



ORIGINAL RESEARCH

Counseling and prenatal diagnosis in facioscapulohumeral muscular dystrophy: A retrospective study on a 13-year multidisciplinary approach

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Abstract

Background and Aims: This is the first national population-based report about prenatal diagnosis for families with a history of facioscapulohumeral muscular dystrophy (FSHD), a complex hereditary disease. The incomplete disease penetrance and the phenotypic heterogeneity observed in carriers of D4Z4 alleles of reduced size, the FSHD molecular hallmark, make the estimate of genetic risk problematic.

Methods: We considered all requests of preconception counseling and prenatal diagnosis received between January 2008 and December 2020 by the genetic counseling service associated with the Italian National Registry for FSHD (INRF). A multidisciplinary team managed the clinical and molecular data of each family.

Results: Between 2008 and 2020, 60 couples required preconception counseling (PC) for FSHD. In 52 couples was observed at least one partner carried a D4Z4 reduced allele (DRA). Out of these 52 couples, 47 had a follow-up visit routine yearly. Out of these 47, 26 (55.3%) couples had children: eight asked for prenatal diagnosis (PND), two had assisted reproduction by heterologous in vitro fertilization (IVF), and 16 did not require further assistance. Regarding PND, 50 prenatal analyses were performed for 36 couples. The test resulted positive in 27 pregnancies, 12 (44.4%) were terminated, and 15 (55.6%) were carried to term.

Conclusion: The different choices made by the couples show the importance of an integrated approach to support genetic counseling for FSHD. These results remark the relevance of the clinical and molecular investigation of the extended family, preferably before conception.

KEYWORDS

chorionic villus sampling, complex genetic disorder, fetal medicine and diagnostic procedures, genetic counseling, prenatal diagnosis

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1 | INTRODUCTION

1.1 | FSHD: Clinical and molecular features of a complex disease

Facioscapulohumeral muscular dystrophy (FSHD, OMIM 158900) is the third most common hereditary myopathy, with an estimated prevalence of 1 in 20,000.¹ Since the first case was described, FSHD was considered an autosomal dominant disorder, with the typical onset and 95% penetrance by the age of 20.² However, FSHD epidemiology shows that the disease course is often unpredictable, ranging from nearly asymptomatic subjects who remain stable in years to patients who become wheelchair dependent. On the low-severity edge of the spectrum, mild-affected patients often have a slight weakness of facial or shoulder, or upper arm muscles, and they may not seek medical assistance until the symptoms become disturbing in everyday life. Age at onset is highly variable, ranging from early onset cases in which disease symptoms appear before 5 years of age,^{3,4} to individuals aged over 70 years with mild muscle weakness or no symptoms.^{5–7} Moreover, large family studies show the reduced penetrance of disease.^{8,9}

FSHD has been associated with the reduction of tandemly arrayed 3.3 kb repeats, named D4Z4, located at the distal end of chromosome 4q, 4q35.¹⁰ There is a second form of FSHD, named FSHD2, in which patients do not carry DRA, instead they might carry mutations in genes that influence the chromatin structure of the FSHD locus at 4q35.¹¹ For almost three decades, the diagnosis of FSHD has been supported by the detection of one D4Z4 allele with a reduced number (<11, alleles ≤41 kb) of D4Z4 repeats at 4q35.¹⁰ However the genetic epidemiology shows that (1) 3% of healthy people from the general population carry one D4Z4 Reduced Allele (DRA)^{12–14}; (2) 2% of healthy subjects carry one DRA combined with the permissive 4A polyadenylation signal (PAS) haplotype¹⁵; (3) 5%–10% of FSHD probands do not carry DRA, and (4) none of the various 4q haplotypes were exclusively associated with the presence of disease in large FSHD studies on families.⁹ These findings generated a degree of uncertainty about the predictive value of detecting a DRA in prenatal diagnosis and make genetic counseling essential in families in which FSHD is present.

In the last 50 years, prenatal diagnosis (PND) procedures have been developed in close association with genetic counseling. As the understanding of many genetic conditions has increased, prenatal tests have been offered to women with a high-risk pregnancy, and nowadays more and more women require some type of counseling even with low risk.¹⁶ As for FSHD, there are two main options for prenatal testing: chorionic villus sampling (CVS), performed between 11 and 13 + 6 weeks of pregnancy, and amniocentesis, performed after 15 weeks of pregnancy, both followed by Southern blot analysis.¹⁷ Preimplantation genetic diagnosis (PGD) based on single nucleotide polymorphisms (SNPs) or microsatellites centromeric to the D4Z4 repeat can be offered, but it is associated with a high risk of

misdiagnosis and needs to be followed by prenatal diagnosis confirmation.^{18,19}

Here, we present the reproductive decisions and the uptake of prenatal diagnosis for FSHD resulting from an integrated multidisciplinary process conducted by the Italian National Consulting Center for FSHD (INCCF) in Modena, Italy in the period 2008–2020.

