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FSHD type 2 and Bosma arhinia microphthalmia syndrome: two faces of the same mutation

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List of abbreviations

FSHD – facioscapulohumeral muscular dystrophy

BAMS – Bosma arhinia microphthalmia syndrome

SMCHD1 - structural maintenance of chromosomes flexible hinge domain containing 1

Abstract

Objective: This study aims to determine whether congenital arhinia/Bosma arhinia microphthalmia syndrome (BAMS) and facioscapulohumeral muscular dystrophy type 2 (FSHD2), two seemingly unrelated disorders both caused by heterozygous pathogenic missense variants in the *SMCHD1* gene, might represent different ends of a broad single phenotypic spectrum associated with SMCHD1 dysfunction.

Methods: We examined and/or interviewed 14 FSHD2 patients and 4 unaffected family members with N-terminal *SMCHD1* pathogenic missense variants to identify BAMS sub-phenotypes.

Results: None of the FSHD2 patients or family members demonstrated any congenital defects or dysmorphic features commonly found in patients with BAMS. One patient became anosmic after nasal surgery and one patient was hyposmic; one man was infertile (unknown cause) but reported normal pubertal development.

Conclusions: These data suggest that arhinia/BAMS and FSHD2 do not represent one phenotypic spectrum and that *SMCHD1* pathogenic variants by themselves are insufficient to cause either of the two disorders. More likely, both arhinia/BAMS and FSHD2 are caused by complex oligogenic or multifactorial mechanisms which only partially overlap at the level of *SMCHD1*.

Introduction

Identical pathogenic variants in the *'structural maintenance of chromosomes flexible hinge domain containing 1' (SMCHD1)* gene are associated with two seemingly unrelated disorders: facioscapulohumeral muscular dystrophy type 2 (FSHD2)[1], a rare form of adult onset muscular dystrophy, and arhinia, a severe congenital malformation often accompanied by reproductive and ocular defects, a triad called Bosma arhinia microphthalmia syndrome (BAMS).[2-4]

FSHD2 has a complex etiology that involves *SMCHD1* (18p11.32) and the D4Z4 macrosatellite repeat array (4q35).[1] Loss of SMCHD1 repressive activity leads to partial relaxation of the D4Z4 chromatin structure and de-repression of the normally suppressed *DUX4* retrogene in the D4Z4 unit. Only specific 4q35 haplotypes provide a poly-adenylation signal (*DUX4*PAS) that stabilizes the DUX4 mRNA, permitting translation of the myotoxic DUX4 protein.[1, 5] Contraction of the D4Z4 repeat array to 1-10 units can also relax the D4Z4 locus and de-repress DUX4 expression; this is the mechanism underlying the more common form of FSHD called FSHD type 1 (FSHD1).[5]

In contrast to FSHD2, where missense and loss-of-function variants are distributed along the entire *SMCHD1* locus, in BAMS patients, the variants are all missense and clustered within or immediately downstream of the ATPase domain.[2, 3] While D4Z4 hypomethylation akin to FSHD2 in BAMS patients suggests a loss-of-function mechanism[2], a gain of function mode of action has also been proposed.[3]

To date, only one patient with both arhinia and FSHD2 and one multiplex family with both conditions have been reported.[2] There has yet to be a systematic investigation of BAMS-associated features in FSHD2 patients. Therefore, we performed phenotypic and genotypic studies in FSHD2 patients and their family members with pathogenic missense variants in the N-terminal region of *SMCHD1* to identify potential areas of overlap.

Materials and methods

Patients

We identified 23 FSHD patients with heterozygous pathogenic missense variantsnear the ATPase domain of *SMCHD1* in the FSHD genetic database in the Human Genetics department of the Leiden University Medical Center. Family members of one patient were recruited through a cohort-study (FSHD-FOCUS study) by the Neurology department of the Radboud University Medical Center, Nijmegen. Another 10 sporadic cases were recruited for participation by referring clinicians from the USA, France, United Kingdom and the Netherlands.

