

CHAPTER FIVE

CONCLUSION

The clinical diagnosis of individuals affected with FSHD is complicated due to extreme clinical variability. Even the differentiation between various muscular dystrophies can be challenging on the clinical level, complicating specific diagnosis of a particular muscular dystrophy. This emphasises the need and relevance of molecular diagnoses of all muscular dystrophies, but in particular for those muscular dystrophies that are as clinically heterogeneous as FSHD.

In this study FSHD was investigated for the first time on a molecular level in South Africa and enabled definitive diagnoses of multiple individuals. The molecular study included both haplotype analysis, utilising nine STRP markers, and Southern blot analysis with probe p13E-11 to detect DNA rearrangements at locus D4Z4 in individuals from five extended FSHD families (F10, F20, F30, F40 and F60). Although the initial aim of this study was to genotype 74 individuals with five STRP markers, a total of 100 individuals were eventually genotyped utilising nine STRP markers. The exact order of the nine STRP markers was also mapped in this study via several recombination events observed within the families investigated.

In the group of 100 individuals, 12 individuals were related to their respective families by marriage and 83 individuals were definitively diagnosed for the first time. As discussed in paragraphs 4.4.2 and 4.4.4 a definitive diagnosis could not be made for the remaining five individuals (40-10, 40-26, 40-27, 21-69 and 21-75). These individuals were clinically diagnosed with FSHD and all harboured the *Eco* RI deletion fragment segregating in their respective families. The *Eco* RI fragments were digested with *Bln* I signifying a chromosome 10 origin. No *Bln* I resistant deletion fragments could therefore be confirmed for these individuals. It thus seems as if the deletion fragments observed in these five individuals resides on chromosome 10 and are therefore not pathogenic.



5.1 SOUTH AFRICAN FSHD FAMILIES INVESTIGATED

Family F10 was the largest South African FSHD family investigated in this study. A definitive molecular diagnosis could be made for all the individuals of this family who were included in this study. A *Bln* I resistant deletion fragment of 24 kb was observed to segregate with the FSHD associated haplotype as well as the FSHD phenotype (Figure 4.12). Genotype-phenotype discordance was observed in only one individual (14-113) who was clinically phenotyped to be affected, but who did not inherit the deletion fragment. This individual was diagnosed in his late fifties, an age at which the phenotype should have been fully penetrant. The clinical diagnosis was, however, made by a clinician not involved in the study and it is therefore suggested that this individual should be clinically re-evaluated by a clinician who specialises in FSHD. The clinical re-evaluation of individual 14-113 will be essential to substantiate his molecular diagnosis.

The first compound heterozygote individual (15-31) in the South African population was also observed in family F10 (Olckers *et al.*, 2001; Van der Merwe *et al.*, 2001b). This highlights the importance of appropriate genetic counseling for this individual as well as his children, who will all inherit FSHD from their father. The chromosomal origin of the deletion fragments was verified with chromosome 10 haplotype analysis in a separate study (Olckers *et al.*, 2001; Van der Merwe *et al.*, 2001b). The presence of two different sizes *Bln* I resistant deletion fragments within one family might be useful in the elucidation of the phenotypic-genotypic correlation in FSHD. Clinical re-evaluation by a clinician specialised in FSHD is therefore also essential for this family.

A 34 kb *Bln* I resistant deletion fragment, and a distinct FSHD associated haplotype from that in family F10 was observed to segregate in family F20, as presented in Figure 4.14. Genotype-phenotype discordance was also observed in this family. Five individuals (21-65, 21-69, 21-71, 21-75 and 21-76) who were clinically diagnosed with FSHD did not inherit the haplotype or the deletion fragment. It can therefore be argued that the phenotype, haplotype and deletion fragment observed are not associated with FSHD in this family. These five individuals were, however, clinically diagnosed at a young age (before the age of 20), and it has been observed that the clinical diagnosis of children is much more complicated than that of adults, thus necessitating re-examination of these individuals on a clinical level at a more advanced age. It is likely that these individuals, who are now in their twenties, will be negative upon clinical examination, confirming the molecular analysis. If they are, however, observed to be clinically affected, further

molecular analysis will be necessary to elucidate the molecular nature of the phenotype-genotype discordance in this family. For this reason, the clinical evaluation of these individuals is a high priority during the next phase of this ongoing project.

Sub-family F21 of family F20 was genotyped as a representative family for the extended family. It was, however, revealed by the presence of a compound heterozygote individual in sub-family F15 of family F10, that one sub-family does not provide sufficient representation of the entire family. If sub-family F15 was for example not included in this study, the compound heterozygote individual would not have been observed and the spectrum of molecular complexity present in this family might not have been revealed and appreciated. Individuals from the other sub-families of family F20 will therefore be included in an extended study in the future.

