The “Sardinia-IDDM study”: an attempt to unravel the cause of insulin-dependent diabetes mellitus in one of the countries with the highest incidence of the disease in the world

Gian Franco BOTTAZZO (a), Andrea LOVISELLI (b), Fernanda VELLUZZI (b), Stefano MARIOTTI (b), Efisio COSSU (c), Rocco CIRILLO (c), Angelo BALESTRIERI (d), Giuseppe DELITALA (e), Vincenzo SEPE (a), Marco SONGINI (f)
and the “Sardinia-IDDM study” groups (*)

(a) Department of Immunology, St Bartholomew’s and the Royal London School of Medicine and Dentistry, London, United Kingdom
(b) Cattedra di Endocrinologia; (c) Cattedra di Malattie Metaboliche; (d) Istituto di Medicina Interna, Università degli Studi, Cagliari, Italy
(e) Istituto di Patologia Medica, Università degli Studi, Sassari, Italy
(f) Centro Malattie Dismetaboliche, Ospedale Brotzu, Cagliari, Italy

Summary. - Sardinia and Finland have the highest incidence of IDDM in the world. Thus, both regions represent ideal observatories for investigating the environmental, genetic and immunological factors, which have led to this dramatic increase. We have concentrated our efforts in Sardinia. Among several projects, there is the mapping of the Island for hot and cold spots for overt IDDM. In order to map the Island for pre-IDDM, we have collected and bled around 10,000 school children (age 6-14 years) and we are now in the process to enroll around 30,000 newborn. We report here our initial results, which show that progression to IDDM is accompanied in both cohorts by the presence of a combination of ICA with either GAD and IA-2 antibodies or both. This approach should lead to design reliable models of IDDM prediction in the general population, which will benefit an early insulin treatment and, hopefully, an effective prevention of the disease.

Key words: insulin-dependent diabetes mellitus, epidemiology, prediction.

Riassunto (Lo “Studio Sardegna-IDDM”: un tentativo di svelare la causa del diabete mellito insulino dipendente (IDDM) in una delle regioni con la più alta incidenza della malattia al mondo). - Sardegna e Finlandia sono le due regioni con la più alta incidenza di IDDM al mondo. Per questo motivo si prestano come osservatorio ideali per lo studio dei fattori ambientali, genetici ed immunologici che hanno portato ad un aumento drammatico di questa malattia. Nelle abbiamo concentrato i nostri sforzi in Sardegna. Tra i molti progetti in corso, c’è quello che riguarda la compilazione di una mappa della Sardegna in relazione ai “cold” e “hot spots” per il diabete manifesto. Allo scopo di produrre una mappa della Sardegna per il pre-diabete, abbiamo raccolto campioni ematici provenienti da circa 10.000 scolari (età compresa tra 6 e 14 anni) ed adesso stiamo anche raccogliendo campioni ematici da 30.000 neonati. In questo documento riportiamo i risultati preliminari del nostro studio, che mostrano che la progressione verso l’IDDM è accompagnata, in entrambe le corse, dalla presenza di una combinazione di ICA e anticorpi anti-GAD e IA-2 da soli, o insieme. Questa strategia di studio dovrebbe portare alla creazione di modelli di predizione applicabili alla popolazione generale, che potrebbe beneficiare così di un trattamento insulinico precoce e, eventualmente, di una efficace prevenzione della malattia.

Parole chiave: diabete mellito insulino-dipendente, epidemiologia, predizione.

Introduction

Insulin-dependent diabetes mellitus (IDDM) represents the most serious form of clinical DM. The disease affects primarily children, but it can also develop in the elderly [1]. The aetiology of IDDM is still largely unknown, but the most widely accepted hypothesis is that the disorder is multifactorial in origin, involving a complex interaction among genetic predisposition, immunological determinants and environmental agents [2, 3]. This results in the autoimmune destruction of the insulin-producing β-cells in the pancreatic islets [4], primarily being mediated by autoreactive T lymphocytes [5]. In IDDM, certain HLA genetic combinations are now well established [6] and the anatomopathological lesions have also been well defined [7]. However, it is still unclear what primary event(s) trigger the immune

(*) See the list before the references in this article.
system to initiate the aggression against the β-cells, thus producing the autoimmune aberration.

