Translational research network and patient registry for auto-inflammatory diseases

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Abstract

Objective. Auto-inflammatory diseases (AIDs) are characterized by recurrent self-limiting systemic inflammation. In a multicentre effort, we set out to register genetic, epidemiological and clinical features as well as prognostic factors of these diseases by prospective longitudinal and long-term documentation, in order to define novel AIDs and to better understand treatment responses and outcome.

Methods. In 2009, a federally funded clinical and research consortium (AID-Net) was established, including an online registry for AIDs (http://www.aid-register.uk-essen.de). Inclusion criteria are disease-associated mutations for hereditary periodic fever syndromes [FMF, hyperimmunoglobulinaemia D and periodic fever syndrome (HIDS), TNF receptor 1-associated periodic syndrome (TRAPS) and cryopyrin-associated periodic syndrome (CAPS)], or, alternatively, clinically confirmed AID, systemic-onset JIA (SoJIA) and periodic fever, aphthous stomatitis, pharyngitis and adenopathy (PFAPA) syndrome with unknown genetic background. Patients were recruited to the registry and patient material was deposited in biomaterial banks (DNA/serum). In addition, basic research projects were initiated that focus on molecular mechanisms of AID.

Results. During the first 9 months, 117 patients (65 males, 52 females; age 1–21 years) have been recorded and classified as FMF (n = 84), HIDS (n = 1), TRAPS (n = 3) and CAPS (n = 1); clinically confirmed AID (n = 5); SoJIA (n = 22); and PFAPA (n = 1). One hundred and fifty blood samples of 18 patients were included in biomaterial banks.

Conclusion. Recruitment and follow-up of patients with AID will enable us to comprehensively address the correlation between clinical and epidemiological data, genetics and biomarkers. The translational approach may help to identify genetic or inflammatory markers relevant for the course and outcome of diseases.

Key words: Registry for auto-inflammatory diseases, Biomaterial bank, Hereditary periodic fever, FMF, TNF receptor 1-associated periodic syndrome, Hyperimmunoglobulinaemia D and periodic fever syndrome, Cryopyrin-associated periodic syndrome, Pharyngitis and adenopathy syndrome, Systemic-onset JIA.

Introduction

Auto-inflammatory diseases (AIDs) are characterized by recurrent, self-limiting episodes of fever and inflammation [1–3]. This group of disorders encompasses hereditary periodic fever (HPF) syndromes such as FMF, hyperimmunoglobulinaemia D and periodic fever syndrome (HIDS), TNF receptor 1-associated periodic syndrome (TRAPS) and cryopyrin-associated periodic syndrome (CAPS) [4–6]. Systemic-onset JIA (SoJIA) and periodic fever, aphthous stomatitis, pharyngitis and adenopathy syndrome (PFAPA), in contrast, are AIDs with unknown genetic background [7–9].

Recently, in some of the AIDs, elucidation of the molecular pathophysiology related to the inflammasome has enabled clinicians to both classify these diseases more precisely and treat them successfully at least in a subset...
of patients [10–12]. Some of the AIDs are extremely rare, e.g. TRAPS, which we have estimated to occur with 5.6 cases per 10^7 person-years [13]. Yet genotype–phenotype correlation, clinical characteristics and response to treatment as well as natural disease course are very difficult to analyse on a single-centre level dealing with solitary cases. Therefore, sufficient data can only be gathered in a multicentre effort.

This has prompted us in 2009 to initiate an online registry for AID in children, in order to evaluate genetic and epidemiological characteristics, clinical presentation, therapeutic strategies, outcome and prognostic factors, as well as the relationship between genotype and phenotype. Ultimately, the establishment of larger, standardized data inventories may enable us to develop guidelines for treatment of such rare diseases. Specifically, this will help to adjust dosages of medication for the reduction of long-term toxicity, while at the same time avoiding complications such as amyloidosis. These efforts are expected to improve treatment quality and outcome in the future and to reduce expenses in health care. The national AID-Net consortium is unique when compared with other registries such as Eurofever since it pursues a translational approach.

**Methods**

**Translational AID-Net**

In 2009, the research initiative AID-Net (Network for Inflammatory Diseases), funded by the German Federal Ministry of Education and Research (BMBF project 01GM08104) was established. The network is structured into five basic research and three clinical research projects located at 12 institutions all over Germany. The main unit of the collaborative clinical research core of AID-Net is the patient registry. Recruitment of patients with AID is prompted via this online registry, additionally patient material is collected and stored in two central biomaterial banks for DNA and serum. Data of the registry and the bio-banks are connected in the online user interface. The patients’ biomaterial is used for the identification of genetic or serological markers of AID. We longitudinally monitor inflammation markers such as acute-phase proteins and relevant biomarkers such as S100A8/A9 (also known as myeloid-related protein 8 and 14 or MRPL8/14) and S100A12, which have recently been described in the context of AID in the course of treatment with, e.g. biological drugs, which is a largely unexplored field [14, 15]. The basic research section of the consortium integrates five research projects, which are specifically dedicated to analyse mechanisms of activation of the innate immune system, with a specific focus on inflammasome-related genes and alternative secretion mechanisms of IL-1β, IL-18, annexin A1, fibroblast growth factor 2 (FGF2) and S100 proteins.

