A novel FKRP-related muscular dystrophy founder mutation in South African Afrikaner patients with a phenotype suggestive of a dystrophinopathy

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Background. Fukutin-related protein (FKRP) muscular dystrophy is an autosomal recessive disorder caused by mutations in the FKRP gene. The condition is often misdiagnosed as a dystrophinopathy. A previously unreported mutation, c.1100T>C in exon 4 of FKRP, had been identified in homozygous form in two white South African (SA) Afrikaner patients clinically diagnosed with a dystrophinopathy.

Objectives. To investigate whether the c.1100T>C mutation and the common European FKRP mutation c.826C>A are present in other patients of Afrikaner origin with suspected dystrophinopathy, and whether a founder haplotype exists.

Methods. The c.1100T>C mutation was initially tested for using an amplification refractory mutation system technique in 45 white SA Afrikaner patients who had tested negative using multiplex ligation probe amplification screening for exonic deletions/duplications in the dystrophin gene. Sequencing analysis was used to confirm the c.1100T>C mutation and screen for the c.826C>A mutation. Two cohorts (each numbering 100) of Afrikaners and other white controls were screened for the c.1100T>C and c.826C>A mutations, respectively.

Results. Of the 45 patients, 8 patients (17.8%) were homozygous for c.1100T>C, 2 (4.4%) were compound heterozygotes for c.1100T>C and c.826C>A, and 1 (2.2%) was heterozygous for c.1100T>C with a second unidentified mutation. The c.1100T>C mutation was found in 1/100 controls, but no heterozygotes for the c.826C>A mutation were identified. Linked marker analysis for c.1100T>C showed a common haplotype, suggesting a probable founder mutation in the SA Afrikaner population.

Conclusion. FKRP mutations may be relatively common in Afrikaners, and screening should be considered in patients who have a suggestive phenotype and test negative for a dystrophinopathy. This test will be useful for offering diagnostic, carrier and prenatal testing for affected individuals and their families. As FKRP muscular dystrophy is autosomal recessive in inheritance, the implications of a positive diagnosis in a family differ significantly from those of an X-linked dystrophinopathy.
patients who were referred for dystrophinopathy testing but tested negative for deletions or duplications in the dystrophin gene. The specific objectives were to determine whether SA Afrikaner patients clinically diagnosed with a phenotype suggestive of a dystrophinopathy and negative for dystrophin mutations have the c.1100T>C and c.826C>A FKRP mutations, and whether the c.1100T>C mutation is a founder mutation in the SA Afrikaner population based on the presence of a common haplotype.

**Subjects**

**Patients**

A total of 45 white SA patients of Afrikaner origin (based on their surnames) were included in this study. The patients were routine referrals to the Division of Human Genetics, National Health Laboratory Service (NHLS), for dystrophinopathy diagnostic testing from 1994 to 2013 and who tested negative for exonic deletions/duplications on multiplex ligation probe amplification (MLPA) screening using SALSA MLPA kits and P034 and P035 probe mixes (MRC-Holland, Netherlands). Patients with a family history suggestive of an X-linked pattern of disease were excluded.

The majority of referrals were from the state hospitals in Johannesburg (Charlotte Maxeke Johannesburg Academic Hospital, Chris Hani Baragwanath Hospital, Rahima Moosa Mother and Child Hospital) and from medical geneticists at the NHLS genetic counselling clinics. A small number of cases were referred from other genetic centres around SA and from doctors in private practice. Blood samples from the patients were sent in with a referral form stating the possible diagnosis and in some cases the clinical features observed.

**Controls**

A total of 200 unrelated and unaffected randomly selected white individuals whose DNA samples were taken from the DNA bank in the Division of Human Genetics were screened. Of these, 100 were selected based on their Afrikaner surnames and screened for the c.1100T>C mutation, and a further 100 white controls were screened for the c.826C>A FKRP mutation.

**Methods**

All 45 patients were screened for the c.1100T>C mutation using the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method, and positive results were confirmed on Sanger sequencing. The c.826C>A mutation was tested using Sanger sequencing only.

**Techniques used**

**ARMS-PCR for FKRP (c.1100T>C) mutation**

A multiplex ARMS-PCR method was designed for detection of the c.1100T>C mutation using four primers: primers FKRP Ex4-Control (F-outer) 5’-CGCCCGCCTACCTCTAGGAG-3’ and FKRP-ARMS-MUTANT (R-inner) 5’-GCCACAGCTTCCAAAGTTGG-3’, which amplified the mutant sequence (209 base pairs (bp)), and primers FKRP-ARMS-NORMAL (F-inner) 5’-GGACTACAGCGTTCAGCTGGG-3’ and FKRP Ex4-Control (R-outer) 5’-AGAAGGACCACAACGTCCAAC-3’, which amplified the normal sequence (186 bp). An internal control fragment of 351 bp was amplified using the FKRP Ex4-Control (F-outer) and FKRP Ex4-Control (R-outer) primers. The PCR products were separated on a 3% agarose gel.

**Sequencing**

Sanger sequencing was performed to confirm the presence of the two FKRP mutations. Products were run on an ABI 3130xl genetic analyser (Applied Biosystems, SA) and results were analysed using the SeqMan II application, DNASTAR Lasergene programme (DNASTAR, USA).

