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## Are low sun exposure and/or vitamin D risk factors for type 1 diabetes?

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The global variation in type 1 diabetes (T1D) incidence rates is one of the most significant observed for any non-communicable disease. Geographical patterns in incidence suggest that low sun exposure may contribute to the wide disparity, with incidence rates generally increasing with distance from the Equator. T1D development is associated with hyperactivity of the adaptive immune system leading to autoimmune destruction of insulin-secreting pancreatic  $\beta$  cells. Both exposure to ultraviolet radiation (UVR) and vitamin D, with their known immunosuppressive effects, have the potential to delay or inhibit the disease. Efforts to confirm the role of UVR by vitamin D dependent and independent pathways in the pathogenesis of T1D have been challenged by inconsistent results among studies. Human observational studies and animal and *in vitro* experiments indicate that at least some of the benefits of sun exposure come from improved vitamin D status. There is no evidence of benefit for T1D risk of vitamin D supplementation during pregnancy at current recommended levels (400 IU per day); but some evidence supports that higher sun exposure and/or vitamin D sufficiency in pregnancy, or supplementation in early life, decreases T1D risk. Further research is required to confirm an association between UVR exposure and T1D and clarify the mechanisms involved.

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## Introduction

Type 1 diabetes (T1D) is the most common autoimmune disease of childhood.<sup>1</sup> The incidence of T1D is increasing worldwide – an annual increase of approximately 3%<sup>2</sup> – and there is evidence of a trend towards earlier age of onset.<sup>2–5</sup> These relatively rapid changes implicate alterations in exposure to environmental agents as risk factors for the disease. Geographic patterns of increased incidence with greater distance from the Equator (higher latitude) provide a clue to environmental influences that may be important.<sup>6</sup> Among several factors that vary according to latitude, levels of ultraviolet radiation (UVR) have been of particular interest because UV irradiation of the skin is the primary source of vitamin D. The active form of this pre-hormone has known effects on immune function that make vitamin D deficiency a plausible candidate risk factor for T1D.<sup>7</sup> Increasing prevalence of vitamin D deficiency in children,<sup>8</sup> occurring in parallel with

increasing incidence of T1D, and seasonal variation in the onset of T1D, have provided additional evidence that vitamin D deficiency may be a risk factor for T1D.

While observational studies support a link between vitamin D deficiency during pregnancy, typically measured as a serum/plasma level of serum 25-hydroxyvitamin D (25(OH)D) less than 50 nmol L<sup>-1</sup>,<sup>9</sup> and risk of T1D,<sup>10,11</sup> trials of vitamin D supplementation have returned largely null results.<sup>12</sup> The most important determinant of serum 25(OH)D levels is sun exposure (including time in the sun and the intensity of UVR).<sup>13,14</sup> This has led more recently to considerations of whether the 25(OH)D levels measured in observational studies (and related to T1D risk) are specific for vitamin D status or a proxy for recent sun exposure. UVR exposure of the skin suppresses adaptive immunity in ways that are similar to those of vitamin D.<sup>15</sup> Thus exposure to UVR may modulate immune function relevant to the onset of T1D through both vitamin D and non-vitamin D pathways. Vitamin D supplementation improves only the former.

Here we review the evidence that low sun exposure, or vitamin D deficiency specifically, are associated with an increased risk of T1D. We restrict our analysis to T1D in children as the risk factors in this age group are likely to be clearer, with less effect of risk exposures later in life. We begin with an overview of the clinical, genetic and immune characteristics of T1D.

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## Type 1 diabetes

T1D results from the progressive autoimmune destruction of insulin-producing beta ( $\beta$ ) cells in the pancreas<sup>16,17</sup> sufficient to decrease and ultimately cease insulin production.<sup>18</sup> Although genetic, serological and biochemical testing can be used to identify at-risk individuals,<sup>19</sup> there is no established mechanism for slowing or preventing the progression of  $\beta$ -cell destruction in humans.<sup>20</sup> Similarly, there is no cure for T1D.<sup>21</sup>

T1D can present at any age, but onset is typically in childhood or adolescence.<sup>22</sup> Children with T1D commonly present to health services with acute clinical symptoms including polydipsia and polyuria, nocturia, enuresis and weight loss (which can occur despite polyphagia), and blurred vision, or more severe conditions such as diabetic ketoacidosis, severe dehydration or shock.<sup>19</sup> These symptoms occur only once the majority (90%) of the  $\beta$ -cells are destroyed or significantly compromised, rendering the child insulin dependent.<sup>18</sup>

The incidence of T1D among children aged 0–14 years varies significantly worldwide with rates ranging from 0.6 per 100 000 in China to 62.3 per 100 000 in Finland.<sup>2</sup> In 2015, the International Diabetes Federation reported that over half a million children worldwide are living with T1D, and an additional 86 000 are estimated to be diagnosed each year.<sup>2</sup>

Children with T1D are more likely to develop a range of disabling and life threatening conditions when compared to children without the disease,<sup>2</sup> of which diabetic ketoacidosis is most common.<sup>1</sup> Many of the complications associated with T1D are the result of chronic hyperglycaemia and are now being observed at a younger age as a consequence of the earlier onset of the disease.<sup>1</sup> In addition to the physical health problems, T1D also places psychological pressure on both those diagnosed and their families.<sup>1</sup> Following a diagnosis significant vigilance is required to minimise glucose variability and sustain optimal diabetes management.<sup>23</sup> A recent large population based cohort study out of Sweden has shown that children with T1D were 2.1 times more likely to be diagnosed with a psychiatric disorder and 1.7 times more likely to attempt suicide when compared with controls.<sup>24</sup> There are also considerable financial costs associated with having the disease. Direct costs associated with treatment, monitoring and health service utilisation, in addition to more indirect costs resulting from carer support, disability and loss of productivity are shared by individuals, their families, health services and government bodies.<sup>2</sup> Globally the cost of treating and managing T1D in 2015 was estimated to be between USD 673–1197 billion.<sup>2</sup>

Children diagnosed with T1D have a 2–4 fold increased mortality<sup>25–28</sup> most commonly the result of acute complications, such as diabetic ketoacidosis or other diabetes related events, death due to unnatural causes and sudden unexpected death. Despite improvements in the treatment and care provided to those with T1D, the average life expectancy of those diagnosed in childhood is still reported to be approximately

10 years less than that observed in the general population.<sup>18,29</sup> This is infinitely better than the prognosis without insulin therapy, and a vast improvement from the 20 year reduction in life expectancy reported some 50 years ago.<sup>30</sup>

The aetiology of T1D is complex. Both genetic and environmental factors are involved and possibly gene-environment interactions. The rapid rate at which T1D is increasing worldwide and the trend towards earlier onset point to a significant role for environmental influences.<sup>1,31,32</sup>

Multiple genes have been linked with increased susceptibility to T1D; most of the risk loci are associated with immune cells and their function.<sup>33</sup> Major susceptibility genes are in the HLA region.<sup>18</sup> From this region *DR3-DQ2* and *DR4-DQ8* haplotypes are most strongly associated, evident in up to 90% of those with T1D.<sup>34</sup> However, less than 10% of genetically susceptible individuals go on to develop T1D<sup>35</sup> and only 13–34% of monozygotic twins are pairwise concordant for the disease.<sup>36–40</sup>

Putative non-genetic/environmental risk factors include maternal and child enterovirus infection, increased maternal age, rapid growth in early childhood, obesity, stress<sup>32</sup> and vitamin D deficiency in early life.<sup>41</sup> The idea that modifiable risk factors may be involved in the pathogenesis of T1D introduces great hope and purpose for preventative efforts. There is mounting evidence that non-genetic and environmental factors may act *in utero*, in early infancy and/or in childhood to modify the risk of T1D, particularly for those who are not genetically susceptible.<sup>42</sup>

T1D development is associated with hyperactivity of the adaptive immune system (T and B lymphocytes) leading to autoimmune destruction of insulin-secreting pancreatic  $\beta$  cells. Amongst the inflammatory cells of insulinitis lesions, CD8<sup>+</sup> T lymphocytes are the most prominent, followed by macrophages, CD4<sup>+</sup> T lymphocytes, B lymphocytes and plasma cells. The first three mentioned cell types can enhance immune responses by production of molecules, enzymes and cytokines with tissue destructive properties, as well as chemokines to attract further inflammatory cells. The demise of pancreatic  $\beta$  cells may be an off-target effect of activated cytotoxic CD8<sup>+</sup> cells. Activation of B lymphocytes and plasma cells is already evident at the time of first diagnosis of T1D. As reviewed elsewhere,<sup>43</sup> ninety percent of newly diagnosed patients with T1D already have antibodies reactive to at least one antigen expressed by  $\beta$  cells, namely to insulin (anti-insulin antibodies, IAA), glutamic acid decarboxylase (GADA), insulinoma-associated autoantigen 2 (IA-2) or zinc transporter 8. Of interest, T regulatory lymphocytes, an important cell type that would normally control over-zealous inflammatory responses, are rare in inflammatory regions of the pancreas.<sup>43</sup>

While a wide range of environmental factors are implicated in risk of T1D, the immunopathology of the disease and the known immunomodulatory effects of vitamin D and other molecules produced in skin following exposure to UVR, make these exposures of particular interest as potential regulators of development and progression of T1D.



