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Aberrant DNA methylation of calcitonin receptor in Fabry patients treated with enzyme replacement therapy

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Fabry disease is as a result of a deficiency of the enzyme alpha-galactosidase A which leads to a gradual lysosomal accumulation of globotriaosylceramide (GB3) in cells of the body. Neuropathic pain is one of the prominent symptoms of Fabry disease in the initial stages of the disease [1,2,3], and a possible mechanism involves the release of inflammatory molecules such as calcitonin gene related peptide (CGRP) which activates both calcitonin receptor-like receptor (CRLR) and calcitonin receptor (CALCR) on cell membranes in target tissues, generating signals that regulate various functions within the cell such as neuropathic pain. The involvement of CGRP and CGRP receptors in pain transmission and modulation in central and peripheral nervous systems has been demonstrated in several studies and they appear to play an essential role in peripheral inflammation and development of neuropathic pain [4,5]. While the mechanism of symptom reduction through enzyme replacement therapy (ERT) is not known [6,7], an emerging evidence suggests that DNA methylation changes can affect important CpG (cytosine phosphodiester bond guanine) sites for pain hypersensitivity in neuropathic pain [8].

We therefore carried out a retrospective investigation of the DNA methylation status of the promoter region of the calcitonin receptor in Fabry patients (6 non-ERT treated and 3 ERT treated) and 6 healthy controls respectively, using bisulphite modified DNA isolated from blood leukocytes, methylation specific PCR, high resolution melting, and sequencing as previously described [9]. The inclusion criteria for Fabry patients were: the presence of acute unexplained periods of pain or chronic pain in the limbs, hypohidrosis, angiokeratomas, and an enzyme activity of GLA <2.8 μmol/l/h [1]. An ethic approval for this study was available (EK478/2009).

At position −78504 CpG in the promoter region of CALCR, 3 ERT treated Fabry patients showed a total methylation (GGAGAAGTGCGTA) on both alleles (Fig. 1, Panel B), 3 non-ERT treated Fabry patients and 5 healthy controls respectively showed a demethylation on both alleles (GGAGAAGTGTGTA) (Fig. 1, Panel A), 3 non-ERT treated Fabry patients and 1 healthy control respectively showed a partial methylation on one allele (GGAGAAGTGC/TGTA) (Fig. 1, Panel C). The specific methylation affected a DNA sequence (GTGCG) that is similar to a consensus binding sequence (R-CGTG) for a hypoxia inducible factor 1 α protein (HIF-1 α). HIF-1 α is important in nerve inflammation and is involved in the development of neuropathies [10,11].

The methylation at −78504 CpG on both alleles of the promoter region of CACLR in ERT treated Fabry patients is predicted to prevent the transcription factor HIF-1 from binding to its responsive element (GTGCG), and as a result, we speculate that pain transmission could be interrupted. This hypothesis is supported in the literature by the fact that HIF-1 alpha regulation of erythropoietin expression requires a methylation free HIF-1 binding site in order for HIF-1 to bind to it and thus regulate its target gene [12,13]. In addition, the overexpression of HIF-1 is associated with increased excitability and pain transmission, and an interruption of HIF-1 α pathway by therapeutical inhibition has been shown to reduce neuropathic pain [14,15]. However, an epigenetic mechanism was not suggested.

Concluding, the relationship of a novel methylation at −78504 CpG on both alleles to ERT treated Fabry patients is underlined by the fact that none of the non-ERT treated Fabry patients and healthy controls respectively showed this methylation change. This strongly suggests that methylation at −78504 CpG on both alleles could be used as an epigenetic biomarker of ERT. However, it also needs to be considered that ERT is mainly intended for patients with progressive disease and extensive clinical presentation [16]. Therefore it could be argued that the CALCR methylation on both alleles in ERT treated Fabry patients rather indicates the severity of disease manifestation than therapy, in which case the CALCR methylation could be an epigenetic biomarker for disease severity. In order to systematically and precisely investigate these possibilities, a design of a prospective study of more Fabry patients before and after ERT for a methylation at −78504 CpG in the promoter region of CALCR gene would be necessary. Therefore, it would be possible to directly compare the methylation status of CALCR of the same Fabry patients before and after ERT, thus enabling a concrete statement about the significance of a CACLR methylation at −78504 CpG as an epigenetic biomarker.

Conflict of interest

The authors do not have any conflict of interests.

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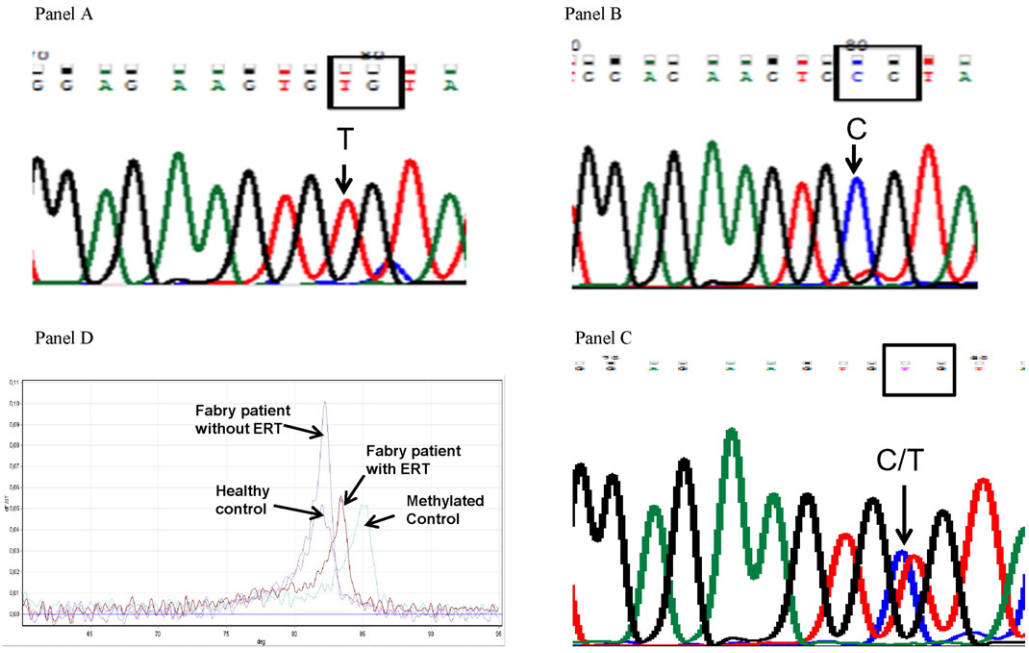

Fig. 1. Panel A: Demethylation at −78504 CpG on both alleles. Panel B: Total methylation at −78504 CpG on both alleles. Panel C: Partial methylation at −78504 CpG on one allele. Panel D: High resolution melting shows methylation peak shifts indicating different degrees of methylation at −78504 CpG.

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