

## SUPPLEMENTARY INFORMATION

**Title:** CYP26B1-related disorder: expanding the ends of the spectrum through clinical and molecular evidence

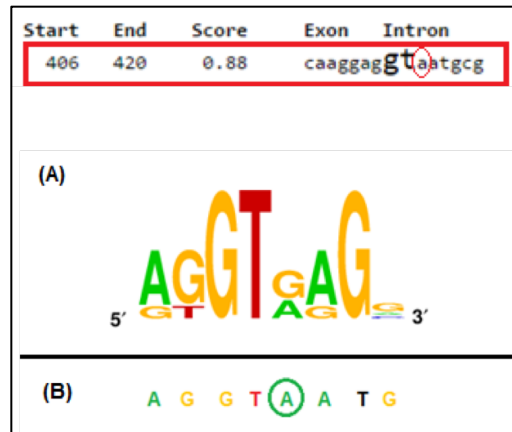
### *Human Genetics*

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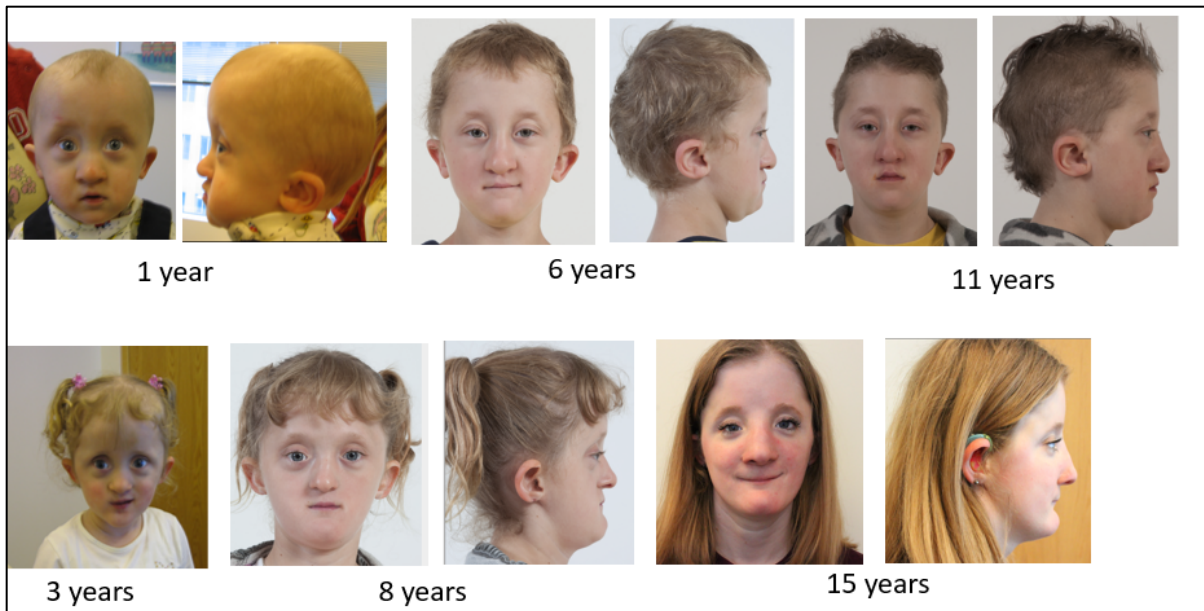
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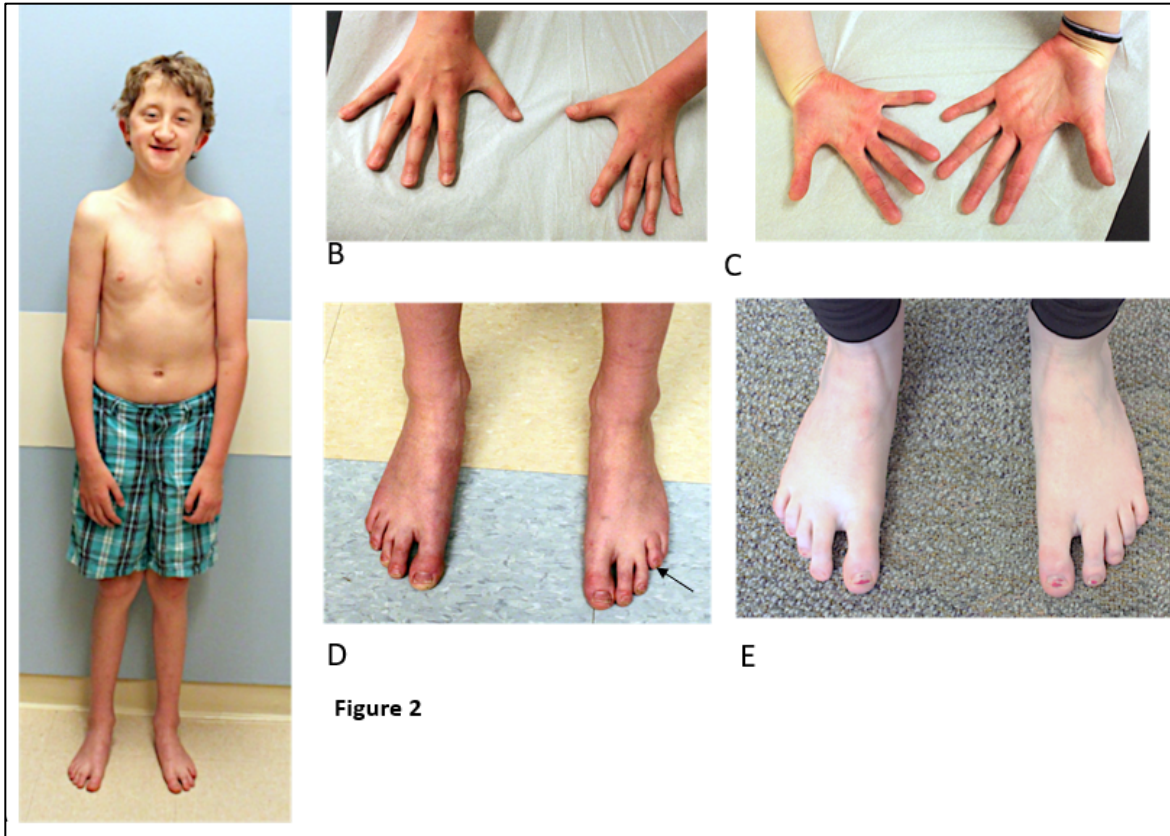
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**Fig S1:** *CYP26B1* donor splice site consensus. The c.1083C>A variant changes the consensus region creating an alternative splice site right before it (circle). A) Consensus region. B) new sequence with the variant c.1083C>A in exon 5.