2 | PATIENTS AND METHODS

2.1 | Setting and clinical evaluation

The Miogen Laboratory of the University of Modena and Reggio Emilia is the reference diagnostic laboratory of the Italian Clinical Network for FSHD (ICNF). It accrued DNA samples of over 3300 DRA carriers including 1634 probands from all over Italy. The ICNF is composed of 14 clinical centers, including expert neurologists and one diagnostic laboratory. The Italian National Registry for FSHD (INRF) is also located at the University of Modena and Reggio Emilia, thus allowing molecular and clinical data to be stored, updated, and investigated in the same location. Since 2016, the clinical evaluation of each individual who undergoes molecular analysis is performed with the systematic use of the Comprehensive Clinical Evaluation Form (CCEF), a validated tool of proved inter-rater reliability, which provides well-defined clinical categories and a wide range of typical and atypical features.²⁰ The CCEF separates individuals in four categories based on the clinical phenotype as follows: category A, including subjects with facial, scapular, and humeral weakness, typical FSHD features; category B, representing subjects with weakness limited to scapular and humeral (subcategory B1) or facial (subcategory B2) muscles; category C, describing subjects with no motor impairment; and category D comprising subjects with atypical FSHD, including uncommon features with or without facioscapulohumeral muscular weakness. The severity of the muscular impairment is measured using the FSHD score, a scale from 0 to 15, the clinical category is assessed after a detailed analysis of patient's phenotype on the basis of the CCEF evaluation protocol.²¹ Patients who receive a score ranging between 5 and 10 display moderate to severe disease, with limited ability to lift their arms, to completely close eyes, smile or protrude lips, and to walk on their tiptoes and/or heels. Patients with a general impairment of all muscle groups, associated with the loss of ability to walk unaided, receive a score ranging between 11 and 15.

2.2 | Multidisciplinary team

Preconception and prenatal counseling were given by a multidisciplinary team, composed of a medical geneticist, a neurologist, a gynecologist, and a biologist who performed prenatal analysis.²² They all contributed and discussed together each case, to guarantee the best service and the most adequate planning for each couple.

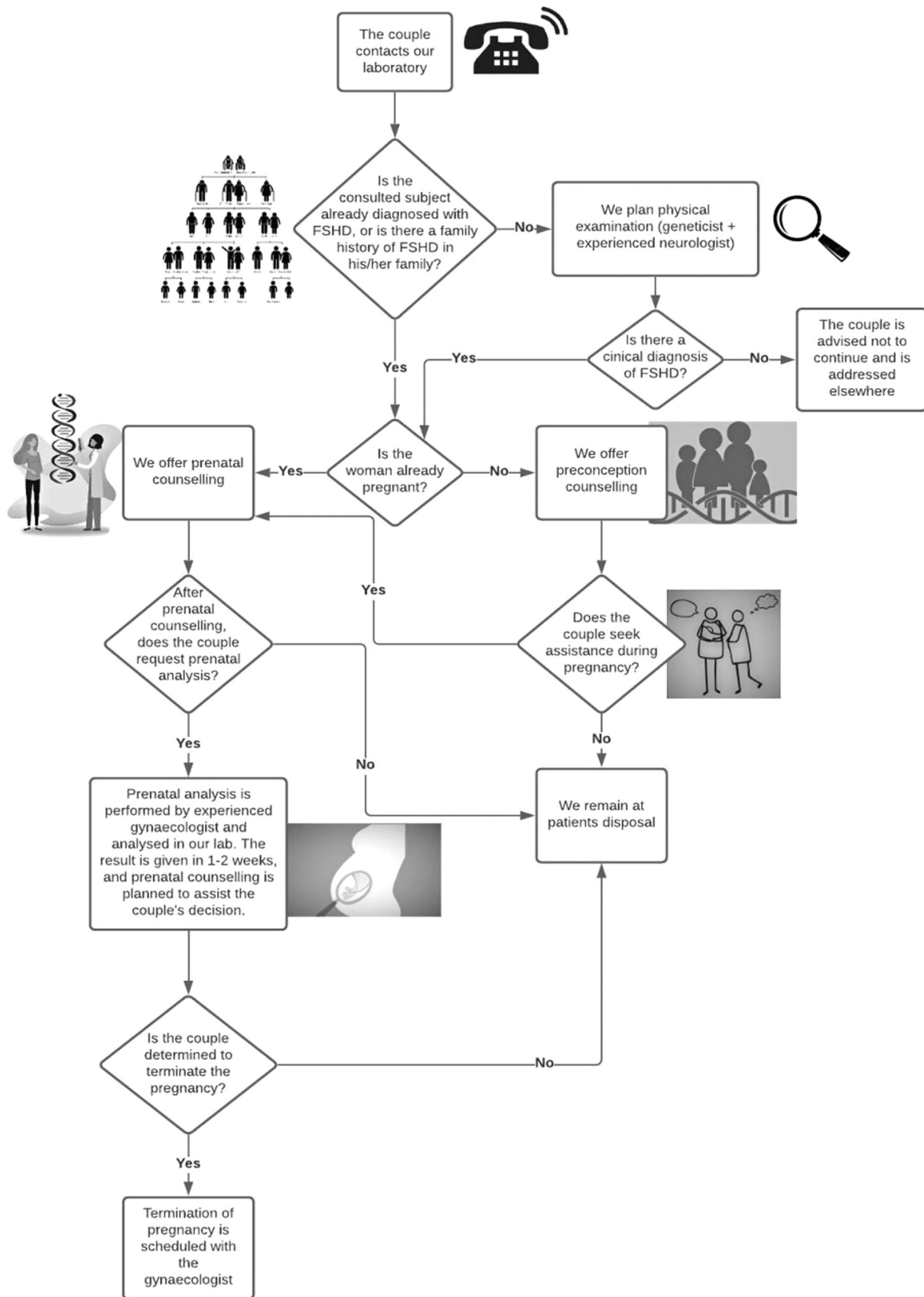


FIGURE 1 Counseling processes in preconception and prenatal diagnosis for FSHD. FSHD, facioscapulohumeral muscular dystrophy

