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Fabry or not Fabry: From genetics to diagnosis

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CHAPTER 1

General introduction

Genetic basis of disease

After Friedrich Miescher first isolated Deoxyribonucleic acid (DNA) in 1869 ^{1,2}, several decades passed before the double helix structure of DNA was discovered by Francis H. Crick and James D. Watson in 1953 ³. But it was not until 1966 that the genetic code, 4 nucleotides coding for 20 different aminoacids, was unraveled, and in 2003, the full human genome sequence was uncovered by the Human Genome Project ⁴. In the meantime, understanding of genetic inheritance had developed substantially, and many inherited disorders had been described. The metabolic disorder alkaptonuria was one of the first diseases ascribed to a genetic cause. For a detailed history see ^{5,6}.

Especially in the last decades, tremendous developments have been achieved, and numerous genes and mutations related to inherited disorders were discovered. Furthermore, the recent development of high throughput DNA sequencing technologies (Next Generation Sequencing, NGS), have made it possible to sequence the full human genome in a short amount of time. Costs of NGS have diminished significantly in the past 15 years: less than \$2000 for coding regions (whole exome sequencing (WES)) and less than \$5000 for the complete human genome (whole genome sequencing (WGS)) ^{7,8}, **figure 1**.

Simultaneously, it became evident that sequence variants in the genome cannot fully explain different (disease) expressions. Epigenetic factors have an important influence through complex regulatory mechanisms ⁹. Although several factors are identified (such as DNA methylation and genomic imprinting), this field of study is relatively new, and the exact mechanisms by which regulation occurs are largely unknown.

Nowadays, genetic testing has shifted from research to daily clinical practice, and the genetic cause for previously unexplained clinical disease is more frequently discovered through the use of gene arrays to test multiple genes at once, or by WES and WGS ^{10,11}.

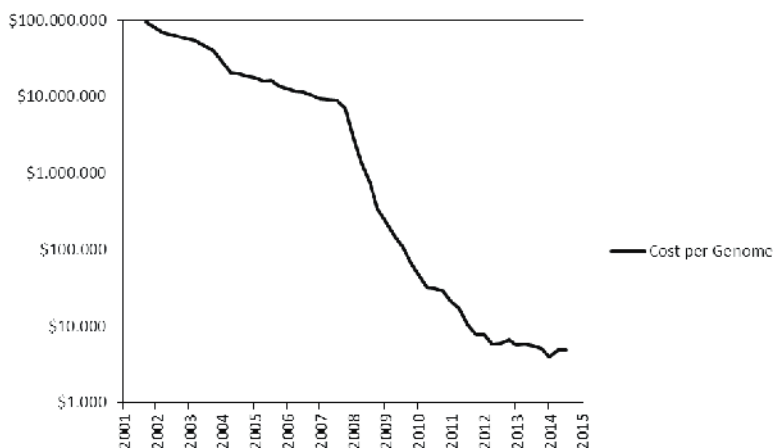


Figure 1. Sequencing costs per genome (adopted from ⁷).

The identification of disease related genes and mutations, and the development of faster and less expensive methods to test for genetic disorders have led to a better understanding of the basis of genetic disease, improved diagnoses and new opportunities for the management of genetic disorders.

The interpretation of genetic data is often challenging. The application of population screening (studies) for genetic diseases, as well as individual case finding has resulted in the identification of an overwhelming amount of single nucleotide variants ⁸. A genetic variant may be clearly related to a disease and may therefore explain the phenotype of a given patient, but accidental findings of other diseases or risk factors occur on a regular basis ¹¹. The prediction of the pathogenicity of a certain variant is not always straight forward, and genetic variants may be disease causing, non-pathogenic (neutral variant), or may only be seen as a risk factor for development of disease. Furthermore, epigenetics and environmental factors may influence pathogenicity on an individual basis.