Genetic testing

DNA was extracted from blood samples and analyzed for D4Z4 repeat size, chromosome 4q and 10q haplotypes, as described previously[6], and for *SMCHD1* pathogenic variants by Sanger sequencing.[1] CpG methylation at the D4Z4 repeat was determined by Southern blot and the methylation sensitive restriction enzyme FseI. Detailed protocols are freely available from the Fields Center website (www.urmc.rochester.edu/fields-center). The Delta1 score, a measure of the degree of D4Z4 hypomethylation, was calculated as described previously.[7] The Delta1 threshold for FSHD-associated *SMCHD1* pathogenic variants lies below -21%.

Clinical assessment

All participants were interviewed regarding nasal and olfactory abnormalities, pubertal development, fertility, eye anatomy and vision, history of maxillofacial surgery, and presence of cleft lip/palate. Photographs were available for 10 participants, which were independently assessed by three clinicians.

In addition, ten members of one FSHD family were examined in person for (subtle) signs of arhinia or associated comorbidities by a clinical geneticist (MK) who was blinded to mutation status. Olfactory function was assessed using the Sniffin' Sticks Screening Test (Burghart, Medizintechnik, GmbH, Wedel, Germany) which assigns a sex and age-adjusted olfactory score. One family member was examined using Skype.

Ethical approval

This study was conducted according to the principles of the Declaration of Helsinki (version October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO). Participants were consented under a protocol approved by the local ethics committee of the Radboud University Medical Center, Nijmegen.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Genetic results

In the large Euro-Caucasian FSHD family, 8 family members carried a pathogenic missense variant in *SMCHD1* (c.320T>C; p.Leu107Pro) (fig 1 and 2, table 1). Importantly this pathogenic variant was previously reported in an unrelated, African-American female with BAMS.[2] All pathogenic variant carriers showed profound hypomethylation at the D4Z4 locus on chromosome 4q with Delta1 scores below -26%.[7] The 5 affected individuals had the FSHD-permissive, 4qA haplotype that contains the somatic *DUX4* PAS.[6] In addition, four of them had a D4Z4 repeat array of 9 units, compatible with an additional molecular diagnosis of FSHD1[5], but also found in 1-2% of the Caucasian control population.[8-10] The three unaffected

individuals were homozygous for the 4qB haplotype, which is not FSHD-permissive because of the absence of a somatic *DUX4* PAS. One family member that tested negative for the *SMCHD1* pathogenic variant, did carry a 9 unit repeat on a 4qA haplotype. Her Delta1 score was -9%.

We identified 23 other sporadic FSHD2 patients in the FSHD genetic database with a pathogenic missense variantin close proximity to or identical to those previously identified in patients with arhinia or BAMS ([2, 3], Shaw, unpublished observation). All FSHD2 patients had a permissive haplotype and D4Z4 hypomethylation (table 2). Seventeen of the 20 pathogenic variants in these FSHD2 patients involved the same *SMCHD1* exon as in arhinia patients and three pathogenic variants were identical to those found in arhinia patients (figure 3). We also identified one family with a heterozygous 3-bp deletion in exon 6 (c.729_731delCTT; p.Phe244del) resulting in the deletion of a single amino acid just two positions downstream of an amino acid affected in BAMS patients.

Clinical characteristics

In the large FSHD family, six individuals with an N-terminal SMCHD1 pathogenic missense variant were examined (individuals II:1 and II:4 were deceased at the time of the study). None of them had microphthalmia, congenital cataracts, coloboma, nasolacrimal duct atresia, mid-face hypoplasia, or cleft lip/palate (table 3). Several family members had narrow nares and/or hypoplastic alae nasi (rounded prominence of nostril) but these features did not segregate with the *SMCHD1* pathogenic variant, suggesting they were unrelated, familial traits. One family member with FSHD1 and 2 (II-3) developed anosmia shortly after surgery for a deviated nasal septum. A second affected patient with both FSHD1 and 2 (III-3) was hyposmic (Sniffin' Sticks Screening Test result below the 10th percentile). All family members who were questioned reported normal pubertal timing and denied infertility.

