
EPIDEMIOLOGY OF RARE ANAEMIAS IN EUROPE

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Summary
Data concerning registry and epidemiology of Rare Anaemias (RAs) in Europe is in almost cases still incomplete or partially documented. For very rare erythroenzymopathies, only few individual cases have been described worldwide and for membranopathies, hereditary spherocytosis (HS) is the most well known cause of congenital haemolytic anaemia with a prevalence from 1 to 5 cases per 10,000 individuals. Other RAs exhibit a much lower prevalence, ranging from 0.5 to 7 cases per million births. Diamond-Blackfan Anaemia (DBA) with 4 to 7 per million live births and Fanconi Anaemia (FA) from 1 to 5 cases per million live births. Congenital Dyserythropoietic Anaemia (CDA), a genetically heterogenous group, exhibits a large variability of frequency depending on the European region: 0.1 to 3.0 cases per million births. In addition many cases are known from a large autosomal dominant family in Sweden. Paroxysmal Nocturnal Haemoglobinuria (PNH) although its incidence in Europe is still unknown; data collection from different sources has given quotes of 1 case per 100,000 individuals to 5 cases per million births.
Introduction

Rare Anaemias (RA) are diseases with a prevalence of less than 5 cases per 10,000 individuals (rare diseases) associated with anaemia as the most relevant clinical manifestation. In Europe, many people, including some health professionals, don’t know of the existence of RA because in the majority of cases the cause is unknown and there is no treatment, exception made of some special types of RA. More than 80% of RA are hereditary, and in dominant pattern, the gene defect can be passed on from parents to their children with the probability of 50%. In recessive hereditary cases, parents or other relatives can be healthy, because only the occurrence of two mutated genes causes the disease and the disease can occur with the probability of 25% in each pregnancy. As in other rare diseases (RD), due to the reduced number of patients, there is a need to mobilise resources and their study can be only efficient if done in a coordinated European way.

Since only the knowledge of the distribution of patients with RA through the Member States and the creation of a European Registry will allow the development of preventive plans and epidemiological surveillance, in October 2002, the European Commission (EC) through its Public Health and Consumer Protection Directorate (DG SANCO), approved for financing a Project entitled European Network for Rare Congenital Anaemias (ENERCA) addressed to improve the status of hereditary RA in Europe. After 2005, ENERCA Project was extended with another Project (European Network for Rare and Congenital Anaemias) where, in addition to congenital anaemias, rare anaemias of acquired origin were also included (1). Accordingly, one of the most important objectives of ENERCA is the improvement of epidemiological surveillance of RA in Europe. To achieve this important objective, ENERCA partners and other experts in RA, have been invited to complete the epidemiological data from their personalized clinical registries, and/or to collect the required data from other sources to pursue its completion.

For obvious reasons, it has been impossible to include epidemiological information from all the known RA. This is due to the large number of RA so far identified (about 90), to the unavailability of data, which is still incomplete or partially documented and to the extreme rarity of some hereditary anaemias (ie RBC enzymopathies) with only a
few cases described in the literature (2). Here the six different RA have been selected for epidemiological description due to their relevant clinical and/or social impact in the European populations: 1. **Haemoglobinopathies** are the most frequent inherited disorders of RBCs characterized by structural defects of haemoglobin molecule leading to chronic or acute haemolytic anaemia. Their epidemiological interest is growing in Europe due to the increasing frequency of the severe sickle cell disease (SCD) as a consequence of immigration. Beatrice Gulbis, from the ENERCA Consortium, describes the current situation of epidemiological aspects of haemoglobinopathies in Europe. 2. **Thalassemia** is a classical Mediterranean haemoglobin disorder and much of its epidemiological information has been provided by the Thalassemia International Federation (TIF), a beneficiary of ENERCA Project. Androulla Eleftheriou and Michael Angastiniotis are TIF members and ENERCA partners and they describe here the current situation of Thalassaemia epidemiology in Europe. 3. **Diamond Blackfan Anaemia (DBA)** is a very rare congenital aplasia where National registries indicate an incidence of 4 to 7 cases per million live births. Since sometimes DBA is difficult to identify in the clinical practice, Sarah Ball, provides us here, in addition to updated information on its epidemiology in Europe, a brief summary of the most important clinical features.. 4. **Fanconi Anemia (FA)** is a very rare genetic disease of erythropoiesis with an estimated frequency of 1-5 cases per million newborns. FA differs from DBA in that the anaemia is associated with additional clinical manifestations due to a progressive bone marrow failure, congenital abnormalities and cancer predisposition. FA research in Jordi Surrallés’ laboratory is undertaken by participation of several important research organizations in Spain: the Catalan Government (Generalitat de Catalunya), the Spanish Government (CIBERER, FIS and SAF), the European Regional Development Funds (FEDER) and Genoma España (FANCOGENE Project). 5. **Congenital dyserythropoietic anaemias (CDA)** comprise a group of rare hereditary disorders of erythropoiesis firstly described by Hermann Heimpel and others in the late 60’s. It is still impossible to determine the precise number of cases per live births but due to the long survival of patients, after a 42 year cumulative incidence, the number of cases per life births should be close to 0.1 to 3.0 cases per million births. 6. **Paroxysmal Nocturnal Haemoglobinuria (PNH)** is a very rare disease characterised by intravascular haemolysis, venous thrombosis and is associated with aplastic anaemia. Unfortunately, its incidence and prevalence in Europe is still unknown but Anita Hill, an outstanding expert in this disorder, after
collecting information from different sources quoted the incidence of PNH between 1 per 100,000 to 5 per million individuals