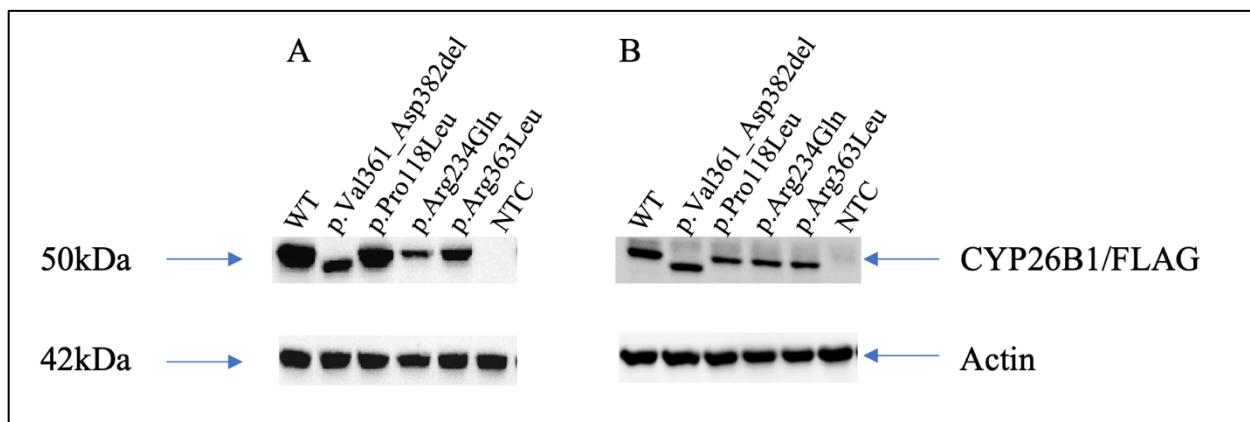


**Fig S2:** Family 1 follow-up.

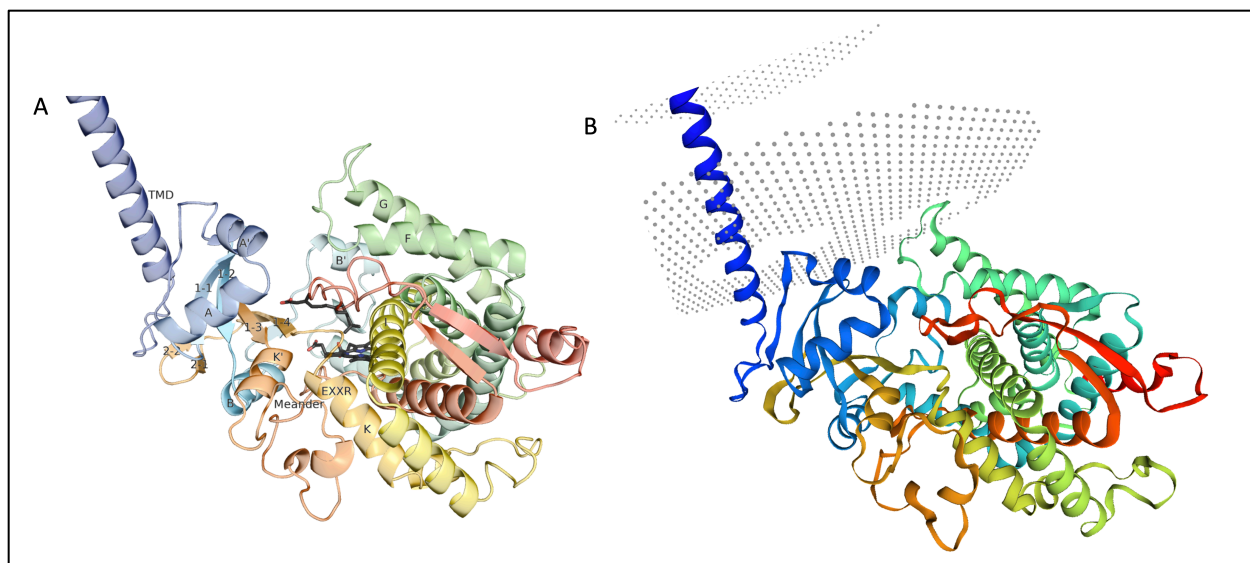


