

Type 1 diabetes in Sardinia: facts and hypotheses in the context of worldwide epidemiological data

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Abstract Type 1 diabetes (T1D) results from an autoimmune destruction of insulin-producing beta cells that requires lifelong insulin treatment. While significant advances have been achieved in treatment, prevention of complications and quality of life in diabetic people, the identification of environmental triggers of the disease is far more complex. The island of Sardinia has the second highest incidence of T1D in the world (45/100,000), right after Finland (64.2/100,000). The genetic background as well as the environment of the island's inhabitants makes it an ideal region for investigating environmental, immunological and genetic factors related to the etiopathogenesis of T1D. Several epidemiological studies, conducted over the years, have shown that exposures to important known environmental risk factors have changed over time, including nutritional factors, pollution, chemicals, toxins and infectious diseases in early life. These environmental risk factors might be involved in T1D pathogenesis, as they might initiate autoimmunity or accelerate and precipitate an already ongoing beta cell destruction. In terms of environmental factors, Sardinia is also particular in terms of the incidence of infection with *Mycobacterium avium* paratuberculosis (MAP) that recent studies have linked to T1D in the Sardinian population. Furthermore, the unique geochemical profile of Sardinia, with its particular density of heavy metals, leads to the assumption that exposure of the

Sardinian population to heavy metals could also affect T1D incidence. These factors lead us to hypothesize that T1D incidence in Sardinia may be affected by the exposure to multifactorial agents, such as MAP, common viruses and heavy metals.

Keywords Type 1 diabetes · Sardinia · Registry · Epidemiology · Incidence

Introduction

Type 1 diabetes (T1D) results from an autoimmune destruction of insulin-producing β -cells that requires lifelong insulin treatment. While significant advances have been achieved in treatment, prevention of complications and quality of life of diabetic people, the identification of environmental triggers of the disease is far more complex. Currently, genotyping for HLA class II HLA-DR and HLA-DQ loci, combined with the family history, and positivities for autoantibodies against islet cell antigens are predictive of the disease [1, 2]. However, most genetically predisposed individuals do not develop T1D [3], and it is agreed that epigenetic and environmental factors might contribute to the individual risk. Furthermore, heterogeneity of the disease, with age-related variability in β -cell failure progressing to insulin dependence, has also been shown and age at onset might be an indicator of the strength of genetic susceptibility. Indeed, in Finland the cumulative T1D incidence in the offspring decreased in parallel with the increase in the age at onset of diabetes among parents [4]. Moreover, in Italy, the effect of having one or both parents of Sardinian heritage on risk of type 1 diabetes declined with increasing age [5]. Epidemiological studies have shown that exposure to environmental risk

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factors has dramatically changed over time, including nutritional factors, pollution, chemicals, toxins and infectious diseases in early life. These factors might be involved in T1D pathogenesis, initiating autoimmunity or accelerating an already ongoing β -cell destruction [3].

The island of Sardinia has the second highest incidence of T1D in the world after Finland [6]. The largely homogeneous genetic background of Sardinia's inhabitants, consequent to centuries of isolation, low immigration and expansion and to the selective environmental pressures like malaria, makes the island an ideal epidemiological observatory for investigating environmental, immunologic and genetic factors related to the etiopathogenesis of T1D [5]. In this review, worldwide epidemiological data on T1D will be discussed in the context of Sardinian data.

Epidemiology of T1D (0–14 years) in Sardinia

Sardinia has the second highest incidence of T1D in the world (45/100,000) [6, 7] (Fig. 1), after Finland (64.2/100,000) [8], and both geographical areas have been defined as “hot spots” [9]. In 1990, the Insulin-Dependent Diabetes Mellitus Sardinia Project was launched in order to map the geographical distribution of T1D on the island and to enquire about the preclinical phases of T1D in a large cohort of people genetically at risk. Since then, numerous studies have been conducted [2, 5], such as EURODIAB PCS, TRIGR, Conscript Studies, Migrant Studies, The Newborns Sardinia Insulin-Dependent Diabetes Mellitus Study, the Sardinian Schoolchildren Insulin-Dependent Diabetes Mellitus Study, Environmental Factor Study and the Ecological/Environmental/Veterinarian Variables Type 1 Diabetes Mellitus Study, which allowed to point out some peculiarities compared to peninsular Italy.