1. Haemoglobinopathies

Haemoglobinopathies are inherited disorders of haemoglobin and are the most common monogenic disorders in humans. The term “haemoglobinopathies” contains two main groups of disorders: the thalassaemias and the haemoglobin variants. The thalassaemias are quantitative defects which arise from a decreased production of structurally normal globin chains, whereas the haemoglobin variants are most often qualitative defects which arise from an alteration in the globin gene structure. Several haemoglobin variants conduct to a thalassaemic phenotype. Haemoglobinopathies are autosomal recessively transmitted affections which mean that the heterozygous individuals are near asymptomatic carriers while the homozygous or compound heterozygous individuals have varying degrees of symptoms. The most severe clinical forms of these disorders are met in the \( \beta \)-thalassaemia major, the haemoglobin Bart hydrops fetalis and the sickle cell disorders (SCD), respectively.

The frequency of different haemoglobinopathies varies in different ethnic groups. Since the carrier condition confers a protection towards the severe forms of malaria, this is the reason why these disorders were first confined on the endemic countries for the malaria. For example the thalassaemias are endemic to the entire Mediterranean area and the frequency of the carriers reaches rates of 15% in Cyprus. Due to population movements the haemoglobinopathies are now encountered in almost every country in the world.

Based on country prevalence estimates of haemoglobin disorders, a chart of the frequencies of the diseases by European (EU) country could currently be: comparable frequencies of haemoglobulinopathies throughout the EU with SCD more frequent than thalassaemias and more frequently encountered in Northern and Western EU countries (1)

Based on neonatal screening, other data have been obtained (Table 1) In the EU, five countries or capital-cities have adopted a neonatal screening program financed by the authorities in public health: England, France, Belgium (Brussels, Liège), Spain (Madrid) and recently The Netherlands (2-6). From those programs, the prevalence of SCD has been confirmed to be high in Northern and Western countries.
Nevertheless, quite high SCD numbers have been demonstrated in Madrid (5); other studies carried out in Spain have confirmed those results (7). The last decade, there has been increasing immigration flows especially from Northern and sub-Saharan regions of Africa all over Europe. Those new immigrants allow to explain the data obtained by a recent prenatal screening study or those reporting the number of living patients in various EU countries (1, 8, 9). Remarkably, the prenatal study conducted in Portugal reported a prevalence of 4.2 ‰ carriers of HbS and 12.6 ‰ of β-thalassaemia carriers (8). Around half of the German citizens come from countries where sickle cell disease and thalassaemias are frequent; the number of living patients with SCD and β-thalassaemia major is estimated to be around 1,250 and 450, respectively (9). Those data confirm that SCD and β-thalassaemia should be recognized as a public health problem all over Europe.

Although facilities for control and management of haemoglobinopathies are available in European countries, providing national programs for prevention and clinical management of SCD as well as of β-thalassaemia major is a challenge. The reason is that haemoglobinopathies are not officially recognized as a significant health problem in all EU countries. However recently, haemoglobinopathies have been recognized as a public health priority by the World Health Organization (10) and European dedicated networks like for example the “Euromediterranean network of research centres conducting molecular and clinical research of thalassaemia and related haemoglobinopathies” (http://www.ithanet.eu), the “European Network for Rare and Congenital Anaemias” (http://www.enerca.org), the portal for rare diseases and orphan drugs “Orphanet” (http://www.orphan.net) and the European Organisation for Rare Diseases (http://www.eurordis.org) are supported by the European Commission.

The distribution of immigrants at risk for haemoglobin disorders is very heterogeneous and differs in each EU country widely from one region to another. It seems reasonable to adapt the prevention strategy to the local situation encountered in each EU country. In those concerned by haemoglobinopathies a clear message should be delivered at a national level: an integrated program should be implemented. But one should be always aware that it outlines many challenges since it implies to implement effective procedures for primary and secondary prevention, diagnosis, education, information, and clinical care.

2. Thalassaemias

Thalassaemia has long been regarded as a disorder affecting Mediterranean
populations, the Middle East, Asia and generally the developing world. It has been known that migration has brought thalassaemia to Europe beyond its southern coast. This has introduced a challenge to the highly developed medical services which need to cope effectively with the newly imported chronic condition. Experience has demonstrated however that this challenge has been variously met and has left many thalassaemia patients in Europe unassisted and prevention services unable to effectively reach the population at risk.