The autoimmune attack often initiates several years before the clinical onset of IDDM. This concept has been substantiated after screening, for the presence of cytoplasmic islet cell antibodies (ICA), large cohorts of first degree relatives of IDDM patients, identical twins discordant for IDDM, autoimmune polyendocrine patients and normal school children [8]. Subsequently, insulin autoantibodies (IAA), autoantibodies to GAD (GADA) and to the islet 37kD/40kD, islet tryptic antigens have attracted much attention, as additionally predictive markers of the disease [9]. Recently, several independent groups have shown that these two latter autoantigens are related to the protein tyrosin phosphatase family, IA-2β or phogr and IA-2α, respectively [10-14].

As mentioned, IDDM develops in association with well defined HLA haplotypes, a finding confirmed and extended at the molecular level [15, 16]. In general, more than 90% of IDDM children in the Caucasian population bears the phenotypes HLA-DR3 and HLA-DR4 or both, whilst the phenotype HLA-DR2 is rarely found in them, apparently conferring resistance against IDDM. Subsequent studies have shown that there is an even stronger association between IDDM and the DQ alleles, where the absence of aspartic acid in position 57 on the HLA-DQB1 chain (DQB1*05:01) and the presence of arginine in position 53 of the HLA-DQA1 chain (DQA1*03:01) confer a higher susceptibility to the disease [17-20]. It is important to point out, however, that these phenotype combinations are frequently present in Caucasoids, but only a relatively small proportion of these individuals develops IDDM.

In reference to the involvement of environmental factors in the aetiology of IDDM, it remains to be firmly proved that common viruses (e.g., Coxsackie viruses, CMV, etc.) indeed play a relevant role [21], even though Coxsackie viruses have been recently indicated as possible causative agents in very young cases of the disease [22, 23]. It has also been suggested that viruses belonging to the slow-virus family could be responsible to prime the autoimmune aggression against the β-cells [5]. Preliminary data obtained in other autoimmune diseases, e.g., Graves' disease [24-27], Sjögren syndrome [28, 29] and rheumatoid arthritis [30], tend to support this hypothesis, but further confirmatory evidence is still awaited. The same applies to the possibility that superantigens may also play a role in the pathogenesis of IDDM [31].

The Finnish-Sardinia IDDM connection

In 1989, the European Union has supported a project, whose objective was to define the epidemiologic characteristics of IDDM in a number of European countries with a high degree of ascertainment. This study, called EURODIAB-ACE, remains on course for the analysis of the data which will define precisely the incidence of IDDM in a potentially “at risk” population of children aged 0-14 years within a European population of 30,000,000. Recently, the age range has been extended to 29 years. The overall results continue to confirm the North-to-South European gradient, with the highest incidence of IDDM in Finland and in the other Scandinavian countries, but with the lowest in the Mediterranean area [32]. This is in accordance with the observation that the incidence of IDDM declines progressively from North-to-South and it is low in equatorial countries [33]. It has been estimated that the Finnish children now have a ten fold higher risk to develop IDDM than the Greek children [34]. Unexpectedly, the same EURODIAB-ACE data have also shown a high incidence of IDDM in Sardinia, an island placed in the middle of the “cold” Mediterranean area for IDDM [32, 35], but with rates of occurrence of the disease very similar to those of Finland. Interestingly, our subsequent data have shown that the incidence of IDDM in Sardinia has been progressively increasing since the mid 1960's [36], thus strongly supporting the hypothesis that, if an environmental change has occurred on the Island, this took place around the time of the Second World War (calculating the long latency period preceding the onset of clinical symptoms). A similar type of temporal events has also been recorded in Finland, where the incidence of IDDM there began to climb around the 1970's [37].