**AID registry**

**Inclusion criteria**

We include patients with:

- disease-associated mutations for HPF syndromes, OR
- clinically confirmed AID with more than three self-limited episodes of fever >38.5°C and increased inflammation markers and asymptomatic intervals between episodes in an otherwise healthy patient, OR
- SoJIA with arthritis and fever (intermittent, period >2 weeks) and at least one of the following criteria: erythematous rash, lymphadenopathy, hepatosplenomegaly, serositis, OR
- PFAPA, AND
- informed consent of parents and patient.

**Exclusion criteria**

Patients with inflammatory disorders of other origin, e.g. infectious diseases such as bacterial or viral infection, malignancies, chronic IBD, non-systemic rheumatic diseases, vasculitis syndromes, primary immunodeficiency and autoantibody-mediated diseases.

**Participating centres**

All members of the GKJR (German Society for Pediatric Rheumatology) are invited to participate in the project. In addition, immunological and molecular genetics laboratories have their own access to the online system for documentation of sample management.

**Statistical analysis and data protection in the AID registry**

The proposed strategy for statistical analysis is mainly descriptive and exploratory, which is due to the limited knowledge of the investigated rare diseases. The evaluation will be stratified by clinical appearance of patients at the time of diagnosis (unchangeable), severity of disease, start of treatment, course, long-term follow-up, prognostic factors, prognostic importance of different genotypes, evidence of trigger and outcome. Statistical analysis is possible via SPSS (SPSS Inc., Chicago, IL, USA) export. Exploratory analysis will be performed for associations that have been reported in the literature. Sample size is limited because these diseases are extremely rare and as the statistical analyses are non-confirmatory throughout, no formal sample size calculations were performed.

The AID registry has been approved by the ethics committee and the data protection responsible at the University of Duisburg-Essen. Parents and young patients aged ≥14 years provide informed consent.

**Biomaterial bank**

DNA is collected from patients at the time of inclusion into the study. AID-associated genetic variations will be tested in all patients. In addition, genome-wide genotyping is planned for those without findings in known AID-related genes. If there is a medical indication for taking blood, at the first and the following visits of patients, serum will be collected for analysing standard markers (such as CRP or serum amyloid A) and exploratory markers (such as S100A8/A9, S100A12 and cytokines) to monitor inflammation longitudinally. This biomaterial bank has been approved by the ethics committee at the University of...
Results

Design of the AID registry

The online registry for AID is a German registry operating by electronic data collection (EDC) with easy handling. All data are entered via remote data entry software ProMiSe (Project Manager Internet Server) version 2.0, which has been developed by R Brand. The only technical requirement for participation is a computer with Microsoft Internet Explorer. Our web site is accessible via http://www.aid-register.uk-essen.de and http://prst.gpoh.de/aid. The server identifies with a limited certificate. Users are required to log in with username and password. We have established a standardized system for coding patient and sample data in an online registry, serum bank and genetic database. We unify the pre-existing structures such as the personal identifier (PID) generator as an appropriate tool for reliably identifying trial participants in medical research networks.

The online questionnaires are designed in a simple fashion and the forms are found on the web site http://prst.gpoh.de/aid/formulare.htm. To achieve longitudinal data, a follow-up for every patient is established by updating the registry after each presentation in either hospital or practice, at least four times per year. The following data are currently documented after informed consent of patient and parents: baseline data (initial date of registration, dates of presentation as in- or outpatient, running number of presentation, acute discomfort, height, weight, diagnosis and ethnic origin); symptoms and signs (general condition, fever, period of fever, serositis, skin involvement, abdominal involvement, joint involvement, musculoskeletal involvement, amyloidosis, lymphadenopathy, conjunctivitis, neurological problems, dystrophy, dysmorphism and trigger factors); genetic analysis (if available); diagnostic parameters (leucocyte count, acute-phase proteins, immunoglobulins D and A, ESR, creatinine, serum amyloid A and urine analysis); and medications, interventions, side effects and complications of therapy (World Health Organization (WHO) toxicity), and medical assessment of disease activity as well as free comments by the investigator. The participants choose from a list of several responses or respond with yes or no. In case of an unchanged condition of the patient, online registration is very simple, because only two statements are necessary for the questionnaire. Check routines are implemented to accomplish a complete record. To ensure validity of data, there are plausibility tests for the user (participating centre) during data input. In addition, new data will be reviewed by the investigators before they are forwarded to the database, which is used for evaluation. A central messaging system serves to facilitate communication between user and controller, so that most of the problems can be resolved easily.