Europe is a continent that goes beyond the EU, and cannot be regarded as a single unity with similar standards in patient care and responses to health problems. There is diversity in the frequency and prevalence of thalassaemia, as well as diverse, standards of care, health systems and ability to respond to the needs of thalassaemia as a health issue.

In this respect the continent may be divided into different thalassaemia areas:

1. The high prevalence countries of the Mediterranean coast, typified by Italy, Greece and Cyprus. These countries have taken the lead in developing services and their results justify their characterisation as models for the control of thalassaemia and other genetic disorders. (11)

2. Lower prevalence countries (1-2% carriers) where thalassaemia has a regional distribution in the indigenous population. These are typified by Romania, Bulgaria, Russia, Portugal and Spain. The services in these countries, especially for prevention, are largely underdeveloped and unevenly distributed. Portugal and Spain are regarded as advanced compared to the others of this group and are more able to respond to public health needs. The others have additional economic and public health handicaps which make service provision difficult. Portugal for example has a total population of 10.5 million with only 40 registered patients (a prevalence of 1/263400 of the population) (12)

3. Low prevalence countries (1/1000 carriers in the population) where migrants from high prevalence areas have settled in significant numbers, creating minority groups carrying a high risk for thalassaemia births. These are typified by Germany, Belgium, the Netherlands and Scandinavia. Prevalence in Germany for example is 450 patients (1/183333) and Belgium with 71 patients (1/145437). These countries have the ability to respond but face organisational and cultural problems inhibiting service delivery for this rare and relatively new disease (2) (12).
4. Low prevalence countries (1/1000 carrier rate) where the thalassaemia problem has not yet penetrated through migration to a significant degree, typified by Poland, Hungary and the Czech Republic. These are potential future targets which must not be forgotten but should be monitored as far as immigration is concerned.

5. The UK and France: these two countries belong to category 3 but differ in that they received migrants many years ago and have responded to a great extent to the needs of the thalassaemia community and have accumulated experience and developed services which may serve as an example to the rest of Europe. Also they have taken the lead in research activities for many years (13).

Each of these groupings presents different needs but explores similar solutions. The first category will not be discussed, except to point out that its experiences should be shared by all, even though they cannot be exactly imitated.

Category 2 presents a challenge similar to that of many underdeveloped countries. The patient support associations are weak, inexperienced and under-funded. The services are poor and need development at a very basic level e.g. blood donation and banking. Planning for upgrading services with the support of international NGOs such as the Thalassaemia International Federation (TIF) includes:

1. Forming national support associations as members of TIF.
2. Identifying interested physicians.
3. Organising educational programmes for health professionals, locally or regionally. There is need for funding of these activities.
4. Gathering all available epidemiological information.
5. Presenting the necessary information to Health Authorities with a plan of action preferably drawn up by a medical advisory panel and with WHO confirmation.
6. Providing political support to local associations and physicians.
7. Encouraging the local associations to join other European groupings such as Eurordis so that a constant flow of information as well as advocacy may be attained.
8. Encouraging WHO regional office to support, morally or otherwise, all development efforts.

In category 3 there are countries which should learn from the experience of UK and France since basically the problems are very similar. They have a model on which to base their programmes and so a North Western European collaborating grouping may be appropriate. TIF has taken the initiative to form such a group, in order to unite the
patient associations initially and then to encourage medical contacts by organising Pan-European conferences. These efforts are coordinated with other rare disease groups in Europe. It must not be assumed that this part of the world does not face difficulties in service provision and assume that they belong exclusively the so called underdeveloped world.

Thalassaemia in the European setting is classified as an ‘immigrant’ health problem and as a ‘rare’ disorder. This creates the illusion of not being important in public health. Rare is defined arbitrarily as affecting fewer than 1 in 2000 citizens. The chronicity of thalassaemia and sickle cell disease and the need for multidisciplinary care with expensive medication, which is beyond the reach of individual families, also the need for prevention programmes and early detection (e.g. neonatal screening) and specialised laboratories, all contribute to making these disorders a major public health concern which the EU must recognise and deal with.

Countries of category 4, in which the immigration from high prevalence areas has not yet reached significant levels, should be closely monitored to detect demographic changes early.

In conclusion Thalassaemia represents a major challenge to health services in Northern Europe even though the prevalence is not as high as it is in the Mediterranean coast.