**Figure S3:** Feet and hands from individuals from family 1.

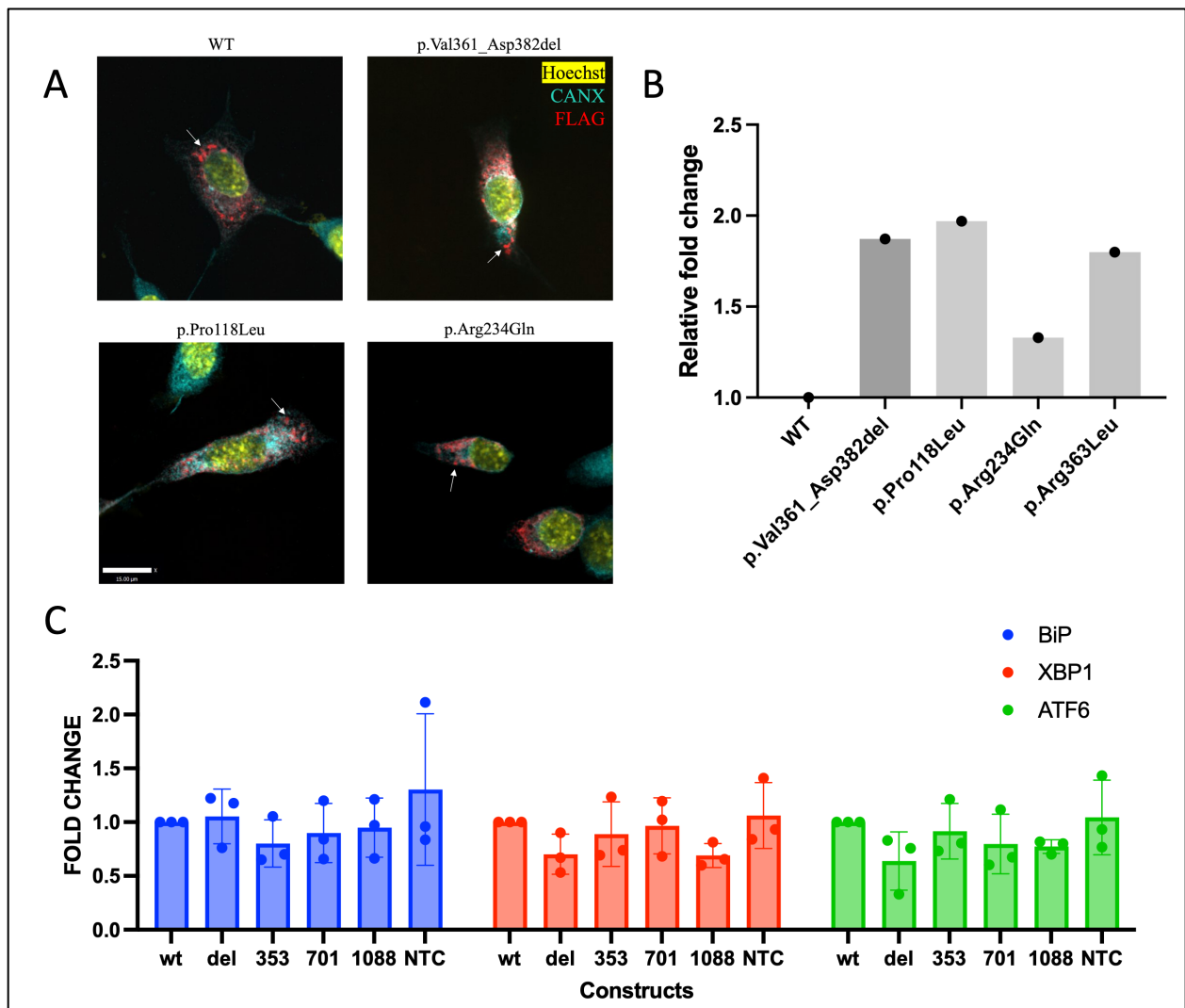




