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Supplemental Data

Exome Sequencing Identifies a Recurrent De Novo *ZSWIM6* Mutation Associated with Acromelic Frontonasal Dysostosis

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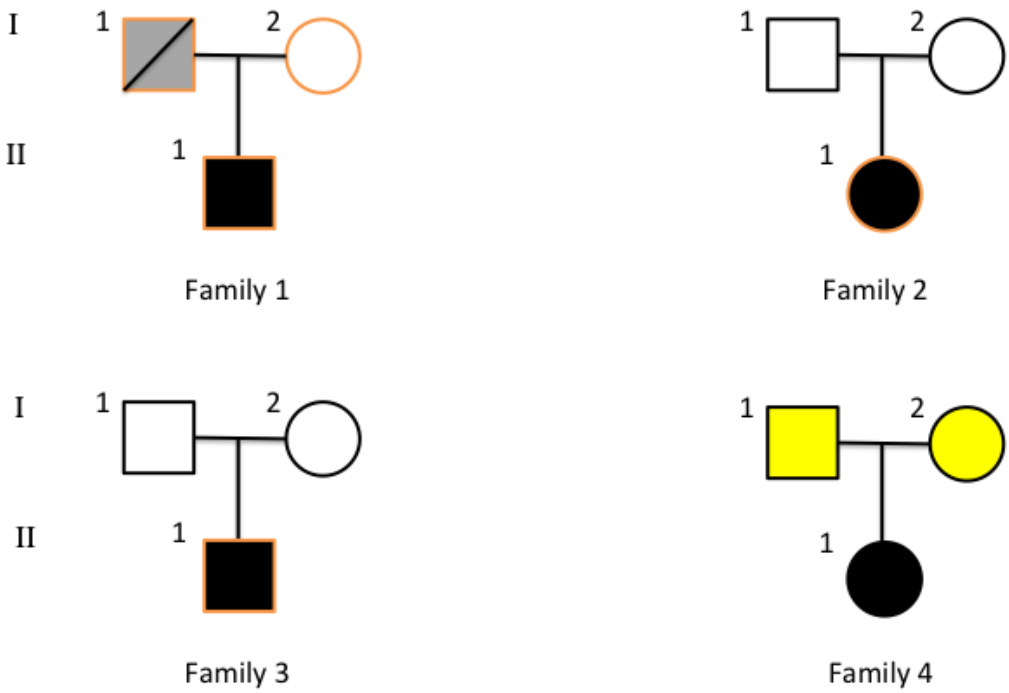

Figure S1. Pedigrees of AFND Families

Individuals that have orange trim were selected for whole exome sequencing. Individual I.1 in Family 1 (gray shading) was previously described as being mildly affected. Individual II.1 in Family 1 and Individual II.1 in Family 3 correspond to Cases 1 and 2, respectively in Hing et al.[1] Parents of Individual II.1 in Family 4 are unknown as patient was adopted (yellow shading) and, therefore, cannot be screened for the ZSWIM6 mutation.

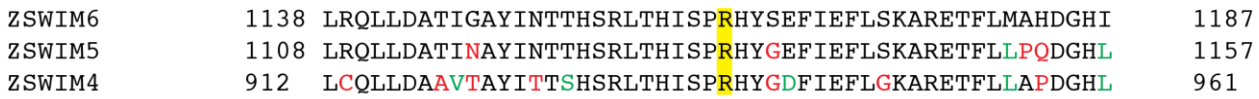

Figure S2. Conservation of Variant Region in Gene Family Members

The aligned 50 amino acid window that spans the ZSWIM6 p. Arg1163Trp substitution is highly conserved in two ZSWIM family members ZSWIM5 and ZSWIM4. Divergent residues are marked in red and conservative substitutions are marked in green. The two paralogs have high homology across the protein suggesting that they resulted from a recent expansion of the genome as determined by evolutionary conservation.