If it is true that Finland and Sardinia head the rank order of incidence of IDDM in Europe by a considerable margin, it is also true that this is certainly intriguing. In fact, the inhabitants of these two distant geographical areas are genetically different, as shown in studies aimed to ascertain, through the analysis of several genetic markers, ancestral settlements of populations following migration patterns and the establishment of proper linguistic characteristics [38-40]. At the HLA genetic level, differences do also exist. Although DR3 and DR4 are both increased in the Finnish and Sardinian IDDM groups, their respective values are reversed, i.e., DR4 occurs in 84% of Finns and only in 44% of Sardinians, whereas DR3 occurs in 42% of Finns and 73% of Sardinians [41]. It is interesting to note that DR3 is detectable in 50% of Sardinian controls compared to only 16% in Finns. The haplotype associations with IDDM are even more disparate in these population groups: A30, B18, DR3 and A2, B18, DR2 and DQ1 occur in IDDM in Sardinians, whereas A1, B8, DR3 and A2, B62, DR4 and DQ8 occur in IDDM in Finnish patients. As previously emphasized, HLA DR2 confers protection to IDDM and other autoimmune diseases (e.g., Graves' disease) in most Caucasoid populations, including the Finns [42, 43]. However, there is an
exception here, again represented by Sardinia, where this allele is often detected in patients affected by these two disorders [44, 45]. Interestingly, even within the Island there is a genetic heterogeneity for the association of DR2 with IDDM, where patients living in the Southern part are positively associated with the allele, while those in the central eastern area are negatively associated with it [46]. In reference to DBP*07:01-01 and indeed all the 31 Sardinian IDDM patients, who were typed in one study, have this phenotype [47], compared to only 73% in Caucasoids, 74% in Black Americans and in 5% Japanese IDDM patients [42, 43]. When one then looks at the homozygous status, DQα2:05:01/DQβ2:04:01, the heterodimer combination, which confers the highest susceptibility to IDDM (decreasing when other amino acids are present in the same positions), has also been detected at high frequency in Sardinian IDDM patients. However, it has to be pointed out that a similar combination has also been found in populations of the same latitude, i.e., Spaniards, where the prevalence of IDDM is much lower than that on the Island [47].

The “Sardinia-IDDM study”

If Finland and Sardinia represent the “hottest” areas of IDDM in the world, efforts have to be clearly concentrated in these two locations, so to be in an advantageous position to gain decisive insights into the aetiopathogenesis of the disease. We have concentrated our efforts in Sardinia. Being an island, Sardinia offers a rare ethnical homogeneity which has been maintained throughout the centuries, thus representing an unique epidemiological observatory, where the dynamics of the events leading to IDDM could be intensively investigated. The ultimate objective of our study is to map the Island for “hot and cold spots of IDDM”, in order to identify areas with a high or low prevalence of overt IDDM and pre-IDDM. For this and other related purposes, several projects are at present being conducted on the Island.

The continuation of the EURODIAB-ACE survey

The data collected so far have shown that the incidence of IDDM in Sardinia is higher than in the other Italian regions (i.e., Lombardy, Latium, and Western Sicily), which maintain low levels similar to those reported in the Mediterranean area (7 to 11 new cases x 100,000 inhabitants per year) [34]. In the total population studied (0-29 years) in Sardinia, the cumulative incidence, x 100,000 inhabitants per year, is 26 for the years 1989-94, while it raises to 35 in the age group 0-14 years. Interestingly, the incidence varies in the 4 Sardinian provinces: for the 0-29 year age group, Cagliari has an IDDM incidence of 28, Nuoro 26, Oristano 26 and Sassari 31, while for the 0-14 year age group Cagliari registers 38, Nuoro 35, Oristano 45 and Sassari 30. In general, the males aged 0-14 years are more affected than females. Even if these data confirm those reported in other countries with a high risk for IDDM (i.e., Finland), they do not explain the apparent concentration of new cases in one area rather than in another. We are currently comparing the geographical distribution of the cases of IDDM reported on the Island in 1989-1994. While we can verify the overlapping of IDDM incidence in the same geographical areas, we may also observe a migrating behaviour, with a "wave-like" movement from one area to another [48], as it has again been observed in Finland after retrospective analysis of the epidemiologic data of IDDM in that country (H. Akerblom, personal communication).

The Sardinian school children-IDDM project (SSIP)