Four family members had symptoms of FSHD: the two older individuals (50 years and older) displayed severe muscle weakness and were wheelchair-dependent, whereas the two younger individuals had facial weakness, an early sign of FSHD. Three of them had both FSHD1 and 2, and one of the younger individuals only had FSHD1.

The 10 sporadic FSHD2 patients who were phenotyped did not have physical features consistent with arhinia/BAMS (table 4). One male reported normal pubertal development but had infertility of unknown etiology. He denied other signs of hypogonadism such as cryptorchidism or micropenis and had never required testosterone replacement. Photographs of this patient revealed no signs of a craniofacial defect.

Discussion

We assessed FSHD patients with pathogenic missense variants in the N-terminal region of SMCHD1, which were recently shown to cause arhinia/BAMS, to determine whether FSHD2 and BAMS might represent the opposite ends of one broad, phenotypic spectrum or if each condition is caused by SMCHD1 dysfunction in the presence of a genetic background unique to each condition. Only one patient with arhinia has been identified thus far who meets clinical and genetic criteria for FSHD2[2], and until now, FSHD2 patients had never been specifically assessed for BAMS-like features.

Detailed examination of a large FSHD family with an *SMCHD1* pathogenic variant identical to one found in BAMS patients did not uncover any congenital defects or dysmorphic features commonly found in patients with BAMS. We identified one patient in this family who developed cataracts in her 70's and lost olfaction after nasal surgery. These findings are unlikely to be related to BAMS as cataracts are very common with aging secondary to cumulative photo-oxidative insults (e.g. ultraviolet-B) and she did not have congenital anosmia as occurs in BAMS patients; rather she lost olfactory function after nasal surgery

which is a recognized, albeit rare, potential side effect of septoplasty.[11, 12] We also observed several family members with nasal hypoplasia. The power of our combined genetic and phenotypic approach, however, allowed us to confidently classify this phenotype as a familial rather than SMCHD1-related trait as it did not segregate with the *SMCHD1* pathogenic variant.

All other FSHD2 patients included in this study reported normal olfaction, no craniofacial or ocular abnormalities and normal pubertal development and those of reproductive age were fertile with the exception of one male patient with infertility of unknown cause. Thus, we find no evidence for phenotypic overlap in FSHD2 and BAMS patients.

The phenotyping protocol for this study was intentionally simple and non-invasive in design such that all study procedures could be performed by patients from afar. Although we performed detailed, structured interviews to collect phenotypic data on the sporadic cases, it is possible that patients were not fully aware of any subtle BAMS-associated features. Future studies will be required to confirm our findings in a larger number of FSHD patients using more sophisticated tools such brain imaging to assess the integrity of the olfactory bulbs and tracts, dilated eye exams, and reproductive hormone testing.

Our data support the hypothesis that arhinia/BAMS and FSHD2 represent two distinct oligogenic disorders. In both conditions, SMCHD1 dysfunction appears to be necessary but not sufficient to cause disease. In FSHD2, a permissive 4q35 haplotype is one known requirement, but the variability in muscle weakness that is seen among family members with the same *SMCHD1* pathogenic variant (and D4Z4 repeat size) suggests that there are other genetic or environmental modifiers yet to be discovered. Incomplete penetrance of *SMCHD1* variants in the form of nasal hypoplasia or isolated anosmia has also been observed in multiplex arhinia/BAMS families.[2] Modifier genes have not been identified in arhinia but SMCHD1 binding partners and/or downstream targets are rational candidates. Thus, in the

extremely rare chance that a patient has an N-terminal SMCHD1 pathogenic variant and meets the genetic requirements unique to arhinia/BAMS and to FSHD2, he/she can demonstrate both conditions.