Family F30 was observed to harbour the same 24 kb *Bln* I resistant deletion fragment and FSHD associated haplotype as family F10, as illustrated in Figure 4.15. Only two individuals (30-104 and 30-106), who were clinically phenotyped to be FSHD positive, did not inherit the deletion fragment. These individuals were younger than 20 years of age upon clinical examination and need to be clinically re-evaluated to confirm the proposed discordance.

A 27 kb *Eco* RI deletion fragment corresponding to a 24 kb *Bln* I resistant deletion fragment was also observed to segregate in family F40. The 24 kb *Bln* I resistant deletion fragment was observed to segregate with the same FSHD associated haplotype, indicated in Figure 4.16, as in families F10 and F30. No definitive molecular diagnosis could be made for three individuals (40-10, 40-26 and 40-27) from this family, although these individuals all harboured a 27 kb *Eco* RI deletion fragment. This fragment was digested with *Bln* I, therefore displaying the characteristics of a chromosome 10-type deletion fragment. Individual 40-10 is, however, the proband of this family, and was recently clinically diagnosed with FSHD by a neurologist specialising in FSHD. In the diagnosis of FSHD on a molecular level, PFGE enables the visualisation of all four alleles. The importance of PFGE analysis was confirmed with the presence of *Bln* I sensitive fragments in these clinically affected individuals. Diagnosis of these individuals was thus postponed until PFGE analyses could be utilised to confirm the chromosomal origin of the 27 kb *Eco* RI deletion fragment.



Family F60 was observed to harbour the same FSHD associated haplotype and 34 kb *Bln* I resistant deletion fragment as those segregating in family F20. This indicates that families F20 and F60 should thus have a common ancestor, which will be further investigated in future studies. It was evident from the results obtained for individual 60-6, as discussed in paragraph 4.4.5 and illustrated in Figure 4.18, that it will be necessary in the future to determine the exact allele size threshold, and therefore the pathogenic range, in the South African population to provide accurate molecular diagnosis. In addition, utilisation of PFGE instead of conventional horizontal gel electrophoresis will undoubtedly increase both the specificity and sensitivity of the molecular diagnosis.

5.2 GENETIC HETEROGENEITY

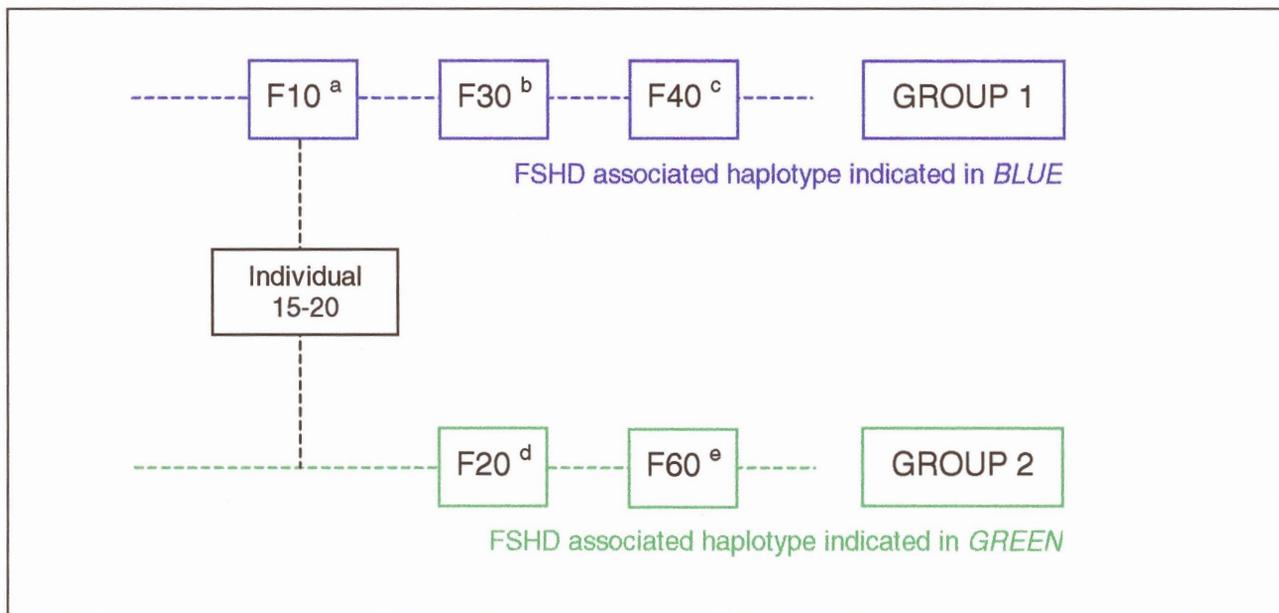
The utilisation of *Bln* I was observed to improve the molecular diagnosis of FSHD in the South African population. *Bln* I resistant deletion fragments associated with the FSHD phenotype were detected in all five families investigated (Olickers *et al.*, 2000; Van der Merwe *et al.*, 2000b). From the results generated in this study, it was concluded that no evidence for genetic heterogeneity was observed in the group of South African families included in this study. To determine whether genetic heterogeneity exists in the South African FSHD population, an even larger group of clinically well characterised families should be investigated. This is currently one of the aims of an extended FSHD research programme.

