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European Drug-induced Agranulocytosis Consortium

- EuDAC -

The EuDAC Study

Research plan

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INTRODUCTION

Studies have shown that adverse drug reactions (ADRs) are one of the most common reasons for hospitalisation in the adult population [1,2,3]. It has also been proposed that ADRs are the fourth to sixth leading cause of death in hospitalised patients [4]. Most ADRs are dose-dependent and pharmacologically predictable (type A reactions), while others have no known pharmacological cause (type B reactions) [5]. Agranulocytosis (unless due to chemotherapy) belongs to this second type that commonly is serious and sometimes leads to withdrawal of the drug from the market [6]. Most research on the genetic basis of susceptibility to ADRs has focused on type A reactions, where there has been considerable progress in identifying genes that determine the metabolism of the drug. There has been less research into the genetic basis of susceptibility to serious type B reactions such as agranulocytosis. These reactions are necessarily rare - a drug that commonly causes these reactions will not be approved by regulatory authorities. Nevertheless, they are of great importance since they may severely limit the use of an otherwise effective drug. Examples of drugs with restrictions due to risk of agranulocytosis are the antipsychotic clozapine, where the risk is handled by monitoring patients carefully during induction of therapy, and the analgesic metamizol (Novalgin), where the risk has led to its removal from the market in many countries.

PURPOSE AND AIM

As the current knowledge about possible genetic causes of drug-induced agranulocytosis is minimal, the aim of EuDAC is to identify possible genetic markers that can predict the risk of drug-induced agranulocytosis. This will be done through a multicentre, multinational European case-control study.

SURVEY OF THE FIELD

Though agranulocytosis presents a low incidence (3.5 per million per year), it is a serious condition with a case-fatality rate of around 10%. In a high proportion of cases, it is associated with drugs; metamizol and beta-lactamic antibiotics are the most frequently involved drugs [7]. The fact that so few patients develop agranulocytosis after ingesting a potentially causative drug may indicate that genetic factors could play a role in its pathogenesis. Significant genetic associations have been identified in the major histocompatibility complex for hypersensitivity reactions associated with several drugs, and some of them have been recognized in drug induced agranulocytosis. The HLA system has been the main hypothesized region: a) gene variants of the HLA-DQB1 has been implicated in the pathogenesis of clozapine [8]; b) HLA A, B7 DQ1 was suggested to be associated with metamizol [9] although it has not been replicated.

THE CONSORTIUM

EuDAC consists of a European web of research groups with the aim to study possible genetic causes of agranulocytosis.

THE EUDRAGENE NETWORK

Eudragene (www.eudragene.org) is a collaborative network of investigators aiming to establish a collection of DNA samples as a resource for studying genes which influence serious ADRs, such as agranulocytosis.

A) Sweden

The SWEDEGENE project, which collaborates with Eudragene, was established in 2008 and aims to set up a database consisting of clinical data and DNA from cases with specific ADRs such as agranulocytosis. Regarding agranulocytosis, drugs under investigation can be of any type except anti-cancer agents. The Department of Clinical Pharmacology at Uppsala University Hospital in collaboration with the Swedish Medical Products Agency (MPA) and the Department of Clinical Pharmacology at Karolinska Institutet are responsible for the collection of cases. Patients are recruited on a nation-wide basis by the use of spontaneous reports of ADRs sent to the MPA by Swedish physicians. Clinical data are collected through interviews utilizing a standardised questionnaire, and information from medical and laboratory records. DNA is collected by extraction from whole blood. Causality assessment is done with the WHO algorithm.

B) Spain - Barcelona

The center at the University of Vall d'Hebron has established a population-based case-control scheme for the surveillance of agranulocytosis in the Metropolitan Area of Barcelona, with 17 participating hospital units of Hematology. It covers a population of 3.3 to 4.1 million inhabitants since 1980. Clinical data are collected through interviews and a standardised questionnaire, and information from medical and laboratory records. DNA is collected by extraction from whole blood. Causality assessment is done with the WHO algorithm.

C) Spain - Valladolid

The Centre at the University of Valladolid (Spain) [Centro de Estudios sobre la Seguridad de los Medicamentos] is in charge of the pharmacovigilance activities in the region of Castilla y León and in this manner acts as a centre of pharmacovigilance for this region. It is the coordinating centre in Spain for the Eudragene network; within this framework, we identify patients in Spain who develop the adverse drug reactions of interest, including agranulocytosis. We collect clinical data and blood samples of the patients according to the Eudragene protocols; thus, clinical information is introduced in the Eudragene database and samples are sent for storing to Erasmus University in Rotterdam —in the last years samples are stored in our premises at the University of Valladolid. Additionally, we have blood samples and clinical information of hospitalised patients who were used as controls in other studies.

