



Gamma-sarcoglycanopathy (LGMD 2C) with Del 525T mutation: report of the first familial case in Niger

H. MOUMOUNI^{1*}, H. ASSADECK¹, S. GUIDA¹, B. S. SOUNA¹, R. ADAL¹,
J. A. URTIZBEREA², F. LETURCQ² and R. B. DARBOUX³

¹Faculté des Sciences de la Santé de Niamey, Laboratoire d'Histo-Embryologie, Neurologie, Radiologie
Traumato-Orthopédie, Niger.

²Hôpital Marin de Handaye, France.

³Faculté des Sciences de la Santé de Cotonou, Laboratoire de Biologie Humaine, Bénin.

*Corresponding author, E-mail: mohassa_ne@yahoo.com; BP 10896 Niamey, Niger.

ABSTRACT

We are reporting a familial case of limb-girdle muscular dystrophy (LGMD) upon 5 out of 6 siblings from parents showing no evidence of muscular dystrophy. The pedigree of the family up to five generations did not reveal any known case in the past even though consanguinity was reported. The clinical observations revealed wheelchair bound or difficulties for walking in all affected subjects, due to muscular dystrophy involving mainly the pelvic girdle. Creatine phosphoKinase (CK) was higher than normal values in both affected children and their parents. The scanning of thigh showed in all patients, an atrophy of the quadriceps with fatty conversion. Molecular analysis was carried out, first using western blot, which revealed gamma-sarcoglycan deficiency and second, by gene screening, which showed Del 525T mutation. This mutation is most widespread in arabo-berbères tribes including Touaregs. The present cases are in our knowledge the first reported in that part of Africa, south of Maghreb. We make a focus on histological and molecular bases of the LGMD.

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INTRODUCTION

The myopathy referred mainly to Duchenne muscular dystrophy that was described by Duchenne in the middle of the 19th century. This anomaly, recessive X-linked transmitted, affects exclusively men. Nowadays, many types of muscular diseases are known, based on their clinical and histopathological aspects, and the molecular bases of most of them are elucidated (Weiler and al., 1999; Leturcq and Kaplan, 2000;

Urtizbera, 2001; Authier et al., 2008). A particular interest in investigating these pathologies was raised in developed countries around 1980 following the era of advances in molecular genetics (Gilchrist and al., 1988; Beckmann et al., 1991; Ginjaar et al., 2000; Sandona and Betto, 2009). It has been shown that these pathologies derive from anomalies in proteins involved in muscle cell structures or functions. The contractibility of muscle cells is enabled by the presence of filaments

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like actin, myosin, tropomyosin, and intermediate filaments, desmin particularly. They contain myoglobin, a pigment which fixes oxygen. Many other proteins are present in the cell plasma under the sarcolemma: integrins, spectrin, and the complex Dystrophine / Dystrophine Associated Glycoproteins (DAGs). The complex Dystrophine/DAGs is particularly involved in the occurrence of myopathies. The DAGs contain about 15 proteins which are linked to dystrophine. These proteins can be divided into 4 subgroups: dystroglycans, sarcoglycans, syntrophines and dystrobrevine. Although their functions are only partially known, it is clear that the complex dystrophine/DAGs participates in the stability of the sarcolemma. Moreover, deficiency in one element of this complex has been shown to alter all the system, then leading to fiber necrosis and fibrosis (Fanin et al., 1997; Leturcq and Kaplan, 2000; Authier et al., 2008; Sandona, and Betto, 2009). The diversity of the proteins that are involved in this phenomenon explains the numerous types of myopathies. The majority of myopathies are genetically transmitted, in variable ways: autosomic, X-linked, recessive, dominant (Eymard et al., 1997; Gilchrist et al., 1988; Bonne et al., 1999; Speer et al., 1999). In spite of important similarities, differences also exist in their clinical expression. Nevertheless, several probes exist now and are of great interests in molecular identification of the myopathies (Nigro et al., 1996; Leturcq and Kaplan, 2000; Khadilkar et al., 2009; Sandona and Betto, 2009).