Several strategies are available to investigate the pathogenicity of genetic variants, including segregation within families, functional assays of the gene product, cellular expression models and prediction models. Furthermore, a relatively high allelic frequency in a control population, may indicate that a certain variant is not pathogenic. Despite these options, the pathogenicity of a variant and its relation to the clinical phenotype in an individual patient may often remain unclear. Such variants are referred to in this thesis as genetic variants of unknown significance (GVUS).

Different opinions exist on the use of genetic testing. Some advocate that WES should be available to all, disregarding the suggestion of a genetic disease in the individual or the family history, because of the proposed benefits of risk estimation and early diagnosis. To this end, several commercial companies even exist that offer genetic testing. On the other hand, there is a strong resistance to this approach, as others recognize the many difficulties that arise with genetic testing. The detection of genetic variants is no longer difficult, but their interpretation, in contrast, is a major challenge ⁸. Moreover, when genetic testing is applied, it is essential to always provide a setting of pre- and post-test counseling and to invest in the clinical evaluation of the individual with a suspected genetic disease.

The BRCA1/BRCA2 genes (hereditary ovarian and breast cancer) serve as an example. A significant number of GVUS are identified in patients who are tested for variants in these genes. Testing has become widespread, and the advised setting of pre- and post-test counseling is not always applied ¹². This has led to difficulties in identifying the correct approach to patients and family members at risk when a GVUS is found. On the other hand, variants in these genes are often identified as accidental finding with WES or WGS ¹¹, revealing a potential risk factor with immense consequences for the patients and their families. For the BRCA genes, an international collaboration has been established to facilitate the pooling of resources to address the issue of GVUS ¹³.

Similar issues arise for many inherited disorders, including lysosomal storage disorders. Improved understanding of the phenotypical spectra and increased genetic testing, has clearly

demonstrated that a variant in the related gene, will not lead to evident disease in all cases. Especially in Fabry disease, GVUS are frequently identified, posing a real problem with respect to counseling, family screening and treatment options. A standardized and applicable approach is warranted to address these difficulties.

Fabry disease

Background

Fabry disease (OMIM 301500, FD) was first described independently by William Anderson in the United Kingdom and Johannes Fabry in Germany^{14,15}. While initially described as a dermatological disorder, both Anderson and Fabry also noticed the presence of other abnormalities, such as proteinuria and lymphedema. Follow-up of the originally described patients and an autopsy report of a Fabry disease patient 50 years later, confirmed the hypothesis that the disease is not limited to the skin, but is in fact a multi-organ disease, as we also view the disease today¹⁶.

FD is a lysosomal storage disorder, a group comprising of more than 50 inherited metabolic disorders, characterized by the impaired degradation or processing of certain macromolecules with subsequent accumulation in the lysosome. The lysosomal storage disorders are named after the lysosome, the cellular organelle responsible for the degradation of large molecules. The lysosome was discovered in 1955 by the Belgian scientist Christian de Duve, for which he received the Nobel prize in physiology or medicine¹⁷. The individual lysosomal storage disorders are rare, but combined, they have an estimated prevalence of approximately 1 in 7000 live births^{18,19}.

In FD, a deficiency of the lysosomal hydrolase α -Galactosidase A (α GalA) leads to impaired degradation and lysosomal accumulation of its substrates, mainly the glycosphingolipid globotriaosylceramide (Gb3). Degradation of other glycosphingolipids with a terminal α -1,4-galactose is also impaired, but its contribution to disease development is not well known. The α -Galactosidase A (GLA) gene, encoding for α GalA, is located on the long arm of the X-chromosome.