2.3 | Counseling procedure

Prenatal counseling was not always performed in Modena, as CVS was sent to the Miogen laboratory by various gynecologists and health professionals. Starting in July 2009, prenatal genetic counseling was carried out with an integrated procedure at the Policlinico Hospital in Modena. It was accompanied or preceded by molecular investigation and clinical examination of all the available members of the family. Figure 1 summarizes the flow of the FSHD consultation process at the INCCF. Couples contact the laboratory for an appointment. PC is proposed to couples that contemplate pregnancy, whereas prenatal counseling is arranged for couples in which the woman is already pregnant (the terms “preconception counseling” and “prenatal counseling” will be used hereafter with these meanings respectively). The clinical team composed of a medical geneticist and a neurologist with experience in neuromuscular disorders work together and discuss the case. In all cases extended clinical investigation and molecular analysis of the D4Z4 locus of all the available family members are suggested to define disease penetrance and the phenotypic spectrum among carriers in the family of the at-risk consultant. Since 3% of the general population carries D4Z4 reduced alleles, molecular analysis, and clinical evaluation is always performed in the consultant's partner.¹⁵ If the woman is pregnant, the possibility of termination of pregnancy are discussed, and the will of the couple is clarified, with the help of genetic counseling. If the couple expresses the will to proceed with molecular analysis of the D4Z4 locus, the first examination with embryonic ultrasound is performed on the same day at the center for Prenatal Medicine of Gynecology and Obstetrics Department, Policlinico Hospital, Modena. An explicative report of the genetic counseling is written and sent to the couple, according to the European recommendations for reporting results of diagnostic genetic testing²³ together with a detailed summary of the family history, analysis of FSHD penetrance in the family and description of the observed phenotypes in light of the most recent epidemiological studies.^{7,9,24–30} The genotype-phenotype correlation in each single-family is based on the CCEF clinical categories observed in DRA heterozygotes in combination with the DRA size, to provide an estimation of the risk of disease in offspring. Chorionic villus sampling (CVS) is scheduled promptly based on the pregnancy stage with facilitated access to the service. CVS is performed by a specialist in maternal-fetal medicine in Policlinico Hospital, Modena, following the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) guideline. An informed consent explicating risk and margin of error related to the procedure is signed before sampling. Molecular analysis is carried out as described below and results are given in 7–14 days, with genetic counseling. In case the couple decides to terminate the pregnancy, an appointment is immediately scheduled to carry out the procedure. In case the woman expresses conflicting wills, a psychiatric consult is arranged with an expert psychiatrist. During each step, the team of the INCCF was available for any questions or concerns of the patients (Figure 2). All the performed analyses are covered by the Italian National Health System.

2.4 | Villus sampling

Before the procedure, an ultrasound examination is performed to determine fetal viability, the crown-rump length (CRL), and the position of the uterus and trophoblast. The procedure is performed under continuous ultrasound guidance with a Voluson E8 and Voluson E 10 ultrasound machine (GE Medical Systems, Kretz Ultrasound). A double-needle technique 17/19G with a fixed guide in a sterile field, under ultrasound guidance, is used. The 17G needle is introduced quickly through the maternal abdominal wall and across the myometrium until the tip reached the borders of the chorion frondosum.^{31,32} A 19G needle is inserted through the first needle into the trophoblast. The internal needle is then connected to a 30-ml syringe that contained 5 ml of culture medium and a vacuum was created. The 19G needle is then moved slowly backward and forwards within the long axis of the chorion and samples are aspirated by an assistant.

2.5 | Molecular analysis

For the molecular analysis of the D4Z4 locus at the first trimester, 30–60 mg of CVS biopsy is required. For the molecular assessment at least three individuals (affected parent, not affected parent, and CVS biopsy) and, whenever possible, grandparents are analyzed.

High molecular weight DNA is obtained from the chorionic villus sampling and from 10 ml of fresh blood sample of each parent, and relatives resulting informative for the PND. Because of the high homology between 4q35 and 10q26, the chromosomal origin of D4Z4 repeat arrays is based on EcoRI/BlnI and XapI chromosomal DNA digestion. This procedure increases the procedure specificity for FSHD diagnosis, which varies between 94% and 100%.³³ After DNA enzymatic digestion and separation by pulsed-field gel electrophoresis (PFGE), southern blot analysis and hybridization with the radiolabelled p13E-11 probe are performed. Restriction fragments are detected by autoradiography. To establish the precise size of the DRA, EcoRI, EcoRI/BlnI, and XapI digested genomic DNA of the villus and other family members, are electrophoresed on a 0.4% agarose gel and analyzed by Southern hybridization with the probe p13E-11 and detected by autoradiography. Haplotypes of the FSHD locus at 4q are assessed using D4S139 and D4S163 markers proximal to the D4Z4 repeat.

