Acromelic frontonasal dysostosis and ZSWIM6 mutation
phenotypic spectrum and mosaicism

Twigg, Stephen R F; Ousager, Lilian Bomme; Miller, Kerry A; Zhou, Yan; Elalaoui, Siham C; Sefiani, Abdelaziz; Bak, Geske Sidsel; Hove, Hanne; Kjærgaard Hansen, Lars; Fagerberg, Christina Ringmann; Tajir, Mariam; Wilkie, Andrew O M

Published in:
Clinical Genetics

DOI:
10.1111/cge.12721

Publication date:
2016

Document version
Publisher's PDF, also known as Version of record

Document license
CC BY

Citation for published version (APA):
Acromelic frontonasal dysostosis and ZSWIM6 mutation: phenotypic spectrum and mosaicism


Acromelic frontonasal dysostosis and ZSWIM6 mutation: phenotypic spectrum and mosaicism.

Acromelic frontonasal dysostosis (AFND) is a distinctive and rare frontonasal malformation that presents in combination with brain and limb abnormalities. A single recurrent heterozygous missense substitution in ZSWIM6, encoding a protein of unknown function, was previously shown to underlie this disorder in four unrelated cases. Here we describe four additional individuals from three families, comprising two sporadic subjects (one of whom had no limb malformation) and a mildly affected female with a severely affected son. In the latter family we demonstrate parental mosaicism through deep sequencing of DNA isolated from a variety of tissues, which each contain different levels of mutation. This has important implications for genetic counselling.

Conflict of interest
All authors declare no conflict of interest.


A Clinical Genetics Group, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK; bDepartment of Clinical Genetics, Odense University Hospital, Odense, Denmark; cHuman Genomics Center, Faculty of Medicine and Pharmacy of Rabat, Rabat, Morocco; dDepartment of Medical Genetics, National Institute of Health, Rabat, Morocco; eDepartment of Obstetrics and Gynecology, Odense University Hospital, Odense, Denmark; fDepartment of Clinical Genetics, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark, and gDepartment of Paediatrics, Hans Christian Andersen Children’s Hospital, Odense University Hospital, Odense, Denmark

†These authors contributed equally to this work.

Key words: frontonasal malformation – mosaicism – preaxial polydactyly – ZSWIM6

Corresponding author: Dr Stephen Twigg, Clinical Genetics Group, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK.
Tel.: +44 1865 222353
fax: +44 1865 222500
e-mail: stephen.twigg@imm.ox.ac.uk

Received 24 November 2015, revised and accepted for publication 22 December 2015

CLINICAL GENETICS
doi: 10.1111/cge.12721

© 2015 The Authors. Clinical Genetics published by John Wiley & Sons A/S. Published by John Wiley & Sons Ltd. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
Acromelic frontonasal dysostosis (AFND; MIM 603671) is characterized by a combination of characteristic frontonasal malformation (FN) with limb defects and anomalies of the brain and usually occurs as a sporadic disorder. Following initial description in a review of the diverse presentations of FN (1), Verloes et al. proposed AFND as a distinct entity (2); subsequent reports have highlighted characteristic features of severe hypertelorism, ptosis, median cleft face with distinctive nasal bifurcation and widely separated nasal alae, parietal foramina, variable brain abnormalities including dysgenesis of the corpus callosum, hydrocephalus and interhemispheric lipoma, limb anomalies with preaxial polydactyly of the feet, tibial aplasia or hypoplasia, and talipes equinovarus (3–5). Although mainly arising sporadically, possible vertical transmission (5) suggested a dominant mechanism, subsequently confirmed by identification of the underlying heterozygous mutation in four AFND cases (6). These four cases were found to carry an identical mutation of ZSWIM6 (MIM: 615951; c.3487C>T, p.Arg1163Trp), all apparently de novo in origin. In one subject, a reduced ratio of mutant to wild-type allele indicated that the mutation was present in mosaic state and had likely arisen post-zygotically; in another family, mild phenotypic features in the father were speculated to have arisen coincidentally to the AFND phenotype. DNA was extracted from peripheral blood samples (Subjects 1-1, 1-2, 2 and parents of Subjects 2 and 3), an aminocentesis sample (Subject 3), and buccal brushings, saliva, urine and skin (Subject 1-1). The resequencing panel consisted of 27 individuals with mild to severe FNMs, with or without extracranial abnormalities, and lacking a molecular diagnosis.

