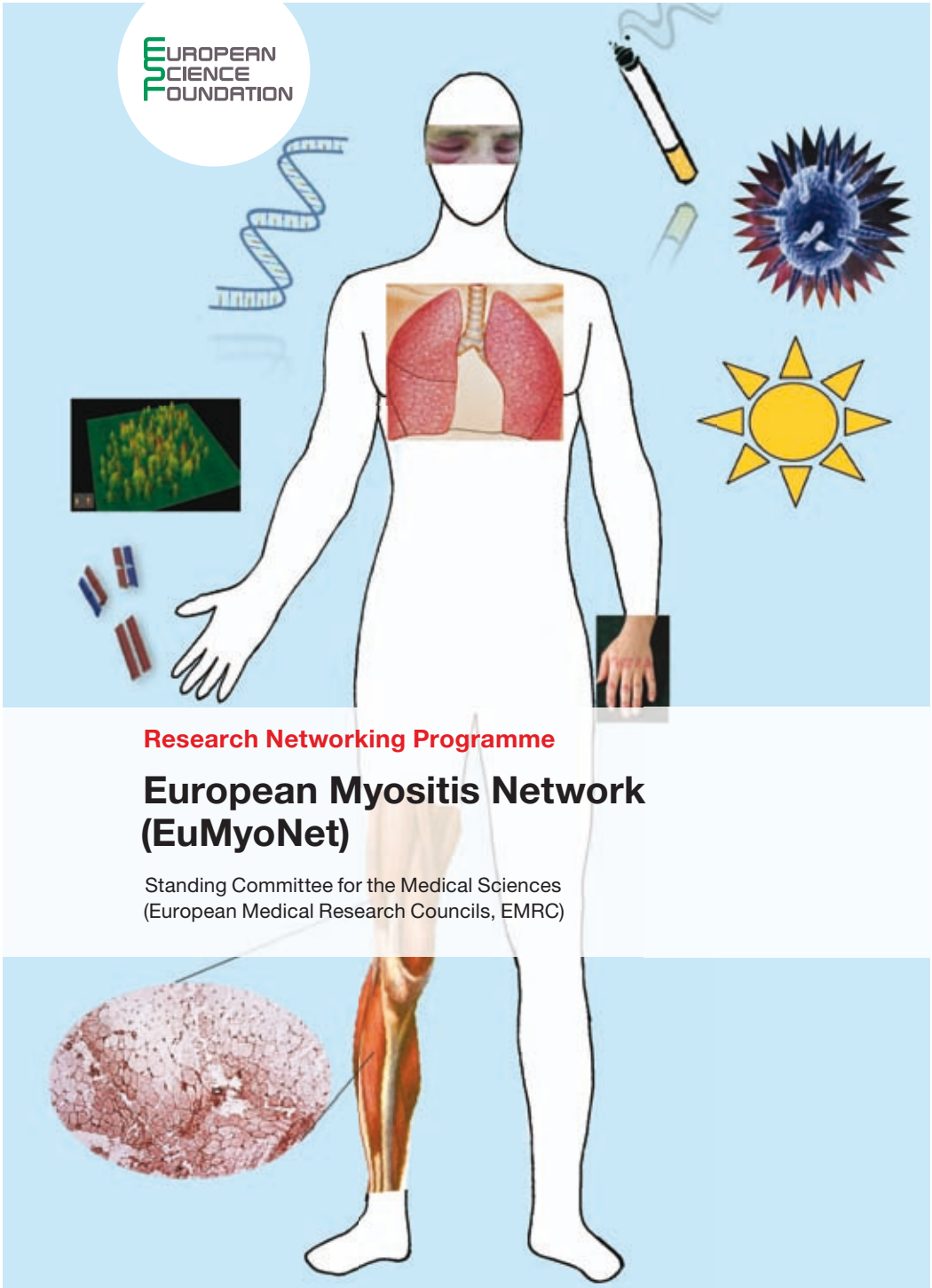


Research Networking Programme

European Myositis Network (EuMyoNet)

Standing Committee for the Medical Sciences
(European Medical Research Councils, EMRC)

European Myositis Network

The aim of this network is to establish an interdisciplinary network to share knowledge and expertise in various scientific fields of adult and juvenile myositis, inflammation of the skeletal muscles, and to establish a European myositis database with longitudinally followed patients and a biobank with DNA, serum samples and muscle biopsies in order to achieve improved knowledge of molecular pathways of myositis.