D) Spain - Malaga

Universidad de Málaga participates according to the Eudragene protocols and identifies patients in Spain who develop the adverse drug reaction agranulocytosis.

E) France

The Department of Clinical Pharmacology at Toulouse University Hospital (Dr Emmanuelle Bondon-Guitton) is responsible for the collection of French cases. Cases have been selected in the French PharmacoVigilance Database according to the Eudragene protocol, with 25 French participating centers of Pharmacovigilance.

Germany

The Berlin Case-Control Surveillance Study (FAKOS) was initiated in the year 2000 to study serious rare toxicity of drugs. Study region was Berlin with an adult source population of 2.8 million inhabitants. Agranulocytosis was one of the target diseases of FAKOS. Patients with agranulocytosis were identified through regular active inquiry in two- to three-week intervals in more than 180 Departments of Internal Medicine, Neurology, Psychiatry, or Anaesthesiology of all 50 Berlin hospitals until September 2009.

CASE SELECTION

Cases are collected on a nation-wide general population basis and there are no restrictions to specific patients with particular diseases, except for such which may potentially introduce bias (listed under exclusion criteria).

INCLUSION CRITERIA

- ✓ Absolute neutrophil count <0.5 * 10^9/L (<500 per µL) during therapy with the suspected drug or within 7 days of stopping it.
- ✓ Complete recovery after cessation of the drug with absolute neutrophil count >1.0 * 10^9/L (>1000 per µL) or a compatible bone marrow aspirate or biopsy. This may be relaxed to show evidence of recovery where available.
- ✓ Causality assessment according to the WHO algorithm of at least possible.
- ✓ Age ≥18 years and ability to give informed consent.

EXCLUSION CRITERIA

- ✓ Recent chemotherapy, radiation therapy (within one month of ADR onset) or previous haematopoetic stem cell (bone marrow) transplantation.
- ✓ Ongoing infectious diseases: EBV viral hepatitis A, HIV, CMV, parvovirus B19 or other infection affecting bone marrow (e.g. preceding sepsis, miliar tuberculosis).
- ✓ Chronic neutropenias (congenital cyclic, idiopathic).
- ✓ Immunosuppressive therapy with cytotoxic drugs.
- ✓ Malignant infiltration of bone marrow.
- ✓ Haematological diseases (e.g. myelodysplasia, aplastic anaemia, pancytopaenia, other blood dyscrasias, e.g. haemoglobin ≤100 g/L and platelets ≤100* 10^9/L).
- ✓ Systemic lupus erythematosus.

CONTROL SELECTION

SWEDEN

As controls, a population cohort of 6,500 unrelated individuals with genome-wide data from the Swedish Twin Registry is used [10].

SPAIN

Controls come from two previous studies. The first is a case-control study on genetic determinants of upper gastrointestinal bleeding for whom blood samples are available (n=347). They are patients admitted to hospital for acute conditions such as trauma (not drug related) or elective surgery. They gave informed consent to participate in future studies on adverse drug effects. The other is a cohort of 400 population control subjects from Malaga where whole genome scan data are available.

GERMANY

Sex- and age-matched hospital controls have been collected (n=100).

DATA STORAGE

All data, both clinical and genetic, will be stored in a common computer database in anonymized form prior to analyses. The database is located at the Swedish research group's location at Uppsala University Hospital and backed-up daily. Log-in to the database requires a unique personal identifier and password only known to the selected persons within the research group. The hospital applies strict network control with high-end firewall protection.

DATA QUALITY ASSURANCE

GENETIC DATA

Markers which fail the following quality control criteria will be discarded: (a) call rate <95%, (b) minor allele frequency (MAF) <1%, (c) a p-value for Hardy–Weinberg equilibrium (HWE) <0.0000001 in controls, (d) cryptic relatedness or sample duplication tested by estimating the identity-by-descent for all possible pairs of individuals, and (e) samples failing test for sex.

CLINICAL DATA AND CASE ADJUDICATION

All clinical data entered into the database are double-checked by a study team member. As clinical data are based on medical records and interviews with patients, missing or conflicting data are expected. Uncertainties which can't be resolved will be treated as missing data. All cases will undergo adjudication by a specialist in clinical hematology to ensure that all inclusion criteria are met and that no exclusion criteria are present.

STATISTICAL ANALYSES

DESCRIPTIVE STATISTICS

Gender and ethnicity, and clinical variables, including indication for treatment, concomitant diseases and drug therapies (ATC code) will be described as proportions for both cases and controls. Age, doses of suspected drugs and time to onset of agranulocytosis will be described with mean values and standard deviations.

NULL HYPOTHESIS

The null hypothesis is that there are no genetic determinants of drug-induced agranulocytosis.