Molecular analysis
A 370 bp fragment covering the ZSWIM6 (Refseq NT_034772.7) exon 14 c.3487C>T variant was amplified using primers E14F 5′-GCTATAATCCTTCTGG TGGTCAAAGGTG-3′ and E14R 5′-CCCCGAACCAAC ATCATCAGTTTC-3′. Amplification was carried with 0.5 U of FastStart polymerase (Roche Diagnostics, Burgess Hill, UK) in a total volume of 20 μl containing 15 mM Tris–HCl (pH 8.0), 50 mM KCl, 2.5 mM MgCl2, 100 μM each dNTP and 0.4 μM primers. Cycling conditions consisted of an 8 min denaturation step at 94°C, followed by 33 cycles of 94°C for 30s, 63°C for 30s and 72°C for 30s, with a final extension at 72°C for 10 min. This product was sequenced using BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA, USA). Deep sequencing on the Ion Torrent PGM platform was used to quantify the proportions of wild-type to mutant allele in genomic DNA. Fragments of 220 bp spanning the c.3487C>T variant were generated (ZSWIM6-specific primers: Exon14F 5′-GCTACATCAACACACGCTACG-3′ and Exon14R 5′-CATACAAAGATCTATCAACAAAACGTT CCCC-3′ with a 10 bp barcode incorporated into either the reverse or forward oligonucleotide and flanked by Ion Torrent P1 and A adapter sequences). The P1 and A adapter sequences were flipped so that Ion Torrent sequencing could be carried out in both directions. The high-fidelity Taq polymerase Q5 (NEB, Hitchin, UK) was used for amplification (0.02 U/μl) in a reaction volume of 25 μl containing 0.5 μM primers, 25 mM Tap-HCl (pH 9.3), 50 mM KCl, 2 mM MgCl2, 1 mM β-mercaptoethanol and 200 μM each dNTP. Cycling was carried out as described above except the cycle number was reduced to 30 and the annealing temperature was 60°C. Amplification products were purified with AMPure beads (Beckman Coulter, High Wycombe, UK) and emulsion polymerase chain reaction (PCR) and enrichment performed with the Ion PGM Template OT2 200 Kit (Life Technologies) according to the manufacturer’s instructions. Sequencing of enriched templates was performed on the Ion Torrent PGM (Life technologies, Carlsbad, CA, USA) for 125 cycles using the Ion PGM Sequencing 200 kit v2 on an Ion 316 chip. Data were processed with Ion Torrent platform-specific pipeline software v4.2.1. As the variant must be present at a level of 50% in a heterozygous individual, and at 0% in a normal control, the forward and reverse deep sequencing read counts were separately normalized using the data from Subject 1-2 and a control, and the average of the two corrected percentages calculated.

Results
Clinical description
The clinical features of Subjects 1-1, 1-2, 2 and 3 are summarized in Table 1 and shown in Figure 1. Subject 1-2 was born at 32 weeks’ gestation (birth weight: 1580 g) and diagnosed with AFND due to the association of severe FN and limb abnormalities. Currently aged 7 years, he has severe neurocognitive and motor delay.

Materials and methods
Subjects
The study was approved by Oxfordshire Research Ethics Committee B (reference C02.143) and Riverside Research Ethics Committee (reference 09/H0706/20); written informed consent was obtained from all participants by the referring clinicians. Karyotyping of all subjects was normal; although array comparative hybridisation (aCGH; Agilent 244K) in Subjects 1-1 and 1-2 showed a 3.2 Mb duplication of 16p12.3-p13.1 that had arisen de novo in the mother, this appears to be coincidental to the AFND phenotype. DNA was extracted from peripheral blood samples (Subjects 1-1, 1-2, 2 and parents of Subjects 2 and 3), an aminocentesis sample (Subject 3), and buccal brushings, saliva, urine and skin (Subject 1-1). The resequencing panel consisted of 27 individuals with mild to severe FNMs, with or without extracranial abnormalities, and lacking a molecular diagnosis.
Table 1. Clinical features of subjects with ZSWIM6 c.3487C>T; p.Arg1163Trp