2.6 | Communication of results

The analysis result was given to each couple in 1–2 weeks at maximum. A report of the genetic counseling was written, according to the European recommendations for reporting results of diagnostic genetic testing,²³ together with a detailed summary of the family history, FSHD penetrance in the family, and associated phenotypes.

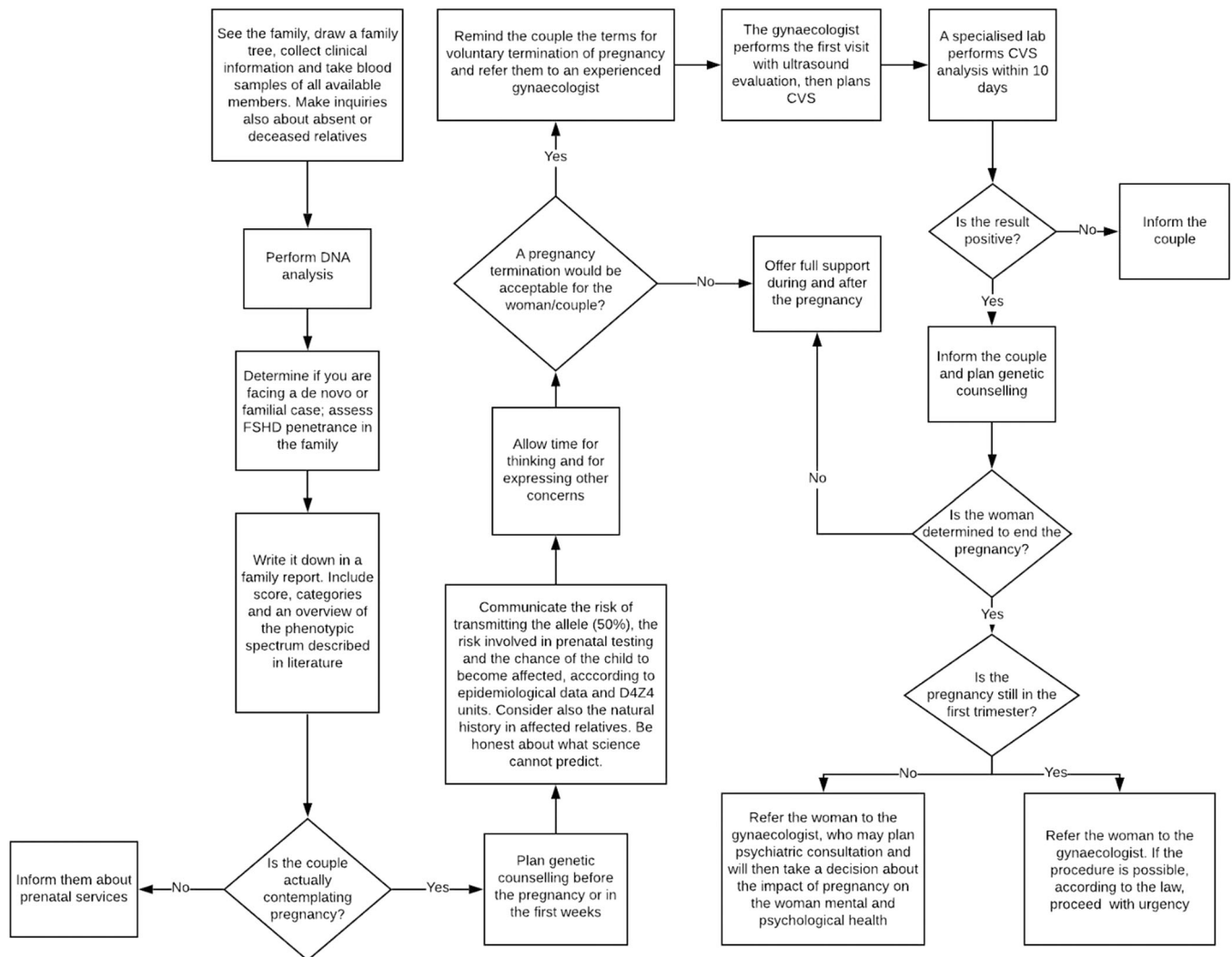


FIGURE 2 Genetic counseling procedure with a multidisciplinary approach.

2.7 | Follow-up

During routine follow-up visits performed every year, the couples were asked whether they had children after preconception/prenatal counseling procedures, and data were used for the purpose of this paper. The follow-up visits took place at the centers of the ICNF.

2.8 | Ethical approval

Ethical approval was not required for this study. All individuals involved gave informed consent for data publication.

3 | RESULTS

From 2008 to the end of 2020, 94 couples belonging to 91 unrelated families requested consultation at the INCCF: 60 couples from 58 unrelated families came for a genetic consultation concerning their

reproduction choices and to be informed about the risks of having children affected by FSHD (PC), while 36 couples belonging to 33 unrelated families requested prenatal diagnosis. The reasons were various: (1) because one partner was affected; (2) because there were one or more people with FSHD in one partner's family; and (3) because a DRA segregates in one partner's family.