3. Diamond-Blackfan Anaemia (DBA)

Diamond Blackfan Anaemia (DBA; OMIM 205900) is a rare congenital aplasia, classically presenting in infancy with profound aregenerative anaemia, often in association with growth retardation and congenital anomalies. Associated physical anomalies are present in 50% of affected children. Craniofacial abnormalities are most commonly described, with cleft or high arched palate, broad flat nasal bridge and hypertelorism. Abnormal thumbs may be present, ranging from flat thenar eminence to absent radii, and including the classic triphalyngeal thumb of DBA. The anaemia responds to treatment with corticosteroids in 60-70% of cases, but remission is usually associated with a residual erythropoietic defect, characterised by persistent mild anaemia and macrocytosis, and increased erythrocyte adenosine deaminase (eADA) activity. Patients with severe anaemia who do not respond to steroids enter a life-long transfusion programme, with chelation therapy to manage transfusion-associated iron overload, unless they have a suitable donor for haemopoietic stem cell transplant (HSCT) (reviewed in (14,15)
National registries indicate an incidence of classical DBA of 4-7 per million live births, with neither gender nor ethnic bias (16-20). In 20% of cases there is a clear family history, most commonly with an autosomal dominant pattern of inheritance. However, it is now accepted that the phenotypic spectrum of DBA encompasses a wider range of severity than originally described, and that an isolated increase in eADA may be the sole manifestation of DBA (21,22). The true prevalence of DBA is thus likely to be higher than predicted from registry data. Similarly, a higher proportion of cases are now believed to be familial; haematological abnormalities were identified in 31% of otherwise phenotypically normal first-degree relatives of children in the UK with apparently sporadic DBA (22). The existence of clinically silent DBA complicates genetic counseling, and represents a particular hazard in donor selection for sibling HSCT (23), especially in association with pre-implantation HLA-typing.

A definitive diagnosis of DBA may be confirmed on genetic testing if a mutation can be identified, currently possible in around 50% of cases. 25% of probands have a mutation affecting RPS19 (19) the first DBA gene to be identified (24). Mutations affecting a further four genes encoding ribosomal subunit proteins have subsequently been reported: RPS17 (25), RPS24 (26), RPL5, RPL11 (27) and RPL35a (25,28). In all cases to date mutations have been heterozygous, affecting a single allele, consistent with an autosomal dominant pattern of inheritance.

The median survival in a longitudinal study of patients treated in Boston Children's Hospital over a 60-year period was 38 years (29), although with a significantly worse prognosis for patients presenting before the introduction of steroid therapy. A high proportion of deaths could be attributed to the consequences of transfusion-transmitted hepatitis or to iron overload (29), results echoed in French (30) and North American registry data (20). Neutropenia and thrombocytopenia often develop after the first decade, and patients with DBA are at risk of progression to severe global bone marrow failure (aplastic anaemia). Acute myeloid leukaemia developed in 4 of 76 (5%) patients in the Boston study (29), with further cases reported in the literature (reviewed in (14,15). Possible reporting bias and incomplete registry data currently preclude an accurate assessment of the risk of malignancy, but a low median age for the development of cancer, and high proportion of cases of sarcoma (31) are consistent with the reported cases reflecting a true increased risk of cancer, in common with other inherited syndromes of bone marrow failure.
4. Fanconi Anaemia

Fanconi Anemia (FA) is a rare genetic disease characterized by congenital abnormalities, progressive bone marrow failure and cancer predisposition. It was originally described in the late twenties by the Swiss pediatrician Guido Fanconi. He reported 3 siblings of the same family with anemia, malformations, recurrent infections and bleedings, resulting in premature death (29). Over thirty years later, the German geneticists Schroeder described spontaneous chromosome fragility and the recessive autosomal inheritance of this syndrome (30). In 1969, Schuler and co-workers provided the first diagnostic test for FA based on chromosome fragility (31), which was later improved and extended by Auerbach and colleagues (32). In 1992 the first FA gene, FANCC, was cloned by the Canadian geneticists Buchwald and his team (33). FANCC was followed by 11 additional genes, the two latest ones being reported in 2007. The diagnostic tests for FA relies on the fact that patient’s cells are hypersensitive to the chromosome breaking activity of DNA interstrand cross linking agents like mytomicin C, diepoxybutane, photoactivated psoralens or cisplatin. The chromosome fragility test must be done in highly experienced laboratories and is usually performed on peripheral blood, but it can also be done on fibroblasts or amniocytes, if required. Interestingly enough, these same agents serve as important drugs in cancer chemotherapy, placing FA research in the center of molecular oncology.

FA is a very rare disease with an estimated frequency of 1-5 cases per million newborns (34). The number of patients in western European countries ranges from hundred to few hundreds in Spain, Germany or France, up to over 1000 patients reported in the North American register. The estimated frequency of otherwise unaffected FA mutation carriers in the general population is close to 1/300. The incidence of FA is, however, abnormally high in some consanguineous ethnic groups such as the Spanish gypsies, where nearly all FA patients share the 295C>T mutation in the FANCA gene, in part explaining the overrepresentation of FA-A patients (>80%) in Spain (35) Another example are the Ashkenazi Jews, where all FA patients are homozygous for the IVS4+4 A>T mutation in FANCC gene (36) or the white Afrikaner of South Africa, all sharing a large deletion in FANCA (37).