Pathogenic variants in the N-terminal region of *SMCHD1* play a critical role in the pathogenesis of both FSHD2 and arhinia/BAMS and. The complete absence of phenotypic overlap between these two disorders, however, suggests that these variants are, by themselves, insufficient to cause either disorder. The current study instead supports an oligogenic or multifactorial disease mechanism for both FSHD2 and arhinia/BAMS.

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Tables

Table 1. Genetic characteristics of FSHD family members.

ID Fig 1	Sex	Age	4q35 locus				SMCHD1 variant and D4Z4 methylation (%)			At risk for FSHD
			4q_1 units	4q_1 haplotype	4q_2 units	4q_2 haplotype	FseI methylation	Delta1 methylation	SMCHD1	
II-1	F	†	20U	4B163	23U	4B163	**3%**	**-42%**	+/-	No
II-2	M	80y	27U	4A161	28U	4B163	58%	12%	+/+	No
II-3	F	75y	9U	4A161	20U	4B163	**4%**	**-33%**	+/-	FSHD1+2
II-4	M	†	9U	4A161	20U	4B163	n/a	n/a	+/-	FSHD1+2
II-5	M	†	20U	4B163	23U	4B163	**25%**	**-16%**	+/+	No
III-1	F	52y	20U	4B163	28U	4B163	**11%**	**-34%**	+/-	No
III-2	M	61y	39U	4A161	45U	4B168	n/a	n/a	+/+	No
III-3	F	51y	9U	4A161	27U	4A161	**6%**	**-35%**	+/-	FSHD1+2
III-4	M	47y	20U	4B163	28U	4B163	**7%**	**-39%**	+/-	No
IV-1	F	21y	18U	4B163	66U	4A161	**22%**	**-29%**	+/-	No
IV-2	F	18y	9U	4A161	45U	4B168	36%	-9%	+/+	FSHD1
IV-3	M	16y	9U	4A161	39U	4A161	**16%**	**-26%**	+/-	FSHD1+2
IV-4	M	19y	28U	4B163	40U	4B168	52%	6%	+/+	No
IV-5	F	14y	20U	4B163	40U	4B168	47%	3%	+/+	No

M: male; F: female; n/a: not available; 4q_1 and 4q_2 represent the two alleles on chromosome 4q35; †: deceased; U: units. IDs correspond to those in the pedigree (Figure 1).

340 Table 2. Genetic characteristics of FSHD2 patients.

ID **Fig 3**	**Sex**	**4q35 locus**				**SMCHD1 variant and D4Z4 methylation (%)**					
		4q_1 units	4q_1 haplotype	4q_2 units	4q_2 haplotype	FseI methy-lation	delta1 methy-lation	SMCHD1 cDNA (NM_015295.2)	SMCHD1 variant (NP_056110.2)	Position relative to known BAMS mutation	exon
2		see Table 1				see Table 1		c.320T>C	p.Leu107Pro	identical to p.Leu107Pro	3
7	M	13	A	33	B	12	-28	c.580C>T	p.Leu194Phe	23 aa distal to p.Phe171Val	5
8	M	11	A	39	B	5	-33	c.610A>G	p.Lys204Glu	33 aa distal to p.Phe171Val	5
10a	M	no genotype data (no DNA)				3	n/a	c.729_731delCTT	p.Phe244del	2 aa distal to p.Ala242Gly	6
10b	F	no genotype data (no DNA)				NA	n/a	c.729_731delCTT	p.Phe244del	2 aa distal to p.Ala242Gly	6
10c	M	no genotype data (no DNA)				NA	n/a	c.729_731delCTT	p.Phe244del	2 aa distal to p.Ala242Gly	6
12	F	13	A	n/a	n/a	5	n/a	c.848A>G	p.Tyr283Cys	41 aa distal to p.Ala242Gly	7
14	M	17	A	47	A	9	-41	c.1058A>G	p.Tyr353Cys	5 aa distal to p.His348Arg	9
15	F	14	A	15	A	1	-37	c.1273G>A	p.Gly425Arg	5 aa distal to p.Asp420Val	10
18	M	11	A	35	B	7	-29	c.1474T>C	p.Cys492Arg	19 aa distal to p.Glu473Gln	12
19	F	17	A	18	A	11	-31	c.1556T>C	p.Phe519Ser	1 aa distal to p.Lys518Glu	12