At first, an intriguing and still unsolved issue in the epidemiology of T1D is the heterogeneity of risk between the sexes. Whereas in most of the countries incidence is higher in males than in females among young adults only [3], Sardinian boys have a statistically significant 31 % higher risk than girls of the same age [7]. This finding is not chromosome Y-linked, but until now no other hypothesis has been tested [10]. Second, geographical heterogeneity within Sardinia was evident, with the highest incidence rates in the middle western part of the island (Oristano Province) followed by Cagliari and Nuoro, and the lowest incidence in the northwestern part of the island (Sassari Province) (Fig. 2) [11]. Due to numerous migratory flows, there are genetic and cultural differences within Sardinia. For instance, the population of Sassari is influenced by a Genoese domination, the population of the area of Alghero (northwest) by the Catalans and the populations of Gallura (northeast) by people coming from Corsica.

Other migratory flows are those of the Ligurian population in the Carloforte island (southwest), Piedmont, Bergamaschi, Tuscany and Romagna miners in the Sulcis–Iglesiente (southwest), the Venetians in the Oristano area (west) and, finally, the Dalmatians–Istrians who settled in Fertilia near Alghero (northwest).

Third, the pattern of temporal trend of the disease is peculiar. At the start of the twentieth century, childhood diabetes was rare and rapidly fatal, but prevalence steadily increased with the introduction of insulin treatment. No epidemiological data were available in the middle of the twentieth century, apart from those published by Scandinavian and Sardinian studies [12], which suggested that childhood diabetes was an uncommon condition in that period. Data from the Sardinian Conscript Register, where T1D was a cause of rejection, showed that prevalence at the age of 20 years was close to zero for the first ten cohorts (1936–1945) and started to rise around the middle of the twentieth century with a 29-fold increase between the late 1930s and the late 1960 birth cohorts [13]. Unfortunately, no data on birth cohorts of conscript residents in peninsular Italian regions are available.