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Gender</th>
<th>Eyes</th>
<th>Nose</th>
<th>Mouth</th>
<th>Skull</th>
<th>Morphology</th>
<th>Development</th>
<th>Limbs</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1b</td>
<td>F</td>
<td>Hypertelorism</td>
<td>Wide nasal bridge, short nasal ridge,</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>bifid nasal tip</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>M</td>
<td>Severe, hypertelorism, downslanting</td>
<td>Wide nasal bridge, widely spaced</td>
<td>Carp-shaped mouth, midline notch in upper</td>
<td>Bony defect of anterior cranial fossa,</td>
<td>Interhemispheric lipoma,</td>
<td>Severe motor and neurocognitive delay</td>
<td>Normal upper limbs, bilateral tibial hemimelia, bilateral bifid first toe, bilateral clubfoot</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>palpebral fissures, downslanting palpebral</td>
<td>nasal alae, widely separated slit-like</td>
<td>lip, midline notch in upper lip, cleft palate</td>
<td>parietal foramina</td>
<td>partial agenesis of the corpus callosum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>nares</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>Hypertelorism, bilateral ptosis,</td>
<td>Wide nasal bridge, short nasal ridge,</td>
<td>Carp-shaped mouth, long philtrum, midline</td>
<td>Bony defect of anterior cranial fossa</td>
<td>Anterior interhemispheric</td>
<td>Severe psychomotor delay, absence of speech, does not walk aged 8 years</td>
<td>Normal</td>
<td>Microgenesis, cryptorchidism, scoliosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>downslanting palpebral fissures,</td>
<td>bifid nasal tip, widely spaced nasal alae,</td>
<td>notches in upper lip, cleft palate</td>
<td></td>
<td>lipoma</td>
<td></td>
<td>Normal upper limbs, bilateral tibial hypoplasia, bilateral clubfoot</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>bilateral cataract</td>
<td>widely separated slit-like nares</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3c</td>
<td>F</td>
<td>Hypertelorism, downslanting palpebral</td>
<td>Aplasia/hypoplasia of the nasal bones,</td>
<td>Midline notch in upper lip</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Normal upper limbs, bilateral club foot</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fissures</td>
<td>wide nasal bridge, bifid nasal tip</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aF, female; M, male.

bMosaic for the mutation.

cPregnancy terminated at 20 weeks’ gestation.
Acromelic frontonasal dysostosis and \textit{ZSWIM6} mutation

Molecular analysis

Screening for \textit{ZSWIM6} c.3487C>T in a cohort of 27 FNM cases revealed the presence of this variant in three individuals, Subjects 1-2, 2 and 3 (Fig. 2a). The ratio of mutant to wild-type allele was approximately 50:50 in each case. As the variant was not detected in blood from Subject 1-1, the mildly affected mother of Subject 1-2 and in whom mosaicism was suspected, we analyzed DNA from four other tissues (buccal brushings, saliva, skin and urine). Sanger sequencing was inconclusive although a subtle drop in peak height of the wild-type allele was evident in the two buccal samples, suggesting the presence of the variant allele (data not shown). This prompted us to undertake more sensitive Ion Torrent-based deep sequencing, which identified the mutant variant in all five tissue samples from Subject 1-1 (Fig. 2b). The average sequencing depth obtained was >152,800 with a lowest read number of 55,173. The percentage of variant allele was highest at \(\sim11\%\) in the buccal scrapings (equivalent to \(\sim22\%\) mutant cells), at 3\% in saliva, around 2\% in urine and blood and lowest at under 1\% in skin. The sensitivity of mutation detection using Sanger sequencing is around 6\% (7) providing an explanation for why testing of DNA from peripheral blood of Subject 1-1 was negative.