M: male; F: female; n/a: not available; nl: normal; 4q_1 and 4q_2 represent the two alleles on chromosome 4q35. IDs correspond to the mutation number in Figure 3.

344 Table 3. Clinical findings in FSHD family with a pathogenic *SMCHD1* variant.

ID Fig 1	**Sex**	**Age (y)**	**SMCHD1 variant**	**Signs of FSHD**	**Interview and assessment of dysmorphic features**	**Pubertal development**	**Sniffin' sticks test**	**Other**
II-1	F	†	+/-	n/a (not at risk)	n/a	n/a	n/a	
II-2	M	80	+/+	n/a (not at risk)	n/a	nl; fertile	n/a	
II-3	F	75	+/-	severe FSHD, wheelchair bound	narrow nares; high nasal bride; hypoplastic alae nasi; bilateral cataracts at age 73y; dystopia canthorum; elongated philtrum	nl; fertile	anosmia	anosmia after nasal septum surgery
II-4	M	†	+/-	severe FSHD, wheelchair bound	n/a	n/a	n/a	
II-5	M	†	+/+	n/a (not at risk)	narrow nares; hypoplastic alae nasi	nl	n/a	assessment using photographs
III-1	F	52	+/-	nl (not at risk)	hypoplastic alae nasi; unilateral epicanthal fold; glasses (-0.25 and -4.25)	nl; fertile	n/a	assessment using Skype
III-3	F	51	+/-	severe FSHD, able to walk a	narrow nares and nose; high nasal bridge; hypoplastic and asymmetrical alae nasi; micrognatia	nl; fertile	hyposmia	

				few steps with support				
III-4	M	47	+/-	nl (not at risk)	high nasal bridge; asymmetrical alae nasi; long philtrum	nl; fertile	normosmia	
IV-1	F	21	+/-	nl (not at risk)	asymmetrical alae nasi	nl	normosmia	
IV-2	F	18	+/+	mild facial weakness	tendency to hypertelorism; short philtrum	nl	normosmia	
IV-3	M	16	+/-	facial weakness	coarse facial features; thick and asymmetrical alae nasi; strabism; tendency to hypertelorism; retrognatia	nl	normosmia	mild learning disability
IV-4	M	19	+/+	nl (not at risk)	none	nl	normosmia	
IV-5	F	14	+/+	nl (not at risk)	midline raphe	nl	normosmia	

F: female; M: male; †: deceased; y: years; n/a: not available; nl: normal. IDs correspond to those in the pedigree (Figure 1).

348 Table 4. Clinical findings in sporadic FSHD2 patients as determined by interview and photographs

ID Fig 3	**Sex**	**BAMS-associated phenotypes**										**Photographs**
		Smell	Nasal abnormalities	Nasal surgery	Open nostrils	Vision	Eye anatomical abnormalities	Tear production	Pubertal development	Fertility	Cleft lip/ palate	
7	M	nl	no	no	yes	nl	no	nl	nl	nl	no	n/a
8	M	nl	no	adenoid removal	yes	glasses	no	nl	nl	nl	no	n/a
10a	M	nl	no	no	yes	Astigmatism, hypermetropy	no	nl	nl	nl	no	n/a
10b	F	nl	no	no	yes	nl	no	nl	nl	nl	no	n/a
10c	M	nl	no	no	yes	nl	no	nl	nl	nl	no	n/a
12	F	nl	no	no	yes	nl	no	nl	n/a	n/a	no	n/a
14	M	nl	Difficulty clearing secretions	no	yes	glasses	no	Decreased (Schirmer's test score 4)	Decreased body hair	Infertile	no	no abnormalities
15	F	nl	no	no	yes	nl	no	nl	nl	nl	no	n/a
18	M	nl	no	no	yes	nl	no	nl	nl	nl	no	n/a
19	F	nl	no	no	yes	glasses	no	nl	nl	nl	no	no abnormalities