## Vitamin D as a risk factor for T1D

In this section we begin by providing an overview of the vitamin D metabolic pathway and then review the evidence of a link between vitamin D and risk of T1D, beginning with human observational studies and then exploring possible pathways of action using animal and *in vitro* studies. As associations with serum 25(OH)D levels do not allow differentiation of specific vitamin D effects rather than those of sun exposure; the focus of this section is therefore on vitamin D intake, including supplementation and evidence from associations between disease risk and polymorphisms in genes of the vitamin D pathway. The effects of environmental exposures on disease risks may depend on the age of exposure; we thus consider exposure at different age groups separately.

### The vitamin D metabolic pathway

Endogenous synthesis of vitamin D in the skin begins with the photoconversion of 7-dehydrocholesterol (7-DHC) to pre-vitamin D. Single nucleotide polymorphisms within the *DHCR7* gene that encodes 7-dehydrocholesterol reductase (that converts 7-DHC to cholesterol) and the *NADSYN1* gene that produces NADPH that is required in this reaction, influence serum 25(OH)D levels.<sup>44</sup> Vitamin D is converted in the liver to 25(OH)D by the 25-hydroxylase enzyme encoded mainly in humans by the *CYP2R1* gene, with conversion to the active metabolite, 1,25(OH)<sub>2</sub>D catalysed by the 1 $\alpha$ -hydroxylase enzyme encoded by *CYP27B1*. Within the blood, vitamin D metabolites are mainly tightly bound to a vitamin D binding protein, with a smaller proportion loosely bound to albumin or circulating as the free metabolite. The vitamin D binding protein gene (*GC*) has been repeatedly shown to be a major determinant of 25(OH)D levels,<sup>44</sup> as has the *CYP24A1* gene that encodes the breakdown enzyme, 24 hydroxylase. The active metabolite exerts its effects on gene transcription through a nuclear vitamin D receptor (*VDR*); polymorphisms within this gene may affect its activity and therefore its downstream functions. The most commonly studied *VDR* polymorphisms are *Fok1*, *Bsm1*, *Apa1* and *Taq1*. Their alleles are commonly referred to as letters (or as nucleotides), such as *Fok1* F/f (rs2228570 C/T, previously rs10735810), *Bsm1* B/b (rs1544410, A/G), *Taq1* T/t (rs731236, T/C) and *Apa1*, A/a (rs7975232, G/T). The *Fok1* "F" allele is more transcriptionally active than the "f" allele resulting in higher *VDR* production;<sup>45–48</sup> the *Taq1* "t" allele may confer increased responsiveness to 1,25(OH)<sub>2</sub>D.<sup>45–47,49</sup>

### Maternal dietary intake of vitamin D during pregnancy

In human observational studies, maternal intake of vitamin D supplements does not reduce risk of T1D in offspring. A protective association between maternal consumption of cod liver

oil supplements (400 IU vitamin D) and T1D was found in one pilot population-based case-control study<sup>50</sup> but was not replicated in a larger follow-up study.<sup>51</sup> In both studies the use of vitamin D supplements was not significantly associated with a reduced risk of T1D and the authors were unable to establish whether the association found in the pilot study was a direct result of the 400 IU of vitamin D or the n-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid) found in the cod liver oil, or the combination of both. Consistent with these findings, a Finnish study<sup>52</sup> based on a cohort of participants with genotypes for T1D conferring moderate or high risk, found no association between self-reported supplement use of vitamin D and presence of multiple autoantibodies/clinical diabetes. Similar findings were reported in a recent Swedish study.<sup>53</sup> However, in both studies the vitamin D intake reported from supplements was low, with only 15% of mothers consuming the recommended daily dose of 400 IU in the Finnish study and in the Swedish study the highest dose reported was 300 IU. For both studies, the baseline 25(OH)D levels of mothers were not known. The absence of such data limits the interpretation of the results. For example, if the majority of participants were deficient at baseline, then the small supplement doses reported would be unlikely to increase 25(OH)D levels to sufficient levels and an effect may not be expected. Equally, if mothers already had sufficient levels of 25(OH)D at baseline, then supplementation would equally not be expected to reduce risk. A meta-analysis of three studies confirmed that there was inadequate evidence to indicate an association between intake of vitamin D supplements in pregnancy and risk of T1D in the offspring.<sup>12</sup>

Higher maternal dietary intake of vitamin D *via* food or supplements during pregnancy has however been weakly associated with a decreased risk of developing T1D-related autoimmunity (positive for one or more of the three islet autoantibodies (GADA, IA-2, IAA)) in offspring in two cohort studies, though both reported notable discrepancies in their findings. The ABIS study indicated that the mothers' use of vitamin D-containing supplements (400 IU) during pregnancy reduced the odds of their offspring developing T1D-related autoimmunity by 29% (OR 0.71; 95% CI 0.52–0.96, *n* = 8694) at 1 year of age, but this effect was no longer apparent at 2.5 years (OR 1.25; 95% CI 0.09–1.73, *n* = 7766).<sup>54</sup> The authors were unable to explain why the association was lost at 2.5 years. The other study, of 233 American mothers (cases = 16), showed that the risk of T1D-related autoimmunity in offspring halved for each increase of 155.6 IU of vitamin D consumed during pregnancy *via* food per day (adjusted HR 0.37; 95% CI 0.17–0.78), but not supplements.<sup>55</sup> As the intake of vitamin D *via* food was considerably lower than the recommended adequate daily intake in the affected group, it is possible that it is deficiency that increases risk of autoimmunity rather than sufficiency being protective. Curiously, the results indicated an apparent 3-fold increase in risk among offspring of mothers who consumed vitamin D (400 IU) *via* supplements, which the authors did not discuss further. The data suggest that maternal supplement use increased the risk of offspring devel-



oping T1D antibodies; however there is no indication that the definitive analyses required to examine the combined effects of diet and supplement use were conducted. In the face of conflicting results and lack of detailed information on some facets of the analysis, the role of maternal dietary intake of vitamin D on T1D-related autoimmunity is still unclear.

There have been no human randomised controlled trials conducted to date despite numerous calls for such studies<sup>12</sup> and evidence of their potential feasibility.<sup>56</sup> Prevention trials would be challenging given the size of the study that would be required to have T1D as an outcome, the time to disease onset, and insufficient evidence that the high doses of vitamin D supplementation thought to be required to modulate T1D are safe during pregnancy. In summary, although there has been considerable interest in the potential preventative effects of vitamin D supplementation during pregnancy to reduce T1D-related autoimmunity or T1D risk, there is currently very limited evidence to support a beneficial role.

## Dietary intake of vitamin D in infancy and childhood

Supplementation with vitamin D or cod liver oil in the first year of life has been found to be protective against the development of T1D in several studies (see Table 1); however as neither dose nor treatment regimen is well-quantified, findings must be interpreted with caution. The first study to examine the relationship between vitamin D supplementation in infancy and reduced risk of T1D in later life was from the EURODIAB project.<sup>16</sup> The case-control study involved 3155 participants (820 cases and 2335 controls) over seven centres in Europe. There was a 33% decrease in odds of T1D among those who received vitamin D supplementation in the first year of life compared with those who received no supplements, after adjustment for a number of potential confounding factors (OR 0.67; 95% CI 0.53–0.86). Stene and colleagues' (2003) nationwide case-control study in Norway of 1668 control subjects and 545 cases with T1D is the largest population-based study conducted to date that has examined the impact of vitamin D and cod liver oil use during infancy and risk of childhood-onset T1D. In Norway, vitamin D supplementation is recommended from infancy in the form of cod liver oil, with a daily dose of 5 ml cod liver oil reported to contain 400 IU of vitamin D. The study found that consumption of cod liver oil in the first year of life was significantly associated with reduced odds of developing T1D in childhood up to the age of 15 years that persisted after adjustment for a large number of potential confounders (adjusted OR 0.74; 95% CI 0.56–0.99). This study collected data at multiple time points giving some insight into the optimal exposure window for vitamin D supplementation. Infants who were given cod liver oil at 7–12 months of age had lower risk of developing T1D in later life compared to those who were supplemented between 0 and 6 months of age.<sup>51</sup> The study

compared risk for those who consumed cod liver oil and those who consumed other types of vitamin D supplement; there was no significant association between intake of other types of vitamin D supplements and reduced risk of T1D.