In African countries such as Niger, muscular diseases are poorly known and are considered as a punishment from the nature. Here we report 5 cases of gamma sarcoglycanopathy in a consanguineous family of 6 siblings with clinical, histopathological and molecular confirmation. The objectives of the work were to enhance the knowledge of these muscular diseases as

regards both clinical expression and molecular supports.

MATERIALS AND METHODS

The study population is a touareg family from Agadez (North of Niger), living in Niamey. Five of the 6 siblings were affected by a severe muscular dystrophy. The father and the mother were linked by a consanguineous marriage. They were respectively 47 and 38 years old and showed no clinical symptoms. The siblings included 3 females (with a twin pair) and 3 males with ages ranging from 8 through 18 years (Figure 1). We performed a full neurological examination followed by a general examination for the whole family. Then we used all clinical and laboratory investigation data after 10 years follow up. Laboratory investigations included cardiac ultrasonography, electrocardiogram, CT scan of the thigh and creatine phosphokinase (CK). A muscular biopsy was performed for western-blot using specific antibodies against dystrophine, dysferlin, calpain and sarcoglycans. The western blot test was followed by direct gene sequencings. During the study, regular home visits were organized to be in touch with the family and provide counselling and helps.

RESULTS

The pedigree allowed consanguinity rate evaluation (coefficient 0.0625). Only the hypothesis of autosomic recessive transmission can be accepted for this disease. The heterozygote of the subject V.6 has been added after the results of molecular biology test.

Data provided by Bio-clinical investigations showed for all affected children an onset age around 9-10 years, an evidence of pelvic deficiency at age of 11 and wheelchair bounds at 13. CK were high in all family members, even in the heterozygotes. Cardio-respiratory and intelligence evaluations were normal. CT scan of thigh in

affected subjects revealed atrophy of quadriceps with fatty conversion (Figure 2). The details of the main tests that were performed are summarized in Table 1.

Deficits in calpain and dysferlin have been tested by Western-blot and gene sequencings they are also responsible for limb-girdle muscular dystrophies transmitted in autosomic recessive way.

According to the touareg origin of the family, the delta 525T mutation that was known in arabo-berbères was first researched and confirmed in all the family members. Subjects V.1, 2, V.3, V.4 and V.5 were studied while the 2 parents and V.6 were heterozygote.

The Western blot test revealed a deficiency in gamma-sarcoglycan (Figure 3).