349 n/a: not available; nl: normal. IDs correspond to the mutation number in Figure 3.

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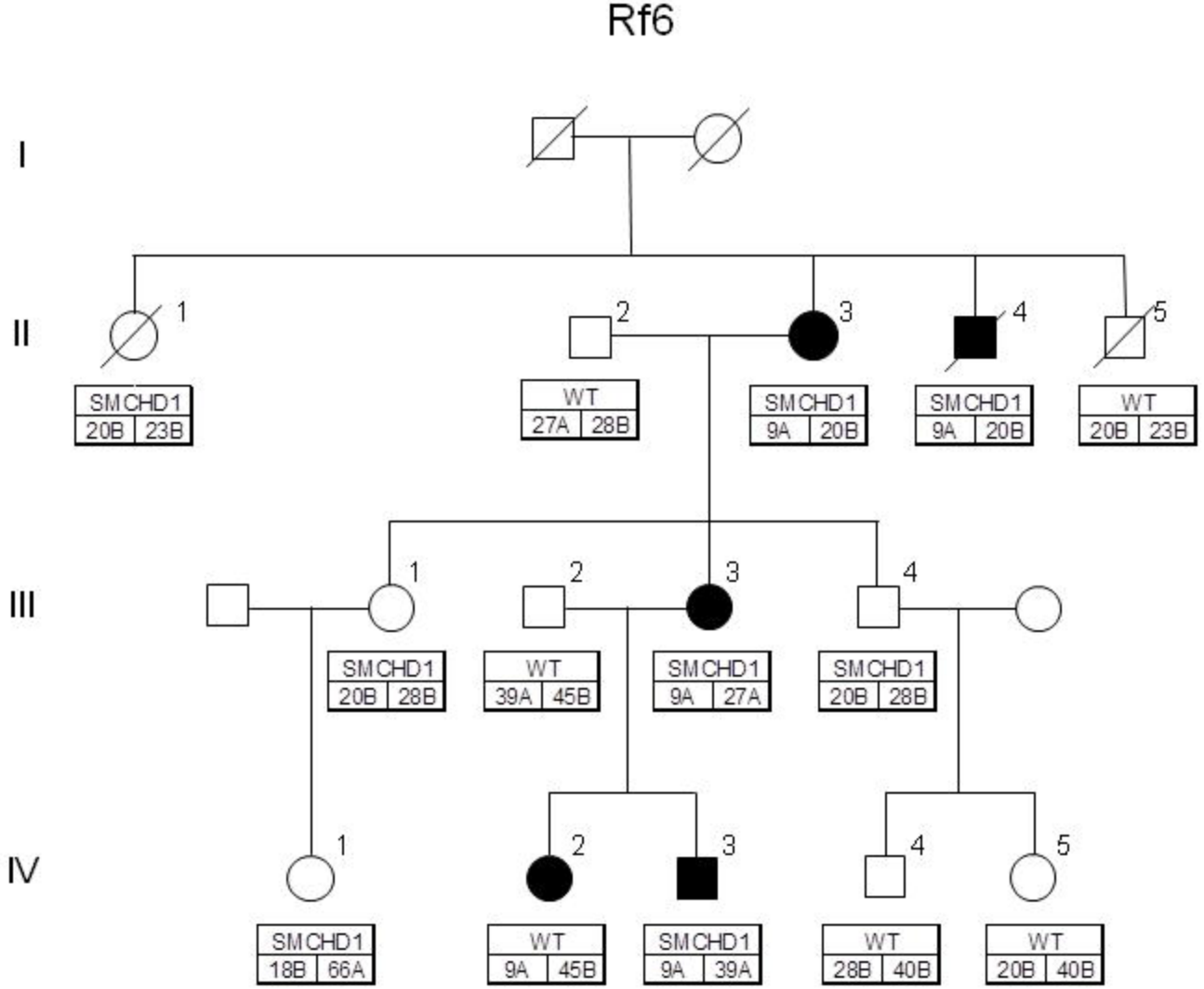
Figures legends