In the same period, 1003 DRA heterozygotes born between 1962 and 1990 were assessed by molecular analysis. Of these 52 (5.2%) requested a PC.

3.1 | Preconception counseling

From January 2008 to December 2020, 60 couples with a history for FSHD (two couples are part of two already existing families) requested a PC at the INCCF (Table S1). The molecular analysis was extended to all the family members recruited for the family study. Overall, molecular analysis was extended to 315 relatives, of which 158 resulted in DRA heterozygotes, and 157 carried D4Z4

alleles of normal size. Among the 60 couples, which came for PC, 8 (13,3%) were excluded from this study because molecular analysis showed that both partners did not carry a DRA. Of the remaining 52 couples, belonging to 50 families, 27 males and 25 females carried a DRA. Among these couples, 23 subjects (44.2%) presented the classical FSHD phenotype (CCEF category A), 8 (15.4%) displayed an incomplete phenotype (CCEF category B), 5 (9.6%) showed an FSHD phenotype with atypical features (CCEF category D), and 16 individuals (30.8%) had no muscle weakness (CCEF category C).

Out of 52 couples, 47 had a follow-up clinical evaluation every year at the centers of the INCCF and five couples did not have a follow-up visit. Figure 3 shows that 26 out of 47 couples (55.3%) had at least one child, and 21 couples (44.7%) did not have children after the PC. Three couples asked for counseling after having had children. Among the 26 couples who had children, 8 (30.8%) asked for prenatal diagnosis (PND), 16 couples (61.5%) did not request PND, and two couples (7.7%) had assisted reproduction by heterologous in vitro fertilization (IVF). At the time of the follow-up, 21 couples had no children.

Results presented above show that after PC different choices were taken by couples carrying a DRA. As FSHD displays wide clinical variability, we tested whether reproductive choices were based on the clinical status of the consultant. As shown in Figure 3, of the 26 couples who had children, 23 (92%) partners carrying one DRA were clinically affected. Their phenotypes ranged from the classical FSHD phenotype (category A) to incomplete phenotype (category B) or complex phenotype (category D). Prenatal diagnosis was requested by 8 couples, 16 couples did not request any prenatal diagnosis, and 2 couples had heterologous IVF. Of the 21 couples, who had no children, five carrier partners had classical FSHD phenotype, four had

incomplete phenotype, one had a complex phenotype, and 11 were healthy (category C).

We also considered the distribution of clinical categories in families of couples in which at least one partner carried a DRA. Figure 4A shows that in 39 families (78.0%) there was at least one subject presenting the classical FSHD phenotype (category A), in 23 families there was at least one individual assessed with category B (seven families had also one subject with a complex phenotype), in nine families there was at least one member with a complex phenotype, and in two families all the members evaluated were healthy.

3.2 | Prenatal diagnosis

From January 2008 to December of 2020, 49 CVS and one amniocentesis were performed for FSHD PND. The 50 requests were received from 36 different couples. Of these, eight requested prenatal diagnosis after the PC (Table S1). The 36 different couples belonged to 33 unrelated FSHD families. Six couples were part of three different families (in two cases the affected mothers were sisters, in one case the consultants were the affected brother and then his wife, and the affected sister and his husband, as described in Table S2). Ten couples requested prenatal analysis more than once, from two to five times. Familial cases were 33, while three probands carried a de novo mutation. Out of 36 couples, 24 females were DRA heterozygotes. Molecular analysis was extended to all available family members and resulted in 80 DRA heterozygotes and 108 with D4Z4 alleles in the normal size range.