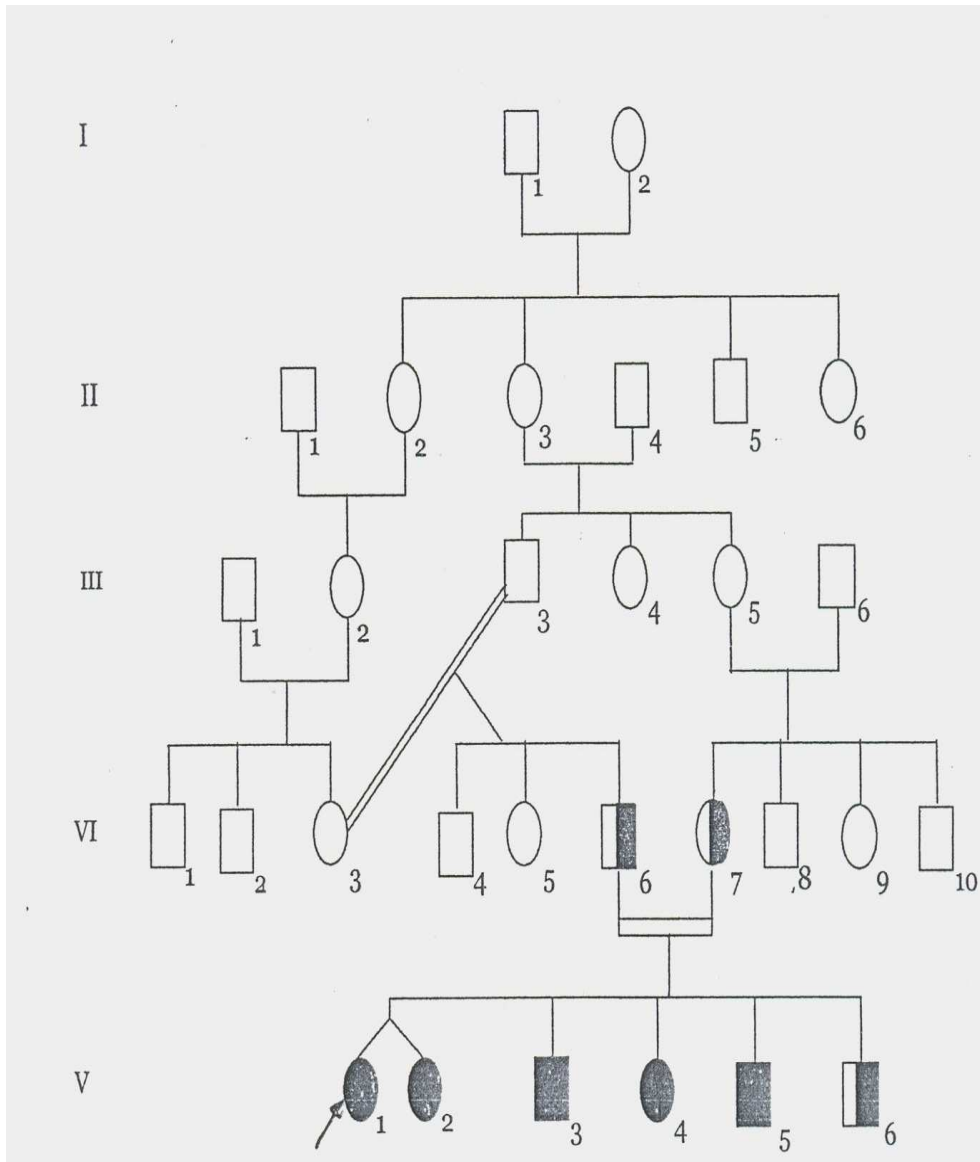
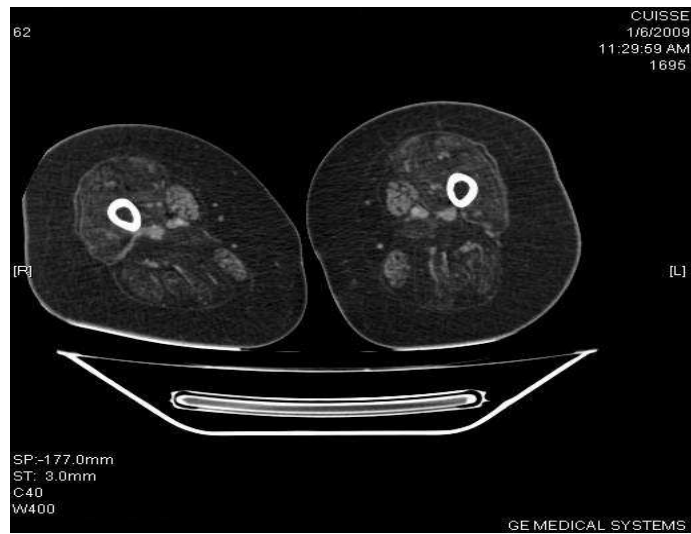


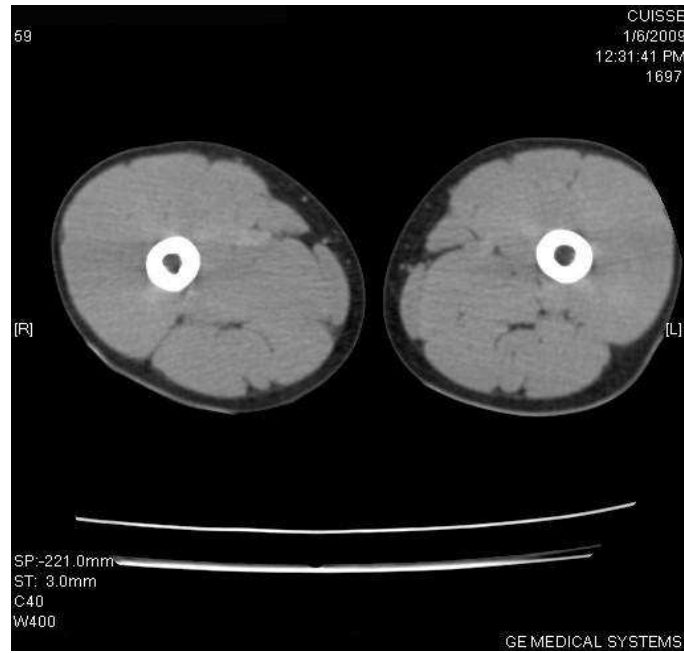
Figure 1: Family's pedigree on five generations.