In Italy, vitamin D supplementation during lactation was associated with reduced risk of T1D (OR 0.33; 95% CI 0.14–0.21) in one study,<sup>57</sup> but there was no association in another<sup>58</sup> when supplementation was given in "early life". In the latter, "early life" was not specifically defined; the null result may be related to the timing of supplementation. The authors proposed that the absence of an association in their study could be a consequence of the high contribution of exposure to UVR to the overall vitamin D status of children. They suggested that the majority of their study population were likely to have sufficient levels of vitamin D resulting in the supplements having little or no additional benefit.<sup>58</sup>

A birth cohort study<sup>59</sup> conducted in Finland has been one of the most influential studies looking at supplement use in early life conducted to date. The study collected data prospectively, thus excluding it from the criticisms that case-control studies have attracted. Furthermore, the study was conducted in a country where sun exposure was likely to have little impact on infants' overall vitamin D status and during a time when the recommended level of daily vitamin D supplementation for infants was five times higher than the current recommendation of 400 IU. The study reported a significant inverse association between intake of vitamin D-containing supplements during infancy and T1D risk by 30 years of age. Infants who received the recommended dose of 2000 IU were 78% less likely to develop T1D than those who received less.<sup>59</sup> A dose-response effect was also evident: among infants thought to have rickets, a condition that results from severe vitamin D deficiency, the risk of developing T1D was increased threefold. Infants who received vitamin D supplements regularly were 88% less likely to develop T1D compared with those receiving no supplementation.<sup>59</sup> The authors highlighted that within the regular supplement group, the risk decreased a further 86% for those who received more than the recommended dose of 2000 IU per day.<sup>41</sup> It is possible that the protective effect of vitamin D supplements observed by Hypponen and colleagues was the result of the high dose of vitamin D (2000 IU), substantially higher than the recommended vitamin D content for multivitamins studied by Stene and colleagues (400 IU).<sup>54,60,61</sup> Interestingly, in the Finnish study, infants supplemented with cod liver oil were categorised as receiving the recommended dose of 2000 IU. These results combined with those of Stene and colleagues suggest that cod liver oil containing 400 IU of vitamin D has superior benefits to 400 IU of vitamin D alone.

A meta-analysis of studies conducted up to 2011 reported that those who received vitamin D supplements in early childhood were less likely to develop T1D compared to those who did not receive supplements (OR 0.71; 95% CI 0.60–0.84,  $n = 668$ )<sup>17</sup> with evidence of a dose-response effect, *i.e.* greater reduction in T1D risk with higher vitamin D consumption.<sup>62</sup> A more recent





**Table 1** The association between vitamin D supplementation in early life and subsequent risk of T1D

Publication	Supplementation	Age range	Dose	Frequency	Duration	Association
EURODIAB Study (1999) <sup>16</sup>	Yes vs. no	Supplements first year of life	Not specified	Not specified	Less than a year vs. more than 1 year	Yes; OR = 0.67 (95% CI 0.53–0.86)
Stene <i>et al.</i> (2000) <sup>50</sup>	Yes vs. no (less than once per week)	Supplements first year of life	Speculated 400 IU	Less than once a week, 1–4 times a week, nearly everyday	Not specified	Cod liver oil – no; OR = 0.82 (95% CI 0.47–1.42) Other vitamin D supplements no; OR = 1.27 (95% CI 0.70–2.31)
Hypponen <i>et al.</i> (2001) <sup>59</sup>	Regular vs. none Irregular vs. none	Supplements first year of life	<2000 IU, within 2000 IU, >2000 IU	Regular, irregular and none	Not specified	Irregular – yes; OR = 0.16 (95% CI 0.04–0.74) Regular – yes; OR = 0.12 (95% CI 0.03–0.51)
Stene <i>et al.</i> (2003) <sup>51</sup>	Yes vs. no (less than once per week)	Supplements first year of life	Speculated 400 IU  Vit D supplements and cod liver oil supplements	Less than once a week, 1–4 times a week, nearly everyday	Not specified	Cod liver oil 1–4 times per week – no; OR = 0.81 (95% CI 0.55–1.19)  Cod liver oil ≥5 times per week – yes; OR = 0.74 (95% CI 0.56–0.99) Other vitamin D supplements 1–4 times per week – no; OR = 0.99 (95% CI 0.69–1.42) Other vitamin D supplements 1–4 times per week – no; OR = 0.97 (95% CI 0.73–1.29)
Visalli <i>et al.</i> (2003) <sup>58</sup>	Yes vs. no	Supplementation during “early years” – no time period provided	Not specified	Not specified	Not specified	No; OR = 1.22 (95% CI 0.82–1.83)
Tenconi <i>et al.</i> (2007) <sup>57</sup>	Yes vs. no	Vitamin D during lactation	Not specified	Not specified	Not specified	Yes; OR = 0.33 (95% CI 0.14–0.81)
Ahadi <i>et al.</i> (2011) <sup>175</sup>	Yes vs. no	Supplements first year of life	Not specified	Not specified	Not specified	Yes; lack of vitamin D supplementation OR = 3.78 (95% CI 1.60–8.89)

*meta*-analysis of eight studies on vitamin D intake in early life supported these findings (OR 0.71; 95% CI 0.51–0.98,  $n = 22\,538$ ).<sup>12</sup> No association between AD-drops (a preparation containing vitamin A and 2720 IU of cholecalciferol per ml) and the presence of T1D-related autoantibodies (GADA and IA-2) was reported in ABIS, although the authors suggested the study was insufficiently powered to detect a link.<sup>54</sup>

Many of the studies providing evidence are of a case-control design. This is efficient for uncommon diseases, but there are a number of limitations, largely involving accurate measurement of (prior) intake and the risk of confounding. Quantitative assessment of vitamin D intake is highly likely to be impacted by recall error (questionnaires concerned intake up to 16 years before study entry), failure to measure vitamin D intake *via* food, and insufficient information on supplement dose. The omission of vitamin D intake *via* food is particularly relevant for studies conducted in countries where diet contributes significantly to overall vitamin D status. No study qualified what combination of dose, frequency and duration of supplementation were used to categorise supplement use *vs.* no supplement use, making it difficult to interpret the odds ratios provided in each of the studies (*e.g.* would an infant be categorised as receiving supplements if they were administered 400 IU of vitamin D three times a week for 9 months of a year or was supplementation required for the full year to qualify?). Most studies did not measure 25(OH)D levels. It is possible that supplementation is only effective, or is maximally effective, when it raises 25(OH)D levels from deficiency to sufficiency. Related to confounding, neither the cohort nor case-control studies quantified the contribution of sun exposure to overall vitamin D status. As a consequence, researchers were unable to control for the production of vitamin D resulting from sun exposure or any independent immunomodulatory effects of UVR.

Only two studies<sup>50,51</sup> reported adjusting for maternal intake of supplements during pregnancy. Given the correlation between supplement use and socio-economic status<sup>59,63</sup> it is likely that children who receive supplementation of vitamin D also had mothers who took vitamin D supplements during pregnancy. Adjusting for maternal supplement use would assist in distinguishing whether the associations found are a culmination of exposure resulting from maternal and infant supplementation or if infant supplementation independently decreases risk. Nevertheless the studies are highly relevant and provide support for randomised controlled trials to determine causality and to provide guidance on the doses required for preventative measures.

## Vitamin D genes and T1D

If vitamin D metabolites are important in risk of T1D, it seems likely that there will also be an association between risk of T1D and genes in the vitamin D pathway that increase/decrease the level of the active metabolite, 1,25(OH)<sub>2</sub>D or activity of the vitamin D receptor.

Studies reporting an association between risk of T1D and vitamin D pathway genes are summarized in Table 2. The evidence for an association with *DHCR7* is contradictory, although the largest study showed a modest increase in risk associated with the G allele (compared to the T allele)<sup>64</sup> and another study showed a link with the presence of insulin antibodies but not T1D.<sup>65</sup> In contrast, there is relatively consistent evidence that the *CYP2R1* gene is associated with T1D risk, with a modest effect size. Genes encoding the VDBP determine 25(OH)D levels,<sup>44</sup> but there is no evidence of an association with risk of T1D. The largest studies support an effect of the *CYP27B1* gene on T1D risk; this may be dependent on the SNP tested, with one study showing an effect for the rs10877012 SNP (−1260 C/A polymorphism) but not the rs4646536 SNP (+2838 C/T polymorphism) in intron 6.<sup>66</sup> Only one small study showed an association between the *Bsm1* and *Fok1* polymorphisms of VDR and risk of T1D,<sup>67</sup> with larger studies consistently returning null results. Similarly, there were no significant associations with the *Apa1* or *Taq1* polymorphisms of VDR. Maternal vitamin D genotype may be important possibly through vitamin D-related effects *in utero*.<sup>68</sup> These findings are consistent with a recent *meta*-analysis that found no significant effect of individual VDR polymorphisms on T1D risk, but a possible association with specific haplotypes (see Table 2).<sup>69</sup>

Recent studies looking at vitamin D status and expression of vitamin D pathway genes in blood,<sup>70</sup> CD4+ memory cells<sup>71</sup> and dendritic cells<sup>72</sup> demonstrated the biological plausibility of vitamin D being involved in the risk of T1D.