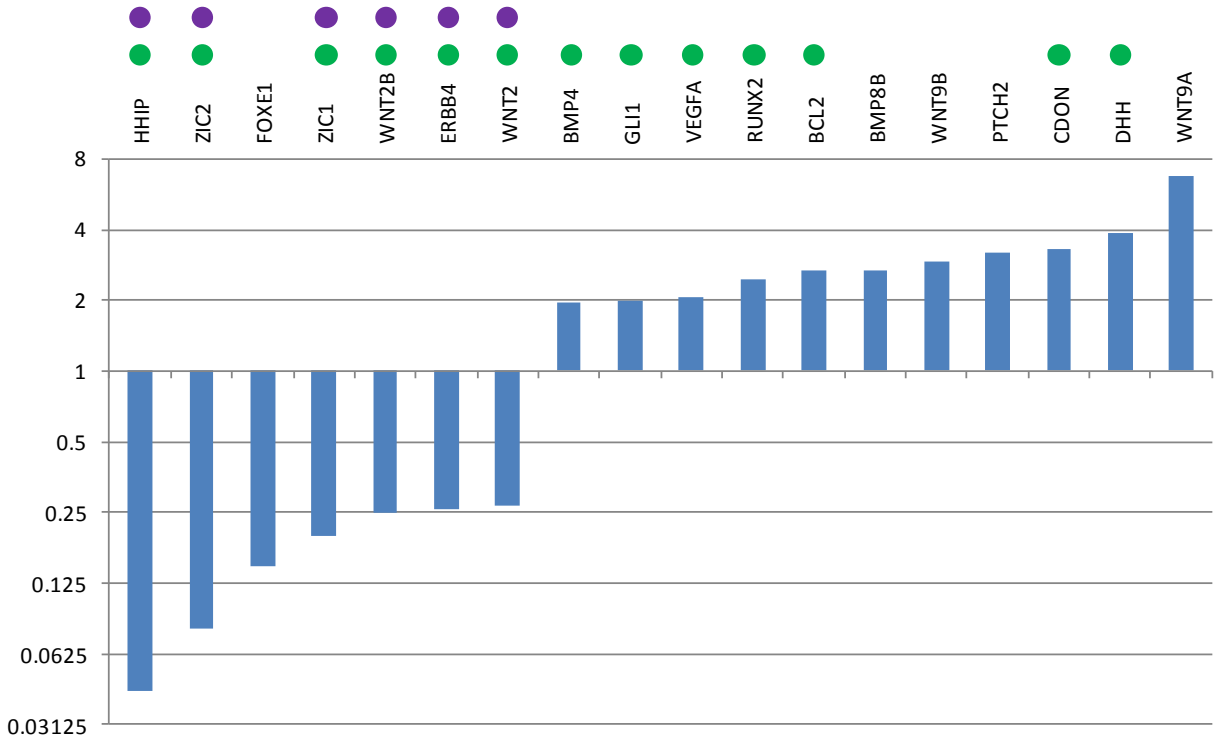

Figure S3. *ZSWIM6* Mutation is Associated with Dysregulation of Hedgehog Pathway Transcripts

Primary osteoblast cell lines demonstrated eighteen transcripts (*HHIP* [MIM 606178], *ZIC2* [MIM 603073], *ZIC1* [MIM 600470], *WNT2B* [MIM 601968], *ERBB4* [MIM 600543], *WNT2* [MIM 147870], *BMP4* [MIM 112262], *GLI1* [MIM 165220], *VEGFA* [MIM 192240], *RUNX2* [MIM 600211], *BCL2* [MIM 151430], *CDON* [MIM 608707] and *DHH* [MIM 605423]) with greater than 1.5 fold up or down regulation in two AFND cases relative to five controls. Thirteen of these transcripts (72%, green dot) support that *ZSWIM6* c.3487C>T results in activation of the Hedgehog pathway with increased expression of activators and reduced expression of repressors.[1-6] Parallel qRT-PCR using two cases and two control primary fibroblast lines demonstrated concurrence in the six down regulated transcripts (purple dot) further supporting our hypothesis that the *ZSWIM6* c.3487C>T mutation results in partial activation of the Hedgehog pathway.