Subject V.3, 17 years old, wheelchair bound since 4 years. Note the muscular atrophy with fatty reconversion.



Subject V.5, 11 years old, showing pelvic deficit, still walking, but with difficulty. Note the importance of atrophy and fatty reconversion.



Subject V.6, 8 years old, heterozygote, walks normally. Images are in normal limits.

Figure 2: CT Scan of the thigh of 2 affected subjects (V.3 and V.5) and of 1 heterozygote.

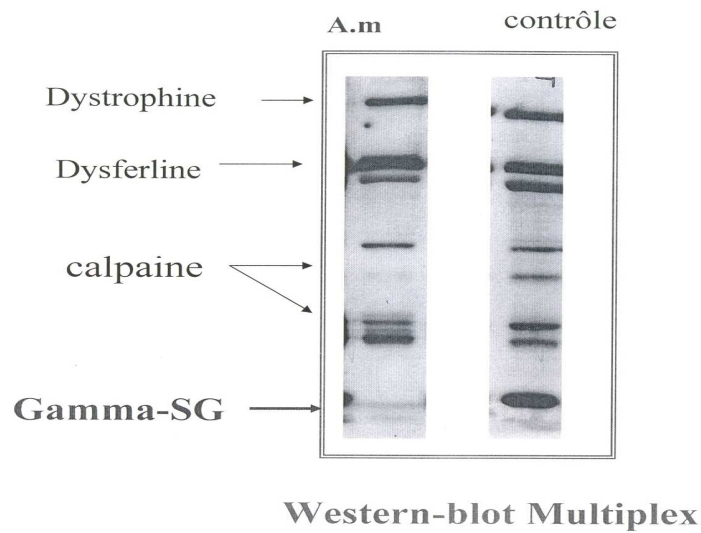


Figure 3: Evidence of gamma-sarcoglycan deficiency by western-blot test in subject V.3.

Table 1: Summary of the main bio clinical observations in the study subjects.

	IV.6 (father)	IV.7 (mother)	V.1, 2* (daughters)	V.3 (son)	V.4 (daughter)	V.5 (son)	V.6 (son)	Observations
walking difficulties onset	None,	None,	9 years	11 years	11 years	11 years (real age)	None, 8 years really	beginning 9-11ans
Pelvic deficiency	None,	None, knees pain	11 years	11 years	11 years	11 years	None	11 years in all cases
Wheelchair bound	No	No	13 years	13 years	13 years	No**	No	13 years for the 4 first affected
Cardio-respiratory examination	Normal	Asthma	palpitations	palpitations	Normal	Normal	Normal	Poor anomalies
CK (UI/L) (Normal value: 0-195UI/L)	257	833	622-789	2317	1849	13790 (at 5 years) 4810 (real)	270 (at 2 years) 499 (real)	High in all cases But always < 1000 in heterozygotes
Thigh CT Scan	-	-	-	Atrophy of quadriceps and posterior muscles of the thigh	-	Atrophy of quadriceps and posterior muscles of the thigh	Normal	Atrophy of quadriceps constant
Molecular biology	Heterozygote Delta 525T	Heterozygote Delta 525T	-	Homozygote Delta 525T	-	Homozygote Delta 525T	Heterozygote Delta 525T	** will probably loss walking capacity while V.6 will be surely free of the disease

* Twins whom clinical evolution is strictly comparable.

Table 2: Summarises some new proteins and genes involved in limb-girdle myopathies.