Myositis is a rare disease with an annual incidence of only 2.2-7.7 cases per million. Therefore in order to collect large cohorts of adults and children with myositis for studies on genetic risk factors and for clinical trials, a European multicentre collaboration is needed. Furthermore, people with myositis come under the care of experts within different medical disciplines, so an interdisciplinary collaboration is essential to enrol representative patients with various clinical characteristics.

EuMyoNet is a novel, unique project. It is the first European multicentre, interdisciplinary research project on inflammatory myopathies, involving neurologists, rheumatologists, neuropathologists, pediatric rheumatologists and basic scientists with expertise in genetics and proteomics. This network will enable us to collect a large number of patients with varying phenotypes which will allow subclassification of myositis. The project will also generate the first interdisciplinary myositis database with prospective *longitudinally* collected clinical and laboratory data as well as biological samples forming a myositis biobank. The longitudinal cohort will be unique and will permit studies of prognostic biomarkers by the use of modern proteomics and metabolomics technologies.

Through an EU-funded project, AutoCure, a European myositis consortium has been founded, which includes a cohort of 850 myositis patients with clinical data, DNA samples and sera. We now aim to extend this cohort by including experts outside AutoCure and by collecting 500 more cases. Data collection will be facilitated by an electronic myositis register (EUROMYOSITIS), which will be implemented in the network. A whole genome-wide scan analysis and serotyping will be performed within the consortium and in collaboration with an international genetic study, MYOGEN. This study will permit us to investigate the role of genes and serological markers for disease susceptibility and prognosis. It will raise new scientific questions on possible molecular pathways in myositis that can be further tested within our network. This network with its unique large database with well-characterised myositis patients will provide a platform for scientific work in order to achieve increased understanding of disease mechanisms. In addition the network will facilitate recruitment of myositis patients to clinical trials.

The ESF EuMyoNet Research Networking Programme will run for five years from May 2010 to May 2015.

Mechanisms underlying the inflammatory myopathies, myositis

The idiopathic inflammatory myopathies (IIM), also known as myositis, are a heterogeneous group of rare autoimmune diseases of varying prognosis. They are characterised by proximal muscle weakness, inflammatory cell infiltrates in muscle biopsies and presence of circulating myositis-specific/associated autoantibodies. The most common subclassification of IIM includes polymyositis, dermatomyositis, myositis overlapping with another connective tissue disorder (myositis/CTD-overlap) and sporadic inclusion body myositis (sIBM). Myositis may also present in children, the most common subgroup being juvenile dermatomyositis (JDM). Myositis subsets may also be subclassified based on autoantibody profiles which are associated with distinct clinical phenotypes[1]. Although glucocorticoids and other immunosuppressive agents can be used to treat myositis, response remains variable and often disappointing. Occasional patients die from disease and/or treatment complications, while survivors often remain disabled[2]. To date there is limited information on prognostic markers that could help in decision making for treatment. Furthermore, given the limited effectiveness of available therapeutic agents in myositis, new treatments are clearly required. To date clinical trials have been limited due to the rarity of the disease and lack of validated outcome measures. Moreover, to facilitate the use and development of novel therapies, the aetiopathogenic mechanisms underlying myositis require further elucidation.