Since 3 out 12 FA genes (FANCD1/BRCA2, FANCI/BRIP1 and FANCN/PALB2) are intimately related to hereditary breast cancer proteins BRCA1 and BRCA2, the biochemical route defective in FA patients is currently known as the FA/BRCA
pathway. The exact role of this pathway is not well understood but current models suggest that FA gene products are essential for repairing DNA lesions that stall DNA replication forks during DNA synthesis \((38,39)\). Improperly processed stalled replication forks lead to DNA breaks that, when left unrepaired or misreported, are the cause of the above described chromosome fragility of FA cells. The 12 genes and their corresponding complementation groups are known as FANCA, -B, -C,-D1,-D2,-E,-F,-G,-I,-J,-L, and -N, being the FANCA gene the most frequently mutated in the majority of populations studied, accounting for almost 60% of all FA patients in USA, whereas FA-C, FA-G represent 10-15% and FA-D1, FA-D2 represent 5% for each one. The other subtypes are extremely rare \((40)\).

Resembling the genetic basis, the clinical symptoms and disease evolution of FA patients are very heterogeneous and include haematological disorders, congenital defects, endocrine pathologies and cancer predisposition. Almost all FA patients suffer progressive bone marrow failure (BMF) with severe trombocytopenia or pancytopenia in the majority of cases. Although the time of onset of haematological disease is highly variable, the majority of patients experience hematopoietic defects during the first decade of life. Further haematological complication of FA patients are myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) with accumulative incidence of 33% by the age of 40 \((41,42)\). The age of onset and progressive evolution of the haematological disease varies between complementation groups, being FAD1 and FAD2 patients more severe than FAA patients \((43,44)\). Some patients undergo a spontaneous recovery of blood counts due to mosaicism. Mosaicism is a very interesting phenomenon that affects from 15 to 25% of all FA patients. It appears when a single hematopoietic stem cell reverts the mutation present in one of the two alleles of the affected FA gene, thus reverting the FA cellular phenotype to a “normal” wild-type. Due to proliferation advantage of this reverted cell, it clonally expands and colonizes the bone marrow of the patient, resulting in clinical improvement in many mosaic patients. Mosaicisms can be detected by the chromosome fragility test when performed by well trained cytogeneticists.

Androgen treatment can delay the decline of blood counts, but the only cure of BMF is hematopoietic stem cell transplantation preferably with a related histocompatible donor as the outcome of unrelated transplants is still poor. However, the majority of patients do not have suitable donors and their only hope is a future implementation of novel therapies including gene therapy \((45)\) and regenerative medicine based on
disease-free hematopoietic progenitors derived from induced pluripotent stem cells, a therapeutic strategy first reported in FA by a consortium of Spanish teams (46).

Two out of 3 FA patients have congenital defects including skin hyperpigmentation with “café au lait” spots (55%), short stature (51%), upper limbs abnormalities, such as radius hypoplasia or abnormal thumbs (43%), abnormal gonads in males (32%), microcephaly (26%), microphthalmia (23%) and urinary tract malformations (21%). However, these defects are common in other genetic diseases and 1 out 3 FA patients do not present any congenital abnormalities at all, suggesting that congenital defects should be considered along other indicators when diagnosing FA (47,48). In addition, the extent of the malformative syndrome also varies among complementation groups. FANCD1 patients present many birth defects and almost 90% of FANCD2 patients are microcephalic and the VACTERL phenotype (vertebral defects, anal atresia, cardiac malformations, tracheoesophageal fistula with esophageal atresia, renal and radial dysplasia and limb malformations) is overrepresented in FA-D1, FA-E and FA-F groups (49).

Eighty percent of FA patients present some endocrinopathology. The most commonly observed are growth hormone deficiency (53%) leading to short stature, hypothyroidism (37%), abnormal glucose/insulin metabolism (39%), obesity (27%) and dyslipidemia (55%). 92% of adult FA patients also present osteopenia or osteoporosis and 65% of them have gonadal dysfunction (50,51).

Apart from above referred AMLs, FA patients have also an extremely high risk of developing solid tumors with an accumulative frequency of 35% by the age of 40. The most frequent cancers are squamous cell carcinomas (SCC) of head, neck, esophagus, and ano-genital region (42% of all solid tumors) and liver cancer (29% of all solid tumors), often as a side effect of androgen treatment (42,52). The incidence of SCC in FA patients is even increased in transplanted versus non transplanted patients, probably as a side-effect of graft-versus-host disease often seen after transplant (53). Thus bone marrow transplant advances the age of onset of SCC up to 10 years in FA patients (54). A recent study demonstrated that only 5% of FA SCCs are positive for human papilloma virus (HPV), at least in European cohorts, suggesting that, unfortunately, the newly developed vaccine against HPV will not prevent the majority of head and neck SCC in FA patients (54). The spectrum and age of onset of cancer is also influenced by the complementation group. An example are FA-D1 patients that present AMLs and solid tumors (medulloblastoma, Wilms tumor) during
5. Congenital Dyserythropoietic Anemias (CDA)