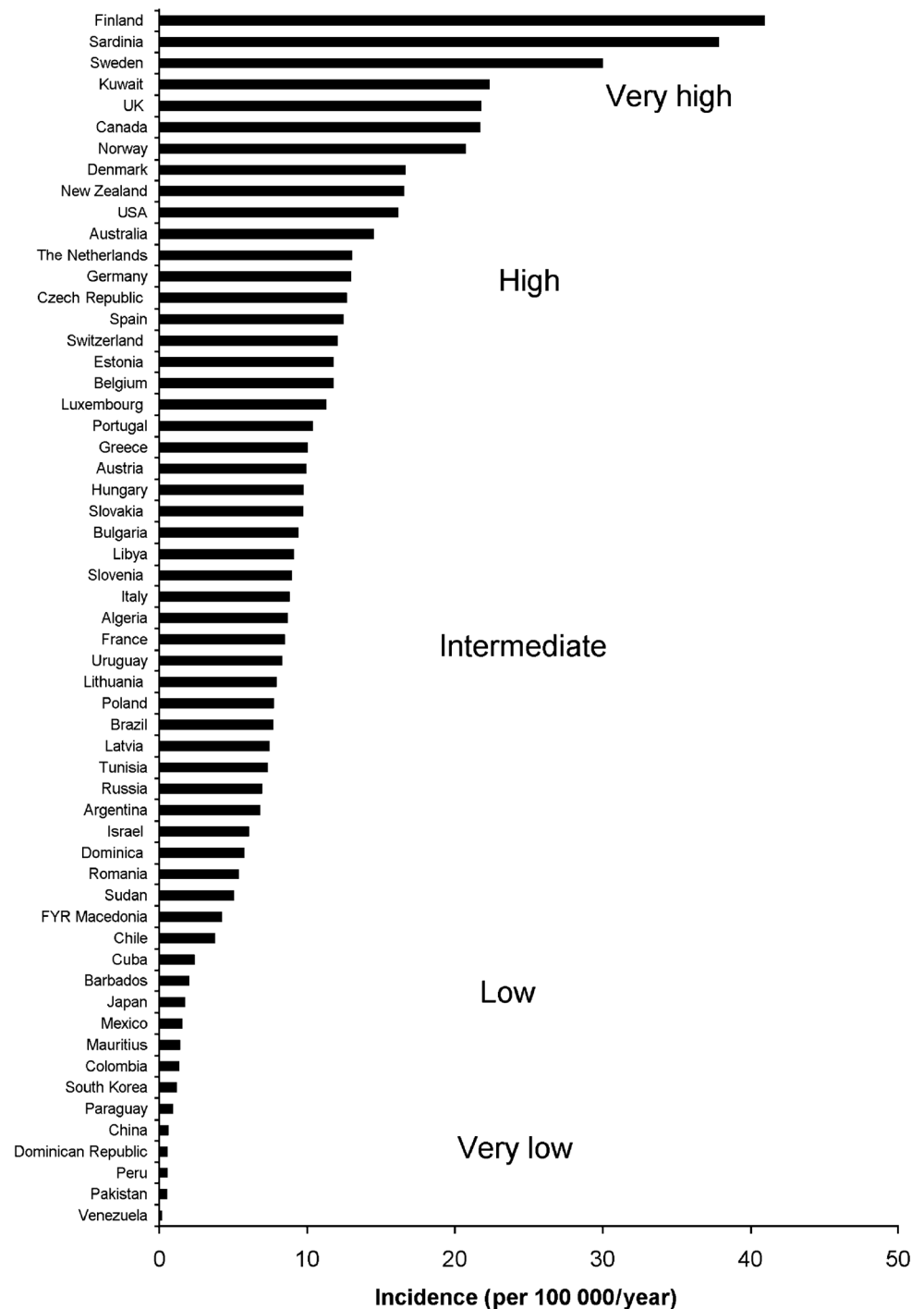
Over the past 60 years, the incidence of T1D has increased by 3–5 % per year [14], doubling roughly every 20 years according to the TEDDY Study Group [15]. A steeper increase has been shown in populations with a lower historical incidence, whereas in the high-risk areas of Finland and Sweden there was a tendency toward a leveling off, which needs to be confirmed over time [8, 16]. In Sardinia, temporal trend analyses on incidence rates in the period between 1989 and 2009 (Table 1) pointed out a significant increase in incidence trends in children (2.12 % per year), which was nonlinear with the birth cohort [7]. In contrast, the increase in incidence according to the calendar period showed signs of regression, when considered more recently (Table 1). The birth cohort effect seems to have started earlier among girls (0–5 years) than among boys (5–10 years). Should the increase in incidence be attributable to the effects of some perinatal environmental factor, this would mean that such a factor acts selectively earlier on females than on males [7]. Such an increase in incidence could possibly be ascribed to the effects of the perinatal environmental factors, but further evidence is needed to confirm it.

Environmental risk factors

Viruses and the hygiene hypothesis

The “hygiene hypothesis” claims that human genes have adapted during evolution in response to a constant and a secular exposure to infectious agents [17]. A very fast decrease in the rate of exposure can give immunological

Fig. 1 Geographic variation of childhood T1D incidence (children under 14 years of age) during 1990–1999 [6]



imbalance and increased susceptibility to the development of autoimmune diseases. Thus, living in very hygienic conditions may lead to a defect in immune regulation and predispose to immune-mediated diseases. This hypothesis was first formulated for polio, as it was observed that increasing hygiene gradually decreased the transmission of polioviruses during the nineteenth century, but paradoxically led to the first clear epidemics of paralytic disease at

the end of the century [18]. The basis of this phenomenon was the observation of a delay in the age of the first infections, with children not exposed to polioviruses until later in childhood, who were no longer beneficiaries of protection by maternal antibodies [18]. Viskari et al. [19] suggested that variation in the incidence rates of T1D might be connected to the geographic spread of enteroviruses. Indeed, a low frequency of enterovirus infection

Fig. 2 Estimated risk of T1D among children aged 0–14 years in the four Sardinian Provinces in period 1989–1999 [11]