Histopathology

The range of clinical and histopathological features suggest that there may be different disease mechanisms in different subgroups of myositis. There are at least four major histopathological features. One is predominated by endomysial inflammatory cellular infiltrates composed of CD8+ and CD4+ T lymphocytes (both positive for CD3+, Figure 1), macrophages and dendritic cells suggesting that the muscle fibres are targets of the immune reaction. The other histopathological phenotype is predominated by perivascular inflammatory cellular infiltrates predominated by CD4+ T cells, macrophages, dendritic cells (and sometimes B cells), suggesting that the blood vessels constitute the target of the immune system (Figure 1). The latter histopathological phenotype is often associated with skin rash typical of dermatomyositis, but can also be found in patients without skin rash, whereas the first pattern is more often associated with patients without skin rash, polymyositis, but can also be seen in patients with another myopathy, sporadic IBM. IBM is further characterised by rimmed vacuoles and inclusions. Given its unresponsiveness to steroids sIBM probably also has another pathomechanism. Another feature is that of a necrotising myopathy with no signs of inflammatory infiltrates and often associated with cancer. Thus the clinical and histopathological features vary but may also overlap suggesting that some pathogenic mechanisms are distinct and some are shared between the different clinical subsets.

Autoantibodies

Conventional clinical techniques, including muscle strength tests, serum levels of serum creatine kinase activity and electromyography, cannot reliably differentiate between IIM and other myopathies. Even muscle biopsy lacks sensitivity and specificity[3]. Here autoantibodies could be an important tool. Autoantibodies are detected in up to

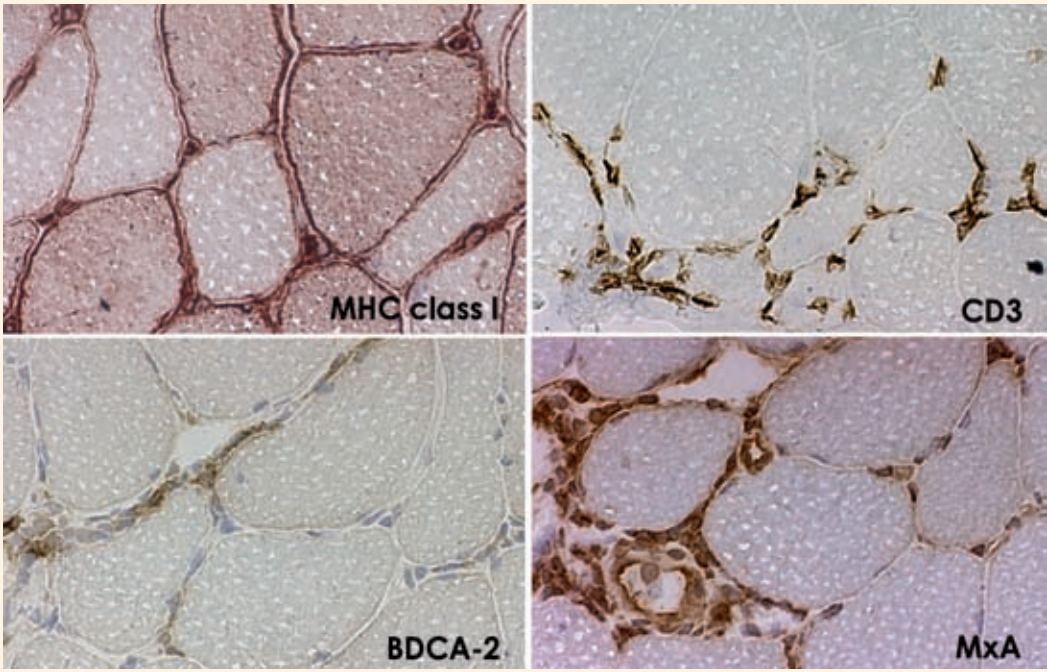


Figure 1. Muscle biopsy of an anti-Jo-1 positive polymyositis patient. Upper panel from left: Positive red staining of major histocompatibility complex (MHC) class I on the surface and in the sarcoplasm of muscle fibres, and brown staining of cluster of differentiation (CD)3+ T cells in endomysial infiltrate. Lower panel from left: positive brown staining of blood dendritic cell antigen (BDCA)-2 positive plasmacytoid dendritic cells, and IFN inducible myxovirus resistance 1 protein (MxA) in capillaries and perivascular infiltrate.
Courtesy of Dr Sevim Barbasso Helmers.