Definition and classification: The congenital dyserythropoietic anemias (CDAs, ICD-10 D64.4) comprise a group of rare hereditary disorders that are characterized by ineffective erythropoiesis as the predominant mechanism of anemia and by distinct morphological abnormalities of erythroblasts in the bone marrow. The term was first used by Crookston et al (56) for cases later classified as CDA II and by Wendt and Heimpel (57) for cases later classified as CDA I. The working classification initially proposed by Heimpel and Wendt is still used in clinical practice. There are, however, families that fulfill the general definition of the CDAs, but do not conform to any of the three classical types (58) (Table 2). In general, the diagnosis of the CDAs requires the presence of all of the four following criteria:

1. Evidence of congenital anemia/jaundice or a positive family history
2. Evidence of ineffective erythropoiesis
3. Typical morphological appearance of bone marrow erythroblasts, and
4. Exclusion of congenital anemias that fulfill criteria 1 and 2, but were classified according to the underlying defect, such as the thalassemia syndromes, certain types of hemoglobinopathies or hereditary sideroblastic anemias.

All types of CDA share a high incidence of splenomegaly, cholelithiasis and iron overload. As in other forms of anemia with ineffective erythropoiesis, this is due to upregulation of iron resorption, mediated by hepcidin. Extramedullary hematopoiesis presenting as paravertebral masses may be observed. Estimates on prevalence, either expressed as lifetime or affected birth prevalence are not available. Cumulative incidence for the time interval 1967 – 2009 of CDA I and II in Europe, including the member States of the European Union, Norway and Switzerland are to be published (Heimpel et al. submitted). The CDAs are not included in mortality or prevalence registries administrated by governments or NGOs such as scientific societies.

In most cases, CDA has no major impact on life expectancy, although being a serious problem for quality of life and social competence. Diagnosis depends on awareness of the medical community and access of the population to hematological diagnosis including bone marrow biopsy and advanced biochemical and/or molecular procedures. Therefore, all estimates on incidence of patients or frequency of
mutations are minimal values and depend on the health system of the population studied. The distribution of the age when the correct diagnosis was made suggests that even today many cases had longtime an erroneous diagnosis or, in cases with moderate of only borderline anemia, remained undetected. In addition, one has to assume that not all cases were the correct diagnosis was made were notified to one of the registries, or were published as case reports. Male/female ratios do not deviate significantly from one. Not only underreporting, but multiple publications of identical cases and multiple notifications in more than one registry or survey are serious methodological problems in as rare disorders as CDA. The reports from the registries mentioned above, and all papers containing patient's data from the same institution or with identity of at least one author were therefore cross referenced.

Source of data: 1. Publications. All publications describing cases of CDA were systematically collected since the first description of CDA in 1967. Completeness was checked by review of online databases (National Library of Medicine, www.ncbi.nlm.nih.gov/pubmed) for key words "congenital dyserythropoietic anemia" or "CDA" last on 31. 07. 2009. All reports were analyzed for citations of previous case reports. In addition, early reports were retrieved from three monographs (59-61). To identify the individuals reported in publications, the authors were asked for additional identifying data (see below) by correspondence, 2. Registries and surveys. The German registry on CDAs collects all cases of CDA, and the International registry in Italy all cases of CDA II. These registries initially tried to recruit all cases from the German speaking countries (Germany, Austria, Switzerland) and Italy by repeated queries in the National hematological societies, respectively. When the competence of these centers became known by publications, they received queries for diagnostic confirmation of suspected cases or were asked for advice for management of patients with CDA from many countries of the world. The same is true for the Department of Pediatrics and the French Center for Inherited Erythrocyte and Erythropoiesis Disorders at Hopital Bicetre (France) (J.D; BB), the Laboratory of the late S. Wickramasinghe, at Imperial College, London (UK), the Hematology Center at the Fundeni Hospital in Bucharest collecting cases from Romania (A.C.), the Oxford CDA Research Initiative in Oxford (UK) and the IRCCS Ospedale Maggiore Policlinico, Divisione di Ematologia 2, Milano (Italy) and 3. Demographic data were obtained from the United Nations Demographic Yearbook (UNDYB) 2008, and Consanguinity data Bittles (62) and www.consang.net
**Cases reported worldwide:** 712 cases from 614 families were included in the identified German CDA Registry from all sources mentioned above. CDA type, sex, date of birth (DOB), date of first diagnosis of CDA and country of residence were first order attributes. Any individual was pseudonymised using a code (three digits for family/ two digits for family member), which does not allow identification of personal data. Not all cases from the Bedouin tribe and of the large Västerbotten family are identified (see below).