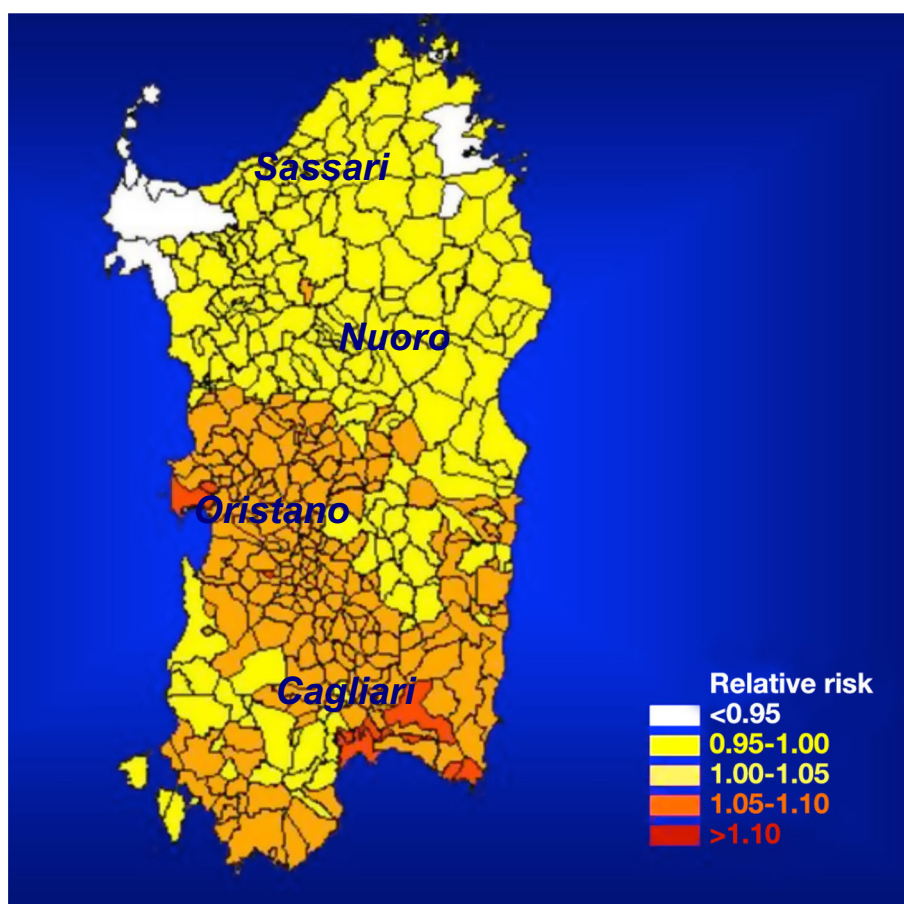


Table 1 Incidence of type 1 diabetes in Sardinia (children under 14 years of age) during 1989–2009 by sex, age-group and calendar period [7]

	Case subjects (N)	Person-years	IR/100,000 person-years	95 % CI
Total	2371	5,286,212	44.8	43.1–46.7
<i>Sex</i>				
Boys	1380	2,725,601	50.6	48.0–53.4
Girls	991	2,560,611	38.7	36.4–41.2
<i>Age (years)</i>				
0–2	320	910,143	35.2	31.5–39.2
3–5	396	961,497	41.2	37.3–45.5
6–8	482	1,034,124	46.6	42.6–51.0
9–11	613	1,127,595	54.4	50.2–58.8
12–14	560	1,252,853	44.7	41.2–48.6
<i>Period</i>				
1989–1991	346	967,116	35.8	32.2–39.8
1992–1991	334	857,969	38.9	35.0–43.3
1995–1997	362	785,852	46.1	41.6–51.1
1998–2000	351	723,217	48.5	43.7–53.9
2001–2003	311	678,325	45.8	41.0–51.2
2001–2006	348	648,162	53.7	48.3–59.6
2007–2009	319	625,571	51.0	46.0–56.9

might increase the risk of T1D by making children more susceptible to enterovirus-induced β -cell damage, whereas infections which occur in the presence of maternal

antibodies induce an immune response but remain mild. This has been called a natural vaccination of the child [20]. In a population with a low prevalence of enteroviruses,

infants lack maternal antibodies because increasing proportions of the mothers has not experienced the virus which is infecting the child. Associations have been suggested between Coxsackie group B viruses and T1D [21]. However, a recent study conducted in Sardinian schoolchildren does not support a role of Coxsackie virus B4 infection in the appearance of pancreatic islet autoimmunity [22].

