Studies of the epigenetic disease mechanism in FSHD

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Summary

Facioscapulohumeral muscular dystrophy (FSHD) is a hereditary muscle disorder that is characterized by weakness and wasting of the muscles of the face (facio), the shoulder (scapulo) and the upper arms (humeral). Usually the first disease symptoms are observed before the age of 20, but these symptoms may vary significantly, in presentation and severity. The severity of the disease not only varies between patients from different families but also between patients from the same family.

Almost 20 years ago it was discovered that FSHD is caused by a loss of a piece of repetitive DNA that is located on the end of the long arm of chromosome 4 (4q35). While in control individuals this piece of repetitive DNA, called the D4Z4 repeat, is present as 11-100 copies on each chromosome 4 end, most FSHD patients carry only 1-10 copies of the repeat on one of their chromosome 4 ends (FSHD1). As a consequence of this discovery, for many years the presence of a gene within the D4Z4 repeat that might explain the occurrence of FSHD in individuals carrying a short repeat array was anticipated. However, until two years ago the identification of a functional gene within the D4Z4 repeat was unsuccessful. Therefore, research has focused on different disease models that might explain the causative mechanism behind FSHD. One of these disease models, which is not necessarily mutually exclusive with the presence of a gene within the repeat, hypothesizes the involvement of an epigenetic or chromatin component in the development of FSHD. Epigenetics concerns the occurrence of hereditary changes in gene function without changes in the sequence of the base pairs of the DNA in our cell nucleus. An example of an epigenetic modification is DNA methylation. Our DNA may contain additional methyl groups and these methyl groups are attached to the cytosine (C) bases in our DNA by enzymes called DNA methyltransferases. The presence of methyl groups on DNA has an influence on the on- or off status of genes.

More than 5 years ago it was observed that patients with FSHD carry less methyl groups on the DNA of the first D4Z4 repeat in comparison with control individuals. What made this observation even more interesting was that low DNA methylation was also observed in a small group of patients with a muscle disease clinically indistinguishable

from FSHD (FSHD2). Because these patients carry more than 10 D4Z4 repeats on each chromosome 4 end, previously the phenotype in these individuals could not be confirmed by genetic tests. Now it was found that a commonality in chromatin structure exists between both patient groups. However, low DNA methylation on the first D4Z4 repeat was also observed in patients suffering from the ICF syndrome. ICF is an abbreviation for Immunodeficiency, Centromeric instability and Facial anomalies syndrome. The most prominent complaints in these patients are repetitive infections as a consequence of an immunodeficiency, but these patients do not have any muscular complaints. Therefore, the exact role of DNA methylation in the disease mechanism of FSHD remained unknown.

FSHD research is complicated by several factors. First, on chromosome 10 a highly similar repeat as on the chromosome 4 end is present. This complicates the DNA diagnosis of FSHD. Also on chromosome 10 less than 11 repeats can be present. However, individuals carrying a short repeat on chromosome 10 do not develop FSHD. Second, several genetic variants of the chromosome 4 end have been identified. Thus far, nine different variants were recognized. Remarkably, in all patients with FSHD the disease chromosome is always of the 4qA161 genetic variant. Besides this variant, 4qB163 and 4qA166 are highly prevalent variants. Individuals with less than 11 D4Z4 repeats on a 4qB163 or a 4qA166 variant do not develop FSHD.

In this thesis several studies are described that focused on the further unraveling of the epigenetic disease mechanism responsible for the development of FSHD. In chapter 2 a study is presented that made a detailed comparison between patients with FSHD and patients with ICF to determine whether there is an additional epigenetic, chromatin structure or clinical overlap besides the low DNA methylation on the first D4Z4 repeat. In chapter 3 a study is described that searched for a chromatin factor that differs between patients with FSHD and patients with ICF. Because this factor is only lowered in patients with FSHD (both in FSHD1 patients with less than 11 D4Z4 repeats and in FSHD2 patients with more than 10 D4Z4 repeats), but not in control individuals and in patients with ICF or in patients with another type of muscle disorder, it is concluded that indeed the chromatin structure of D4Z4 plays an important role in the development of FSHD. In chapter 4 a detailed DNA methylation study is described that tried to determine whether changes in chromatin structure explain the occurrence of FSHD only in individuals with less than 11 D4Z4 repeats on the 4qA161 variant of chromosome 4, while individuals with less than 11 D4Z4 repeats on the 4qA166 and

4qB163 variants and on chromosome 10 are healthy. Finally, in chapter 5 a pilot study is described in which we tried to change the chromatin structure of the D4Z4 repeat in patients with FSHD by folic acid supplementation.

Low DNA methylation is restricted to the D4Z4 repeat in patients with FSHD2 (chapter 2)

To better understand why two completely different diseases, the myopathy FSHD and the immunodeficiency ICF, share an epigenetic component, namely low DNA methylation on the first D4Z4 repeat, a study was performed in which these two patient groups were compared in detail. One of the hypotheses was that especially patients with FSHD2 show an overlap with patients with ICF and that possibly these patients might share a defect in the same DNA methylation mechanism.
Besides low DNA methylation on the D4Z4 repeat, it was shown that in patients with ICF other pieces of repetitive DNA also contain less methyl groups. This may be explained by the presence of a defect in one of the DNA methyltransferase enzymes in some patients with ICF. One of the experiments in the study consisted of the determination of the DNA methylation level of these other repeats with less DNA methylation in patients with FSHD2. However, in patients with FSHD2 the DNA methylation level was not low on these other repeats and thus the overlap in low DNA methylation levels between FSHD2 and ICF seems to be restricted to the D4Z4 repeat. Also other characteristics of patients with ICF, like certain types of chromosomal abnormalities in white blood cells, which are only visible after treatment with phytohaemagglutinin (HA), and low levels of the immunoglobulins IgA, IgG and IgM were not observed in patients with FSHD2. In conclusion, the overlap between patients with FSHD2 and patients with ICF is restricted to the low DNA methylation level on the D4Z4 repeat.