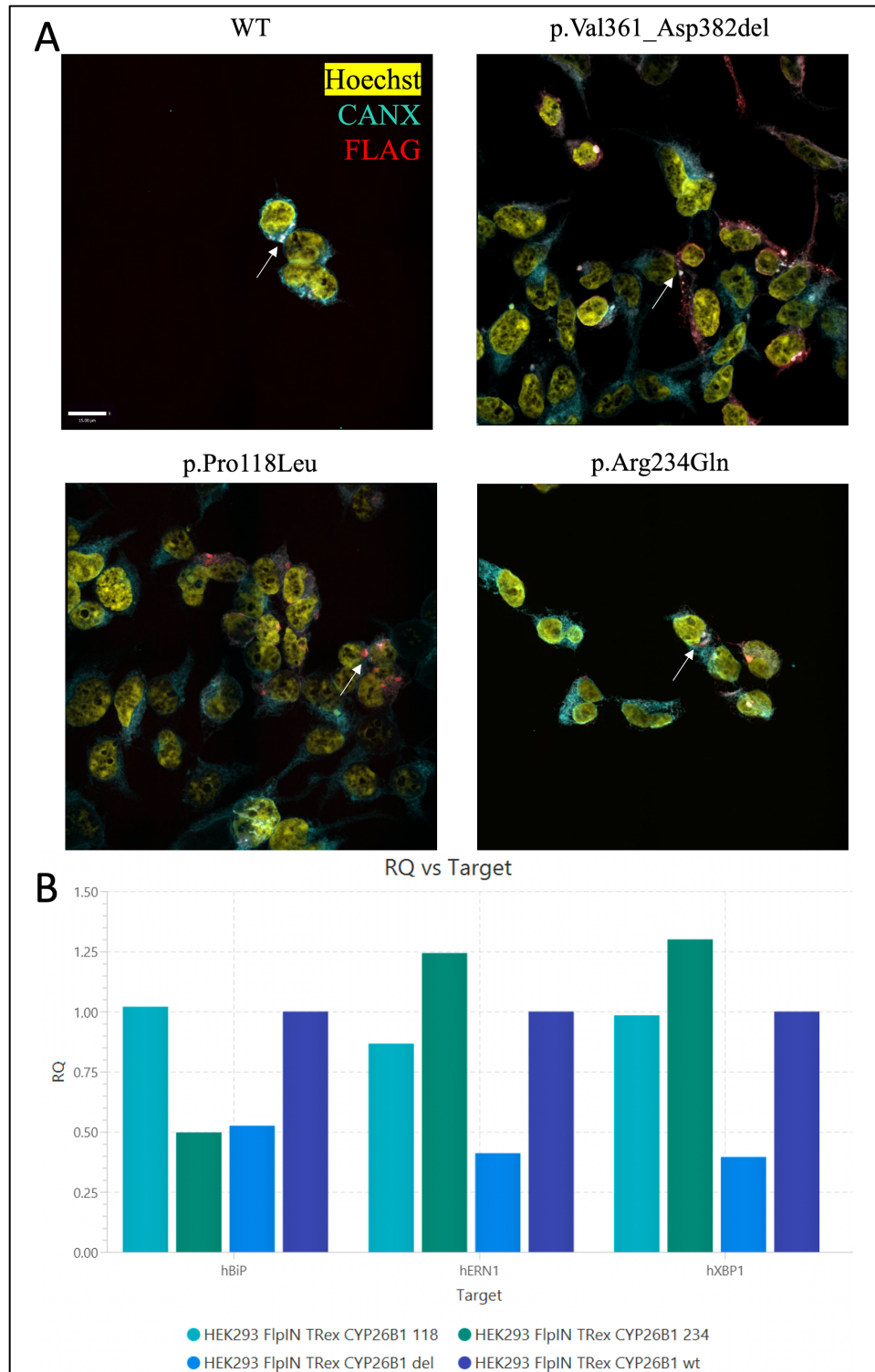
**Fig S4:** Expression vector protein verified by western blot in HEK293T/17 (A) and NIH/3T3 (B).