An Italian study found a significant correlation between incidence of T1D in the period between 1996 and 2001 and mumps and rubella viral infection in the peninsular regions covered by diabetes registries, but no association was evident in Sardinia [23]. Failure to report compulsory notifications of the diseases by general pediatricians, however, might have biased data on the association between T1D and these childhood viral infections.

A possible effect of microbes such as *Helicobacter pylori* and patterns of gut microbiome has been studied in animal models [24]. A few studies have reported an increased prevalence of *Helicobacter pylori* in T1D patients [25] while conversely the prevalence of this infection was similar in Sardinian children and adolescents with T1D and in age-matched healthy controls [26].

There is some evidence indicating that *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection might be linked to T1D in Sardinian patients [27–31]. MAP infections are estimated to affect approximately 60 % of Sardinian cattle, and they can be found in pasteurized milk products and may be asymptotically transmitted to humans. In 2005, Dow postulated a causative role for MAP in T1D [28]. Sechi et al. [29] in 2007 described the DNA of MAP in the blood of autoimmune T1D, but not in non-autoimmune T2D. The link connecting MAP and T1D comes from the concept of molecular mimicry. Protein elements of the pathogen share sequence and/or conformational elements with the host so that immune responses directed at the pathogen also attack the host. For example, mycobacterial heat-shock protein of MAP (HSP65) and pancreatic glutamic acid decarboxylase (GAD) share some identity in amino acid sequence with immune consequences [30, 31]. Furthermore, MAP has been linked to other autoimmune diseases such as Hashimoto's thyroiditis and multiple sclerosis [31–34]. A “perfect storm” has been hypothesized where MAP is the central instigator that infects the intestine and initiates an autoimmune process. T cells migrate to the pancreatic lymph nodes and to the pancreas where they attack β -cells which present antigens structurally similar to those of MAP, thus triggering an autoimmune process. Further studies, however, are needed to confirm these preliminary data in larger cohorts of new-onset T1D children.

A possible role of malaria infection (endemic in Sardinia since 5000 years b.c. and eradicated only in 1950) in facilitating the increasing incidence of T1D has been

postulated. In Sardinia between 1946 and 1951 the Rockefeller Foundation conducted a large-scale study to test the feasibility of the strategy of “species eradication” of an endemic malaria vector, involving an aggressive malaria control program involving 267 metric tons of DDT (dichlorodiphenyltrichloroethane) which were spread over the island [35]. The eradication of malaria significantly helped the subsequent socioeconomic development and public health of the island. The widespread use of DDT apparently did not affect the health of Sardinians. However, data on its environmental effects are limited, and the numbers of male workers exposed to DDT are too small (almost 5000) to distinguish its effects on carcinogenesis from that of other diseases. No other countries have achieved such a rapid eradication of malaria with DDT; moreover, incidence data of T1D in other malarial areas are not available, so that no comparative study can be performed to test the hypotheses that either the rapid decline in incidence of malaria or DDT itself or both of them are related to the increased temporal trend of T1D incidence.

Intestinal infections continue to be a problem worldwide, and helminths, which currently infect a substantial number of the world's population, are primary culprits. Immune responses (helminths and their eggs are probably the most potent stimulators of mucosal Th2 responses) elicited by worms can modulate immune reactions to other parasites, bacterial, viral infections and several unrelated diseases [36]. Helminth intestinal infections in the last 4–5 years have been disappearing in industrialized countries, and the lack of acquisition of “worm experience” could predispose to seemingly unrelated diseases. Although no conclusive evidence exists for this hypothesis, the emerging evidence suggests some causal links between helminth infections, immune modulation and autoimmune disease [36]. Sardinian data seem to confirm a decreasing trend of helminths infections (pinworms) among Sardinian schoolchildren (Songini personal communication).