Discussion

AFND is an extremely rare FNM with fewer than 20 recognizable cases described in the literature (1,
Fig. 2. ZSWIM6 sequence analysis. (a) Sequence chromatograms showing ZSWIM6 c.3487C>T in Subjects 1-2, 2 and 3 (red arrows). The C>T variant is absent in Subject 1-1 (DNA from peripheral blood) and the parents of Subjects 2 and 3. (b) Deep sequence analysis for ZSWIM6 c.3487C>T. The left hand panel shows the percentage of the variant T allele detected in Subject 1-1 and 1-2. The value for Subject 1-2 has been corrected to 50% and all other figures adjusted accordingly. The T allele is shown in red and the C allele in blue. The right hand panel shows the uncorrected read depths achieved for each sample.

2, 4–6, 8–10). The most consistent clinical features are FNM accompanied by preaxial polydactyly of the lower limbs. The nasal deformity is usually severe, with symmetrical clefting and widely separated slit-like nares, while limb anomalies can also include tibial hypoplasia and clubfoot. Recently, a recurrent mutation of ZSWIM6, c.3487C>T encoding p.Arg1163Trp, was identified in four AFND individuals (6). ZSWIM6 is a member of a group of proteins, found in bacteria, archaea and eukaryotes, that all contain a SWIM Zn-finger-like domain that could function both as a DNA binding domain or in protein–protein interaction (11). Very little is known about the role of ZSWIM6, although the missense substitution identified in AFND is likely to disrupt the function of a highly conserved sin3-like domain at the C-terminus of the protein (6). Expression appears to be ubiquitous although higher in the brain, and analysis of AFND patient cells suggests an effect on hedgehog signaling (6). A molecular-developmental explanation for the specific pattern of malformations occurring in AFND is currently lacking.

In this report we screened a phenotypically diverse FNM cohort for this variant and identified three positive individuals, all of whom shared the characteristic nose with symmetrical, widely separate nostril openings and severe hypertelorism (Fig. 1b,e). This included a previously undiagnosed patient with severe FNM but normal limbs. A confident diagnosis of AFND with normal limbs has only been possible in one previous case, one of the two half-sisters reported by Warkany et al. (10), where a diagnosis could be made because of the classically affected relative. Our findings imply that similar cases with isolated severe symmetrical FNM should undergo ZSWIM6 screening. Interestingly, although Subject 3 had lower limb abnormalities, polydactyly was absent, highlighting that this feature may not always be present either.

Although mosaicism had been suspected in the mildly affected parent of a classical AFND patient (6), it was not molecularly confirmed. We prove, through next generation deep sequencing of DNA from multiple tissues, that mosaicism can occur in the mildly affected parents of AFND cases. Notably, the low level of mosaicism found could not be convincingly detected by Sanger sequencing, even of multiple tissues. The use of PCR-based or capture techniques combined with next generation deep
sequencing is an effective method to identify low-frequency mosaic mutations that are missed by conventional techniques (12, 13). In our analysis deep sequencing allowed the convincing detection of mutations at less than a 2% level. The finding of mosaicism has important counselling implications for AFND families and the possibility of mosaicism in one of the parents of a child with a germline mutation, whether they are mildly affected or appear normal, should be considered. The phenotype of Subject 1-1 shows similarities to frontorhiny, a distinct FNM caused by biallelic mutations of \textit{ALX3} (14). We propose that for patients thought to have frontorhiny, but with a negative \textit{ALX3} mutation screen, the possibility of low-level mosaicism for the \textit{ZSWIM6} mutation should be sought by deep sequencing of multiple tissues.

Acknowledgements

We are very grateful to the families for their participation in this study. We thank Sanjena Mithra and Emily Taylor for their assistance and Sue Butler, John Frankland and Tim Rostron for help with cell culture and DNA sequencing. This work was supported by the NIHR Biomedical Research Centre, Oxford and the Wellcome Trust (Project Grant 093329 to A. O. M. W. and S. R. F. T., and Senior Investigator Award 102731 to A. O. M. W.) and Newlife Foundation for Disabled Children (10-11/04 to A. O. M. W. and S. R. F. T.).

References