80% of polymyositis and dermatomyositis patients. Some are almost exclusively found in myositis patients, so-called myositis-specific autoantibodies (MSAs), and others are also found in other autoimmune diseases, so-called myositis-associated autoantibodies (MAAs) such as anti-U1-hnRNP, anti-PM/Scl or anti-Ro[4]. Although 50% of IIM patients have defined autoantibodies (MSAs or MAAs), 70% of patient sera have been reported to contain antibodies that bound to human myocytes, implying that at least a further 20% have as yet unidentified autoantibodies[5]. A number of putative MSAs have been reported in small cohort studies but most of these specificities have yet to be validated in larger cohorts or correlated to other risk factors such as genetics.

Interestingly, the MSAs are often associated with distinct clinical phenotypes. Thus the most prevalent MSAs, the anti-tRNA synthetase antibodies, of which anti-histidyl tRNA synthetase (anti-Jo-1) is the most common and found in 20-30% of myositis patients, is associated with the anti-synthetase syndrome with distinct clinical manifestations including myositis, interstitial lung disease, arthritis, Raynaud´s phenomenon and skin rash on the hands, so called mechanic's hands (Figure 2)[6,7]. Anti-Mi-2 antibodies are associated with typical dermatomyositis skin rash: heliotrope exanthema and Gottron´s papules and anti-SRP antibodies are associated with a necrotising myopathy

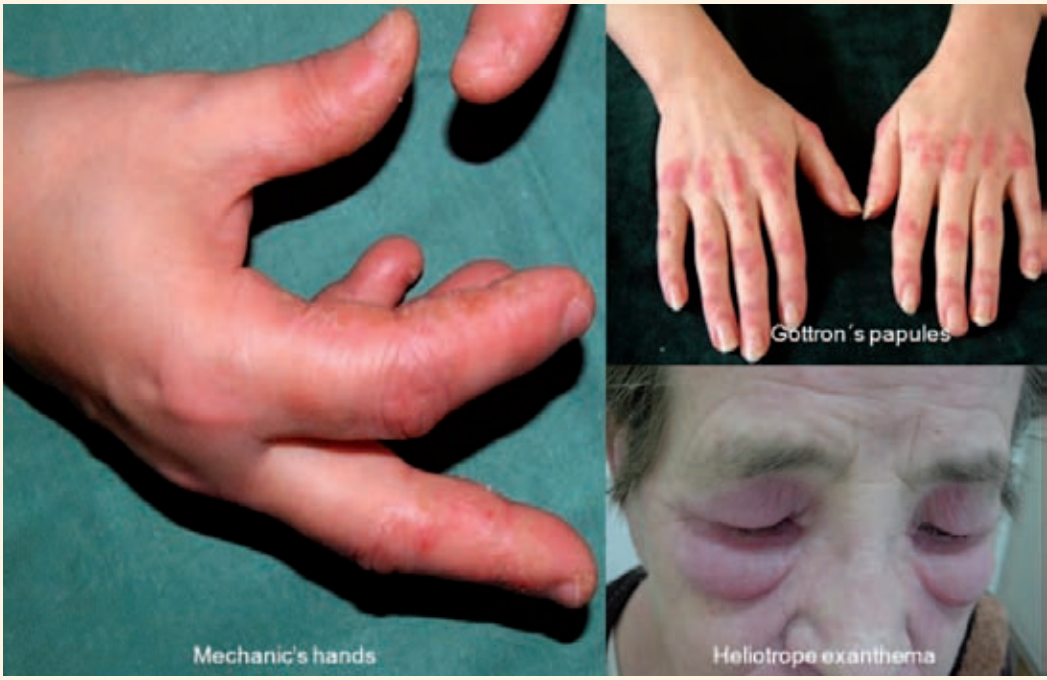
which seems to be fairly resistant to therapy[8,9].