In order to identify the “hot pre-diabetic areas” on the Island, cohort of over 10,000 school children have been recruited. The recruitment started in 1986, after visiting primary and secondary schools in towns of the provinces of Cagliari (CA), Nuoro (NU) and Oristano (OR), and was completed in 1995 (total cohort over 7000 children). The province of Sassari (SS) joined the study in 1994 and the recruitment of the remaining 3000 children has now been completed. Around 10,000 school children have been already tested for ICA, showing an overall prevalence of about 5% (5 JDF-units) [49], the highest in the world together with Finland [50]. A more refined analysis, including the determination of GADA [51] and to IA-2ic (IA-2A) [10], have been recently completed in 7574 school children (Abstract accepted to the IDF Congress, Helsinki, July 1997). They have been recruited in 27 towns of the 4 Sardinian provinces, with the following geographical distribution: Cagliari (7 towns, 2628 school children), Oristano (5 towns, 1727 school children), Nuoro (12 towns, 1908 school children) and Sassari (3 towns, 1311 school children). The sera collected were tested for ICA (7574), GADA (5408) and IA-2A (5411), respectively. Cagliari showed the highest prevalence of ICA for titres ≥ 5 JDFu (5.7%: p < 0.01 vs OR = 2.8% and NU = 3.5%), whereas Cagliari (1.4%) and Oristano (1.2%) had the highest prevalence of ICA for titres > 20 JDFu (p < 0.05 vs NU = 0.5%). Oristano also had the highest prevalence of GADA (2.1%: p < 0.05 vs CA = 1.1%, NU = 0.7% and SS 0.7%) and IA-2A (1.5%: p < 0.05 vs SS = 0.3%). Combination of autoantibody specificities was always higher in Oristano than in the other 3 provinces. The prevalence of
ICA and IA-2A in OR was 0.9% (p < 0.01 vs SS = 0.1%). GADA and IA-2A was 1.0% (p < 0.05 vs CA = 0.2%, NU = 0.0% and SS = 0.1%) and ICA and GADA and IA-2A was 0.7% (p < 0.05 vs CA = 0.2%, NU = 0.0% and SS = 0.1%). The identification of Oristano, as the area with the highest prevalence of islet-related autoantibodies, is consistent with the EURODIAB-ACE data, also showing the highest incidence of IDDM in the same province, compared with the others. The provinces of Nuoro and Sassari, with the lowest incidence of IDDM, had also the lowest prevalence of islet-related autoantibodies, either when analysed as an individual marker or as combined specificities. Our initial data showed that, within the island of Sardinia, the geographical pattern of potential pre-IDDM individuals, identified thought islet-related autoantibody determination in a large school children cohort, seems to follow that of the incidence of the overt disease.

The predictive value of islet-related autoantibodies, as single or combined specificities, was also assessed in 3080 school children, initially recruited from the provinces of Cagliari, Oristano and Nuoro (V. Sepe et al. 1997. Diabetologia, Suppl. 1, A 21, Abstract 75). These children had the longest follow-up (2 to 10 years) and their sera were also tested for ICA, GADA and IA-2A. All were interviewed and, among them, 10 have developed IDDM. There were 5 males and 5 females and the interval, since they were recruited into the study and the time they developed the disease, varied between 2 and 60 months. None of the children, who became diabetic, lived in the province of Nuoro and all, but one, were “sporadic” cases of IDDM, i.e., no IDDM was recorded in their first degree relatives. Of the children who became diabetic, all were positive for ICA, 7 had IA-2A and 4 also had GADA. In reference to sensitivity, specificity and positive predictive value, the overall results are summarized in Table 1.

The data indicates that: 1) ICA alone, ≥ 5 JDFu (100%) or >20 JDFu (80%) showed the highest values of sensitivity for pre-IDDM; and 2) combination of ICA >20 JDFu and IA-2A can be regarded as a promising test for pre-IDDM, with sensitivity 70%, specificity of 99% and the highest positive predictive value (32%). In summary, it is only by consistently following these children that prediction of IDDM, among school children recruited in the general population, will be ultimately assessed in Sardinia.

The Sardinia newborn-IDDM project (SNIP)

The aim of the project is to investigate the natural history of IDDM as early as from birth. Around 30,000 children will be recruited, followed-up and investigated from the clinical, genetic and immunological stand points. Of the 18 paediatric departments scattered in Sardinia, 14 have started the study between November 1993 and November 1994. The remaining 4 centers (3 from the whole province of Oristano and 1 from the town of Sassari) started their recruitment during 1994. For each centre, the calendar year recruitment and collection of samples will coincide with their starting date. All the participating centers have agreed to continue the recruitment for a second calendar year. Over 18,000 cord blood/maternal samples have been collected so far.

In order to study the natural history of IDDM in reference to the appearance of islet-related autoantibodies, between November 1993 and September 1996, 12,858 cord blood sera were tested for ICA (M.F. Mulas et al. 1997. Diabetologia, Suppl. 1, A 81, Abstract 311). Their prevalence was 2.4% (314) for titres ≥ 5 JDFu and 0.7% (86) for titres > 20 JDFu, with a similar trend in the 3 provinces studied (Cagliari, Nuoro and Sassari). Of the 12,858 children initially recruited, 1897 blood samples have been collected at year 1 and 278 at year 2. The sera were tested for ICA (year 1 = 1853; year 2 = 238), GADA (year 1 = 1893; year 2 = 277) and IA-2A (year 1 = 1894; year 2 = 275). The results are summarized in Table 2.