**Congenital dyserythropoietic anemia type I (CDA I, OMIM 224120):** This was the first disorder described under the term CDA (63). Definition is based on the general criteria shown above, and confirmed by the characteristic morphological aberrations seen by light of electron microscopy. The mutated CDAN1 gene was mapped to the long arm of chromosome 15 between 15q15.1q15.3 in four Bedouin families with a high degree of consanguinity (64). From studies in unrelated patients of European, Bedouin, North-American and Asian origin altogether 36 different point mutations, distributed over 13 exons were detected (65-69). In less than 10 % of cases, only one or no mutations were detected, suggesting mutations of other genes (70). Most families have been detected among Western Europeans, Arabs and other Mediterranean populations, but single cases have also been reported from the USA, India, Japan, Australia, New Zealand, Polynesia and China. Two cases of the latter countries had mutations previously found in European patients.

At present, 174 cases from 145 families are recorded. In addition, more than 70 cases from the original Bedouin tribes all being homozygote are known, not collated in the identified registry are known. The cases recorded in Europe are shown in Figure 1. Total frequency is 0.24 per Million, with large variations between 0.01 and 0.6 per Million in different regions. No significant differences according to ethnic origin are observed.

**Congenital dyserythropoietic anemia type II (CDA II, OMIM 224120):** CDA II was first described under the term HEMPAS (Hereditary Multinuclearity with Positive Acidified Serum Lysis Test (56) and independently as CDA II (63). Definition is based on the general criteria shown above, and confirmed by the characteristic morphological aberrations seen as well as by abnormalities of the red cell membrane (71-73). The mutated CDAN1 gene was mapped to the long arm of chromosome 15 between 20p11.23-20p12.1 and identified as SEC23B (74-75). All genotyped cases were homozygote or compound heterozygotes. Most families have been detected
among Western Europeans, Mediterranean populations, but single cases have also been reported from the USA, Canada, India, Japan, Australia, New Zealand and South Africa. At present, 454 cases from 356 families are recorded. The cases recorded in Europe are shown in Figure 2. Frequency is 0.71 per Million, with large variations between 0.1 and 2.5 per Million in different regions. A particular high prevalence is found in Southern Italy. No significant differences according to ethnic origin are observed. A non-significant trend of increased prevalence in some non-indigenous ethnic groups may be explained by their higher consanguinity rate.

**Congenital dyserythropoietic anemia type III (CDA III, OMIM 105600):** CDA III was first described in 1962 under the name of Hereditary Benign Erythroreticulosis (76) or “Västerbotten anomaly” in members of a large family living in Northern Sweden, and designated as type III after Types I and II were classified (77). At present, the fifth generation of this family is being investigated, and most data on CDA III have been described by the investigators from Umea, Sweden (78). There are two more families with similar hematopoietic changes and dominant inheritance living in North and South America, but only a few details are known, and it is not clear whether they share the same genetic basis. In addition, 25 cases from 23 families are known with cases in only one generation, suggesting an autosomal recessive mode of inheritance. No genetic data are reported, and some of these cases may be misclassified.

**Congenital dyserythropoietic anemia type variant (CDA-variants):** These patients fulfill the general definition of CDA, but represent an extremely heterogeneous group. Failure to attribute some of these cases to one of the three types may result from incomplete diagnostic workup. The mode of inheritance is generally autosomal recessive, but nothing is known about the genes possibly involved. There are 98 cases from 81 families known, the vast majority from Europe. Robust estimates of prevalence are not possible.

### 6. Paroxysmal Nocturnal Haemoglobinuria

Paroxysmal nocturnal haemoglobinuria (PNH), first described as a distinct clinical entity in 1882 (79), is characterised by intravascular haemolysis, venous thrombosis and is associated with aplastic anaemia (80). The characteristic symptoms of PNH, abdominal pain, dysphagia, erectile failure and intense lethargy, can be attributed to the intense intravascular haemolysis and the release of free plasma haemoglobin from its intra-cellular compartment (81). PNH arises through a somatic mutation of the phosphatidylinositol glycan complementation class A (PIG-A) gene in a
haematopoietic stem cell followed by a tremendous expansion of this abnormal clone (82). The functions of the GPI-linked proteins are extremely varied. At least two are important in the control of complement. Decay accelerating factor (DAF or CD55) controls the early part of the complement cascade by regulating the activity of the C3 and C5 convertases. CD59 inhibits terminal complement by preventing the incorporation of C9 onto C5b-8 and therefore preventing the formation of the membrane attack complex (MAC). As a result of complement-mediated attack, the survival of PNH erythrocytes in vivo is shortened to about 10% that of normal red cells (83).