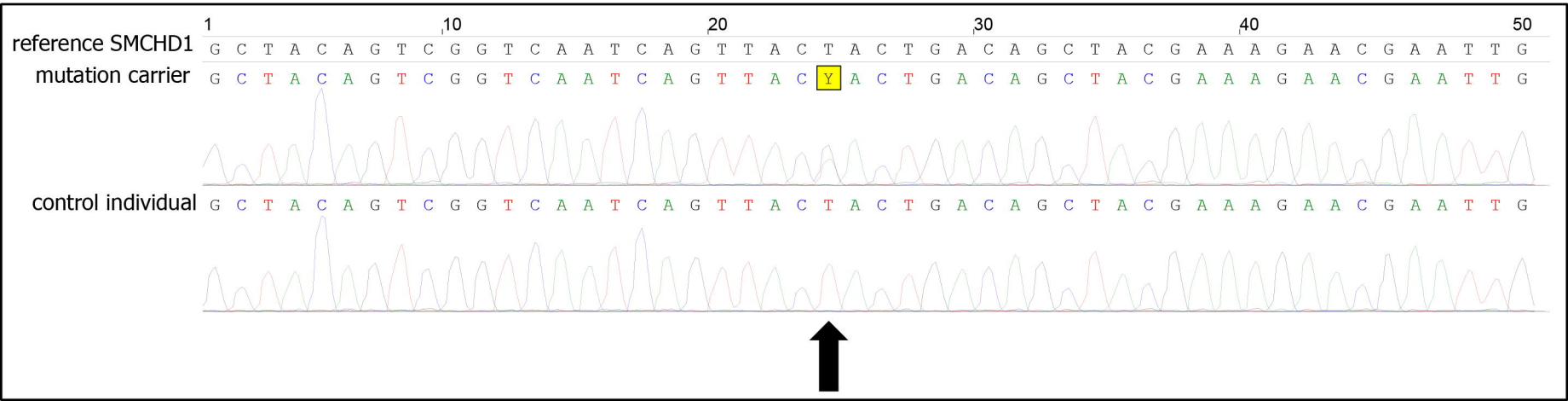
Figure 1. Pedigree for FSHD2 multiplex family with pathogenic variant (p.L107P) in *SMCHD1*. Shaded symbols represent family members meeting clinical criteria for FSHD2. Genetic information is listed below each family member: top box is mutation status (*SMCHD1* variant present or WT- wild type), lower boxes indicate the 4q35 haplotype (A or B) and D4Z4 repeat length (units) for each allele.

