Expanded spectrum of exon 33 and 34 mutations in SRCAP and follow-up in patients with Floating-Harbor syndrome

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Abstract

Background: Floating-Harbor syndrome is a rare autosomal dominant short stature syndrome with retarded speech development, intellectual disability and dysmorphic facial features. Recently dominant mutations almost exclusively located in exon 34 of the Snf2-related CREBBP activator protein gene were identified to cause FHS.

Methods: Here we report the genetic analysis of 5 patients fulfilling the diagnostic criteria of FHS obtained by Sanger sequencing. All of them presented with short stature, speech delay as well as psychomotor delay and typical facial dysmorphism. Three patients showed a good response to growth hormone treatment.

Results: Two patients demonstrate novel, heterozygous de novo frameshift mutations in exon 34 (c.7396delA and c.7218dupT) leading to premature stop mutations in SRCAP (p.Val2466Tyrfs*9 and p.Gln2407Serfs*36, respectively). In two further patients we found already known SRCAP mutations in exon 34, c.7330C > T and c.7303C > T, respectively, which also lead to premature stop codons: p.Arg2444* and p.Arg2435*. In one patient, we identified a novel de novo stop mutation in exon 33 (c.6985C > T, p.Arg2329*) demonstrating that not all FHS cases are caused by mutations in exon 34 of SRCAP.

Conclusions: Our data confirm a mutational hot spot in the final exon of SRCAP in the majority of FHS patients but also show that exon 33 of this gene can be affected.

Keywords: SRCAP, Floating-Harbor syndrome, Short stature, Growth hormone therapy

Background

Floating-Harbor syndrome (FHS, OMIM: #136140) is a genetic disorder characterized by short stature, delayed bone age, retarded speech development and intellectual disability (ID) as well as characteristic facial dysmorphisms (Figure 1).

Recently mutations located in exon 34 of the Snf2-related CREBBP activator protein (SRCAP) gene, encoding the core catalytic component of the multiprotein chromatin-remodeling SRCAP complex, were found to cause FHS in about 50 patients [1–4]. One patient who carried an exon 33 SRCAP mutation has been reported [5]. SRCAP locates to chromosome 16p11.2, comprises 34 exons and encodes 3230 amino acids.

SRCAP is the catalytic component of the homonymous SRCAP complex which mediates the ATP (adenosine triphosphate)-dependent exchange of a variant histone H2AZ/H2B dimer for a canonical H2A/H2B dimer at nucleosomes, leading to transcriptional regulation of selected genes by chromatin remodeling close to promoter regions. SRCAP is one of several proteins that help to activate a gene called CREBBP (Figure 2B). CREBBP plays a key role in regulating cell growth and division and is important for normal development. Mutations in the SRCAP gene may result in an altered protein that interferes with normal activation of the CREBBP gene, leading to a disturbed development. Rubinstein-Taybi syndrome, an autosomal dominant inherited disorder with some phenotypic overlap, is caused by mutations in the CREBBP gene itself [6].
Here we report the clinical and molecular data in 5 patients fulfilling the diagnostic criteria of FHS. All of them presented with short stature, speech as well as psychomotor delay and typical facial dysmorphism including a prominent nose, low-hanging columella and short philtrum.

**Methods**

**Patients**

Written informed consent forms and permission for publication of this report and accompanying photographs were obtained from all participants or their legal guardians.

**Figure 1** Photographs of patients, showing facial characteristics of Floating-Harbor syndrome. A - Patient A at age 5 years. B - Patient B at 22 years. C - Patient C at 5.5 years. D - Patient D at 7 years. E - Patient E at age of 5.5 years. Note overlapping facial dysmorphism such as long-hanging columella, short philtrum, and thin lips.

**Figure 2** Schematic representation of the SRCAP gene and positions of known SRCAP mutations. A - In this study, five de novo mutations have been identified to cause FHS (red frame – novel mutations, red-gray dashed frame – recurrent mutations). B - Expanded SRCAP protein network predicted functional links to several proteins involved in transcriptional regulation of selected genes by chromatin remodeling including CREBBP.
The Charité University Medicine ethics board approved this study.

**Mutation analysis**
Genomic DNA was isolated from peripheral blood using standard techniques. For mutation screening we amplified the coding region of SRCAP [NCBI Reference Sequence: NM_006662.2], including the flanking intronic sequences and the predicted promoter region. Primer sequences and PCR (polymerase chain reaction) conditions are available on request. PCR products were purified using the enzymes exonuclease I and shrimp alkaline phosphatase treatment, and directly sequenced with the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and analyzed on an automated DNA Analyzer (3730 Applied Biosystems).