The brisk intravascular haemolysis commonly leads to haemoglobinuria, dysphagia, recurrent abdominal pain, severe lethargy and erectile failure. PNH is a chronic condition, frequently affecting young individuals, that may persist for many years and which often presents clinicians with difficult management problems. The symptoms associated with ongoing haemolysis and/or insufficient haematopoiesis have a major impact on the patient’s well-being. Patients usually have acute exacerbations of haemolysis on the background of persistent lower levels of haemolysis. The acute exacerbations can occur either regularly or unpredictably, and have a further adverse impact on quality of life. Anaemia and the need for transfusions to sustain haemoglobin levels occur frequently. Haemolysis in patients with PNH can be monitored by levels of the enzyme lactate dehydrogenase (LDH) and levels are frequently elevated, exceeding 20 times the upper limit of normal during severe paroxysms (84-87). The most feared complication of PNH is venous thrombosis which occurs in ~50% of patients with haemolytic disease and is the cause of death in at least one-third (80,88). PNH is known to be a rare disorder, but its incidence and prevalence have so far been poorly defined (89,90) with very few studies. It therefore remains of unknown frequency worldwide with little information on the incidence. Figures of incidence quoted by PNH information websites range between 1 per 100,000 to 5 per million population (91-93). Increased prevalence is reported in some regions, e.g. Thailand and other countries in the Far East (90,94,95), possibly due to a higher incidence of aplastic anaemia (96).

In a study performed to accurately report the incidence and prevalence of PNH in a given population in a well-defined geographical area, survival data were collected on all patients diagnosed with PNH between January 1991 and July 2006 (97). All patients were diagnosed by flow cytometry in one laboratory. This study did not
include routine screening of normal individuals or patients with myelodysplastic syndrome but only the routine diagnosis of all samples referred for exclusion of PNH. The population of the study region was 3,742,835. Seventy-six PNH patients were diagnosed giving an incidence of 0.13/100,000/year. Based on incidence and survival rates, the estimated 15-year prevalence of PNH is 1.59 per 100,000 resulting in a predicted prevalence of 59 patients in the study region. Levels of LDH were elevated in 82.5% of patients. Of the 59 patients in the study region, 33% reported haemoglobinuria. With a population of 57,105,375 (2001 census of Britain), Britain should have an estimated 75 new cases of PNH/year and a predicted prevalence of 908 patients. The U.S.A. will therefore have 4713 cases of PNH based on its July 1, 2005 census bureau population estimate of 296,410,404.

The US definition of a rare disease is one that affects less than 1 in 200,000 individuals; the corresponding number in Japan is 1 in 50,000 and in Australia 1 in 2000. These numbers translate to prevalences of 1-8 in 10,000. The European Community definition is less than 5 in 10,000, and the World Health Organisation has suggested less than 6.5-10 in 10,000 (98). PNH would certainly remain classified as rare regardless of whose definition was used.

References


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91. Orphanet. Paroxysmal Nocturnal Hemoglobinuria. 1-12-2004. Ref Type: Internet Communication

92. HMDS. HMDS. 1-1-2001. Ref Type: Internet Communication

93. Rare Thrombotic Diseases Consortium. Paroxysmal Nocturnal Hemoglobinuria. 1-1-2000. Ref Type: Internet Communication


98. Aronson JK. Rare diseases, orphan drugs, and orphan diseases. Br.Med.J. 333, 127. 15-
Table 1. Neonatal screening program financed by national authorities in Europe

<table>
<thead>
<tr>
<th>Country</th>
<th>Systematic (S) Targeted (T)</th>
<th>Number tested</th>
<th>Period tested</th>
<th>SCD (‰)</th>
<th>β-thal major (‰)</th>
<th>HbAX (‰)</th>
<th>Ref</th>
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<tr>
<td>Belgium*</td>
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<td>191,783</td>
<td>1994 - 2007</td>
<td>0.64</td>
<td>0.025</td>
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<tr>
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<td>2005 - 2007</td>
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<tr>
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<td>1996 - 2007</td>
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<td>24.5</td>
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<tr>
<td>The Netherlands</td>
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<td>2007</td>
<td>0.30</td>
<td>0.044</td>
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<tr>
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<td>2003 - 2005</td>
<td>0.16</td>
<td>NA</td>
<td>5.6</td>
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* Brussels and Liège
** Madrid
*** SCD and homozygous for Hb E
Table 2. Characteristic features of different types of congenital dyserythropoietic Anaemias (CDA)

<table>
<thead>
<tr>
<th>CDA type</th>
<th>CDA I</th>
<th>CDA II</th>
<th>CDA III familial</th>
<th>CDA III sporadic</th>
<th>CDA Variants</th>
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<td>dominant</td>
<td>Variable</td>
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<td>Cases reported</td>
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<td>~450</td>
<td>3 families</td>
<td>~ 20</td>
<td>~70</td>
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<tr>
<td>Morphology</td>
<td>Abnormal chromatin structure, chromatin bridges</td>
<td>Multinuclearity of mature erythroblasts</td>
<td>Giant multinucleated erythroblasts</td>
<td>Giant multinucleated erythroblasts</td>
<td>CDA I-like, CDA II-like, Others</td>
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<td>Sec23B 20p11.23</td>
<td>unknown 15q (21-25)</td>
<td>unknown</td>
<td>unknown</td>
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<tr>
<td>Associated dysmorphology</td>
<td>Skeleton, others</td>
<td>Variable, rare</td>
<td>B-Cells Retina</td>
<td>variable</td>
<td>CNS Others</td>
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