**Fig S5: A) CYP26B1 protein structural overview.** The 3D model of wildtype CYP26B1 chromatically labeled from N terminus (blue) to C terminus (red), helices and strands are labeled as per P450 structural conservation as letters and pairs of numbers respectively. Retinoic acid is positioned above the heme in the core and are both labeled black. **B) CYP26B1 relative to membranes.** Predicted wildtype structure aligned with a membrane annotation identified 3 points of contact on the CYP26B1 architecture. Labelled chromatically from N terminus (blue) to C terminus (red).



**Fig S6: A)** CYP26B1 foci formation (white arrow) in transiently transfected NIH3T3 cells. **B)** Counting of transiently transfected NIH/3T3 cells presenting the foci formation relative to the WT. At least 166 cells were verified for each construct. **C)** UPR genes (*BiP*, *XBP1*, *ATF6*) expression verified in transiently transfected NIH/3T3 cells by qPCR.



**Fig S7: A)** CYP26B1 foci formation (white arrow) in stably transfected HEK293 cells. **B)** UPR genes (*BiP*, *ERN1*, *XBP1*) expression verified in stably transfected HEK293 cells by qPCR.

**Table S1: Primer sequences.**

| Name                                                  | Primer sequence (5'-3')          |
|-------------------------------------------------------|----------------------------------|
| <b><i>CYP26B1</i> primers – PCR/Sanger sequencing</b> |                                  |
| CYP26B1.E1F                                           | gcccctaagcccgcataaag             |
| CYP26B1.E1R                                           | gaccagggcaagtcacatcttg           |
| CYP26B1.E1IntF                                        | AGGGCTTGGATCTGGTGTCTCGGC         |
| CYP26B1.E2F                                           | cagttcttgtgcacgctatgaa           |
| CYP26B1.E2R                                           | agtcttcaagcatggtgtgcaa           |
| CYP26B1.E3F                                           | ccaaagacttgctggcagctg            |
| CYP26B1.E3R                                           | ctttgtccatgtgccctgtgc            |
| CYP26B1.E4F                                           | gcacagggcacatggacaaag            |
| CYP26B1.E4R                                           | agctcagaatggagcctgggt            |
| CYP26B1.E5F                                           | gctcagaggaagcagaattcac           |
| CYP26B1.E5R                                           | tgaagtcctgaaatggaggct            |
| CYP26B1.E6F                                           | aaggcaggtcttgaacatctgg           |
| CYP26B1.E6R                                           | TCCCACACACAGGTTTCTACCT           |
| <b>MUTAGENESIS – Expression plasmid</b>               |                                  |
| CYP.cDNA.g1088tR                                      | tgggcgtgaacaggagcatgacctccttg    |
| CYP.cDNA.c353t.R                                      | cgggtgctgcgaagccactcgggtgc       |
| CYP.cDNA.g701a.F                                      | cttcagtggctaccagcggggcattcagg    |
| CYP.cDNA.g701a.R                                      | cctgaatgccccgctggtagccactgaag    |
| CYP.cDNA.c353t.F                                      | gcaccgagtggtcttcgcagcaccg        |
| CYP.cDNA.g1088tF                                      | caaggaggtcatgctcctgttcacgcca     |
| CYP26B1.delcDNAF                                      | tggggatctggaaccctccttgatgacgcag  |
| CYP26B1.delcDNAR                                      | ctgcgtcatcaaggagggtttccagatcccca |
| <b>MINIGENE ASSAY – genomic DNA</b>                   |                                  |
| CYP26B1.Intron4.F                                     | GCTCAGAGGAAGCAGAATTCAC           |
| CYP26B1.Intron5.R                                     | TCCCACACACAGGTTTCTACCT           |
| <b>MINIGENE ASSAY – mutagenesis</b>                   |                                  |
| CYP26B1.delcDNA.1083C>A.F                             | CGTCATCAAGGAGGTAATGCGCCTGTTCACG  |
| CYP26B1.delcDNA.1083C>A.R                             | CGTGAACAGGCGCATTACCTCCTTGATGACG  |
| <b>MINIGENE ASSAY – transcript amplification</b>      |                                  |
| cDNA specific primer (pET01 vector)                   | GATCCACGATGC                     |
| PCR.CYP26B1.F                                         | ACTGTGCTGGAGAAGCTGCG             |
| PCR.CYP26B1.R                                         | GGTCGAACACGTTTCACGTCTT           |