**Protein network analysis**
An expanded protein network of SRCAP was created by the STRING interaction database (string-db.org). As parameters for the network display were used 'evidence view', 'high confidence 0.700', and 'no more than 10 interactors'.

**Results**
All patients demonstrated heterozygous de novo mutations in the gene SRCAP (Table 1, Figure 2A). For patients A and B, we identified two novel frameshift mutations in exon 34 (c.7396delA and c.7218dupT, respectively) predicted to introduce premature stop codons in SRCAP (p.Val2466Tyrfs*9 and p.Gln2407Serfs*36, respectively). Patients C and D carry previously described point mutations c.7330C > T and c.7303C > T, respectively, which also

| Patient | SRCAP mutation | Sex | Birth weight (SD) | Birth length (SD) | OFC at birth (SD) | Age at first assessment | Short stature (SD) | Age at last assessment | Height (SD) | OFC (SD) | ID | Language impairment | Craniofacial features |
|---------|----------------|-----|-------------------|-------------------|-------------------|------------------------|-------------------|----------------------|--------------|-----------|----|------------------|---------------------|
| A       | Exon 34        | Female | -1                | -2                | -1.5              | 2 years               | -2.5              | 10.5 years | -2          | -1        | +   | +                | Low-hanging columella, short philtrum, thin lips |
| B       | Exon 34        | Female | -1                | -1.8              | -1.5              | 2 years               | -3.2              | 22 years   | 154 cm; -1.8 | -1        | +   | +                | Broad nasal tip, long columella, short philtrum, thin lips |
| C       | Exon 34        | Female | -1.3              | -1                | -0.5              | 4 years               | -3.6              | 21 years   | 140 cm; -3.7 | -1.6      | +   | +                | Low-hanging columella, short philtrum, thin lips |
| D       | Exon 34        | Female | -1.3              | -1.5              | n.d.              | 5 years               | -2                | 7 years 3 months | -2          | -0.4      | +   | +                | Low-hanging columella, short philtrum, thin lips |
| E       | Exon 33        | Female | -1.3              | -1                | Mean              | 5 years 4 months      | -3.4              | 10 years   | 140 cm; -1.7 | -1.7      | +   | +                | Prominent nose, low-hanging columella, short philtrum, thin lips |

*The clinical manifestations of patient B at age of 5 years were published in the AJMG 10:47-52, 2001.
lead to premature stop codons: p.Arg2444* and p.Arg2435*
[1,2]. Interestingly, in patient E, Sanger sequencing identi-

fied a novel stop mutation in exon 33 (c.6985C>T, p.Arg2329*) (Figure 2B, red-framed boxes).

Table 1 and Figure 1 summarize the clinical data. In the

individuals analyzed here birth weights ranged be-

tween mean and -1.3 SD (standard deviation) as well as

birth lengths between -1 to -2 SD (Table 1). The occipi-

tofrontal head circumference (OFC) at birth was normal

in all. Prior puberty bone ages were significantly delayed

when X-rays were available (in patients A, B, C, D).

Postnatal short stature varied from -1.7 to -3.7 SD, how-
ever three patients (patients A, B, E) received growth

hormone (GH) therapy during childhood and their

heights were between -1.7 to -2 SD at time of last assess-

ment. In all individuals, postnatal OFC was lower than

the mean but still in the normal range (-0.4 to -1.7 SD).

Delayed pubertal development and primary ovarian in-

sufficiency were observed in patient B. Patient C showed

normal pubertal development and hypermenorrhea in

adult.

Behavioral difficulties such us aggressive behavior, anx-

iety, sleep disturbances, and rigid mannerisms were ob-

served in two patients starting before and at puberty

(patients A and E) and in adulthood (patient C), respect-

ively. Except one, all patients showed delayed speech de-

velopment as well as reduced cognitive abilities with

schooling at schools for mentally handicapped children.

As adults two patients were able to speak in short sen-

tences and to read and write with simple skills. Patient E

carrying the exon 33 mutation showed only mild speech
delay but has normal cognitive skills and attended a nor-

mal school.

The characteristic facial aspect with a prominent nose

with a broad nasal tip, a low hanging columella, a short

philtrum, and a thin upper lip was present in all and re-

mains constant also in adult patients (Figure 1). Minor

skeletal abnormalities such us brachydactyly (patients A,

B, D), broad fingertips (patients C and E) or pseudoar-

throsis of the clavicles (patient C) were observed in all

patients studied here.