At year 1, 8 children were positive for GADA. At year 2, 4 of them did not attend the pre-IDDM clinic, but 4 were followed-up and confirmed GADA positivity. 3 showed ICA and 2 had also IA-2A. To date, 6 children were not followed up, but 5 showed IA-2A and 2 showed GADA. The data are summarized in Table 2.

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive no. (%)</th>
<th>Se %</th>
<th>Sp %</th>
<th>PPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA ≥ 5 JDF-u</td>
<td>255 (8.3)</td>
<td>100</td>
<td>92</td>
<td>4</td>
</tr>
<tr>
<td>ICA &gt; 20 JDF-u</td>
<td>64 (2.1)</td>
<td>80</td>
<td>96</td>
<td>13</td>
</tr>
<tr>
<td>GADA</td>
<td>37 (2.1)</td>
<td>40</td>
<td>98</td>
<td>11</td>
</tr>
<tr>
<td>IA-2A</td>
<td>45 (2.8)</td>
<td>70</td>
<td>98</td>
<td>14</td>
</tr>
<tr>
<td>ICA &gt; 20 and GADA</td>
<td>19 (1.1)</td>
<td>30</td>
<td>99</td>
<td>16</td>
</tr>
<tr>
<td>ICA &gt; 20 and IA-2A</td>
<td>22 (1.3)</td>
<td>70</td>
<td>99</td>
<td>32</td>
</tr>
<tr>
<td>GADA and IA-2A</td>
<td>15 (0.9)</td>
<td>30</td>
<td>99</td>
<td>20</td>
</tr>
<tr>
<td>ICA &gt; 20 and GADA and IA-2A</td>
<td>14 (0.8)</td>
<td>30</td>
<td>99</td>
<td>21</td>
</tr>
</tbody>
</table>

7574 school children were tested for ICA, 5408 for GADA and 5411 for IA-2A.
developed IDDM. There were 3 males and 3 females, aged between 11 and 29 months old and all, but one, were ICA negative at time of birth (cord blood). At year 1, 2 out of 6 attended the pre-IDDM clinic and both were ICA positive, together with GADA or IA-2A. At the time of onset, sera from 3 newly IDDM children, out of the 6 who developed the disease were available. All were positive for ICA and GADA, but 1 was also positive for IA-2A.

These initial data indicate that: 1) GADA tend to develop before ICA and IA-2A at year 1; 2) there is a significant increase in the appearance of islet-related autoantibodies at year 2, when compared with year 1; and 3) children developing IDDM before the age of 3 years appeared to be ICA negative at birth (cord blood), but islet-related autoantibody positive at the onset of the disease. From the epidemiological view point, it appears that we are witnessing a possible increase of the incidence of IDDM under the age of 3. This unexpected finding is presently monitored in Sardinia.

The long-term objectives of the “Sardinia-IDDM study”

Combining the results of the SSIP and the SNIP, with those obtained from the EURODIAB-ACE epidemiological study for overt IDDM, we should identify not only “hot spots” for high incidence of IDDM, but also “hot pre-diabetic areas”, where the population living there could have an increased risk for developing IDDM. It is only after the completion of the epidemiological, genetic and immunological analyses of both these populations, i.e. that with overt diabetes and that with pre-IDDM, that it will be possible to define whether just a few “hot spots” or several “hot spots” do exist on the Island. We shall then be able to verify whether there is a fluctuation of IDDM, affecting certain areas over different periods of time. As soon as this information is available, a full ecological exploration will be organized and carried out in selective areas with the two distinctive patterns, i.e. “hot” and “cold”; of incidence of overt disease and prevalence of pre-IDDM.

General considerations

Today in Sardinia (population around 1,500,000 inhabitants) there are around 220 new cases of IDDM per year, only in the age group 0-29 years, making a total of around 30,000 IDDM patients, only in this age range. If then one considers that the prevalence of IDDM has not yet been quantified in the age range 30-80 years and that 15-20% of patients with NIDDM are also IDDM cases of slow progression to insulin dependency, the number of IDDM patients on the Island can be estimated around 15,000 to 20,000. Combining the projected figures of patients with IDDM and NIDDM, their number is around 50,000. From these data, the impression is that the Island, like Finland, is facing an “epidemic” of the disease.