Inconsistent findings from these genetic studies may be due to sample size issues, or that effects are location dependent<sup>73</sup> or only apparent for the combination of a less effective polymorphism (*e.g.* in VDR) with low 25(OH)D level, as has been demonstrated for risk of tuberculosis.<sup>74</sup> As yet, genetic studies do not support a causal role of vitamin D deficiency in T1D risk.

## Animal studies

Experiments on the non-obese diabetic (NOD) mouse, the preferred murine model of T1D, have been used to better understand the etiopathogenesis of T1D, test potential therapeutic interventions and understand their mechanisms of action. The mechanisms leading to pancreatic  $\beta$ -cell destruction in the NOD mouse include activation of cytotoxic CD8+ lymphocytes and macrophages which are regulated by IL-12-dependent T-helper 1 (Th1) cells. The active form of vitamin D, 1,25(OH)<sub>2</sub>D, inhibits IL-12 production.

Vitamin D-deficient NOD mice have higher incidence of T1D and exhibit earlier onset when compared to NOD mice maintained on a diet that leads to vitamin D-sufficiency.<sup>75</sup> These findings persist even when the deficiency is subtle and vitamin D status is restored at 100 days.<sup>76</sup>

Experiments on NOD mice have yielded fairly consistent support for the beneficial role of vitamin D in delaying onset and preventing T1D when administered lifelong.



Table 2 Vitamin D pathway genes and risk of T1D

Publication	Case/control	Gene	Allele	Association with T1D risk
Cooper <i>et al.</i> (2011) <sup>176</sup> Frederiksen <i>et al.</i> (2013) <sup>177</sup>	8517/10438 1708 high genetic risk; 148 IA; 62 IA and T1D	<i>DHCR7</i> <i>DHCR7/NADSYN1</i>	rs12785878	G allele ( <i>cf.</i> T allele): OR = 1.07 (95% CI 1.02–1.13) Increased risk of IA but not T1D. HR = 1.36 (95% CI 1.08–1.73) for each additional minor allele
Thorsen <i>et al.</i> (2014) <sup>178</sup> Cooper <i>et al.</i> (2011) <sup>176</sup> Hussein <i>et al.</i> (2012) <sup>179</sup> Ramos-Lopez <i>et al.</i> (2007) <sup>70</sup>	1467 trios (907 cases, 896 sibs) 8517/10438 120/120 203 simplex T1D families ( <i>n</i> = 609)	<i>DHCR7</i> <i>CYP2R1</i> <i>CYP2R1</i> <i>CYP2R1</i>	rs12785878 rs12794714 rs10741657 rs10741657	G allele ( <i>cf.</i> T allele): OR = 0.93, <i>p</i> = 0.21 T allele ( <i>cf.</i> C allele): OR = 1.04 (95% CI 1.00–1.09) GG associated with increased risk Variant G more often transmitted to affected offspring and more frequent in cases than controls
Thorsen <i>et al.</i> (2014) <sup>178</sup> Blanton <i>et al.</i> (2011) <sup>180</sup> Blanton <i>et al.</i> (2011) <sup>180</sup> Thorsen <i>et al.</i> (2014) <sup>178</sup> Cooper <i>et al.</i> (2011) <sup>176</sup> Cooper <i>et al.</i> (2011) <sup>176</sup> Cooper <i>et al.</i> (2011) <sup>176</sup> Bailey <i>et al.</i> (2007) <sup>181</sup> Bailey <i>et al.</i> (2007) <sup>181</sup> Fichna <i>et al.</i> (2010) <sup>182</sup>	1467 trios (907 cases, 896 sibs) 203/153/116 first degree relatives 203/153/116 first degree relatives 1467 trios (907 cases, 896 sibs) 8517/10438 8517/10438 8517/10438 7854/8758 7854/8758 215/236	<i>CYP2R1</i> <i>GC</i> <i>GC</i> <i>GC</i> <i>GC</i> <i>CYP27B1</i> <i>CYP27B1</i> <i>CYP27B1</i> <i>CYP27B1</i>	rs12794714 rs10741657 rs4588 rs7041 rs2282679 rs4588 rs7041 rs10877012 rs10877012 rs4646536	A allele ( <i>cf.</i> G allele): OR = 1.01, <i>p</i> = 0.86 A allele ( <i>cf.</i> C allele): OR = 1.05 (95% CI 0.91–1.20) T allele ( <i>cf.</i> G): OR = 1.07 (95% CI 0.92–1.24) C allele ( <i>cf.</i> A allele): OR = 1.01, <i>p</i> = 0.90 A allele ( <i>cf.</i> C allele): OR = 0.95 (95% CI 0.91–1.00) T allele ( <i>cf.</i> G allele): OR = 0.98 (95% CI 0.93–1.03) A allele ( <i>cf.</i> G allele): OR = 0.93 (95% CI 0.89–0.98) C allele ( <i>cf.</i> A): OR = 1.07 (95% CI 1.02–1.13) T allele ( <i>cf.</i> C allele): OR = 1.08 (95% CI 1.02–1.14) <i>p</i> = 0.67 for difference in allele frequency between cases and controls
Hussein <i>et al.</i> (2012) <sup>179</sup> Lopez <i>et al.</i> (2004) <sup>183</sup>	120/120 252/320	<i>CYP27B1</i> <i>CYP27B1</i>	rs10877012 rs10877012	CC associated with increased risk T1D associated with allelic variation in the promoter (rs10877012) polymorphism ( <i>p</i> = 0.003) but not the intron 6 (rs4646536) polymorphism
Frederiksen <i>et al.</i> (2013) <sup>177</sup>	1708 high genetic risk; 148 IA; 62 IA and T1D	<i>CYP27B1</i>	rs4646536 rs4646536	Increased risk of IA but not T1D HR = 0.59, 0.39–0.89 for A/G <i>cf.</i> G/G
Thorsen <i>et al.</i> (2014) <sup>178</sup> Abd-Allah <i>et al.</i> (2014) <sup>184</sup>	1467 trios (907 cases, 896 sibs) 120/120	<i>CYP27B1</i> <i>VDR</i>	rs4646536 Bsm1; (BB)	C allele ( <i>cf.</i> T allele): OR = 0.96, <i>p</i> = 0.48 Bb: AOR = 2.1 (95% CI 1.1–3.2); bb: AOR = 1.7 (95% CI 1.0–1.9)
Cooper <i>et al.</i> (2011) <sup>176</sup> Garcia <i>et al.</i> (2007) <sup>185</sup>	8517/10438 216/203	<i>VDR</i> <i>VDR</i>	Bsm1 Bsm1 (BB)	A allele ( <i>cf.</i> G allele): OR = 1.00 (95% CI 0.95–1.05) Frequency of b allele and bb genotype significantly lower in T1D cases, <i>p</i> < 0.04
Thorsen <i>et al.</i> (2014) <sup>178</sup> Capoluongo <i>et al.</i> (2006) <sup>186</sup>	1467 trios (907 cases, 896 sibs) 246/246	<i>VDR</i> <i>VDR</i>	Bsm1 Bsm1 (BB)	T allele ( <i>cf.</i> C allele): OR = 0.94, <i>p</i> = 0.22 Bb: OR = 1.01 (95% CI 0.64–1.59); bb: OR = 0.92 (95% CI 0.54–1.57)
Lemos <i>et al.</i> (2008) <sup>187</sup> Abd-Allah <i>et al.</i> (2014) <sup>184</sup>	207/249 120/120	<i>VDR</i> <i>VDR</i>	Bsm1 Fok1; (FF)	G allele ( <i>cf.</i> A allele): OR = 1.01 (95% CI 0.78–1.31) Ff: AOR = 1.7 (95% CI 1.0–2.7); Ff: AOR = 3.8 (95% CI 1.2–9.4)
Hamed <i>et al.</i> (2013) <sup>188</sup> Thorsen <i>et al.</i> (2014) <sup>178</sup> Cooper <i>et al.</i> (2011) <sup>176</sup> Capoluongo <i>et al.</i> (2006) <sup>186</sup>	132/40 1467 trios (907 cases, 896 sibs) 8517/10438 246/246	<i>VDR</i> <i>VDR</i> <i>VDR</i> <i>VDR</i>	Fok1 Fok1 Fok1 Fok1 (FF)	f allele ( <i>cf.</i> F allele): OR = 1.08 (95% CI 0.64–1.85) T allele ( <i>cf.</i> C allele): OR = 0.99, <i>p</i> = 0.85 A allele ( <i>cf.</i> G allele): OR = 0.99 (95% CI 0.95–1.04) Ff: OR = 0.90 (0.60–1.35); ff: OR = 1.64 (95% CI 0.91–2.97)
Lemos <i>et al.</i> (2008) <sup>187</sup> Abd-Allah <i>et al.</i> (2014) <sup>184</sup>	207/249 120/120	<i>VDR</i> <i>VDR</i>	Fok1 Apa1 (AA)	T allele ( <i>cf.</i> C allele): OR = 0.93 (95% CI 0.71–1.22) Aa: AOR = 0.6 (95% CI 0.5–1.0); aa: AOR = 0.6 (95% CI 0.2–1.1)
Thorsen <i>et al.</i> (2014) <sup>178</sup> Garcia <i>et al.</i> (2007) <sup>185</sup>	1467 trios (907 cases, 896 sibs) 216/203	<i>VDR</i> <i>VDR</i>	Apa1 Apa1	C allele ( <i>cf.</i> A allele): OR = 0.99, <i>p</i> = 0.92 <i>p</i> = NS for the difference in allele frequency between cases and controls
Lemos <i>et al.</i> (2008) <sup>187</sup>	207/249	<i>VDR</i>	Apa1	T allele ( <i>cf.</i> G allele): OR = 1.01 (95% CI 0.77–1.31)

Table 2 (Contd.)