Discussion

Our molecular data confirm a mutational hot spot in the

final exons of SRCAP in all patients tested here. Postnatal

short stature with in relation larger OFC together with a

distinct facial aspect and delayed speech development as

important diagnostic criteria of FHS were fulfilled in all

patients reported here. Markedly delayed bone age was
disclosed in all patients (A, B, C, D) where hand radio-
grams were available. Behavioral difficulties were observed

in three patients of our study group and occur in about

one third in a larger study cohort, therefore behavioral

problems should be monitored [4]. Interestingly, growth

hormone treatment led to significant growth improve-

ment toward the low normal range in three patients indi-
cating effectiveness of this therapy in patients with FHS.

One female patient with the clinical diagnosis of FHS has
been reported with precocious puberty following

treatment with gonadotrophin-releasing hormone

anologue and later growth hormone treatment because of

growth hormone deficiency [7]. This patient reached

average adult height.

So far unreported endocrinological abnormalities

delayed puberty and primary ovarian insufficiency in pa-

ten B and hypermenorrhea in patient C) were docu-

mented in the adult females reported here.

The vast majority of affected individuals carries truncat-

ing mutations of exon 34 of SRCAP with two mutations

(p.Arg2444*, p.Arg2435*) which are recurrently identified

(Figure 2A) [1,3,4]. In our cohort we identified these two

recurrent mutations and two novel frameshift mutations

in exon 34. Moreover, we found one novel mutation in the

penultimate exon 33. The phenotype of this patient is in

accordance with the manifestations of FHS, except for ID.

Interestingly, she is the only one in our cohort with only

mild speech delay and normal schooling. One could

calculate that this milder phenotype may be due to partial

nonsense-mediated decay or faster degradation of the pro-
duced altered SRCAP protein. Until now, only one other

mutation in exon 33 of SRCAP (p.Gln2334*) has been
documented in an affected individual with typical features
of FHS [5]. Apart from typical manifestations of FHS this

8 year old patient has not only speech delay but also signifi-
cant intellectual disability in contrast to our patient carrying

the exon 33 mutation. However, average intelligence and

regular schooling have been already reported in a few af-

fected individuals with exon 34 mutations and therefore

the broad range of cognitive skills in patients with FHS

seems to include normal psychomotor development [4].

All hitherto FHS causing mutations are predicted to

cause a truncated SRCAP protein lacking the putative

C-terminal AT-hook DNA binding motif. Due to the ob-
served heredity transmission by heterozygous de novo

mutation a dominant negative disease mechanism has

been postulated [1]. Therefore, truncating mutations out-

side exons 34 and 33 may result in nonsense mediated
decay leading to different phenotypic effects [8]. One ex-

ample of different phenotypes associated with mutations

located in the last exons of a gene versus mutations in

other exons is a lipodystrophy-progeroid phenotype in in-
dividuals carrying mutations in the last exon of the

FBNI gene while mutations of other exons of this gene lead
to Marfan syndrome [9]. However, absence of SRCAP mul-

tations were reported in 3/9 patients investigated by direct

sequencing and may have different explanations, e.g. an

overlapping phenotypic spectrum with Rubinstein-Taybi

syndrome or other syndromes as well as possible genetic
heterogeneity in FHS [3]. Potential candidate genes may include proteins in distinct vicinity to SRCAP and CREBBP with functional links to chromatin remodeling mechanisms (Figure 2B). Thus, further work is required to fully elucidate the pathomechanism of FHS.

Conclusions
In patients with suspected FHS, we strongly recommend that mutational analysis should include not only sequence analysis of exon 34 but should extend to at least exon 33 of this gene. Growth hormone treatment shows effectiveness in patients with FHS.

Endocrinological and gynaecological follow-up is needed for adult patients with FHS to evaluate for further complications.

Consent
Written informed consent was obtained from the patients/parents for publication of this research and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
WS carried out the mutational analysis and drafted the manuscript. DH designed and coordinated the study, and wrote the manuscript. PM participated in design of the study and wrote the manuscript. GR, ER, WH, and AW provided clinical data and blood samples for mutational analysis from patients and their relatives. All authors read and approved the final manuscript.

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Online Mendelian Inheritance in Man (OMIM), http://www.omim.org
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Search Tool for the Retrieval of Interacting Genes/Proteins (STRING), http://string-db.org/

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