Table S1. Clinical Features of Individuals with AFND

Brain	**Individual 1**	**Individual 2**	**Individual 3**	**Individual 4**
Periventricular nodular heterotopia	Not present	Left temporal horn	Not present	Not present
Ventriculomegaly	Mild	Not present	Not present	Mild
Choroid plexus cyst	Present	Present	Not present	Not present
Marked dilated perivascular spaces	Not present	Present - versus periventricular cysts	Not present	Not present
Corpus callosum	Hypoplastic	Aplastic	Normal	Normal
Septum pellucidum	Deficient	Normal	Normal	Normal
Cavum septum pellucidum	Not present	Not present	Present	Not present
Calcification/ossification of the falx	Present	Present	Present	Minimal
Interhemispheric lipoma	Tubulonodular - large*	Tubulonodular	Tubulonodular	Small
Olfactory bulbs	Absent	Absent	Unable to assess	Unable to assess
Pituitary	Enlarged sella	Absent adenohypophysis	Enlarged sella	Enlarged sella
Other		Fenestrated basilar artery; persistent falcine venous sinus		Retrocerebellar cyst

Eyes				
Corneal Dermoid Cyst	None	Left	None	None
Glaucoma	Left	None	None	Unknown
Myopia	None	Yes	Yes	Unknown
Optic Nerve Hypoplasia	None	None	Right, segmental	Unknown
Persistent primary vitrea	None	Yes	None	Unknown

Craniofacial				
Hypertelorism	Yes	Yes	Yes	Yes
Ptosis/Telecanthus	Yes	Yes	Yes	Yes
Median Facial Cleft	Yes	Yes	Yes	Yes

Separation of Nostrils	Yes	Yes	Yes	Yes
Cleft palate	Complete	Submucous cleft palate	Absent	Absent
Parietal Foramina	Bilateral	Bilateral	Bilateral	Absent
Persistent craniopharyngeal canal	Not present	Not present	Not present	Present
Canalis basilaris medianus	Not present	Present	Not present	Not present
Vertical clivus	Present	Present	Present	Absent

Limb				
Preaxial polydactyly - Lower extremity	Right	Left	Bilateral	Left
Tibial Hypoplasia	Right	Mild	Right	Absent
Patellar Hypoplasia	Right (aplasia)	Left	Right	Unknown
Additional abnormalities				
Hypopituitarism	Yes	Yes	Absent	Unknown
Cryptorchidism	Yes	N/A	Normal	N/A
Imaging Studies Reviewed	**Head CT and MRI**	**Head CT and MRI**	**Head CT**	**Head CT**

* Interhemispheic lipoma is contiguous with the floor of the anterior cranial fossa.

Table S1. Clinical Features of Individuals with AFND

Clinical phenotyping, including computed tomography and magnetic resonance imaging, confirmed classic findings of AFND and identified several new phenotypic features in our four probands. The cases demonstrated variable classic features of frontonasal dysplasia, parietal foramina and tibial hemimelia. In addition, interhemispheric lipoma, calcification of the falx, vertical clivus, enlargement of the sella turcica and hypopituitarism were the most consistent additional findings.

Table S2. Orthologous Proteins with Highest Homology to ZSWIM6 Domain

Accession id	Species
NP_065979	Homo sapiens
XP_004275235	Orcinus orca
NP_663431	Mus musculus
XP_004416075	Odobenus rosmarus divergens
XP_004664973	Jaculus jaculus
XP_001234010	Gallus gallus
XP_005281095	Chrysemys picta bellii
XP_005489834	Zonotrichia albicollis
XP_004862962	Heterocephalus glaber
XP_004678517	Condylura cristata
XP_004583864	Ochotona princeps
XP_004374643	Trichechus manatus latirostris
XP_005558916	Macaca fascicularis
XP_003899762	Papio anubis
XP_003827517	Pan paniscus
ELW66262	Tupaia chinensis
XP_535247	Canis lupus familiaris
XP_004789987	Mustela putorius furo
XP_005060742	Ficedula albicollis
XP_002745001	Callithrix jacchus
XP_004058851	Gorilla gorilla gorilla
XP_002916338	Ailuropoda melanoleuca
XP_003926003	Saimiri boliviensis boliviensis
XP_005604291	Equus caballus
XP_003981056	Felis catus
ELK17704	Pteropus alecto
XP_004608592	Sorex araneus
ELR52973	Bos mutus
XP_002696357	Bos taurus
EPQ02547	Myotis brandtii