Classification Type*	Protein	gene	Classification Type*	Protein	gene
1A	Myotilin	5q22	2A	Calpain	15q15
1B	Lamin A/C	1q11	2B	Dysferlin	2p13
1C	Caveolin	3p25	2C	γ -sarcoglycan	13q12
1D	?	6q23	2D	α -sarcoglycan	17q12
1E	?	7q	2E	β -sarcoglycan	4q12

* LGMD Type 1 = dominant
LGMD Type 2 = recessive

DISCUSSION

The pedigree analysis shows in spite of its singularity to assume autosomic recessive transmission, giving orientation towards the subgroup of myopathies to be tested. Pedigree analysis is not easy for genetic counselling even if we consider that the twins were monozygotes, the chance to observe 5 affected subjects in an offspring of 6 is less than 1/60 000 for an expected risk of 1/4. This pedigree gives support of targets for a wide research in the extended family by using these molecular probes. By matching these data and those provided by molecular analysis we confirmed to the parents that their last son (V.6) will not develop the full disease and we notify to them their ability to have unaffected offspring. The touareg origins of the family help us focus first on the Delta 525T mutation, which has been confirmed by gene screening (Ben Hamida et al., 1983; Fanin et al., 1997; Moreira et al., 1998; Leturcq and Kaplan, 2000). Regarding the high rate of consanguinity found in the present study, further research should be extended to the large family. This will help in detecting heterozygotes and in preventing high-risk unions. The Delta 525T mutation was listed first as Delta 521T and is the most frequent cause of sarcoglycanopathy in patients of Maghreb origins. In Maghreb countries, it represents up to 50% of progressive muscular dystrophies in children versus 10 to 15% in Europe or United States. Our cases were the first to be reported in this part of Africa, in the south of Maghreb. Origins of Delta 525T mutation are yet discussed, but the high frequency in Maghreb allows the hypothesis of an existing link with arabo-berbers. The observation of cases in Mediterranean region (Libya, Jordan, Libanon, Saudi Arabia, Tunisia, etc.) and now in Niger can be explained by migration flows in this area (BenHamida et al., 1983; Urtasun et al., 1998; Ginjaar et al., 2000; Merlini et al, 2000; Khadilkar et al, 2009). The line between Touaregs and arabo-berbers is now well established; then it is not surprising to observe the Delta 525T mutation in this ethnic group.

Paraclinic examinations revealed a rise of creatine phosphokinase (CK) in all members of the family but levels were < 1000 U/L in heterozygotes. In addition, a muscular atrophy with fatty reconversion was clear in all homozygotes since the age of 8.

Sarcoglycanopathies belong to the Limb Girdle Muscular dystrophies (LGMD) 2D, 2E, 2F, and 2C in the nomenclature of European Neuromuscular Centre (ENMC) (Nigro et al., 1996; Sandona and Betto, 2009; Urtizbera, 2001). Their clinical expression is so similar that sometimes only immunochemistry or mutation analysis can help distinguishing them. Myocardiopathy is usually rare in sarcoglycanopathies.

Gamma-sarcoglycanopathy (LGMD 2C) results from a molecular deficit in gamma-sarcoglycan, a protein of 25kD, encoded by a gene located on 13q12. Although cases have been reported in numerous countries, 2 ethnic groups are mainly involved: arabo-berbers and gipsy, which are known with important consanguineous marriage. In arabo-berbers the mutation is Delta 525T and C283Y in gipsy. Seven other mutations were reported, involving the same gene (Fanin et al., 1997; Urtasun et al., 1998; Merlini et al., 2000).

Myopathies resulting from anomalies of other proteins have been reported: (calpain, telethonin, dysferlin, myotilin, lamin, caveolin, etc.) (Liu et al., 1998; Mc Nally et al., 1990; Baghdiguan et al., 1999; Bonne et al., 1999; Hauser et al., 2000). Other myopathies rise from more complex disturbances e.g. a repetition of triplets (Steinert myopathy) or as the consequence of abnormal unknown proteins as reported by Speer et al. (1999) in Landouzy-Déjerine, LGMD 1D, LGMD 1E, etc. (Table 2).

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