Loss of methyl groups on lysine 9 of histone H3 and loss of binding of the proteins HP1γ and cohesin to the D4Z4 repeats in FSHD (chapter 3)

Besides DNA methylation other important epigenetic factors are the histone modifications. To fit the enormous amount of DNA in the nucleus of a cell, our DNA is wrapped around specific proteins. These proteins, the histones, may contain methyl groups at their tails, just like DNA. Histone tails can also contain acetyl groups. Depending on the location of the methyl and acetyl groups and the amount of methyl and acetyl groups, genes can be switched on or off. With the study described in chapter 3 we

have tried to map the presence and amount of methyl and acetyl groups on the histone proteins in the D4Z4 repeat. We identified several different histone modifications, both active as well as repressive. In addition, we observed that patients with FSHD1 and FSHD2 have fewer methyl groups on a specific location (the amino acid lysine 9) of histone H3, both in comparison with control individuals and patients with ICF. Finally, we observed a secondary loss of the proteins HP1γ and cohesin. The discovery that these proteins bind to D4Z4 in skin and muscle cells but almost not in blood cells is intriguing, because thus far it is unclear why patients with FSHD present mainly with muscle complaints, while the defect in the DNA and the low DNA methylation and the loss of methyl groups on lysine 9 of H3 seems present in all types of cells.

DNA methylation analysis of the D4Z4 repeats on chromosome 4 and chromosome 10 (chapter 4)

To better understand the role of DNA methylation in the development of FSHD and to determine why the presence of less than 11 D4Z4 repeats only on the 4qA161 variant results in FSHD, a detailed DNA methylation analysis of the D4Z4 repeat on chromosome 4 and chromosome 10 was performed. Contrary to previous studies in which only DNA methylation of the first D4Z4 repeat was determined, in this study also the methylation level of all following D4Z4 repeat units was measured. The results of the study are presented in chapter 4 and it is clear that low DNA methylation on the D4Z4 repeat is not FSHD-specific. Instead, it seems related to the number of D4Z4 repeats present. Also in control individuals with short repeat arrays on a non-4qA161 chromosome 4 end or on chromosome 10, low DNA methylation levels were observed. Thus, the results suggest thus that low DNA methylation on the D4Z4 repeat is necessary but not sufficient to develop FSHD. We now postulate that the presence of the 4qA161 variant critically determines the occurrence of FSHD and that differences in the DNA sequence of the different chromosome 4 ends may explain FSHD development. This hypothesis is supported by other results of the study presented in chapter 4. DNA methylation was examined in detail in patients with FSHD2. Not only on chromosome 4, but also on chromosome 10, very low DNA methylation levels were observed, unrelated to the repeat size. These results suggest the presence of gene defect responsible for DNA methylation of the D4Z4 repeat in patients with FSHD2, both on chromosome 4 and on chromosome 10. The most remarkable result of this study is that all patients with FSHD carry at least one 4qA161 chromosome 4 end with low DNA methylation. In summary,

there seem to be two ways to develop FSHD. In most patients with FSHD a reduction of the amount of D4Z4 repeats is observed on the 4qA161 variant (FSHD1). As a consequence the number of methyl groups present on the D4Z4 repeat is reduced. In a small group of patients with FSHD, although more than 10 D4Z4 repeats are present on each chromosome, because of a yet unknown gene defect very low DNA methylation levels are present on chromosomes 4 and 10, and most importantly also on the 4qA161 variant (FSHD2). The combination of the 4qA161 variant and low DNA methylation seems to be a necessary prerequisite for the development of FSHD.

No effect of folic acid and methionine supplementation on the DNA methylation level of the D4Z4 repeat in patients with FSHD

As presented in this thesis, patients with FSHD show important chromatin structure alterations, namely low D4Z4 methylation and loss of methyl groups on lysine 9 of histone H3 and lower binding of the proteins HP1γ and cohesin to the D4Z4 repeat. Because these epigenetic abnormalities seem to play a very central role in FSHD1 and FSHD2 patients, in the study presented in chapter 5 we tried to restore part of these epigenetic abnormalities. Folic acid is an important vitamin that together with the amino acid methionine can raise the DNA methylation level in our cells. A small pilot study was therefore conducted in FSHD1 patients, FSHD2 patients and control individuals. During three months individuals were supplemented with 5 milligram folic acid once a day and 1 gram methionine three times a day. Blood samples were drawn before and after these three months and several factors were tested in these samples, including serum folate and vitamin B12 levels, DNA methylation on the D4Z4 repeat of chromosome 4 and DNA methylation on the total DNA bases in a blood cell. Although the total amount of methyl groups present on the DNA in a blood cell was raised, the DNA methylation level on the D4Z4 repeat was not changed, neither in patients with FSHD nor in control individuals.