**Haplotype analysis**

Three dinucleotide short tandem repeat (STR) microsatellite markers flanking the FKRP gene, D19S219, D19S412 and D19S606, were typed in affected individuals and their families (where available) to determine whether the c.1100T>C mutation occurs on a founder haplotype.[21] The order of markers is centromere-D19S219-D19S412-FKRP-D19S606-telomere. The distance between D19S219 and D19S606 is ~1.9 Mb, with ~0.72 Mb between D19S606 and the mutation.[21]

**Ethics approval**

The project protocol was submitted to the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg, and an ethical clearance certificate was obtained (ref. no. M120618).

**Results**

**c.1100T>C and c.826C>A mutation screening**

The diagnosis of FKRP muscular dystrophy was confirmed in 10/45 patients tested (22.2%) and was suggestive in another 2/45 heterozygous patients (4.4%) (Table 1). One heterozygous individual with the c.1100T>C mutation was identified in the 100 Afrikaner controls tested. None of the 100 white controls tested positive for the c.826C>A mutation.

<table>
<thead>
<tr>
<th>Mutations identified</th>
<th>Genotype</th>
<th>Patients, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.1100T&gt;C homozygote</td>
<td>c.1100T&gt;C/c.1100T&gt;C</td>
<td>8* (17.8)</td>
</tr>
<tr>
<td>Compound heterozygote</td>
<td>c.1100T&gt;C/c.826C&gt;A</td>
<td>2 (4.4)</td>
</tr>
<tr>
<td>c.1100T&gt;C heterozygote</td>
<td>c.1100T&gt;C/unidentified</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td>c.826C&gt;A heterozygote</td>
<td>c.826C&gt;A/unidentified</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td>c.1100T&gt;C and c.826C&gt;A negative</td>
<td>Negative/negative</td>
<td>33 (73.3)</td>
</tr>
</tbody>
</table>

*This includes the two patients tested in the UK.
Discussion

The FKRP-related muscular dystrophies are caused by different mutations in the FKRP gene and may vary in disease severity.[1] The marked clinical similarity between LGMD2I and dystrophinopathy patients has often resulted in misdiagnosis of an FKRP-related muscular dystrophy.[2]

A study by Schwartz et al.[3] in Denmark involved screening for the common c.826C>A (p.Leu276Ile) FKRP mutation in 102 European patients, who presented with a dystrophinopathy-like phenotype but in whom no dystrophin gene rearrangements were identified. The study found that ~15% of patients were homozygous for a common c.826C>A FKRP mutation and were therefore diagnosed with LGMD2I (an FKRP-related muscular dystrophy) and not with a dystrophinopathy as had initially been thought.

Similarly, before this study, a novel c.1100T>C (p.Ile367Thr) FKRP mutation was first identified in a homozygous state in two unrelated Afrikaner patients who were clinically diagnosed with DMD/BMD, but in whom no deletions or duplications in the dystrophin gene were found. As a result of this finding, a further 43 SA white patients suspected of having a dystrophinopathy were screened for this novel mutation in this study. Eight patients (17.8%) in total were found to have this mutation in a homozygous state and 3 (4.4%) were heterozygous for the mutation, suggesting that FKRP mutations are a significant cause of disease in patients presenting with a dystrophinopathy-like phenotype. Furthermore, since a significant number of patients with Afrikaners ancestry carry the c.1100T>C mutation, this mutation has been reported in other populations, and linked marker analysis revealed a common haplotype, it is probable that the c.1100T>C mutation is a founder mutation in the Afrikaner population. A number of founder mutations for other genetic disorders have been identified in the SA Afrikaner population.[7-11]

Owing to the suggestive clinical features, the 3 heterozygous patients were subsequently screened for the c.826C>A mutation. This mutation was found in 2 of the 3, confirming the diagnosis of FKRP muscular dystrophy. A diagnosis of FKRP muscular dystrophy was therefore confirmed in 22.2% of the patients screened and suspected in another 4.4% who were heterozygous for one of the mutations. It has been reported in one study[12] that 4 cases in 10 families (40%) were compound heterozygotes and carried another missense mutation in the second allele that was said to be pathogenic. In another study that screened for the common c.826C>A FKRP mutation in 20 German patients from 19 unrelated families, 13 of the 20 patients (65%) were found to be homozygous for the mutation and 3 were found to be compound heterozygotes carrying a second heteroallelic mutation.[13]

The FKRP c.826C>A mutation appears to be common worldwide, with a carrier frequency estimated to be 1 in 300.[13,14] In this current study, the carrier frequency of the c.1100T>C mutation was found to be approximately 1 in 100. This suggests that the FKRP c.1100T>C mutation may be more common in the Afrikaner population of SA than elsewhere. The c.826C>A mutation was not found in 100 control individuals tested in the SA population, but was found in the patient cohort, indicating that this is not a particularly common mutation in SA, but does occur.

Conclusion

This study shows that FKRP-related muscular dystrophy accounts for a significant number of patients with a phenotype suggestive of a dystrophinopathy, and that the mutation c.1100T>C is likely to be a founder mutation in the SA Afrikaner population. The c.826C>A was identified in compound heterozygous form, suggesting that this common European mutation also occurs in SA white patients. Patients of possible Afrikaners ancestry with a dystrophinopathy phenotype who test negative for DMD/BMD mutations on MLPA should be screened for FKRP mutations. The findings emphasise the need for caution in the diagnosis of dystrophinopathy-like disorders, to prevent misdiagnosis and inaccurate genetic counselling. Since FKRP-related muscular dystrophy is an autosomal recessive and not an X-linked disorder, the risks to relatives are significantly different. The knowledge derived from this study will be very useful in ensuring more accurate diagnostic, carrier and prenatal testing for SA families with dystrophinopathy-like muscular dystrophies.

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