Publication	Case/control	Gene	Allele	Association with T1D risk
Abd-Allah <i>et al.</i> (2014) <sup>184</sup>	120/120	VDR	Taq1 (TT)	Tt: AOR = 0.6 (0.4–1.2); tt: AOR = 0.7 (95% CI 0.4–1.3)
Lemos <i>et al.</i> (2008) <sup>187</sup>	207/249	VDR	Taq1	T allele (cf: C allele): OR = 0.90 (95% CI 0.69–1.18)
Garcia <i>et al.</i> (2007) <sup>185</sup>	216/203	VDR	Taq1	<i>p</i> = NS for the difference in allele frequency between cases and controls
Garcia <i>et al.</i> (2007) <sup>185</sup>	216/203	VDR	haplotype	BAT higher in cases ( <i>p</i> = 0.002); BAT higher in controls ( <i>p</i> = 0.003); AabbTT and aabbTT associated with higher levels of GADA, IA-2 and TGF- $\beta$
Cooper <i>et al.</i> (2011) <sup>176</sup>	8517/10438	CYP24A1	rs2296241	A allele (cf: G allele): OR = 1.00 (95% CI 0.95–1.05)
Bailey <i>et al.</i> (2007) <sup>181</sup>	7854/8758	CYP24A1		Null ( <i>p</i> = 0.23)
Thorsen <i>et al.</i> (2014) <sup>178</sup>	1467 trios (907 cases, 896 sibs)	CYP24A1	rs6013897	A allele (cf: T allele): OR = 1.03, <i>p</i> = 0.65
Lopez <i>et al.</i> (2004) <sup>183</sup>	187 families with one child with T1D	CYP27B1	–1260 C/A	Haplotype CT (–1260 A/2338 T) was significantly more often transmitted to affected offspring; AT (–1260 C/2838 T) was significantly less often transmitted ( <i>p</i> = 0.02)
Miettinen <i>et al.</i> (2015) <sup>68</sup>	Mothers ( <i>n</i> = 512), family members ( <i>n</i> = 708), & child with T1D ( <i>n</i> = 534) cf: healthy control children ( <i>n</i> = 381), mothers (379) and fathers ( <i>n</i> = 340)	VDR	2338 T/C (31 SNPs)	Maternal rs1544410 and rs731236 significant after adjustment for multiple testing. No difference in allele frequency between case and control children.

Unfortunately, the translation of these beneficial results from NOD mice to humans has been challenging due to the hypercalcaemic and/or bone effects resulting from the large doses that have been required to achieve disease protection.

Administration of 1,25(OH)<sub>2</sub>D<sub>3</sub> can at least partially prevent the development of insulinitis and T1D in NOD mice.<sup>77–80</sup> Daily oral administration of pharmacological doses (50 ng) from weaning to end of life completely prevented the development of the disease.<sup>75</sup> However, a lower calcaemic dose of 10 ng day<sup>–1</sup> offered only partial protection, suggesting that hypercalcaemia may be required for complete protection. This was supported by another study that found reduced disease protection with lower calcium levels, when comparing outcomes of NOD mice treated with 1,25(OH)<sub>2</sub>D<sub>3</sub> and its synthetic precursor 1 $\alpha$ OH D<sub>3</sub>. Similar effects have been demonstrated in other autoimmune disorders such as experimental autoimmune encephalomyelitis (EAE, the animal model of MS).<sup>80,81</sup>

Initially all studies that had shown beneficial effects of vitamin D on T1D incidence administered 1,25(OH)<sub>2</sub>D<sub>3</sub> and the first study to administer the precursor, vitamin D<sub>3</sub>, produced null results.<sup>82</sup> This led to the belief that vitamin D<sub>3</sub> administration was ineffective in reducing disease risk; however, more recent studies have refuted this<sup>83</sup> and it is likely that the short regimen (*in utero* to 70 days) was insufficient to interfere with disease progression in NOD mice.

Different treatment windows for vitamin D supplementation for preventing the onset of T1D have been tested. So far, administration of vitamin D<sub>3</sub> during pregnancy or early life (up to 70 days), even at high doses (1000 IU per day) has had little to no effect on diabetes development in NOD mice or their offspring.<sup>82–84</sup> It has been suggested that during this early period, training of immune cells is yet to occur and thus escapes the immunomodulatory actions of vitamin D.<sup>83</sup> In one study NOD mice were treated with 800 IU of vitamin D<sub>3</sub> during pregnancy, early life or lifelong. The study reported that vitamin D supplementation could only significantly prevent T1D development in both male and female NOD mice if administered lifelong. Lifelong supplementation resulted in a 58% and 52% reduction in diabetes incidence, respectively, when compared with control mice.<sup>83</sup> These findings were corroborated by Gysemans and colleagues (2005) suggesting that only lifelong treatment, initiated immediately after weaning, resulted in significant protection from developing T1D (66% risk reduction; *p* ≤ 0.0001).<sup>85</sup>

A concern from many of the NOD mouse studies was that the doses required to elicit therapeutic benefit resulted in hypercalcaemia and/or bone demineralization. In response there have been concerted efforts worldwide to develop structural analogs of 1,25(OH)<sub>2</sub>D<sub>3</sub> which offer more pronounced immunomodulatory effects without calcaemic side effects. KH1060, a structural analogue of 1,25(OH)<sub>2</sub>D<sub>3</sub>, administered at doses of 200 ng or 400 ng KH1060 per kg intraperitoneally in 0.05 ml arachis oil on alternate days, delayed and prevented onset of insulinitis and T1D in NOD mice,<sup>79</sup> and in another study the 1(OH)D<sub>3</sub> analog provided greater disease protection when compared with 1,25(OH)<sub>2</sub>D<sub>3</sub>.<sup>80</sup> Of the two analogs, only





the low dose of KH1060 mediated some protection without eliciting significant hypercalcaemic effects (60% decrease in incidence). As seen with  $1,25(\text{OH})_2\text{D}_3$ , the increased protection provided by the higher dose of KH1060 (400 ng) and the  $1(\text{OH})\text{D}_3$  analog coincided with elevated serum calcium levels.

In addition to reducing the incidence of T1D, non-hypercalcaemic analogs of  $1,25(\text{OH})_2\text{D}_3$  inhibited the progression of insulinitis to clinical diabetes and prevented recurrence of T1D following islet transplantation. An experiment using NOD mice tested the effectiveness of MC1288 (a non-hypercalcaemic analog of  $1,25(\text{OH})_2\text{D}_3$ ) with and without a short course of the anti T cell immunosuppressant, cyclosporine A (CyA), in inhibiting the progression of insulinitis to clinical diabetes. Monotherapy of CyA or MC1288 was ineffective in preventing the progression to overt disease at 200 days, however, the combined treatment of MC1288 with CyA, significantly reduced diabetes incidence to 35% when compared with the control group (65%).<sup>86</sup> In another study, a  $1,25(\text{OH})_2\text{D}_3$  analog, BXL-219 (formerly RO 26-2198), given to adult NOD mice, prevented progression to overt disease by 38 weeks (90% in the control group *vs.* 16% in the NOD mice treated for 16 weeks). In mice that did not progress to overt disease, there was an increase in the frequency of T regulatory cells (CD4+ CD25+) in pancreatic lymph nodes that specifically inhibited T cell responses to the autoantigen IA-2.<sup>87</sup> BXL-219 can downregulate proinflammatory chemokine production by pancreatic islets leading to reduction in T-cell recruitment.<sup>88</sup>

KH1060 also delayed recurrence of T1D in NOD mice following islet transplantation. Islet survival was significantly prolonged in NOD mice treated with KH1060 or cyclosporine1 (60 days and 50 days, respectively, *versus* 9.5 days in controls;  $p < 0.001$  and  $p < 0.0001$ ); however mice treated with sub-therapeutic doses of both drugs also showed prolonged graft survival (48 days;  $p < 0.0001$ ). Furthermore, 80% of the mice that remained normoglycemic to 60 days post-transplantation remained disease free for more than 15 days after the cessation of all treatment.<sup>89</sup>

