role of infectious agents in the JIA risk, although the scope of infectious agents and the risk relationship (detrimental or protective) is far from clear. The potential role of maternal smoking in disease risk is also interesting, but the two studies to date have been conflicting, and the study showing a risk relationship, whereas large in total sample size, was very small in terms of the number of JIA cases that could be drawn from the overall number. Studies that are specifically designed to further test these, and other hypotheses, in terms of large sample sizes and data collected are vital to achieve further clarity.

Untested hypotheses

The studies conducted so far have (inadequately) covered a small subset of plausible hypotheses of environmental determinants of the JIA disease risk. Many more could be hypothesized, particularly when based upon research conducted for other autoimmune diseases. Of the spectrum of autoimmune diseases, one might speculate that JIA might best be compared with adult RA given the clinical similarities, and/or an organ-specific autoimmune disease with a commonly paediatric age of onset, such as T1D. In this section, we review two environmental risk factor hypotheses generated through the study of other autoimmune diseases, with a particular focus on what is known of their role in RA and T1D disease risk.

The hygiene hypothesis

The so-called ‘hygiene hypothesis’ was first put forward by Strachan in 1989 [59]. It was derived from a study of hay fever among 17414 British children born in March 1958 and followed until age 23. It identified a striking relationship between hay fever and family size and position in the household of the child. Hay fever was inversely associated with sibling number at age 11, and the inverse association was stronger in children with older siblings. Conversely, children with few, and older, siblings would be more likely to come in contact with infection introduced to the family and spread by ‘unhygienic’ contact among the siblings. Conversely, children with few, and younger, siblings would be exposed to far less sibling-transmitted infections. This more-hygienic early life could be extrapolated to the general community where decreasing family size and higher standards of hygiene have co-occurred with increases in the incidence of allergic diseases [59].

This hypothesis has since been extended to include not only allergic diseases, but autoimmune diseases as well, based partly on the steady temporal increase in the incidence of many autoimmune diseases alongside allergic diseases. For example, the incidence of T1D has increased markedly in developed countries over the past decade [57]. This is far too rapid to be attributed to genetic change, strongly suggesting that changes in environment are likely to be driving the increase. In addition, the largest increases were identified in the 0–4-year age group [57, 60], suggesting that changes to the early-life environment may be most relevant. Additionally, the frequency of T1D appears higher in firstborns, compared with subsequent children, in a multiplex family [61]. Therefore, microbial exposure, including infection, in the first few years of life may also be relevant to the risk of developing paediatric autoimmune diseases such as T1D [62] and IBD [63].

The mechanism through which infection might provide protection against allergy and autoimmunity remains to be clarified, but most centre on the idea that exposure to infection in early life serves to better ‘prime’ the immune system. Several hypotheses have been proposed, none of which have been studied in enough detail to allow definitive conclusions. These include a competitive mechanism whereby the strong immune responses elicited by infectious agents might successfully compete with weaker allergens and autoantigens, causing a shift in homeostatic cytokine signals away from those produced by autoimmune or allergic immune system responses. A regulatory mechanism has also been proposed, whereby regulatory cells stimulated by infectious agents might also serve to down-regulate immune responses to autoantigens. Thirdly, stimulation of the innate immune system via toll-like receptors by infection may assist in protecting against autoimmune responses [57]. In the absence of sufficient priming, the immune system of certain individuals (for example, those who are genetically pre-disposed) may more easily tip towards an allergic or autoimmune response.

While there is growing evidence from human and animal model research that the hygiene hypothesis might account, at least in part, for the rapid increases in the incidence of T1D seen in developed countries [64], there is more limited evidence of the role of early-life infection in protecting against the risk of developing RA in adulthood. A study to assess the relationship between the RF (as an autoantibody strongly associated with RA) and markers of infant hygiene was performed using the Hertfordshire Cohort Study [65]. An inverse association was detected, only in women, between RF positivity and sharing a bedroom during childhood (OR = 0.48; 95% CI 0.30, 0.78; P = 0.003). The authors concluded that this relationship might extrapolate to a relationship between early-life exposure to infection and a lowered risk of developing RA later in life [65].

Determining the role of early-life infection on adult autoimmune disease risk is a difficult task, as most studies (unless focused on population-based medical registers or long-term prospective cohorts) will be significantly hindered by a recall bias. It has been proposed that it is important to capture information regarding the full
spectrum of early-life infections, including the common cold and mild gastroenteritis, that may not have been presented to hospitals or even to general practitioners [58]. Thus, the lack of evidence supporting a role for early-life hygiene in increasing the risk of developing RA in adulthood may be a product of the difficulties of collecting comprehensive and accurate measures of early-life infection from adults. For JIA, however, like T1D, the earlier age of onset provides an opportunity to more accurately examine the role of infant hygiene in determining disease risk. Information that should be captured includes the scope of exposure from mild to severe type of infectious illness and frequency of occurrence. Proxy measures of early-life microbial exposure should also be taken, including birth order, sibling number, day-care use and exposure to pets and farm animals [58]. In utero exposures, such as the mother’s exposure to microbes associated with farm animals during pregnancy, are also of demonstrated importance [66]. Measurements of biomarkers of current and past infective illness, such as IgM and IgG seropositivity, should also be considered. Studies that are designed to capture these exposures at the time of diagnosis in JIA cases, and also in a reference set of age-matched, healthy control children representative of the general population from which the cases are drawn, may provide useful contributions to accurately assess the hygiene hypothesis in relation to the JIA disease risk.