5.3 DUAL FOUNDER EFFECT

The possibility of a single ancestral mutation in the South African FSHD population was excluded by the presence of two distinct haplotypes, each co-segregating with a specific *Bln* I resistant deletion fragment (Van der Merwe *et al.*, 2000a). This indicates distinct genetic backgrounds for FSHD in South Africa, and thus two ancestral origins for the current FSHD phenotype observed in the South African population. Results to date indicate the presence of a dual Founder Effect for FSHD in the South African population. This is analogous to familial hypercholesterolemia in the South African Afrikaner population in which multiple founder mutations were observed (Vergotine *et al.*, 2001). Genotype-phenotype comparative studies between the two South African FSHD family groups will shed light on the significance of the ancestral origins of FSHD in the South African population.

Due to elucidation of some aspects of the molecular basis of FSHD in the South African population, the relationship between the five families investigated could be determined. Individual 15-31 was found to be a compound heterozygote with regard to the two FSHD-associated genotypes that segregated in the South African families investigated. Individual 15-20, the mother of 15-31, from family F10 represents the link between the two groups of South African families, namely groups 1 and 2, as indicated in Figure 5.1 (Olckers *et al.*, 2001).

Figure 5.1: Relationship between five FSHD families investigated in this study



a = Family F10 presented in Figure 4.12, b = Family F30 presented in Figure 4.15, c = Family F40 presented in Figure 4.16, d = Family F20 presented in Figure 4.14, e = Family F60 presented in Figure 4.17.

5.4 FUTURE DIRECTIONS IN FSHD RESEARCH

At the time of analysis the *Xap* I analysis had not yet been reported. In cases where no definitive diagnosis could be made, for example individuals 40-10, 40-27 and 40-27, (as discussed in paragraph 4.4.4) the utilisation of *Xap* I analysis might assist in the characterisation of the genetic defect. The translocation frequency between the 3.3 kb repeats on chromosomes 4q35 and 10q26 in the South African FSHD population and in the general population will in the future be investigated via the utilisation of the *Bgl* II/*Bln* I dosage test.

Calculation of LOD scores was not an aim of this study. It could be argued that statistical evidence might be necessary given the various cases of phenotype-genotype discordance that were observed in this study. However, the discordance was primarily observed in young individuals under the age of 20, and the presence of DNA rearrangements due to

the deletion of 3.3 kb repeat units at the D4Z4 locus, has been demonstrated to be causative of FSHD. For these reasons, the calculation of LOD scores may only become an aim of this study after the re-evaluation of these individuals by a specialised clinician.

In the future a phenotype-genotype correlation study needs to be performed on clinically well characterised individuals. As mentioned previously, the clinical diagnosis of FSHD is very difficult due to extreme clinical variability of the FSHD phenotype. It will be essential to get one clinician, specialised in FSHD diagnoses, to clinically evaluate all individuals in order to exclude clinical bias. In addition the aspect of anticipation has to be addressed. As mentioned in paragraph 2.1.2.4, a shortcoming of all the papers reporting anticipation in their populations was that they investigated only affected parent-offspring pairs, thus only two generations. It is foreseen that the extended FSHD families in the South African population will enable the investigation of anticipation over several generations, most likely substantiating the study reported by Flanigan *et al.* (2000).

Results generated in this study indicated that it was extremely useful to have utilised both haplotype and Southern blot analyses in our elucidation of the molecular basis of FSHD in the South African population (Van der Merwe *et al.*, 1999; Olckers *et al.*, 2000). The combination of haplotype and Southern blot analysis definitively identified the relationship between the families investigated. Moreover, haplotype analysis also supported the presence or absence of *Bln* I resistant deletion fragments in the families investigated. Future studies will reveal whether all South African FSHD families have only the two ancestral origins reported here. In addition, the detailed haplotype data generated via this study will allow comparison of the South African FSHD population with other populations. This will undoubtedly facilitate determination of the global ancestral origins of FSHD.

Several international groups have made significant advances in FSHD research since the identification of the FSHD associated DNA rearrangements at the D4Z4 locus in 1992. This study represents the first attempt to unravel the molecular basis of FSHD in the South African population. Only via collaboration across several disciplines will we be able to provide effective support to those affected by this complex and highly variable phenotype. To date several structures and resources including a molecular diagnostic service, an FSHD clinic, a physiotherapy clinic and a specific support group have been put in place to support individuals affected by FSHD. Identification of the dual Founder Effect of the FSHD phenotype in the South African population paved the way for an effective molecular diagnostic FSHD service in this population.