Streptozotocin (STZ), an antibiotic that causes destruction of pancreatic  $\beta$  cells, can be used to create rodent models of T1D. The model has been useful in elucidating associations between insulin and various stages in the vitamin D metabolic pathway. In the STZ diabetic rat, induction of T1D was associated with reduction of  $1,25(\text{OH})_2\text{D}$  levels that was reversible with insulin therapy.<sup>90</sup> Furthermore, induced diabetes resulted in a decrease in vitamin D binding protein concentration, so that total  $1,25(\text{OH})_2\text{D}$  was reduced. Notably, the concentration of free  $25(\text{OH})\text{D}$  and  $1,25(\text{OH})_2\text{D}$  were unchanged.<sup>91</sup>

In this model, insulin has a direct stimulatory effect on the  $1\alpha$ -hydroxylase enzyme that converts  $25(\text{OH})\text{D}$  to the active form, and also alters the responsiveness of renal  $1\alpha$ -hydroxylase to the usual cue of lowered phosphate levels and changes in PTH.<sup>92,93</sup> In the chronic insulin-deficient state there is significantly reduced  $1\alpha$ -hydroxylase activity and enhanced renal  $24$ -hydroxylase activity. Similar actions in humans could explain the lower  $1,25(\text{OH})_2\text{D}$  levels and increased  $24,25(\text{OH})_2\text{D}$  levels that are commonly seen in chil-

dren with T1D,<sup>94,95</sup> *i.e.* changes in vitamin D metabolism are the result of insulin deficiency, rather than the cause.<sup>96</sup>

In summary,  $1,25(\text{OH})_2\text{D}_3$ , vitamin  $\text{D}_3$  or their structural analogs can delay or inhibit the development of T1D when administered at pharmacological doses from weaning to end of life. Daily oral administration appears to be more effective in preventing the onset of T1D than intraperitoneal administration given on alternate days. Elevated calcium levels may be required for maximal benefit from vitamin D supplementation. Structural analogues provided alone or in conjunction with an immunosuppressant agent can reduce progression to overt disease after the development of autoantibodies, and recurrence after islet transplantation.

## In vitro studies

Human pancreatic tissue expresses  $1\alpha$ -hydroxylase and VDR<sup>97</sup> and a vitamin D response element has been identified in the human insulin receptor gene promoter.<sup>98</sup> Suspensions of rat islet cells can convert  $25(\text{OH})\text{D}$  to  $1,25(\text{OH})_2\text{D}$  and acute application of high dose  $1,25(\text{OH})_2\text{D}$  ( $50 \text{ nmol L}^{-1}$ ) causes a marked rise in calcium levels, confirming that the cells are responding to  $1,25(\text{OH})_2\text{D}$ .<sup>97</sup> That is, pancreatic tissue can use circulating  $25(\text{OH})\text{D}$  to make  $1,25(\text{OH})_2\text{D}$  locally, and pancreatic cells are responsive to  $1,25(\text{OH})_2\text{D}$ .

Immune cells from people with T1D have been cultured with  $25(\text{OH})\text{D}$  or  $1,25(\text{OH})_2\text{D}$ . The doses used are often much higher than is seen in serum; it is proposed that local production of vitamin D metabolites can lead to high local levels. In monocytes from patients with T1D and healthy controls, culture with  $25(\text{OH})\text{D}$  ( $125 \text{ nmol L}^{-1}$ ) significantly inhibited the differentiation of monocytes into dendritic cells, increasing the number of intermediate cells (CD11c+ CD14+ CD83+ CD123–/low). These cells had a similar phenotype to previously described DC-10 cells, which produce IL-10 and have tolerogenic characteristics because they can induce T regulatory cells.<sup>99</sup> This increase in response to  $25(\text{OH})\text{D}$  depended on the VDR genotype: smaller increase in intermediate cells in genotype bb (of BsmI) than BB and Bb; and a smaller increase for genotype TT compared to tt (TaqI).<sup>72</sup> In T-helper (Th) cells isolated from patients with T1D and healthy controls that were stimulated with  $25(\text{OH})\text{D}$  ( $125 \text{ nmol L}^{-1}$ ), there was significantly lower VDR expression in cells from patients with T1D compared to those from healthy controls. There were significantly fewer CD4+ cells in  $25(\text{OH})\text{D}$ - and  $1,25(\text{OH})_2\text{D}$ -stimulated Th cells from T1D patients carrying the FF genotype of FokI compared to those with Ff/ff ( $p = 0.02$ ). This suggests that vitamin D supplementation may be more effective for prevention and/or management of T1D in those carrying the FF genotype, through promoting a more regulatory T-cell milieu.<sup>100</sup> However, in another study, in isolated CD4+ memory cells, FOXP3 expression was higher in cells from Estonian compared to Finnish children with T1D even in those who were vitamin D sufficient. The authors concluded that the findings did not support a crucial role for  $25(\text{OH})\text{D}$  as



a regulator of  $\beta$ -cell autoimmunity or FOXP3 expression,<sup>71</sup> although they did not investigate the possible modulating effect of VDR genotype.

The evidence that  $1,25(\text{OH})_2\text{D}$  protects against cytokine-induced  $\beta$  cell death is inconsistent. In an older study, both physiological and supraphysiological concentrations of  $1,25(\text{OH})_2\text{D}$  (ranging from  $10 \text{ pmol L}^{-1}$  to  $10 \text{ nmol L}^{-1}$ ) and vitamin D analogues (KH1060 and MC1288) were ineffective in attenuating the inhibitory effect of IL-1 $\beta$  on insulin release in islet cells of newborn rats.<sup>101</sup> Similarly, Gysemans and colleagues showed that exposure to  $1,25(\text{OH})_2\text{D}$  reduced expression of several cytokines (IL-1 $\beta$ , IP-10 and IL-15) but did not prevent cytokine-induced  $\beta$ -cell death.<sup>85</sup> However, in other studies, exposure of isolated rat pancreatic islets to IL-1 $\beta$  in the presence of  $1,25(\text{OH})_2\text{D}$  (varying concentrations down to  $0.1 \text{ nmol L}^{-1}$ ) or analogues were protective and counteracted the suppressive effects of IL-1 $\beta$  on insulin production.<sup>102</sup> In a series of studies, Riachy and colleagues showed that, in human pancreatic islet cells treated with IL-1 $\beta$ , TNF- $\alpha$  and IFN- $\gamma$ , the addition of  $1,25(\text{OH})_2\text{D}$  ( $10^{-8}$  and  $10^{-6} \text{ mol L}^{-1}$ ) significantly reduced inflammation and the vulnerability of islet cells to cytotoxic T lymphocytes.<sup>103</sup> Later studies showed that, in both rat and human islet cells,  $1,25(\text{OH})_2\text{D}$  induced high levels of an anti-apoptotic protein (A20)<sup>104</sup> and was able to counteract cytokine-induced Fas expression that makes cells susceptible to apoptosis,<sup>105</sup> thereby providing cyto-protection of islet cells. It seems likely that  $1,25(\text{OH})_2\text{D}$  acts on both immune cells and  $\beta$  cells, reducing the expression of key pro-inflammatory genes such as IL-1 $\beta$  as well as protecting the islet cells by making them less inflammation prone, and chemoattractive.<sup>85</sup>

## Latitude and sun exposure in risk of T1D

To our knowledge there are no pre-clinical studies investigating an immunomodulatory effect of UVR on T1D. Also, *in vitro* studies are relevant only to examining the effects of vitamin D. An exception is a study of the direct effects of UV irradiation on skin equivalents. The following section therefore reviews the human studies that have demonstrated a link between T1D and proxies of UVR exposure and vitamin D including latitude and ambient UVR. To date no studies have measured individual UVR exposure or adjusted for dietary intake of vitamin D. Thus these studies using proxies for personal UVR exposure/vitamin D are not able to help in delineating whether observed associations are due to sun exposure, vitamin D, or a related exposure.