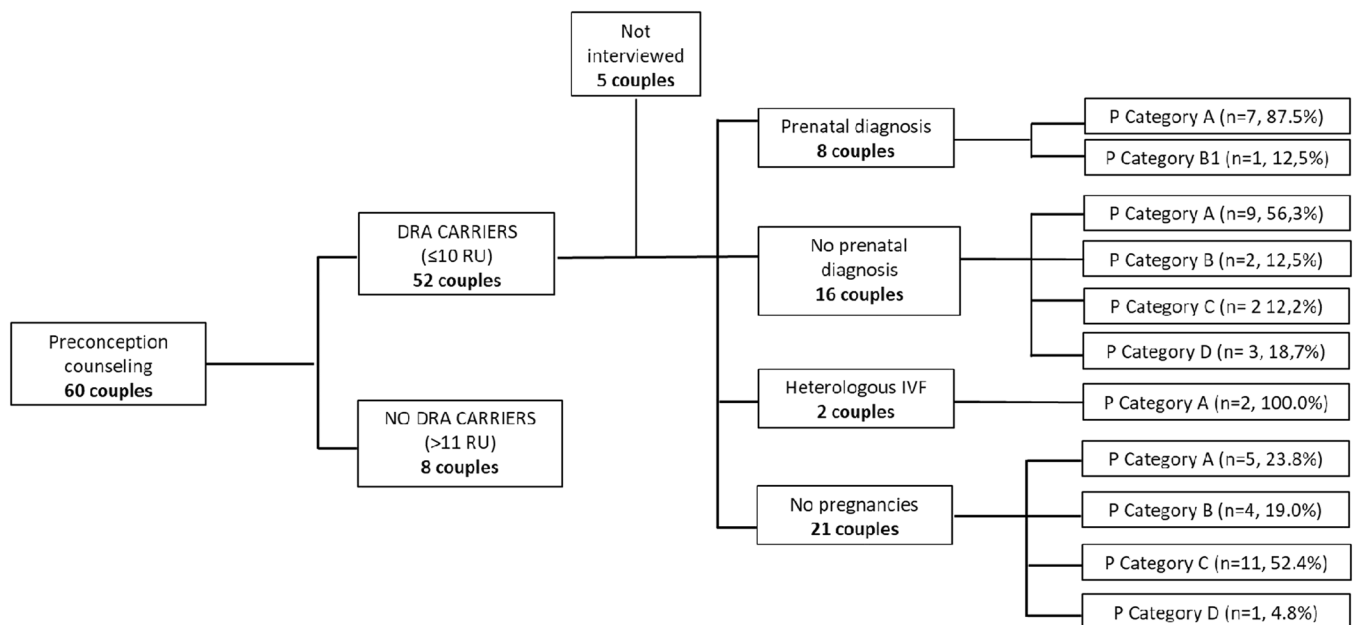


FIGURE 3 Summary of reproductive decisions. Results of the survey on couples who had received preconception counseling, considering the clinical status of the consultant carrying DRA. DRA, D4Z4 reduced allele; IVF, in vitro fertilization

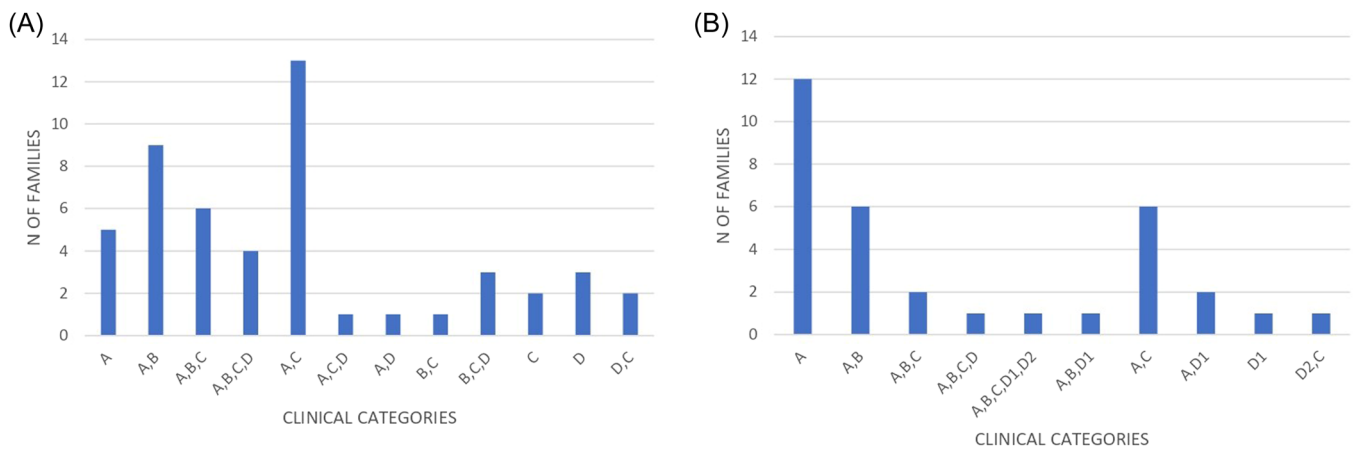


FIGURE 4 Distribution of clinical categories in families of couples requiring prenatal diagnosis. (A) Shows the clinical categories of DRA carriers of families requiring preconception counseling, while (B) shows the clinical categories of DRA carriers of families requiring prenatal diagnosis. DRA, D4Z4 reduced allele

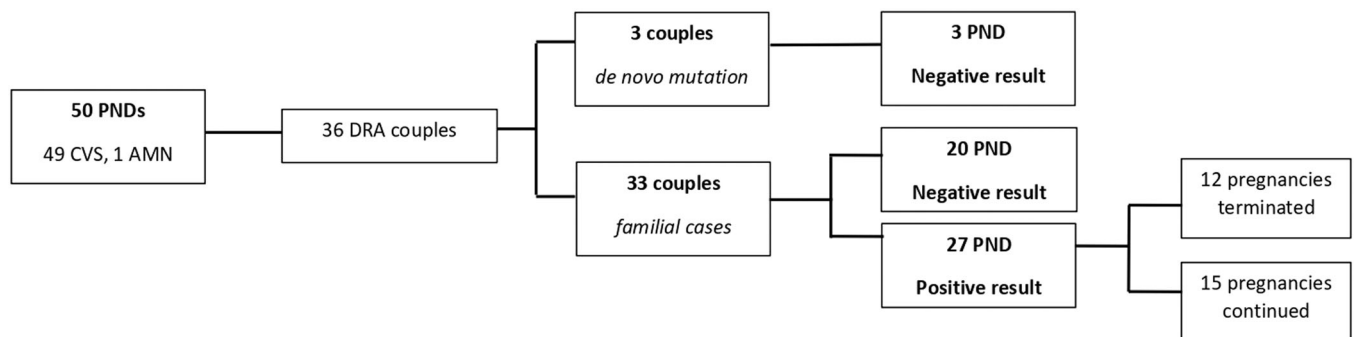


FIGURE 5 Summary of prenatal diagnosis results.