The aetiology of IDDM is still largely unknown, but it is not excluded that the external factors may act as early as in utero. Although still controversial [52-54], the importance of cow’s milk products at the time of weaning has been suggested, as well as the recently revisited role of Coxsackie viruses in very young IDDM patients [23]. However, there is no information on the natural history of the disease as early as from birth. The SNIP will enable us to identify the initial and subsequently appearing of the known islet-related immunological markers in a population living in an area of high incidence of IDDM, such as Sardinia. It is for this reason that this project is unique. As for the SSIP, it will also offer the opportunity to clarify the natural history of IDDM in “sporadic” cases versus “familial” cases and design, for the first time, precise models of prediction to be then applied specifically to these two forms of IDDM.

Table 2. - Prevalence of islet-related autoantibodies, alone or in combination, in the Sardinian newborn cohort, followed at year 1 and year 2. At year 1, 1853 children were tested for ICA, 1893 for GADA and 1894 for IA-2A; at year 2, 238 for ICA, 277 for GADA and 275 for IA-2A.

<table>
<thead>
<tr>
<th>Test</th>
<th>Year 1</th>
<th>Year 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (%)</td>
<td>Positive (%)</td>
</tr>
<tr>
<td>ICA</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>GADA</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>IA-2A</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>ICA and GADA</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>ICA and IA-2A</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>GADA and IA-2A</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>ICA and GADA and IA-2A</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

(a) p < 0.001 vs year 1; (b) p < 0.05 vs year 1.
Long-term advantages for IDDM prediction and prevention

Individuals who are found positive for the various IDDM-related autoantibody markers, especially if in more than one combination, thus at higher risk of developing IDDM, will definitely benefit from an early diagnosis and prompt insulin replacement therapy. There is no doubt that this will influence positively the degree and the quality of the glyco-metabolic control, with a consequently lower risk of developing late diabetic complications. It should be emphasized that one of the practical outcomes of these projects is the establishment of a uniquely large serum bank. This will enable us to perform further laboratory investigations, according to information that will emerge from the initial investigations and from the continuous progress in IDDM-related research around the world. Finally, when “high risk” individuals are identified, they can later enter in new preventive trials (such as nicotinamide or insulin). These trials have already commenced in other Centers, but still carried out on an experimental basis. However, these individuals could also be the first to benefit from any new preventive trial which could be launched in the future.

Acknowledgments

Since its conception, the “Sardinia-IDDM study” has been supported by the Italian Ministry of Health, the Istituto Superiore di Sanità, the British Diabetic Association, the Autoimmune Diseases Charitable Trust and the Associazione Sarda per lo Studio e la Ricerca dell’IDDM in Sardegna. We are very grateful to Mr. Paul Whitick for patiently editing the manuscript.

Submitted on invitation.
Accepted on 14 February 1997.

(*) THE “SARDINIA-IDDM STUDY” GROUPS

General management: Overall scientific and administrative responsibility: G.F. Bottazzo (London), General co-ordinator: M. Songini (Cagliari), Local overall responsibility: A. Balestriero (Cagliari), Secretary/Administrator: E. Solliani (Cagliari).