PID

Our medical research network critically relies on a pseudonymous but unambiguous identification of patients. Therefore, we use a PID generator, which can maintain a comprehensive patient list, match personal data and create PIDs. The PID is composed of eight alphanumeric characters. The data manager provides, for example, first name, family name and birth date of a patient and requests a PID from the separate PID server. This PID can then be used as a pseudonymous identification key for the registry database to avoid duplicates. For statistical analysis, personal data will be processed in an anonymized fashion, without PID. With the information kept on the registry server, it will be impossible to retrieve the identity of individual patients. The development of the PID generator was commissioned to TMF (Telematics Platform TMF e.V., a common platform for medical research networks in Germany), and it has become part of the generic privacy concept of the TMF, which details how patient data should be handled within medical research networks [16, 17].

Recruited cases

Currently, 117 patients (65 males and 52 females) between 1 and 21 years of age have been included by nine participating centres in the first 9 months after initiation of the AID registry. Of these patients, 12 (11%) have been documented only prospectively, and for 105 (90%) patients retrospective and prospective data have been available. The following diagnoses have been identified:

- confirmed mutations for HFG syndromes (n = 89): FMF (n = 84), HIDS (n = 1), TRAPS (n = 3) and CAPS as Muckle–Wells syndrome (n = 1);
- clinically confirmed AID with more than three self-limited episodes of fever >38.5°C and elevated inflammation markers (n = 5);
- SoJIA (n = 22); and
- PFAPA (n = 1).

The patients’ countries of origins are Turkey (n = 64), Germany (n = 22), Arab countries (n = 6), Italy (n = 1), Kazakhstan (n = 1), Persia (n = 1) or unknown (n = 22). The spectrum of mutations in HFG syndromes detected by genetic analysis in the index cases is summarized in Table 1. All the recruited patients will be followed up longitudinally. One hundred and fifty blood samples of 18 patients have been collected in the biomaterial banks (Table 2).

Basic research projects

Innate immunity has come into the focus in immunology research over the last decade, partly due to the discovery of pattern recognition receptors (PRRs). Identification of sensors in the outer cell membrane such as Toll-like receptors (TLRs) or the receptor for advanced glycation endproducts (RAGEs) and intracellular detection mechanisms such as nod-like receptors (NLRs) have amplified the knowledge about how innate immune cells initiate a broad spectrum of defence mechanisms. Some pro-inflammatory molecules have been recently ascribed to the novel group of damage-associated molecular
inflammatory responses in vitro and S100A12, act via PRRs and induce dramatic pro-

Phagocyte-specific S100 proteins, i.e. S100A8, S100A9 proteins and other molecules represent danger signals high-mobility group box 1 (HMGB1) protein, HSPs, S100 pattern (DAMP) molecules. DAMP molecules including

<table>
<thead>
<tr>
<th>Gene mutations</th>
<th>Number of cases</th>
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<tbody>
<tr>
<td>FMF</td>
<td></td>
</tr>
<tr>
<td>MEFV pM694V</td>
<td>61</td>
</tr>
<tr>
<td>MEFV pM680I</td>
<td>10</td>
</tr>
<tr>
<td>MEFV pV726A</td>
<td>4</td>
</tr>
<tr>
<td>MEFV pE148Q</td>
<td>3</td>
</tr>
<tr>
<td>MEFV pL110P</td>
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</tr>
<tr>
<td>MEFV pE167P</td>
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</tr>
<tr>
<td>MEFV pR761H</td>
<td>1</td>
</tr>
<tr>
<td>MEFV pM694I</td>
<td>1</td>
</tr>
<tr>
<td>TRAPS</td>
<td></td>
</tr>
<tr>
<td>TNFRSF1A pR92Q</td>
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</tr>
<tr>
<td>HIDS</td>
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<tr>
<td>MVK pI268T</td>
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</tr>
<tr>
<td>CAPS</td>
<td></td>
</tr>
<tr>
<td>NLRP3 pT405P</td>
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</tr>
<tr>
<td>Total (n)</td>
<td>89</td>
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<table>
<thead>
<tr>
<th>Blood samples</th>
<th>Diagnosis</th>
<th>Number of cases</th>
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<tbody>
<tr>
<td>109</td>
<td>SoJIA</td>
<td>8</td>
</tr>
<tr>
<td>24</td>
<td>CAPS</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>HIDS</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>FMF</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>TRAPS</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Clinically confirmed AID</td>
<td>1</td>
</tr>
<tr>
<td>150</td>
<td>Total (n)</td>
<td>18</td>
</tr>
</tbody>
</table>

pattern (DAMP) molecules. DAMP molecules including high-mobility group box 1 (HMGB1) protein, HSPs, S100 proteins and other molecules represent danger signals that share characteristics of cytokines [18–20]. Phagocyte-specific S100 proteins, i.e. S100A8, S100A9 and S100A12, act via PRRs and induce dramatic pro-

inflammation. Moreover, for many mutations (e.g. TNFRSF1A R92Q), it is still controversial whether there is a significant relationship between genotype and phenotype [13, 33, 34]. In this context, we attempt to evaluate the diagnostic and prognostic value of individual mutations. The heterogeneity of AID is further illustrated by the absence of mutations in the coding region of suspect genes in certain families, compared with others with clinically indistinguishable phenotypes [30, 31].