XP_004423086	Ceratotherium simum simum
XP_005654279	Sus scrofa
XP_006789860	Neolamprologus brichardi
XP_005392704	Chinchilla lanigera
ERE85311	Cricetulus griseus
XP_005694723	Capra hircus
XP_003974842	Takifugu rubripes
XP_004072276	Oryzias latipes
XP_005152146	Melopsittacus undulatus
XP_004017167	Ovis aries
XP_002188354	Taeniopygia guttata
XP_004703761	Echinops telfairi
NP_001129959	Danio rerio
XP_002934254	Xenopus tropicalis
XP_005432754	Falco cherrug
XP_002815636	Pongo abelii
EPY75632	Camelus ferus
XP_005240692	Falco peregrinus
XP_005422070	Geospiza fortis
XP_005720129	Pundamilia nyererei
XP_004566964	Maylandia zebra
XP_005459722	Oreochromis niloticus
XP_005319644	Ictidomys tridecemlineatus
XP_004458914	Dasypus novemcinctus
XP_003462766	Cavia porcellus
EMP39970	Chelonia mydas
XP_005065620	Mesocricetus auratus
XP_006224073	Rattus norvegicus
XP_005356822	Microtus ochrogaster
XP_004623121	Octodon degus
CAG05288	Tetraodon nigroviridis
XP_005500446	Columba livia

XP_005021162	Anas platyrhynchos
XP_005521159	Pseudopodoces humilis
XP_003266152	Nomascus leucogenys
XP_003415610	Loxodonta africana
XP_513126	Pan troglodytes
XP_004318103	Tursiops truncatus
XP_003801268	Otolemur garnettii
XP_002715723	Oryctolagus cuniculus
XP_003759438	Sarcophilus harrisii
ELK26042	Myotis davidii
XP_003216452	Anolis carolinensis
NP_001090431	Xenopus laevis
ELU00951	Capitella teleta
XP_789113	Strongylocentrotus purpuratus
XP_005101541	Aplysia californica
XP_975534	Tribolium castaneum
XP_001661402	Aedes aegypti
XP_319218	Anopheles gambiae
EDS41857	Culex quinquefasciatus
XP_003213507	Meleagris gallopavo
XP_001640202	Nematostella vectensis
XP_002126311	Ciona intestinalis
XP_002114866	Trichoplax adhaerens
EEB16267	Pediculus humanus corporis
EEC14250	Ixodes scapularis
XP_001952608	Acyrthosiphon pisum
XP_003747218	Metaseiulus occidentalis
EFV54576	Trichinella spiralis
EHJ66570	Danaus plexippus
EKC18195	Crassostrea gigas
ENN79439	Dendroctonus ponderosae
XP_004933519	Bombyx mori

XP_002153837	Hydra vulgaris
GAA55992	Clonorchis sinensis

Table S2. Orthologous Proteins with Highest Homology to ZSWIM6 Domains

Ninety-seven orthologous metazoan proteins were aligned with Human ZSWIM6 and >75% conservation was seen from amino acid residues 269-1215.

Supplemental References

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3. **Rohr, K.B., Schulte-Merker, S., and Tautz, D. (1999). Zebrafish zic1 expression in brain and somites is affected by BMP and hedgehog signalling. Mechanisms of development 85, 147-159.**
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5. **Bonifas, J.M., Pennypacker, S., Chuang, P.T., McMahon, A.P., Williams, M., Rosenthal, A., De Sauvage, F.J., and Epstein, E.H., Jr. (2001). Activation of expression of hedgehog target genes in basal cell carcinomas. The Journal of investigative dermatology 116, 739-742.**
6. **Gotschel, F., Berg, D., Gruber, W., Bender, C., Eberl, M., Friedel, M., Sonntag, J., Rungeler, E., Hache, H., Wierling, C., et al. (2013). Synergism between Hedgehog-GLI and EGFR signaling in Hedgehog-responsive human medulloblastoma cells induces downregulation of canonical Hedgehog-target genes and stabilized expression of GLI1. PloS one 8, e65403.**