Milk intake

The hypothesis that early exposure to cow's milk or a lack of breastfeeding may predispose a child to T1D dates to the 1980s. Since then, many researchers have examined this issue, with conflicting results [3]. Sardinia is the Italian region with both the highest incidence of diabetes and intake of cow's milk. However, Sardinia is located away from the regression line between the consumption of cow's milk and the incidence of T1D obtained by European data [5]. The Sardinian Trial to Reduce IDDM in the Genetically at Risk (TRIGR) study center is one of the 50 centers across Europe, Canada and the USA participating in the

main nutritional study of primary prevention of T1D that aimed to understand whether hydrolyzed infant formula compared to cow's milk-based formula decreases the risk of developing T1D in children with increased genetic susceptibility [37]. The study will continue until 2017 when the data will be finally analyzed. Preliminary data published by the Study Group showed no differences in the appearance of autoantibodies to islet cells by the age of 7 years between children who received an extensively hydrolyzed casein formula and those who received a conventional cow's milk-based formula [38]. A systematic review indicated that, although there is some evidence to suggest that breastfeeding is associated with a reduced risk of T1D, this evidence must be interpreted with caution because of the possibility of recall bias or evaluator bias and inadequate methodological quality, including sample size, and suboptimal adjustments for potential confounders in the primary studies [39]. Several environmental determinants operating since the gestational period have also been suggested to affect T1D risk, but evidence is limited and no data have been collected in the Sardinian population [40].

Vitamin D

An inverse relationship between latitude and T1D incidence has been shown [3]. Moreover, a lack of vitamin D or its receptor (linked to the light-colored eye/lack of melanin) has been suggested to be involved in pathogenesis of T1D in European Caucasians [41]. Indeed, a higher frequency of blue eyes and fair skin has been reported in northern people with T1D than in control subjects [42]. This finding may be relevant for explaining geographical differences in T1D incidence as prevalence of blue eyes differs substantially between northern and southern European Caucasians. Even in Sardinia, in spite of the considerable sunlight and dark color of skin and eyes among the background population, the frequency of blue eyes in T1D subjects is still twofold higher than in control [42]. The lack of either vitamin D or its receptor has been suggested as a possible explanation for this finding. In Italy, however, a recent case–control study within the Piedmont Diabetes Registry showed no association between vitamin D levels at birth and risk of having T1D up to 10 years of age [43].

Nitrates

No association between the consumption of bottled water with different concentrations of nitrates and incidence of T1D has been found in an ecological study. Indeed, no correlation between nitrate level in tap water and T1D incidence among the 21 Sardinian health districts has been described in period 1989–1998 [44].

Air pollution, metallic elements

As already reported in other countries, the peak incidence of T1D occurs in the autumn–winter seasons. The Sassari Province, which has the lowest incidence of T1D on the island, has also the lowest average temperature along with the highest rainfall, which might be related to air pollution and metallic elements [5]. It has been reported that long-term exposure to traffic-related air pollution and to heavy metals may contribute to an increased incidence of autoimmune diseases and to the development of diabetes [45, 46]. The unphysiologic balance in the ratio of specific metals could play a role in upsetting normal glucose and insulin metabolism [47]. Alteration in metals status can also increase oxidative stress that may contribute to the insulin resistance and development of diabetic complications [48]. The list of chemical agents capable of producing or exacerbating autoimmune manifestations in susceptible individuals is constantly growing and includes hundreds of pharmacologic agents, hydrocarbon pollutants, pesticides, dietary supplements and heavy metals. Reports of heavy metal-induced autoimmunity in humans including gold-, cadmium- and mercury-induced glomerulonephritis began to emerge four decades ago [49]. A recent study investigated the hypothesis that geochemical elements could create permissive environmental conditions for autoimmune diabetes in Sardinia [50]. An ecological analysis identified a negative association between elements such as Co, Cr, Cu, Mn, Ni, Zn and T1D incidence in Sardinia [50]. The results suggest a possible protective role for some elements. Studies in animal models have observed that Hg, Cr, Pb and Cd might induce a modification of the immune system with activation of anti-self (TB) cell, whereas Au and Ag might induce autoimmunity [49, 51, 52]. There is solid evidence that Hg can induce autoimmune diseases in both humans and experimental animals [53]. Among toxic metals associated with the risk of diabetes, arsenic has received special attention for more than two decades since the publication in 1994 of a cross-sectional study in the historically high-arsenic area of southwestern Taiwan [54]. However, the debate on the causality of the observed association between arsenic and diabetes remains unresolved. Arsenic metabolism has been prospectively associated with increased incidence of T2D [55]. Some studies suggest different ways in which arsenic may influence the pancreatic β -cell function and insulin sensitivity, such as oxidative stress and effects on glucose absorption and transport, gluconeogenesis, adipocyte differentiation and Ca^{++} signaling [56–59]. Data on the outcome of arsenic exposures and T1D are very limited.