PND revealed the presence of DRA in 27 samples out of 50. Of these 27 pregnancies, 12 were terminated (44.4%), and 9 before 12 + 6 weeks (Figure 5). In three cases terminations was performed at 15 + 6, 15 + 4, and 15 + 5 weeks respectively, in accordance with Italian law. One was a bichorial-biamniotic twin pregnancy. Selective termination of the DRA heterozygote was performed, the child who tested negative was then born healthy. No one of the patients had a spontaneous abortion after the procedure (chorionic villus sampling or amniocentesis).

We investigated whether in families requiring PND there is a clear presence of the disease and that moderate to severe phenotypes occur in at least one generation. We measured the degree of muscle impairment using the FSHD scale and used the FSHD score as an indicator of clinical severity. The score of the affected parent in our cohort was on average 4.92, ranging from 1 to 11; consultants with score below 4 had at least one first- or second-degree relative (parents, siblings, and cousins) with FSHD score higher than 5. There was no significant difference in the FSHD score between parents who terminated the pregnancy after a positive PND result and parents who did not terminate the pregnancy (data not shown). As shown in Figure 4B, the majority of families had at least

one member assessed as category A (93.9%), but in 13 families also complex phenotypes and/or asymptomatic carriers were present. In the three cases in which termination of pregnancy after the 12th week was performed, not all the affected parents showed severe phenotypes, but the families have at least one member with FSHD score above 9.

4 | DISCUSSION

Genetic counseling and PND for FSHD have been available since 1993. Since then, no systematic reports have been published regarding the rate of uptake of PND in FSHD. This is the longest-running study reporting national rates of prenatal diagnosis for FSHD. In 2008, the INCCF has established an integrated approach for genetic counseling and PND to provide accurate prenatal assistance to couples from families in which FSHD is present. This is because several clinical evidence make establishing the predictive value of a positive molecular test challenging.

The genetic counseling carried out by the INCCF is based on the epidemiological studies conducted on the Italian population.

As summarized in Table 1, these studies drew light on the genotype-phenotype correlation in FSHD families and represent a point of reference for medical geneticists. Several elements are taken into account when providing genetic counseling to FSHD couples: (1) the size of the DRA, (2) the degree of kinship in relation to the probands, (3) the age at onset, (4) the clinical phenotypes as defined by the CCEF, (5) the disease clinical severity defined by the FSHD score, (6) the sex of the DRA carrier, and (7) the penetrance in the family. Each consultation is tailored to the molecular and clinical information regarding the DRA carrier partner and her/his family on the basis of percentages reported in Table 1.

In the period 2008–2020, of 1003 DRA carriers born between 1962 and 1990, 52 (5.2%) requested a PC. This data looks similar to the percentage of PND and PGD performed for Huntington disease (HD) in the UK, which was 3.0% of the at-risk pregnancies in the year 2015 (estimated on the prevalence and incidence of HD).³⁴ It must be considered that, in most cases, HD becomes overtly symptomatic between the ages of 30 and 50 years,³⁵ often after the reproductive age, and that also FSHD may have a tardive onset.⁹ It is also possible that, in the case of HD, the certainty of the molecular test for this fatal illness with complete penetrance might discourage reproductive decisions for all the connected implication. In the case of FSHD, it is possible that the relatively low severity of the disease might not interfere with the couple's reproductive decision or that the insidious disease onset and progression, together with the uncertain predictive

value of the molecular marker for FSHD diagnosis, discourage patients in considering PND to guide reproductive decisions.

Overall, our analysis indicates that the information given during the PC allowed the couple to take a personal, fully informed decision. In fact, 26 couples had at least one delivery and, of these, eight requested a prenatal diagnosis as well and two resorted to IVF. The remaining 16 decided to have normal deliveries without prenatal testing. Interestingly, half of those couples with one partner presenting a classic FSHD phenotype who decided to have children did not request PND. Instead, a vast majority (84.6%) of the DRA heterozygotes with no muscle impairment (category C phenotype) had no children after the PC (the average age of the consultants was 36.9).

Prenatal diagnosis could be offered to all FSHD couples. In our study, 55.6% of pregnancies with a positive test were continued. This percentage is far distant from that observed in the case of the HD.³⁴ In a UK study, 9.8% of the HD pregnancies with a positive test were continued. The different reproductive decisions made in the two diseases most likely depend on the clinical heterogeneity and reduced penetrance of FSHD in comparison with HD.

We also observed that the rate of prenatal genetic diagnoses for FSHD is rising in the last years (Figure 6). This growth in the rate of prenatal tests indicates increased awareness about the risks associated with the disease. To address these needs we consider the clinical evaluation based on the CCEF protocol associated with the family study fundamental.