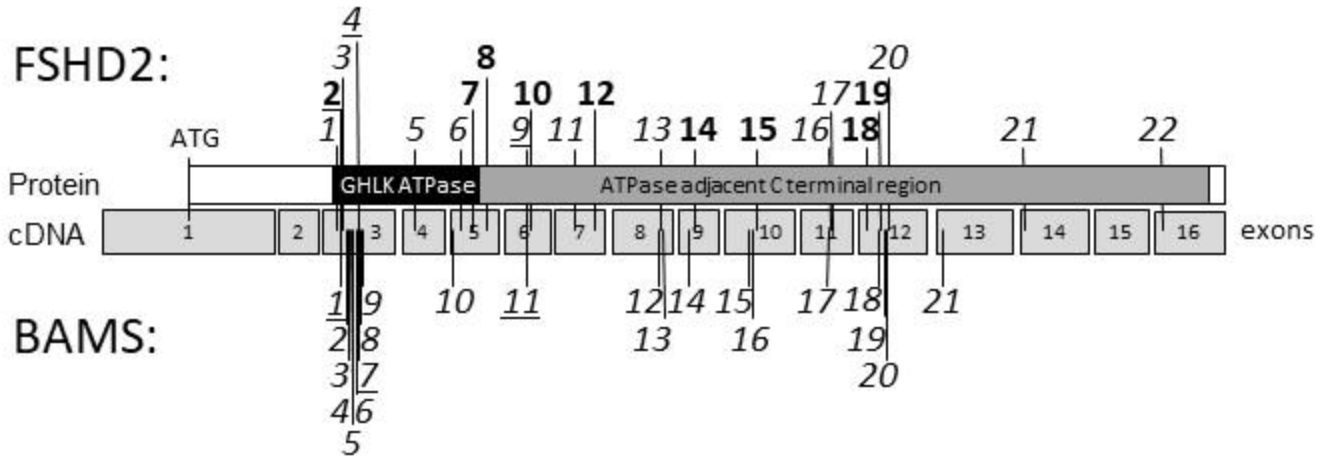
Figure 2. Sequence track of the *SMCHD1* pathogenic variant in the FSHD2 family and in a control sample. The position of the variant in exon 3 is indicated above the sequence traces and is highlighted in yellow. The genomic position is based on reference genome hg19 and the transcript and protein position on accession number NM015295 and NP056110, respectively.

Figure 3. Schematic of pathogenic missense variants in the N-terminal region of *SMCHD1* associated with FSHD2 and/or arhinia/BAMS. Pathogenic variants in the FSHD2 cohort in the current study are in bold (see also table 2), and the pathogenic variants that have been implicated in both FSHD2 and BAMS are underlined.


Rf6
I
II
III
IV
1
2
3
4
5
SMCHD1 20B 23B
WT 27A 28B
SMCHD1 9A 20B
SMCHD1 9A 20B
WT 20B 23B
1
2
3
4
SMCHD1 20B 28B
WT 39A 45B
SMCHD1 9A 27A
SMCHD1 20B 28B
1
2
3
4
5
SMCHD1 18B 66A
WT 9A 45B
SMCHD1 9A 39A
WT 28B 40B
WT 20B 40B

Rf6 (exon 3), chr18:g.2666926T>C, c.320T>C, p.Leu107Pro





FSHD2:			BAMS:		
1. p.N104S	*9. p.A242T*	*17. p.R479P*	*1. p.L107P*	*8. p.N139H*	*15. p.Q400L*
2. p.L107P	**10. p.F244del**	**18. p.C492R**	*2. p.M129K*	*9. p.L141F*	*16. p.D420V*
3. p.A110T	*11. p.H263D*	**19. p.F519S**	*3. p.A134S*	*10. p.F171V*	*17. p.E473Q*
4. p.G137E	**12. p.Y283C**	*20. p.T527M*	*4. p.S135C*	*11. p.A242G*	*18. p.K518E*
5. p.D150H	*13. p.R344Q*	*21. p.V615D*	*5. p.S135N*	*12. p.W342S*	*19. p.T523K*
6. p.M189V	**14. p.Y353C**	*22. pP690S*	*6. p.E136G*	*13. p.Q345R*	*20. p.N524S*
7. p.L194F	**15. p.G425R**	*23. p.L748P*	*7. p.G137E*	*14. p.H348R*	*21. p.R552Q*
8. p.K204E	*16. p.G478E*				

SMCHD1 (exon 1-48)	aa 1-2,005
GHKL ATPase domain (exon 3-5)	aa 111-365
ATPase adjacent C-terminal region (exon 5-16)	aa 365-702