In geographic areas highly exposed to heavy metals, there is a significant positive correlation between barium and sodium oxide and the risk of both multiple sclerosis

and T1D [50, 60]. Co, Cr and Ni seem to have protective effects against the onset of both diseases [60]. Lower amounts of zinc in drinking water have been found to be associated with the risk of developing T1D in childhood [61]. A recent study found that zinc serum levels in T1D were lower compared to controls [61]. A zinc-dependent molecule, found in muscle and adipose tissue, (IRAP) maintains an active glucose transporter; therefore, zinc helps to increase glucose uptake. Zinc has a protective role on β -cells, opposing cytokine-mediated destruction, and in T1D there is an increased excretion of intestinal zinc [62]. The ability of zinc to modulate NFkappaB activation in the diabetogenic pathway may be the key mechanism for its protective effect. Inhibition of the NFkappaB pathway already demonstrated to be effective in preventing the onset of T1D in rodent models may prove to be an important target pathway for nutritional strategies in T1D prevention [63]. Zinc is an essential trace element crucial for the function of more than 300 enzymes, including DNA and protein synthesis, enzyme activity and intracellular signaling. Cellular Zn homeostasis necessitates the compartmentalization of Zn into intracellular organelles, a process that is tightly regulated through the integration of Zn transporting mechanisms [64, 65]. Bosco et al. [66] described how Zn plays a central role in cellular protection against apoptosis and oxidative stress, as Zn is essential for normal insulin production, concluding that the Zn transporter is an important target for autoimmunity in T1D.

Discussion and conclusion

Despite huge efforts from the scientific community and numerous investigations, the exact cause of T1D is still not known. The lively debate on the exact role of genetic susceptibility and environmental factors in determining the disease is still ongoing [67, 68]. Even the TRIGR Study upon which high hopes were invested did not produce definitive results [37].

None of considered factors up to now have demonstrated a strong influence on T1D etiopathogenesis in Sardinia, the Mediterranean island with the second highest incidence rate in Europe, approaching that of Finland. Epidemiological studies indicate that external factors play an important role in triggering or aggravating autoimmune processes in genetically predisposed individuals. Recently, the attention has been focused on environmental pollution and exposure to different metals. Soil heavy metal contamination is a major environmental concern, and the ecological risk associated with heavy metals on human health is increasing. Heavy metals in soil may be harmful to human health through the food as ingestion is the dominant exposure pathway for heavy metals. Another

extremely interesting aspect is represented by the possible protective effect of certain elements. Zinc may possibly represent a beneficial micronutrient supplement in children at risk of diabetes. These hypotheses need to be confirmed by case–control studies—designed to compare blood levels of metal in Sardinian children at onset of diabetes and in age- and sex-matched non-diabetic children—and by large prospective cohort studies of non-diabetic Sardinian children, allowing to stratify the cumulative incidence of T1D by the baseline blood levels of examined metals.

Another peculiarity of the island of Sardinia in terms of environmental factors is the supposed role of MAP that recent studies have linked to T1D and other autoimmune diseases in the Sardinian population. It can be speculated that this “perfect storm” might find fertile ground in subjects made more susceptible by Zn and/or Cu deficiency. Another participant in fostering increased susceptibility could be socioeconomic variables, due to the quite high hygienic conditions and the limited exposure to microbes and breastfeeding. These factors might cause a defect in immune regulation and predispose to immune-mediated diseases. Microbes have been associated with both increased and decreased risk reflecting their possible role as risk or protective factors. The microbial effects offer attractive possibilities for the development of preventive interventions for type 1 diabetes based on the elimination of triggering agents (e.g., enterovirus vaccines) or use of protective microbes as probiotics. At the moment, the limited evidence available is not sufficient to clarify all the relationships outlined herein. Hopefully, in the near future, both researchers and funders companies should attempt to establish population-based epidemiological, physiological, microbiological and environmental studies in Sardinia and peninsular areas covered by T1D registries to establish how these and possibly other environmental factors may determine diabetes susceptibility and onset.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This article does not contain any studies with human or animal subjects performed by the any of the authors.

Informed consent For this type of study formal consent is not required.

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