## Latitude gradient

A positive correlation between latitude and the incidence of T1D has been observed globally, with higher incidences generally observed in locations further away from the Equator. Studies within countries have had more consistent results

than those that compare between countries. Indeed, within country analysis may provide more accurate information,<sup>106</sup> particularly if the residing populations are genetically and culturally homogenous.<sup>107</sup> Importantly, ecological studies provide correlations only and are unable to define which of the many biological, environmental and behavioural correlates of latitude are etiologically important, for example, genetic susceptibility; infection exposure; socioeconomic status; dietary intake of fortified foods, supplements and fatty fish; climatic variation; sun seeking behaviours and clothing habits.<sup>31</sup>

Two studies conducted within Australia have provided valuable insight into the association between latitude and T1D due to the wide latitude range, relatively homogenous population and insignificant contribution of vitamin D intake to overall vitamin D status.<sup>108,109</sup> In a nationwide ecologic analysis of immune disorders there was a positive correlation between T1D prevalence and increasing southern latitude ( $r = 0.77$ ,  $p = 0.026$ ). The prevalence of T1D was almost three-fold higher between the south and north latitude extremes.<sup>110</sup> Similar results were reported in a more recent study of 1571 children on the Western Australian Children's Diabetes Database, which considered the effect of latitude in conjunction with a number of potential confounding factors. The study found that for every 1 degree increase in latitude, the risk of T1D increased by 3.5%, as averaged across the range of  $15\text{--}35^\circ$  South, after adjustment for socioeconomic status, population density, remoteness and ethnicity.<sup>111</sup> The modest latitude effect observed in Australia<sup>31</sup> has also been found in Sweden<sup>112</sup> and Norway,<sup>113</sup> whilst in China the incidence rate in the northern middle regions is 12-fold higher than in the southern middle regions.<sup>114</sup>

Two large studies as part of the EURODIAB Study and the Diabetes Mondial Project Group have conducted between-country analyses and returned interesting results. In the EURODIAB Study, climatological factors such as rainfall, sunshine hours and average temperatures had little relevance to the significant positive correlation between increasing latitude and incidence of T1D, leading the authors to postulate that either wealth or genetic variation were more likely the source of the observed latitude gradient.<sup>115</sup> The Diabetes Mondial Project Group assessed the incidence of T1D among children across 51 countries, and conversely reported that UVB irradiance was inversely associated with T1D incidence, and overall incidence rates were positively associated with per capita health expenditure.<sup>6</sup> Finland, one of the furthestmost northern countries included in the study ( $60^\circ$  North) had an incidence rate over 7 times that of countries approaching zero latitude (at the Equator) which was indicative of the general trend. However, Sardinia was a notable exception to this trend, with its rate (45 per 100 000) substantially higher than its neighbouring countries. Similarly, Estonia, one of the bordering countries to Finland had an incidence rate that was a quarter that of Finland despite being ethnically similar.<sup>116</sup> Such disparity in incidence rates between countries supports the notion that ethnic groups with their varying degrees of genetic susceptibility contribute to the spatial variation in incidence<sup>117</sup> in combination with



environmental and behavioural factors. However, migrant studies which show that the rates among those from low incidence countries assimilate quickly to those of the country they live in<sup>118,119</sup> suggest that environmental effects may be more important than those of ethnicity.

## Seasonal variation in month of birth

Many studies have examined the effect of month of birth on T1D risk. However, whether an association exists remains an open question. A significant difference in birth seasonality pattern in children who were later diagnosed with T1D compared with the seasonal pattern of total live births has been found in several registers worldwide, with most studies reporting an excess in late spring/summer<sup>120–128</sup> and/or a trough in late autumn/winter,<sup>123,124</sup> though this is not completely consistent.<sup>129–131</sup> One study reported that a diagnosis of T1D was 30% more common among those born in spring than in winter.<sup>127</sup> Some studies have reported seasonal variation in sub-populations (such as males and homogenous populations) only,<sup>122,124,131–133</sup> and many have reported no difference in seasonality pattern from that of the general population.<sup>31,134–137</sup>

It has been suggested that the apparent seasonal month of birth pattern among those diagnosed with T1D is due to the well-established relationship between season and serum 25(OH)D levels observed in many countries throughout the world and the immunosuppressive effects of UVR and/or vitamin D.<sup>35</sup> An excess in late spring/summer births coincides with the lowest serum 25(OH)D levels being experienced in the second trimester of pregnancy. Maternal vitamin D insufficiency during the second trimester may affect in utero development of the pancreatic  $\beta$ -cells<sup>138</sup> or regulate the developing immune system in a way that increases the risk of T1D in childhood.<sup>31</sup>

Of note, in countries closest to the Equator, where cutaneous production of vitamin D is possible throughout the year, or countries where dietary intake of vitamin D is significant, there is little seasonal variation in 25(OH)D levels and no evidence of a birth-month effect for T1D. A number of nutritional studies have found that the commonly observed association between season and 25(OH)D levels was negated by increased supplement use<sup>139</sup> or greater consumption of fatty fish<sup>6</sup> in winter months. If vitamin D status were in some way responsible for the seasonal variation in birth rate among those diagnosed with T1D then we would expect that not all studies, particularly those conducted in countries where 25(OH)D levels are relatively constant throughout the year, would observe an association.

An absence of birth-month effect is more commonly reported in countries of low incidence of T1D,<sup>132,137</sup> likely somewhat the result of small sample sizes but also pointing to ethnicity as a potential confounding factor.<sup>106</sup> A large cohort study spanning Europe (37–53°N), Australia (34°N), USA (39–40°N) and Israel (31°N) was one of several studies that found no seasonal pattern in month of birth in low incidence countries, reporting a seasonal pattern only in ethnically homo-

genous populations that had a medium to high incidence rate of T1D.<sup>132</sup> It has been suggested by others that ethnicity may be a modifier due to differences in circulating vitamin D binding protein between ethnic groups,<sup>140</sup> in addition to differences in sun protective behaviours and diet. Beyond mechanisms of UVR and/or subsequent vitamin D production, alternative explanations for a birth-month effect are thought to involve seasonal exposures to viruses and infections.<sup>121</sup> Overall the evidence supporting an association between month of birth and T1D is unclear. Despite some consistency across studies, factors such as ethnicity, latitude and background incidence appear to be important, making it difficult to draw conclusions in relation to an effect of sun exposure or vitamin D.

## Seasonality of diagnosis of T1D

Seasonality of diagnosis of T1D has been extensively studied and reported in both northern<sup>114,122,133,141–145</sup> and southern hemisphere studies.<sup>31,141,146–151</sup> In many countries, seasonality of diagnosis follows a cyclic, sinusoidal pattern<sup>149</sup> with a peak occurring in cooler winter months,<sup>143,148–150,152–154</sup> or late autumn/winter<sup>122,141,155</sup> and a nadir in spring and summer.<sup>122,143,155</sup> Unlike month of birth, seasonality of diagnosis is now a well-recognised feature of T1D in childhood.<sup>141</sup>

Seasonal variation in diagnosis is commonly more marked in boys,<sup>133,141,142,144,145,148,152</sup> older children (5–14 years)<sup>122,141–144,152,156</sup> and in countries which experience higher incidence and prevalence of T1D.<sup>141</sup> Larger studies offering greater statistical power are required to confirm these findings<sup>107</sup> and identify the underlying reasons for the observed differences between sub populations.

While somewhat consistent, the source or sources of the above associations are not clear. It is likely that the birth month effect and seasonal variation in diagnosis of T1D arise by different mechanisms. Given the time that it takes for T1D to manifest, it is more likely that an excess in diagnosis of T1D in cooler winter months is more indicative of a season-dependent precipitating factor that triggers the presentation of symptoms or acceleration of  $\beta$ -cell destruction among those at an advanced stage of disease development, rather than a cause-of-disease relationship.<sup>18</sup> Viral infections, temperature, low UVR exposure, or low 25(OH)D levels are likely influencing factors as they follow similar cyclic patterns to disease onset and have also been linked with the pathogenesis of T1D. However, we need more precisely characterised risk factors to demonstrate that there is variation in disease onset between seasons that persists after adjusting for confounding factors, and to allow a better understanding of the underlying reasons for the association.

## Temperature

Temperature is one of the possible mechanisms underpinning the observed seasonal variation in diagnosis, and recent human observational studies have reported an independent



association between temperature and T1D onset.<sup>145,157</sup> Cooler temperatures affect insulin production by triggering the release of norepinephrine which increases hepatic and peripheral insulin resistance.<sup>157</sup> The increase in demand for insulin places significant load on the remaining operating  $\beta$ -cells causing them to fail. In the advanced stages of disease development when  $\beta$ -cells are limited, this acute overload may lead to the presentation of symptoms and subsequent diagnosis in cooler months.

## Ambient UVR

One environmental factor that varies by both latitude and season is ambient UVR. Latitude, season, and sunshine hours have been used as proxies for UVR, however technological advances have allowed more recent studies to measure ambient UVR. To our knowledge no study has measured personal exposure to UVR and examined its independent effect on T1D risk so here we review studies that have used ambient UVR as a marker of sun exposure.

Data on ambient UVR collected from various satellites have been linked with registers in Australia, Canada, and Sweden and from the Diabetes Mondial Project Group. Two Australian and one Canadian study found that T1D incidence/prevalence was significantly and independently inversely correlated with levels of ambient UVR.<sup>31,110,153</sup> One of the Australian studies showed that the relationship was non-linear and moreover, the inverse relationship appeared to be restricted to low population density areas and with an apparent positive relationship in areas of high population density.<sup>31</sup> It was hypothesised that the relationship between ambient UVR and actual exposure may be lost in cities (high population density) because of tall buildings and a less outdoors lifestyle. Data from the Diabetes Mondial Project Group showed that there was an inverse association between incidence of T1D and UVB irradiance which persisted after controlling for per capita health expenditure.<sup>6</sup> However, while there was an overall inverse trend based on the combined data, there were a number of discrepant regions. These recent studies provide some evidence of a statistically significant inverse correlation between T1D and UVB irradiance.