TABLE 1 Epidemiological genotype-phenotype studies conducted in FSHD families

General assumption	Supporting data	Reference
Considering the cohort of relatives carrying the DRA, they result less affected than probands	32.2% of all relatives (irrespective of the allele size) do not show any functional impairment	Ricci et al. ⁹
First-degree relatives carrying the DRA are more affected than distant relatives	27.5% of first-degree relatives are healthy, against 47.1% of second-degree relatives	Ricci et al. ⁹
Penetrance and severity of the disease (both in probands and in relatives) are inversely correlated with DRA size. 1–3 DRA carriers have on average, lower age at onset and increased severity if compared to larger deletion carriers	Penetrance in relatives is, at the age of 20, 64.3% for 1–3 DRA carriers, 21.8% for 4–6 DRA carriers, and 19.6% for 7–8 U carriers; at the age of 50, penetrance is respectively 88.7%, 55.0%, and 55.7%. In 9–10 U the overall penetrance is 40%. 40% of relatives carrying DRA with 1–3 units are severely affected (FSHD score 57) by age 30. In contrast, no relatives carrying DRA with 4–8 units had an FSHD score higher than 6 in this age window	Ricci et al. ⁹
Females have a later age of onset, and female relatives are less severely affected than male relatives	Male relatives had a significantly higher mean FSHD score (5.4 vs. 4.0, $p = 0.003$) and they developed motor impairment on average 7.3 years before than females ($p = 0.003$)	Zatz et al. ³⁰ ; Ricci et al. ⁹
The typical phenotype (A category), which has a steeper progression than the other clinical categories, is more represented in index cases than in relatives	Clinical category A was much more represented in index cases than in carrier relatives [115 (81%) vs. 37 (35%), respectively]. Whereas the incomplete phenotype (clinical category B) was more frequent in carrier relatives than in index cases (25% vs. 6%) ($p < 0.001$); In the cohort of 7–8 DRA carriers, 52.9% of probands and 10.0% of relatives displayed the classic FSHD phenotype	Vercelli et al. ²⁶ ; Ruggiero et al. ⁷

Abbreviations: DRA, D4Z4 reduced allele; FSHD, facioscapulohumeral muscular dystrophy.

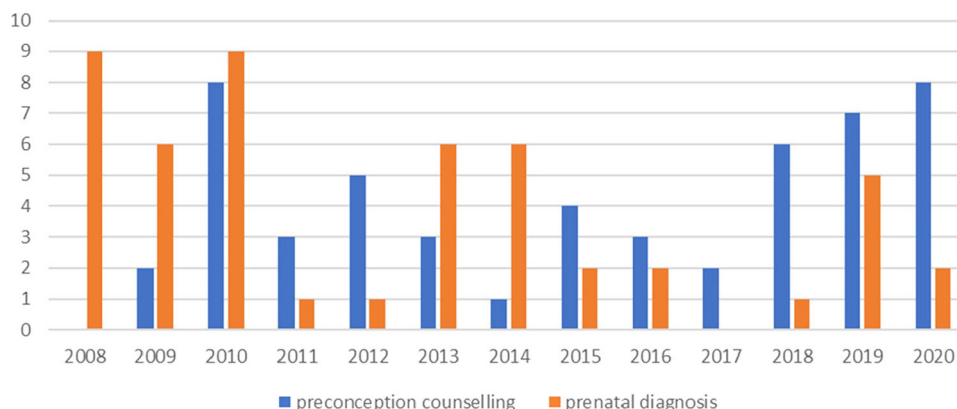


FIGURE 6 Trend in services for FSHD couples, years 2008–2020. Requests for preconception counseling have been increasing in the last years. FSHD, facioscapulohumeral muscular dystrophy

5 | STUDY LIMITATIONS

To date, this is the largest study about preconception counseling and prenatal diagnosis for FSHD couples; however, the numerosity of the cohort can still be increased, and our findings might not be representative of the entire cohort of FSHD patients in Italy. Also, the picture that we outlined in this paper might not reflect the path of genetic counseling and prenatal diagnosis in other health systems.

6 | CONCLUSION

A multidisciplinary approach is needed to offer reproductive counseling for complex genetic diseases. A personalized and detailed consultation can influence the choices of two future parents to procreate. The information received, statistically supported by data collected over the years from a rare disease registry, made the pre-conceptual counseling service valuable.

AUTHOR CONTRIBUTIONS

Maria Francesca Di Feo: Conceptualization; data curation; validation; writing—original draft; writing—review & editing. **Cinzia Bettio:** Conceptualization; data curation; writing—review & editing. **Valentina Salsi:** Formal analysis. **Emma Bertucci:** Conceptualization; writing—review & editing. **Rossella Tupler:** Conceptualization; data curation; supervision; writing—review & editing.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

ETHICS STATEMENT

The INRF database was approved by the Provincial Ethics Committee of Modena (2712/CE). Informed written consent was obtained from all study participants, in accordance with the ethical standards of the 1964 Declaration of Helsinki. All authors have read and approved the final version of the manuscript. Prof Rossella Tupler had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis. Prof Rossella Tupler affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

TRANSPARENCY STATEMENT

Prof Rossella Tupler affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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