FKRP expression in lymphoblastoid cell lines: a human cellular model to study the impact of the mutations on the protein functionality.

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Introduction

Fukutin Related Protein (FKRP) is a transferase involved in the biosynthesis of a phosphorylated O-mannosyl trisaccharide present in α-dystroglycan (DAG1) [1]. Mutations in the FKRP gene are responsible for congenital muscular dystrophy, hyperCKemia, cardiomyopathy and LGMD2I [2]. Since the molecular mechanisms at the base of the great phenotypic variability observed in LGMD2I are not known, it is of great interest to study the cellular expression- functionality of the Fukutin Related Protein both in physiologic and pathologic state.
Aim of this work was then to evaluate the FKRP and DAG1 protein expression in Epstein Barr Virus (EBV) lymphoblastoid cells lines (LCLs) obtained from normal subjects and from patients carrying a FKRP mutation

Methods

LCLs were obtained by EBV infection on mononucleated peripheral blood cells and grown in DMEM + 10% FBS (Fig1). Two normal control subjects and three subjects belonging to the same family with the FKRP Ala114Gly mutation were examined, two of which were heterozygous for the mutation. One of the heterozygous patients have clinical manifestation of Limb Girdle Dystrophy while the other one showed only scapular winging. Western Blot (WB) analysis was employed to evaluate separately the FKRP and DAG1 expression by using specific primary monoclonal antibodies and specie-specific conjugate secondary antibodies.

Results

SDS-PAGE of the in toto cellular extract from all family members did not show qualitative protein differences (Fig 2). Western Blotting of the control cell extract clearly showed the FKRP band at approxymatively 60 kD plus other three unknown bands with slower molecular weight (MW). The patient samples showed the same pattern of bands without quantitative differences (Fig 3). DAG1 also was expressed in LCLs as multiple bands on WB including that one awaited at approximately 150 kD. Very interesting the proband's sample (FK2) carrying the FKRP Ala114Gly mutation and with Limb Girdle Dystrophy showed the absence of two bands at higher MW (B1 and B2) when compared with the control's samples (Fig 4). The sample of the other one heterozygous family member (FK1) showed instead a reduction, not statistically significant, of the intensity of these bands (Fig 4)

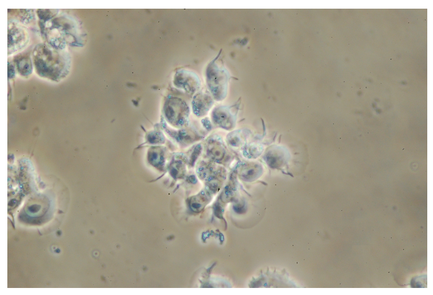

Figure 1 Lymphoblastoid cell lines: phase contrast microscopy (20x)

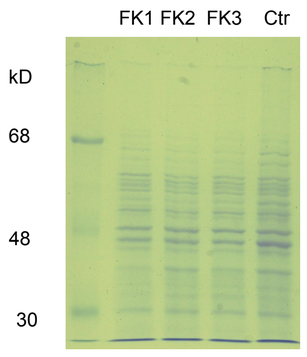

Fig 2 SDS-PAGE of the cellular extract coomassie blue stained

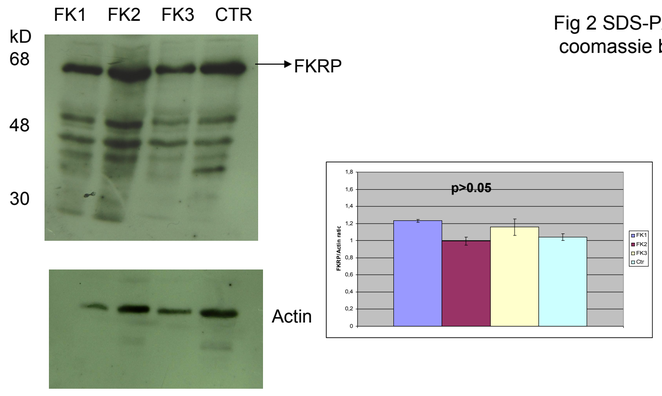

Fig 3 FKRP Western Blotting of the family members (FK1,FK2,FK3) and normal control (CTR) FKRP mutation

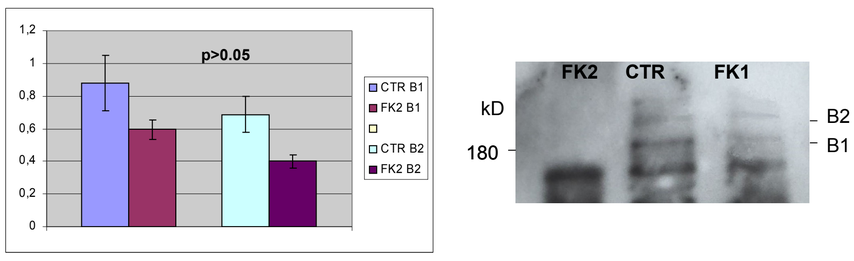

Fig 4 Alpha dystroglycan 1 Western Blotting of the heterozigous family members (FK 1 and FK2) and normal control (CTR) . B1 and B2 are anti-DAG1 immunoreactive bands

Conclusions

Our results clearly show that LCLs express both the protein bands corresponding to Fukutin Related Protein and α-dystroglycan 1 and that the LCLs of patients with FKRP mutations and LGD may show differential patten of expression of the DG1 when compared with normal controls. LCLs may then be considered an useful tool to study the molecular network involved in the genetic disease, FKRP associated, such as the LGMD2I.

Bibliografia

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2) Rasmussen M, Scheie D, Breivik N, Mork M, Lindal S. Clinical and muscle biopsy findings in Norvegian paediatric patients with limb girdle muscular dystrophy 2I. Foundation Acta Paediatrica. (2014), 103: 553-558.

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