The idea that UVR may in some way have a role in the development of the disease seems well supported in the epidemiological literature; however the mechanisms remain unknown. It is unclear whether UVR exposure impacts T1D risk through its subsequent production of vitamin D or whether other immunoregulatory molecules produced in skin following exposure to UVR are responsible. Equally, it may be that the combination of exposure to UVR and vitamin D sufficiency is necessary to reduce risk.<sup>158</sup>

## 25(OH)D levels and T1D

As both dietary vitamin D and exposure to UVR increase blood 25(OH)D levels, 25(OH)D levels are not only an indication of

vitamin D status but also recent UV exposure. For this reason, 25(OH)D levels are addressed here in this perspective. The modulating effects of high 25(OH)D levels during the antenatal period on T1D risk maybe specific to the third trimester. Serum 25(OH)D levels of newborn babies taken at the time of birth were not associated with later risk of T1D in two studies.<sup>140,159</sup> In a recent study, Sorenson and colleagues (2016) measured the serum 25(OH)D levels of mothers at multiple time points throughout pregnancy and subsequent risk of T1D in their offspring up to the age of 15 years.<sup>10</sup> The results were consistent with findings of two previous studies in the field, indicating a significant association between low 25(OH)D levels during late pregnancy and increased T1D risk<sup>11</sup> and no association with levels in early pregnancy.<sup>160</sup> It is not known whether the apparent reduction in risk with high levels of 25(OH)D during the third trimester is associated with the large transfer of 25(OH)D through the placenta that occurs during this time<sup>161</sup> or some other unknown biological mechanism unique to the third trimester.

In the absence of a single study that has quantified maternal supplement use and measured 25(OH)D levels at multiple time points throughout pregnancy, it is not possible to uncover why there is an apparent association between reduced risk of T1D and high levels of 25(OH)D during the third trimester but not with maternal intake of vitamin D supplements during pregnancy. It is possible that higher doses of supplementation than are currently recommended may be required to achieve a protective effect in offspring, or that benefits of high levels of 25(OH)D in the third trimester are due to factors other than vitamin D *per se*, such as exposure to UVR.

Studies that measure 25(OH)D levels in addition to both dietary intake of vitamin D and sun exposure are required to determine whether any reduction in risk is the result of the actions of vitamin D specifically, or exposure to UVR more generally.<sup>162</sup> In a related autoimmune disease, multiple sclerosis (MS), UV irradiation inhibits development of symptoms of EAE with only a small, transient rise in 25(OH)D levels. Human studies also show that higher exposure to UVR decreased first demyelinating event risk (a common precursor to MS) independently of vitamin D, suggesting that supplementation alone may be insufficient to reduce the risk of MS.<sup>163</sup>

## Mechanisms of potential control of T1D by sun exposure and vitamin D

To study the immunoregulatory mechanisms of UVR exposure, it is preferable to irradiate an intact epidermal surface. This is usually the skin of a shaved experimental rodent although changes within human skin biopsies have also contributed to our understanding. Skin dendritic cells are affected both directly and indirectly by UVR exposure.<sup>164,165</sup> It is necessary that they reach the draining lymph nodes where they can present antigens in such a way as to stimulate reduced, less proliferative responses. UVR-exposed dendritic cells (possibly with UVR-induced pyrimidine dimers) secrete down-regulatory





cytokines and express markers to control the differentiation programme of interacting T lymphocytes, particularly for the induction and stimulation of T regulatory cells and generation of further immunoregulatory molecules. In another UVR-regulated pathway, it has been shown that UVR-induced prostaglandin E2 can affect myeloid cell progenitors in the bone marrow such that their daughter dendritic cells have reduced immunogenic properties.<sup>166,167</sup> We propose that this epigenetic imprinting of the progenitor cells contributes to the prolonged systemic immunoregulatory properties of UVR exposure. Other studies suggest that dermal mast cells are important for immunoregulation by UVR exposure. UVR was unable to suppress systemic contact hypersensitivity responses in mast cell deficient mice, and re-implantation of IL-10-producing mast cells into skin restored immunoregulation by UVR.<sup>168</sup> The mast cell is also important to the ability of UVR to suppress antibody production. In experimental mice, UVR exposure blocked germinal centre formation, antibody secretion and T follicular helper cell function by processes dependent on mast cell-derived IL-10.<sup>169</sup> These findings may contribute to potential control by UVR of autoantibody production in T1D.

Much of what is known about the immunoregulatory effects of vitamin D has been generated from *in vitro* analyses, principally with a focus on control of those cells that activate auto-reactive T and B lymphocytes. Dendritic cells are antigen presenting cells that regulate the level of activation, and the type of cells that subsequently respond and proliferate to their signals. It is generally accepted that exposure of dendritic cells to 1,25(OH)<sub>2</sub>D during their differentiation stimulates the development of regulatory, less mature and less immunogenic dendritic cells (for review<sup>170</sup>). These dendritic cells express inhibitory surface markers (e.g. PD-L1, ILT3) and secrete mediators (e.g. CCL2, CCL22) that encourage less recruitment and activation of inflammatory cells. In turn, fewer effector cells develop; this is complemented by increased production of, and proliferation by, T regulatory cells. Topical application of 1,25(OH)<sub>2</sub>D also increases the moderating properties of T regulatory cells in draining lymph nodes.<sup>171</sup> It is hoped that similar responses occur *in vivo* in humans, and that sufficient levels of 1,25(OH)<sub>2</sub>D are produced locally from circulating 25(OH)D to execute these responses (many immune cells including dendritic cells express *Cyp27B1* for rapid autocrine and paracrine conversion of 25(OH)D to 1,25(OH)<sub>2</sub>D). 1,25(OH)<sub>2</sub>D can also affect T and B cells directly (most immune cells express the VDR), causing a reduced immune response. For example, 1,25(OH)<sub>2</sub>D reduces their production of IL-2, interferon-gamma, IL-17 and IL-22 and thus ensures reduced Th1 and T-helper 17 responses. 1,25(OH)<sub>2</sub>D can down-regulate B lymphocyte activity by reducing plasma cell generation, IgM and IgG secretion and generation of B cell memory. The VDR is also expressed by other immune cells like mast cells that can respond to 1,25(OH)<sub>2</sub>D for increased production of the immunoregulatory cytokine, IL-10.<sup>172</sup> We are just starting to better understand other variables that may control the immunoregulatory properties of 1,25(OH)<sub>2</sub>D *in vivo*, particularly in

the context of T1D. The unknowns include the role of variants of both the VDR and vitamin D binding protein, as well as the activity of vitamin D catabolising enzymes (e.g. *CYP24A1*). Further, the concentrations of 1,25(OH)<sub>2</sub>D required for immunomodulation *in vivo* are uncertain, and whether they can be attained after UV irradiation of skin or pharmacological supplementation.

Vitamin D is only one of multiple immunoregulatory molecules produced in skin exposed to UVR that may negatively regulate the development of T1D.<sup>164,165</sup> Molecules or receptors in skin responding to UVB photons include *trans*-urocanic acid that isomerises to the more soluble and more immunoregulatory *cis*-urocanic acid. Nerves in the skin are activated by UVB stimulating the release of immunoregulatory neuropeptides (Nerve Growth Factor, Substance P, Calcitonin Gene Related Peptide). Membrane lipids and DNA of many skin cell types can also absorb UV photons and generate signals stimulating immunological pathways.<sup>164,165</sup>

In summary, immune responses may be down-regulated *via* both vitamin D-independent and vitamin D dependent pathways following exposure of skin to UVR. With relevance to regulating autoimmunity in T1D, both pathways can stimulate the development of regulatory, less immunogenic dendritic cells and T regulatory cells, and signal decreased plasma cell antibody production. The question remains as to the relative extent of immune regulation by UV-induced vitamin D or those other molecules and pathways stimulated by UVR exposure. Studies in rodents suggest that neither 1,25(OH)<sub>2</sub>D nor the VDR are necessary for UVR to suppress contact hypersensitivity responses.<sup>173,174</sup> In humans, further experimentation is required to determine the relative importance of the molecules produced in skin following UVR exposure that can modulate the development and progression of T1D.

## Conclusions

T1D incidence varies worldwide and appears to be positively associated with latitude. The significant inverse relationship between ambient UVR and T1D indicates that the latitude effect is in part explained by UVR. Animal and human studies of the role of vitamin D, particularly those focused on maternal intake have provided minimal support for the role of vitamin D supplementation during pregnancy. The epidemiological studies have had many methodological issues however, and have potentially been impacted by insufficient doses of vitamin D. The apparent contrasting findings that high blood levels of 25(OH)D during pregnancy (particularly in the third trimester) reduce subsequent risk of T1D in offspring, but vitamin D supplementation is not protective signifies either that there is an important role for UVR exposure, or perhaps a threshold effect requiring higher doses of vitamin D in supplements than is usually given. In contrast, association studies show a dose-dependent inverse relationship between higher vitamin D intake in early infancy and subsequent risk of developing T1D, suggesting specific benefits of vitamin D.



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