Pathophysiology

Accumulation of Gb3 is still regarded as the primary cause of the clinical phenotype of FD. However, the exact mechanisms that are involved in the development of the disease are not completely understood²⁰. Accumulation of Gb3 can be found in several cell types including vascular cells, the endothelium, cardiomyocytes, podocytes and neurons in dorsal root ganglia and the central nervous system. Besides Gb3 itself, globotriaosylsphingosine (lysoGb3), a deacylated form of Gb3, has been identified as a hallmark of FD by Hans Aerts and coworkers²¹. LysoGb3 induces smooth muscle cell proliferation in vitro, and it has been suggested that lysoGb3 is involved in the pathophysiology of FD²¹. The mechanisms by which lysoGb3 is formed and how it causes its possible pathological effect is yet unknown^{21,22}. Plasma lysoGb3 has also been demonstrated to reliably identify FD patients with a classical FD phenotype, while Gb3 is sensitive for males only²³. As a consequence, lysoGb3 is a promising diagnostic marker. Furthermore, Gb3 and lysoGb3 are often used in the follow-up of patients, especially to assess the treatment efficacy. While a reduction of Gb3 and lysoGb3 is achieved with enzyme replacement therapy (ERT)^{24,25}, the value of these markers in this context is not evident²⁶.

Phenotype

In hemizygous males, FD may already present during childhood or adolescence with features that characterize what is now referred to as classical FD: neuropathic pain, angiokeratoma, cornea verticillata and hypo- or anhidrosis. During childhood, gastrointestinal symptoms and (micro)albuminuria may also occur²⁷. At a later age, progressive chronic kidney disease (CKD), left ventricular hypertrophy (LVH) and cerebrovascular disease will often develop, ultimately leading to the necessity of renal replacement therapy, heart failure, stroke or cognitive decline²⁷. Biochemically, males are characterized by absent or near absent α GalA enzyme activity in leukocytes, and a marked increase of Gb3 and lysoGb3 in plasma and urine. Accumulation of Gb3, demonstrated by characteristic lamellated lysosomal inclusions, can be found in all tissues, including heart, kidney and skin.

Besides this classical phenotype, an increasing number of individuals with an attenuated phenotype are identified. These individuals present later in life, often with isolated LVH, CKD or stroke. The characteristic clinical and biochemical features are mostly absent in these cases, and residual leukocyte α GalA enzyme activity is generally present in males²⁸.

Due to the X-linked nature of FD, females have long been considered to be carriers only. However, females can develop considerable FD related signs and symptoms, although the phenotype is generally less severe^{29,30}. Moreover, they show more variable disease, most likely due to differences in X-chromosome lyonization³¹⁻³⁴.

Treatment

Management of FD patients comprises of the combination of supportive care and ERT. As FD affects the vascular system, other cardiovascular risk factors may also influence the risk of complications. It has become apparent that supportive treatments, such as platelet aggregation inhibitors, should be considered. It has been shown that renal function decline is more rapid with higher levels of proteinuria²⁶. Angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) reduce proteinuria, and are likely to reduce kidney function decline as a result^{26,35}. Carbamazepine, gabapentin and amitriptyline are the most frequently used medications for the management of neuropathic pain. Unfortunately, evidence for the efficacy of the suggested supportive care is scarce. However, supportive care has improved substantially over the years, and it is most likely that optimal supportive care has a substantial benefit for the patients.

Two enzyme preparations are available (agalsidase alfa, Shire HGT, and agalsidase beta, Genzyme, a Sanofi company). Both products are administered intravenously every other week, agalsidase alfa at a dose of 0.2 mg/kg, and agalsidase beta at 1.0 mg/kg. The initial clinical trials showed beneficial effects on neuropathic pain, cardiac mass and kidney function, and biopsies showed a clear reduction of Gb3 in tissues^{36,37}. However, it has been shown that despite ERT, disease complications may still occur²⁶. Furthermore, the development of antibodies against the infused enzyme may diminish the effect of ERT in males^{25,38}. Although speculative, it is questionable if ERT has a substantial added value to optimal supportive care, especially for patients with antibodies and patients with a non-classical phenotype.

Pathogenicity of GLA variants

While the prevalence of FD has long been estimated to be around 1 in 40.000 live male births²⁷, recent findings have suggested that this is an underestimation.