Vitamin D and sun exposure

Vitamin D is traditionally thought of as a hormone that is essential for bone and mineral homeostasis. However, there is mounting evidence that vitamin D also plays an important role in modulating the immune system [67]. The mechanism through which the active form of vitamin D, 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], exerts its effects on immunity is currently the subject of much research effort [68]. Findings to date have been recently reviewed in detail [67, 68]; however, in brief, 1,25(OH)₂D₃ appears capable of enhancing the innate immune system. It has been shown to activate an anti-bacterial response via stimulation of the production of anti-microbial peptides [69], and the production of 1,25(OH)₂D₃ is up-regulated in wound repair [70]. In relation to adaptive immunity, 1,25(OH)₂D₃ seems to influence the differentiation and maturation of dendritic cells to antigen presenting cells, and down-regulates the expression of cytokines such as IL-12 that are associated with the Th1 cells [71]. Th1 cells are known to mediate the autoimmune response in diseases such as MS, T1D and RA [67], and this appears true also of JIA [72]. Vitamin D can also up-regulate regulatory T cells [73]. Therefore, 1,25(OH)₂D₃ seems to promote an immune system with tolerogenic properties [73].

Recently, the newly described Th cell Th17, which secretes the pro-inflammatory cytokine IL-17, has been implicated in autoimmune pathogenesis, including adult RA [74, 75]. Interestingly, an excess of Th17 cells have been detected in the joints of JIA patients, particularly in those with a more severe clinical course [76]. There is evidence to suggest that 1,25(OH)₂D₃ inhibits the production of Th17 cells via a number of potential pathways, including a reduction in the expression of the Th17 stimulatory factor IL-6 [77–79]. In experimental models of autoimmune uveitis, a comorbidity often associated with JIA [80, 81], it has been shown that 1,25(OH)₂D₃ suppresses the autoimmune response both by inhibiting the production of Th17 cells, and the production of IL-17 from those Th17 cells that are produced [82]. This research provides strong impetus for examining the role of vitamin D in JIA pathophysiology.

While some vitamin D is obtained through diet, the majority of this hormone is obtained through exposure of the skin to the sun. When bound to the active vitamin D ligand, the vitamin D receptor (VDR) acts as a transcription factor, regulating transcription of vitamin D-responsive genes. Thus, the VDR mediates the biological effects of vitamin D [83]. The VDR is widely expressed within the cells of the immune system including T cells and dendritic cells, adding further weight to the importance of vitamin D in immunomodulation [84].

Latitude is an important determinant of Ultra Violet Radiation (UVR) levels, prompting studies that have examined the incidence of autoimmune diseases in populations of different latitudes. In MS, much higher prevalence is observed at latitudes >50° than at the equator [84]. Even within individual countries such as Australia, differences related to latitude have been identified, with prevalence 6-fold higher in Tasmania (43° south) than in north Queensland (19° south) [67, 85]. There is evidence of a relationship between the latitudinal gradient and T1D [86, 87], paediatric Crohn’s disease [88], WG [89] and possibly RA [67, 90], but the role of latitude in the JIA disease risk has not been studied.

Season of birth may also be related to autoimmune disease risk, suggesting that exposure to vitamin D during relevant developmental periods of gestation might be of critical importance. Vitamin D levels reach a peak in autumn, and a nadir in spring [91]. For MS, an excess of autumn births among cases has been reported by a number of studies including a large meta-analysis analysing over 42 000 individuals across Canada and Europe [92]. For T1D, there is a mixed opinion resulting from inconsistent study findings. A British study found an excess of spring and summer birth dates in T1D cases compared with the general population [93]. This relationship has been further identified by some studies [94, 95] but not by others [96, 97].

Few studies have been conducted to determine the relationship between the season of birth and RA. One study, conducted in 1987, suggested no association [98]. However, season may influence RA disease activity, with higher activity reported in spring compared with autumn [99] or age at onset [100]. It should be noted that studies identifying the association of disease with seasonality have also hypothesized that such associations might equally support the aetiological involvement of climatic environmental factors other than UVR exposure that vary by season. Importantly, viral outbreaks may be more common in colder months when UVR exposure is.
also low. Conversely, research suggesting virus-related increased risk of JIA might instead indicate an important role for vitamin D, which could be lowest during seasons of higher infection risk. Studies aimed at measuring both vitamin D and the viral load in autoimmune disease will be required to unravel the possible contributions of these factors.