The presence of autoantibodies in myositis offers the most compelling evidence to date for the role of aberrant adaptive immunity in IIM, although the specific antigens that are the target of the immune reactions are still unknown. One feature of the autoantibody response within myositis is that it is usually confined to a single antigenic target within a single patient. Although ubiquitously expressed, the target antigens may be up-regulated and/or modified in regenerating, apoptotic or de-differentiated muscle tissue[10]. A non-specific muscle injury might trigger this autoimmune response, which leads to myositis. Cross-reactivity with cancer neo-antigens might explain how muscle becomes a secondary target of immune attack in cancer-associated myositis. Accumulating data suggest that anti-histidyl tRNA synthetase antibodies may have a role in the disease mechanisms. Anti-Jo-1 antibodies may occur years before onset of clinical symptoms and sera from patients with anti-Jo-1 antibodies may activate the type I interferon system and the B cell activating factor (BAFF)[11-13]. Subclassifying patients according to autoantibody profile may be a new way forward to get a better understanding of patterns of variation in IIM and their disease mechanisms. However, the role of autoantibodies in the pathogenesis in IIMs as well as their use as a prognostic marker still needs to be determined.

Genetic and environmental risk factors

Similar to other autoimmune disease, *e.g.* rheumatoid arthritis, genes and environment are likely to contribute to susceptibility to myositis. The best established environmental risk factor in inflammatory myopathies is UV light. There is an observed correlation between the occurrence of dermatomyositis and particularly the subset with anti-Mi-2 autoantibodies and UV-light exposure[14]. The association between UV-light exposure and this subtype of myositis could suggest that UV light is an exogenous modifier that can influence the clinical phenotype in two closely related diseases, poly- and dermatomyositis. There are also case reports on associations to viral and parasitic infections, although large epidemiological studies to confirm these associations are lacking.

More studies have a focus on genetic risk factors and both MHC and non-MHC genes are associated with myositis. HLA-DRB1*0301 and DQA1*0501 are major risk factors for both polymyositis and dermatomyositis[15]. Within our consortium, based on the UK Adult Onset Myositis Immunogenetic Collaboration (AOMIC), for the first time genetic differences were demonstrated between polymyositis and dermatomyositis. Work at the Centre for Integrated Genomic Medical Research (CIGMR) showed that HLA-DRB1*07 proved a risk factor for dermatomyositis but was protective for polymyositis[16]. Alleles of the 8.1 ancestral haplotype (AH) (HLA-B*08-DRB1*03-DQA1*05-DQB1*02, 8.1 AH) show even stronger associations for certain IIM serotypes, particularly anti-histidyl-tRNA synthetase (anti-Jo-1) antibody. Similarly, the DRB1*07-DQA1*02-DQB1*02 haplotype is associated with dermatomyositis, but it is even more strongly associated with possession of the dermatomyositis-specific anti-Mi-2 Ab. Interestingly, in children the genetic/serology associations are equally strong but the relative frequency of autoantibodies is different and certain clinical features are more prevalent (*e.g.* calcinosis) and can be associated with specific antibodies[17].



These cumulative data suggest that molecular disease mechanisms may differ in myositis subsets and genotyping/serotyping may identify homogenous patient subsets for proteomic and metabolomic molecular studies and for future clinical trials using targeted therapies. Furthermore, genotyping/serotyping/proteomics may identify new prognostic markers for disease outcome.

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Figure 2. Skin rash on the hands, so called mechanic's hands, is one of the manifestations of the anti-synthetase syndrome associated with anti-tRNA synthetase antibodies. Typical dermatomyositis skin rash: Gottron's papules and heliotrope exanthema.
Courtesy of Professor Jiří Vencovský.

Specific objectives

- To implement the use of a myositis register (EUROMYOSITIS) centred on a novel, electronic, web-based format to facilitate longitudinal collection of clinical and laboratory data in a standardised form using outcome measures that have been validated and are recommended by international myositis expertise within the IMACS (International Myositis Assessment and Clinical Studies).
- To develop patient-administrated electronic forms to facilitate data collection in the EUROMYOSITIS.
- To implement the use of this electronic system in clinical practice amongst myositis research centres.
- To collect longitudinal clinical data from at least 1350 European patients with myositis.
- To create a database with genetic data from a genome-wide associationanalysis (GWAS) and serology data from at least 1350 European patients with myositis and to link this to the clinical database.
- To define autoantibody profiles concerning myositis-specific and myositis-associated autoantibodies and to identify new autoantibodies.
- To identify diagnostic/prognostic biomarkers from the database and the register by integrating and analysing the clinical data with genetic and serology data.
- To use the network and myositis register to facilitate new clinical trials.
- To create a platform for functional cellular and molecular studies.
- To facilitate training of young clinician scientists within this research field.