ASSOCIATION ANALYSES

Association analyses with genetic and clinical factors will be performed for all cases as a group, and stratified for each drug or class of drugs. Since cases are recruited from multiple European countries, we will correct for population stratification by utilising data from controls from each country, and principal component analysis will be performed. To correct for multiple testing, the level of significance will be set at around $p<1*10^{-8}$, which is equivalent to a Bonferroni correction for 1 million independent tests.

We will perform single SNP tests with logistic regression with adjustment for age, sex and population stratification by including significant principal components as covariates in the logistic-regression model. Results are illustrated with Q-Q-plots and Manhattan plots, and with odds ratios. Analyzes will be made under the assumption of different genetic models (dominant, additive, and recessive). We will draw an LD map including SNPs which show significant associations with agranulocytosis.

POWER AND SAMPLE SIZE ESTIMATION

Case sample size estimations in table 1 are based on 80% power to detect association at $p<1*10^{-8}$ with 6000 controls.

Frequency of disease-associated genotype	Frequency of the same genotype in controls	Required number of cases/controls
30%	20%	150/6000
30%	18%	100/6000
30%	16%	50/6000
30%	10%	25/6000

Table 1. Case sample size estimation.

VERIFICATION AND REPLICATION

The 10-20 top hits will be verified in cases through Taqman SNP genotyping. We will make an effort to collect 100 new cases and controls for replication of the 10-20 top hits. We will then only need to correct for 10-20 multiplied tests, i.e. a p-value of 0.0025 - 0.005 will be sufficient.

IMPUTATION

As controls from the Swedish Twin Registry used genotyping chips with less density (Illumina Omni Express Chip 700K), imputation to predict the missing SNPs will be required, i.e., replacing missing genotypes with predicted values that are based on the observed genotypes at neighboring SNPs.

FURTHER ANALYSES

As SNPs identified through GWAS are commonly ‘‘associated’’ variants rather than causative, further experimental or informatics analyses on genes and variants ‘‘linked’’ to the associated variant may be required to determine the specific protein(s) directly involved in the ADR variant. In order to determine the specific proteins directly involved in drug response, further experimental or informatics analysis must be performed on genes and variants ‘‘linked’’ to the associated variant. Such analyses will be the subject of follow-up studies requiring separate research plans and is beyond the scope of the present investigation.

TIME-LINE OF THE PROJECT

Figure 1 outlines the estimated time-line of the current project.

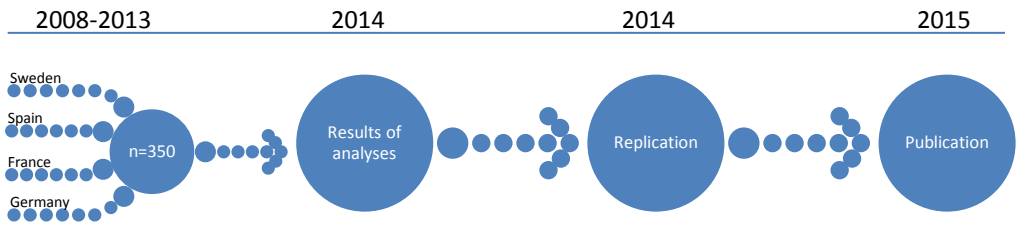

Figure 1. Time-line of the EuDAC Study.

APPROVAL FROM ETHICS COMMITTEES

Approval for the SWEDEGENE project has been received (EPN Uppsala Dnr 2010/231). We comply with Sweden's Data Inspection Board regulations, Personal Data Act, and have Biological Specimen Banks (Biobanks) approval. Corresponding approvals have been received for each participating center in EuDAC.

SIGNIFICANCE AND CLINICAL RELEVANCE

EuDAC is a unique effort that brings several European countries together, which is necessary when competing with larger countries such as USA or China. Our aim is to find genetic variants and other factors that influence the risk of drug-induced agranulocytosis.

Our findings may enable testing and prediction of the individual risk of drug induced agranulocytosis before starting a drug treatment. This would benefit the patient, health care and society and it is the most obvious incentive for our project. It is also possible that our findings would increase knowledge about the development of agranulocytosis when not drug-induced. This might enable drug companies to screen molecules for potential risk of agranulocytosis at an early stage and thus design safer drugs. Furthermore, understanding the mechanism of agranulocytosis might render ideas for the development of new drugs to treat agranulocytosis.

In summary, knowledge about causes of agranulocytosis may minimise the risk of this reaction in the future, and have a positive effect on drug safety, patient health, health costs and promote the development of new drugs.

GENERALIZABILITY

As the current study includes patients on a population basis, results derived from it is expected to be of wide generalizability. The Swedish collection of cases and controls are nation-wide, and the Spanish collection is derived from three different metropolitan regions with multi-million inhabitants.

PROTOCOL AMENDMENTS

Any protocol amendments made after publication will be documented under this section.

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