Especially in the last decade, the awareness of FD has increased substantially, most likely related to the approval of the enzyme preparations by the European Medicines Agency (Europe) and Food and Drug Administration (USA, agalsidase beta only) in 2001. FD may present with signs or symptoms that are relatively common in the general population (such as LVH, CKD and stroke). Subsequently, FD is increasingly considered as part of the differential diagnosis for these signs or symptoms, especially when they cannot be explained by more prevalent causes. Furthermore, improved technological possibilities to test for rare genetic disorders have resulted in the increased identification of patients with a GLA variant. Testing for FD among patients with a hypertrophic cardiomyopathy (HCM) serves as an example. Several genes are associated with HCM and often a cardiochip (a customized gene-specific DNA-based microarray for a number of genes related to HCM, including the GLA gene) is applied in order to identify the genetic cause for the disease. In The Netherlands, a cardiochip is used to test over 40 genes related to HCM at once. This has already led to the identification of more than 10 families with a GLA variant in the past years.

This increased awareness of FD is also illustrated by the number of screening studies performed in so called 'high-risk' groups, studying the prevalence of FD in cohorts with for example LVH, CKD or stroke. The studies demonstrated a much higher prevalence of patients with a GLA variant than expected based on previous calculations³⁹. Furthermore, screening studies among newborns revealed a prevalence of GLA variants up to 1 in 2500⁴⁰⁻⁴². While most population screening initiatives are yet performed within the framework of a scientific study, FD is already implemented in the newborn screening program in the state of Missouri in the USA⁴³, and is considered for implementation elsewhere.

The increased identification of individuals with a GLA variant has revealed many new variants, and to date, more than 600 variants in the GLA gene are described⁴⁴. Interestingly, patients who are identified to have a GLA variant do not always have the characteristic clinical and biochemical features as described above. They may have an attenuated phenotype of FD, often referred to as atypical, late onset, or non-classical FD in the literature. However, it is important to realize that the presence of a GLA variant does not necessarily mean that this patient has FD, and that other risk factors or diseases may cause the clinical phenotype of that patient in the presence of a neutral GLA variant. It is of utmost importance to distinguish the true FD patient from those with a non-pathogenic GLA variant. The true FD patient needs regular assessments of kidney, heart and brain. Supportive care and ERT should be considered in some cases. On the other hand, a wrongful diagnosis of FD in patient with a non-pathogenic GLA variant may lead unnecessary treatment with ERT. And equally important: it may cause considerable but unnecessary distress for patients and their families. This thesis focuses on the approach of patients who have a GLA variant and in whom there is uncertainty about the FD diagnosis.

Aims of this thesis

'The Hamlet study: Fabry or not Fabry' was initiated to evaluate clinical and laboratory assessments in order to improve the diagnosis of FD ⁴⁵.

With a systematic review of the literature, we studied the clinical phenotype of individuals with a GLA variant who were identified by screening, in order to assess the prevalence of patients with a classical FD phenotype and of patients with a GVUS in the GLA gene (**chapter 2**).

Diagnostic algorithms were subsequently developed through modified Delphi consensus procedures in collaboration with international experts. First, criteria for a definite diagnosis of FD, to identify those in whom there is no doubt about the diagnosis, were defined (**chapter 3**). Potential diagnostic criteria were identified in the literature for patients with an uncertain diagnosis of FD, in the presence of a GLA gene, who presented with LVH (**chapter 3**), CKD (**chapter 4**), transient ischemic attacks, stroke of white matter lesions (**chapter 5**) or with neuropathic pain, angiokeratoma or cornea verticillata (**chapter 6**). The potential diagnostic criteria were assessed by the experts and additional analyses were performed to improve evidence. Finally, diagnostic algorithms were constructed based on the results of the Delphi consensus procedure.

Assessment of cornea verticillata (**chapter 7**), small nerve fibers (**chapter 8**) and lysoGb3 (**chapter 9**) were studied separately as possible tools to aid in the diagnosis of individuals with an uncertain diagnosis of FD.

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