The epidemiology study group: Responsible: M. Songini (Cagliari), Territorial co-ordinators: R. Cirillo (Cagliari), A. Filigheddu (Sassari), F. Mastino (Oristano), G. Pala (Nuoro), Participants: L. Addis (Tempio), S. Amedda (Cagliari), E. Angius (Cagliari), F. Atzeni (ADIG-Sardegna), S. Atzeni (ADIG-Sardegna), L. Bellu (Alghero), P. Bulcioli (Tempio), A. Cabras (ADIG-Sardegna), S. Cabras (Oristano), G.M. Cambosu (Oristano), L. Carboni (Cagliari), L. Carreras (Gusinzu), M. Cessa (Cagliari), M.A. Cipressa (Carbonia), A. Ciràco (Nuoro), R. Cirillo (Cagliari), C. Claudi (Nuoro), D. Cocco (Cagliari), M.T. Contigliani (Alghero), A. Corda (Iglesias), E. Cossu (Cagliari), G. Deltita (Iglesias), G. Fanciulli (Alghero), A. Farris (ADIG - Sardegna), F. Fenou (Cotzia) (ADIG-Sardegna), A. Filigheddu (Sassari), G.D. Filigheddu (Tempio), O. Frongia (Oristano), P. Frongia (Cagliari), T. Frati (Sassari), Z. Gambula (Carbonia), A. Gigante (Sorso), G. Idda (Bosa), N. Landis (Iglesias), S. La Scala (ADIG-Sardegna), A. Loi (Elmas), S. Losta (Cagliari), M. Maioli (Sassari), A.M. Marinaro (Sassari), C. Marinii (Cagliari), U. Marongiu (Lanusei), A. Massida (Lanusei), G. Melis (Carbonia), T. Meloni (Sassari), S. Mercu (Sorso), A.F. Milla (Nuoro), T. Muntoni (Cagliari), E. Murena (ADIG-Sardegna), B. Nieddu (Muravera), A. Pacifico (Sassari), G. Pala (Nuoro), G. Pippone (Tempio), M. Pippa (Cagliari), G. Piredda (Osieri), G. Pisano (Isili), G. Poddighie (ADIG-Sardegna), R. Ricciardi (Cagliari), F. Sanscu (Olbia), S. Sassu (Tempio), M. Scarpa (Cagliari), E. Secci (Osieri), F. Sechi (Osieri), G. Sera (ADIG - Sardegna), V. Sica (Gaslini), M. Silvetti (Cagliari), P. Simula (ADIG - Cagliari), M. Sittia (Gaslini), F. Solinas (Alghero), M. Sor (Oristano), P. Tronci (Cagliari), L. Vinc (Carbonia).


The newborn study group: Responsible: R. Cirillo (Cagliari), Co-ordinators: E. Angius (Cagliari), G. Cambosu (Oristano), T. Meloni (Sassari), C. Pintor (Cagliari), Central co-ordinators: E. Cossu, G. Gualt, M.F. Mulas, I. Pelligra, M. Saba (Cagliari), Laboratory personnel: E. Cianchetti, S. Cocci, A. Floris, A. Lai, P. Pisani, P. Pitfalls, C. Forra, T. Rovamo, A.M. Satteneri, Territorial co-ordinators: V. Barra (Cagliari), L. Bello (Alghero), P. Bulcioli (Tempio), F. Cadoni (Cagliari), M.A. Chessa (Carbonia), G. Delitala (Iglesias), S. Erre (Osieri), M. Falchi (Muravera), N. Flamme (Sassari), G. Idda (Bosa), N. Landis (Iglesias), C. Lichter (La Maddalena), O. Limongelli (S. Gavino), M. Lei (Cagliari), A.M. Marinaro (Sassari), U. Marongiu (Lanusei), A. Masta (Olbia), G. Meloni (Sassari), A.F. Milla (Nuoro), L. Murtas (Oristano), G. Pilo (Sassari), G. Piredda (Osieri), G. Pisano (Isili), S. Pulina (Tempio), R. Ricciardi (Cagliari), E. Sequi (Sorso), M. Sor (Oristano), R. Tradu (Cagliari), M.A. Zedda (Cagliari), A.M. Zalatore (Cagliari) + Paediatric GPs in Sardinia (about 230), Field workers: 12 professional nurses.


The emigrant study group: Responsible: M. Songini (Cagliari), Co-ordinator: A. Olianas (Pavia), Consultants: E. Bosi (Milan), R. Lorini (Pavia), A.M. Tencori (Pavia), F. Velluzzi (Cagliari), Laboratory personnel: A. Vitale, External support: Federazione Associazioni Sardi in Italia (FASI) and Circoli Emigranti Sardi Lombardia.

The environmental study group: Responsible: C. Palmas, M. Songini (Cagliari), Co-ordinators: A. Contu, A. Vona (Cagliari), Consultant: D. Dolei (Sassari), Laboratory personnel: G. Trova (Sassari).

Veterinary study group: Responsible: G. Pintori (Sassari), co-ordinator: M. Padda (Sassari).

Scientific committee: S. Carta (Rome), R. Del Baglivo (Rome), S. Del Giacco (Cagliari), A. Maida (Sassari), M. Pironi (Rome), G. Pozza (Milan), G. Vicari (Rome).
REFERENCES