Activities

Specifically, the proposed activities, key targets and milestones of EuMyoNet are:

- Finalising the electronic myositis register.
- Implement the electronic myositis register into myositis clinical research centres within Europe.
- A website for the European Myositis Consortium.
- Collection of at least 500 more patients with clinical longitudinal data, sera and DNA samples.
- DNA extraction, and GWAS analyses of the additional 500 cases.
- Serotyping of the additional 500 cases + remaining 300 cases.
- A database with serology data and genetic data.
- Combine the clinical analyses with genetic and antibody profiles.

Milestones year 1

- A website for EuMyoNet.
- Finalise the electronic myositis register (EUROMYOSITIS).
- Implement the electronic myositis register into clinical research centers.
- Enter data from the first 850 patients into the electronic European Myositis register and start to follow them longitudinally.

Milestones year 2

- Continued collection of longitudinal data and sera from the first 850 myositis patients.
- Collection of at least 500 more patients with clinical data, sera and DNA samples, and DNA extraction of 500 samples.

Milestones year 3

- Continued collection of longitudinal data, and sera from 1350 myositis patients.
- GWAS analyses of the additional 500 cases.
- Serotyping of the additional 500 cases.
- A database with serology data and genetic data will be created.

Milestones year 4

- Continued collection of longitudinal data, and sera from 1350 myositis patients.
- Analyses of clinical data in combination with genetic and antibody profiles.

Milestones year 5

- Clinical analyses with genetic and antibody profiles.
- Prognostic biomarkers to be identified.
- Manuscript prepared.

In addition, EuMyoNet will have annual steering committee meetings and scientific workshops. Further, EuMyoNet will organise at least one summer school course on muscle inflammation for PhD students, post-docs and MDs.

Expertise within EuMyoNet

The expertise within EuMyoNet covers the following research areas.

- All clinical groups will provide expertise on clinical medicine.
- **Clinical and laboratory data**: on the IMACS outcome measures, on a web-based myositis register, and on muscle biopsy analyses for research.
- **Serology**: serotyping and testing for new autoantibodies and development of new technology for autoantibody testing.
- **Genomics**: DNA sampling and extraction, HLA-typing and fine mapping of genes, and genome wide scan (GWAS).
- **Proteomics and metabolomics**: identify potential biomarkers.
- **Bioinformatics**: integrate the clinical database with genetic and antibody databases. Analyse and identify new subgroups of myositis patients according to genetics, serology, muscle biopsy features and clinical outcome. Identify prognostic biomarkers.
- **Epidemiology**: environmental risk factors and gene environment interaction. Expertise and facilities including statisticians, epidemiologists and computer programmes.

EuMyoNet welcomes new participants with an interest in understanding the pathogenesis of myositis.

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For the latest information on this Research Networking Programme consult the EuMyoNet websites:
www.esf.org/eumyonet
www.eumyonet.org

Cover:
The cover picture depicts the long-term aim of EuMyoNet: to achieve increased knowledge on risk factors and molecular disease mechanisms, and to improve treatment in inflammatory myopathies – disease of the muscle, also known as myositis. A person's genetic make-up (depicted in the picture by the double helix of DNA) together with exposure to environmental factors (examples illustrated are UV light from the sun, infectious agents and smoking) are likely to contribute to susceptibility to myositis and will be investigated. We also plan to identify molecular pathways (the image of the bioarray is courtesy of Dr Thomas Häupl) that may lead to muscle weakness and the involvement of other characteristic organs such as the skin in dermatomyositis (illustrated by the hand and eyes highlighted in the picture; courtesy of Professor Jiří Vencovský) and lung in both polymyositis and dermatomyositis. The role of autoantibodies, detected in up to 80% of polymyositis and dermatomyositis patients, will be investigated as well as the role of T lymphocytes, macrophages and dendritic cells, typically found in skeletal muscle.
Image courtesy of Dr Sevim Barbasso Helmers, who also designed the cover picture.

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