Cardiolipin metabolism in Barth syndrome
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Chapter 1

Introduction
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Cardiolipin metabolism in Barth syndrome

Barth syndrome (BTHS, MIM#302060) is a severe X-linked recessive disorder, first described in the early 1980’s [1]. The classical clinical hallmarks of BTHS include cardiac and skeletal myopathy, neutropenia and growth abnormalities. With the identification of more BTHS patients in recent years, however, it has become clear that the phenotype is highly variable, even within a single family, and patients have been identified with only one of the classical features of BTHS. In 1996, an Italian group discovered that BTHS is caused by mutations in the tafazzin gene (located on chromosome Xq28), which is named after a masochistic comic character from an Italian television sports show [2]. As a result of alternative splicing, the tafazzin gene is thought to encode multiple tafazzin proteins [2]. Based on sequence homology, it was hypothesized that tafazzin functions as an acyltransferase involved in the remodeling of phospholipid acyl chains [3]. In 2000, Peter Vreken and colleagues showed that cells from BTHS patients have abnormalities in the mitochondrial phospholipid cardiolipin (CL) [4]. In the BTHS cells, CL levels were lower and the incorporation of linoleic acid in CL, normally the primary CL acyl chain in humans, was severely reduced, suggesting that tafazzin is involved in remodeling of CL acyl chains [4]. The CL abnormalities were confirmed in later studies using model systems for BTHS [5,6] and the biochemical phenotype was broadened by the finding that cells from BTHS patients also have higher levels of the CL breakdown product (and remodeling intermediate) monolysoc-CL (MLCL; i.e. lacking one acyl chain) [7]. CL is synthesized at the mitochondrial inner membrane from its precursors phosphatidylglycerol and CDP-diacylglycerol by the enzyme CL synthase [8], followed by acyl chain remodeling, in part by tafazzin. In mitochondria, CL is involved in several processes, such as oxidative phosphorylation and mitochondrial apoptosome-mediated apoptosis [9]. Because of the abnormal CL profile in cells from BTHS patients, one would expect that these CL-associated processes are disturbed, and this was confirmed recently [10-12]. The pathogenesis of BTHS, however, is still not (fully) explained by these observations, as is the clinical heterogeneity.

In the work presented in this thesis, we aimed to elucidate the function(ality) of tafazzin proteins and to get more insight in the clinical variability, genotype-phenotype relation and the pathogenesis of BTHS, processes that are not only dependent on tafazzin and/or CL, but also on modifying factors. In chapter 2, a detailed overview is provided of the current state of knowledge on CL. Synthesis and remodeling of CL, its function and its role in various pathological conditions, notably BTHS, are discussed.

Since at the time no direct proof was available that tafazzin functions directly in the remodeling of CL acyl chains, and it can be hypothesized that tafazzin could function in the remodeling of the acyl chains of one of the CL precursors, we identified and characterized the human CL synthase (chapter 3). We showed that the CL synthase enzyme has no substrate preference for 18:2-containing precursors, which makes it more likely that tafazzin indeed functions at the level of CL remodeling. Chapter 4 describes the development of a new diagnostic method for the diagnosis of BTHS. We validated this HPLC-mass spectrometry analysis of CL and MLCL in various cell types and tissues providing a confirmatory method for the diagnosis of BTHS and a retrospective screen in which we identified a patient that was previously undiagnosed
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(chapter 4). In chapter 5, we analyzed the functionality of the different tafazzin splice variants by expression in a tafazzin-deficient yeast mutant. By complementation analysis, both at the level of growth phenotype and CL composition, we showed that only human tafazzin lacking exon5 is able to fully restore the tafazzin defect of the yeast mutant (chapter 5). We further investigated the functionality of the tafazzin variants, as well as its role in CL metabolism, by the analysis of tafazzin mRNA expression levels in various human tissues and in cells from BTHS patients (chapter 6). We also expressed the tafazzin variants in (human) BTHS cells in order to check whether the yeast studies could be confirmed in human cells and that tafazzin lacking exon5 is indeed the active tafazzin (chapter 6). Finally, we used the tafazzin-deficient yeast mutant to perform a proteomics analysis to investigate the changes that occur at the protein level due to the tafazzin deletion (chapter 7). Proteins that are affected by tafazzin deletion could serve as modifying factors involved in the clinical variability and the pathogenesis of BTHS.

References