The overall concept of the AID-Net consortium reflects a translational research approach. A network for research of AID—combining biomaterial banks, patient registry and basic research projects—is a novel approach and will be beneficial in several ways. Basic research may be using information about newly identified genetic aberrations or biomarkers for a better understanding of the molecular basis of disease, while on the other hand novel findings evolving from the research projects can be easily tested for clinical relevance by using patient material available in the biomaterial banks. This will allow translation of

Discussion

Clinically manifest AIDs are rare disorders in children [5, 6]. The lack of widely accepted and standardized diagnostic and therapeutic criteria for AID, either laboratory or clinical, remains a considerable problem [27, 28]. Our previous experience with an ESPED (German Paediatric Surveillance Unit) survey shows that only long-term follow-up may finally reveal whether clinically or genetically diagnosed AID cases belong to the spectrum of AID [13]. By means of continuous documentation, AID can be investigated systematically, providing a better understanding of the course of disease, as well as of therapy and prognosis [13, 27–29]. For this purpose, an online registry for AID has been established (accessible via http://www.aid-register.uk-essen.de). This registry serves to identify patient groups that might be interesting for subgroup analysis on an epidemiological level and also for research analysis of their samples. Registry data are biased because cases are recorded only with the consent of the patient. Data are not comparable with a prospective randomized study and it is not possible to answer pathophysiological questions based on this registry alone. Furthermore, a population-based approach to the collection of epidemiological data is hampered by the fact that complete recruitment of patients is hard to achieve.

Our understanding of the pathophysiology of AID is still limited. Most significantly, a useful laboratory test for the overall function of the inflammasome is lacking [6, 8, 14, 30–32]. As a result, screening has to rely on labour and cost-intensive genotyping for mutations in all genes that may cause auto-inflammation. Moreover, for many mutations (e.g. TNFRSF1A R92Q), it is still controversial whether there is a significant relationship between genotype and phenotype [13, 33, 34]. In this context, we attempt to evaluate the diagnostic and prognostic value of individual mutations. The heterogeneity of AID is further illustrated by the absence of mutations in the coding region of suspect genes in certain families, compared with others with clinically indistinguishable phenotypes [30, 31].

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Translational research network for auto-inflammatory diseases (AID-Net)

newly discovered pathogenic mechanisms of the innate immune system into improvements in patient care by assessing the potential of novel biomarkers or genetic tests for monitoring disease activity of patients. This may finally provide an option to develop guidelines for treatment such as dosages of medication optimized to reduce long-term toxicity, complications and side effects. With long-term data about treatment becoming available, case–control studies or randomized trials to address specific questions, e.g. failure and response of novel therapeutics, may be initiated. Therapeutic protocols will be established by the study group for hereditary periodic fever syndromes of the GKJR, so that in Germany all children will be treated in a standardized way.

Networking of AID research is necessary because of the rarity of these diseases [27, 29, 35–37]. For international cooperation and interdisciplinary considerations, the AID registry may be electronically linked with other international databases. Currently, there exists a link to the national DRFZ (German Rheumatism Research Centre). In principle, international networking is expected to further increase the data pool, with concomitant improvements in the significance of statistical findings. A link with the Eurofever registry is planned. The latter is particularly critical with respect to therapeutic questions due to more experience in the use of new medications (anakinra, etanercept and other immunosuppressive drugs) is clearly required. The connection with bio-banks will allow national and international networking of AID research in both clinical and pathophysiological fields.

Conclusion

Basic research projects were initiated that focus on molecular mechanisms of AID. By long-term documentation, AIDs are investigated systematically, providing a better understanding of the course of these diseases as well as of therapy and prognosis. We are creating a novel integrative database (accessible via http://www.aid-register.uk-essen.de), which finally comprises all epidemiological, clinical, genetics and inflammation parameters. The translational approach of AID-Net may help to identify genetic or inflammatory markers relevant for the course and outcome of disease. By online links, national and international networking of AID research is possible in a simple way.

Rheumatology key messages

- Translational approach of AID-Net combines projects on epidemiology, clinical and immunological features as well as molecular genetics.
- By online links, an AID registry provides a platform for international networking of AID research.

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