There is more direct evidence for the role of vitamin D on autoimmune disease risk. Studies have demonstrated that vitamin D insufficiency or deficiency is associated with T1D [100] and RA [101, 102], and that supplementation may be inversely associated with the onset of these diseases [103–6]. Moreover, intervention trials of high-level vitamin D supplementation during RA disease activity may relieve pain symptoms and reduce the production of the inflammatory disease activity biomarker CRP [99, 107].

Taken together, there is significant evidence to suggest that vitamin D may play an important role in reducing the autoimmune disease risk and severity through modulation of the immune system towards higher tolerogenic capacity. The positive results of the vitamin D intervention trials for RA suggest that this is a high priority area for future JIA research.

**Future directions in the identification of environmental risk factors for JIA**

It would be true to say that research aimed at identifying environmental risk factors for JIA lags considerably behind that of many other autoimmune diseases. Although this has to date limited our understanding of the JIA aetiology, there are advantages in beginning large-scale studies at this point in time. The first advantage is that knowledge generated through research into other relevant autoimmune diseases such as T1D and RA greatly assists in formulating plausible hypotheses to test in studies of JIA. The second is that, in the past several years, we have gained a greater understanding of the architecture of complex diseases, and we understand that aetiological factors, whether they be genetic or environmental, are individually likely to confer modest risk. Therefore, large sample sizes are necessary to identify causative factors with statistical certainty. We also appreciate that genes and the environment are likely to significantly interact. Therefore, measuring the genetic and environmental factors simultaneously will provide the opportunity to examine such interactions, and help us understand the likely complex relationships between genes and the environment to determine individual disease risk.

All of the factors so far studied in JIA, including breastfeeding, infection and smoking, require detailed further scrutiny in studies designed specifically to test their role in disease risk. Additionally, there is strong impetus to examine as-yet untested hypotheses, including the role of a hygienic early-life environment, and the importance of sun exposure and vitamin D levels, in determining the JIA disease risk.

In considering the best experimental design for such research, there are several factors that require consideration. The first are the advantages, but the inherent difficulties associated with the prospective collection of environmental data in diseases of relatively low incidence. Given the likely impact of early-life environment on disease risk, the most ideal way of collecting such data is to recruit participants prior to birth, and to follow them longitudinally through childhood to disease onset. However, for a disease such as JIA where prevalence appears to approximate 1 in 1000 [2], hundreds of thousands, even millions, of participants would be required by such a study to ensure that sufficient cases arose from the sample to provide adequate statistical power to detect differences between cases and the remaining cohort [108].

In weighing up cost and time vs benefit, case–control studies that aim to gather environmental data at, or close to, disease diagnosis, can provide a useful compromise. This approach is particularly useful for childhood-onset disease research, where inaccurate recall of early-life environmental factors by parents is reduced compared with adult onset diseases. Disease-related changes in recall, lifestyle or biological measures are less problematic for incident compared with prevalent cases. Also data that may be relevant to disease onset, for example, presence of infection or levels of vitamin D, can be collected at diagnosis. Comparative control reference groups are also required, and these should be drawn from the same population as the cases, and be matched for ethnicity, age and sex, among other factors. This provides a design in which cases and controls have emerged from the same broad pool of potential environmental exposures [109], and exposure to causative environmental factors should be more common in cases than in controls. Large sample sizes that provide sufficient statistical power to detect risk factors conferring small, or subtype-specific, effects might be more swiftly achieved through co-ordinated multicentre recruitment studies. Accurate environmental measures coupled with large sample sizes and minimal recall bias in this setting have the capacity to robustly test risk-factor hypotheses. The collection of biological specimens from such case–control samples adds much value, providing the opportunity to measure biomarkers, seek out genetic risk factors and examine gene–environment interactions.

In seeking to address the paucity of knowledge of the JIA risk factors, both environmental and genetic, we have established the ChiLDhood Arthritis Risk factor Identification sTudY (CLARITY) at the Royal Children’s Hospital, a tertiary paediatric hospital in Melbourne, Australia. In CLARITY, we are collecting extensive data relevant to early-life environment and environment at disease onset, and a blood specimen for genetic and biomarker analyses, from JIA cases and from healthy controls.

In conclusion, this review has highlighted that although the evidence base for the environmental determinants of JIA remains to be strengthened, the findings to date
indicate that environment does impact on disease risk. Additionally, the lessons learned in other more extensively studied immune disorders should be taken into account to progress knowledge in this important area of JIA research.

**Rheumatology key messages**

- Research to identify environmental risk factors for JIA is lacking.
- Breastfeeding, infection and maternal smoking may play a role in JIA.
- An examination of early-life infection and Vitamin D is warranted.

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