**Table S2:** Physical characteristics of our patients from family 1 compared with a previously described individual with biallelic variants in the *CYP26B1* gene who has survived infancy.

|                                 | <b>Proband<br/>Family 1</b> | <b>Sister<br/>Family 1</b> | <b>Morton <i>et al.</i> (1)</b> |
|---------------------------------|-----------------------------|----------------------------|---------------------------------|
| Height, percentile              | 75                          | 95                         | 50                              |
| Weight, percentile              | 25-50                       | 50                         | U                               |
| Head circumference, percentile  | 25-50                       | 25                         | <3                              |
| Turribrachycephaly              | +                           | +                          | +                               |
| Hypertelorism                   | -                           | +                          | +                               |
| Wide orbital fissures           | +                           | +                          | U                               |
| prominent nose                  | +                           | +                          | +                               |
| Malar hypoplasia                | +                           | +                          | +                               |
| bulbous nose tip                | +                           | +                          | +                               |
| small mouth                     | +                           | +                          | +                               |
| protruding ears                 | +                           | +                          | +                               |
| low set ears                    | +                           | +                          | +                               |
| Conductive hearing loss         | +                           | +                          | +                               |
| high palate arch                | +                           | +                          | +                               |
| crowded teeth                   | +                           | +                          | U                               |
| Facial asymmetry                | -                           | -                          | -                               |
| Pointy Chin                     | +                           | +                          | -                               |
| Craniosynostosis                | -                           | -                          | +                               |
| Skull defect/Cranium bifidum    | -                           | -                          | +                               |
| Abnormal spine curvature        | -                           | +                          | +                               |
| Radio-ulnar limitation          | +                           | +                          | -                               |
| Radio-humeral limitation        | -                           | -                          | +                               |
| Pectus excavatum                | +                           | -                          | -                               |
| Prominent pisiform bone         | +                           | +                          | U                               |
| Advanced bone age               | -                           | -                          | U                               |
| Short forth metatarsal          | +                           | -                          | +                               |
| Arachnodactyly                  | +                           | +                          | +                               |
| Camptodactyly                   | -                           | -                          | +                               |
| Fusion metatarsal and cuneiform | +                           | +                          | U                               |
| Bone mineral density            | N                           | N                          | N                               |
| Developmental delay             | +                           | +                          | +                               |
| Learning disability/ID          | +                           | +                          | +                               |
| Autism and behavioral issues    | -                           | -                          | -                               |

(+): present; (-): not present; U: unknown; N: normal; NA: not applicable

**Table S3:** Comparison of the clinical and radiological findings of the fetuses reported by Laue et al. (2011) and the fetus of family 2 presented in this study.

|                                   | Laue et al., 2011 (2)<br>Family 1 |                |                | Present study<br>Family 2 |
|-----------------------------------|-----------------------------------|----------------|----------------|---------------------------|
|                                   | II.1                              | II.2           | II.3           | IV.1                      |
| Parental consanguinity            |                                   |                |                | y                         |
| Gestational age (weeks)           | 36                                | 15             | 12             | 33                        |
| Neural tube defects               | + <sup>a</sup>                    | + <sup>a</sup> | + <sup>a</sup> | + <sup>b</sup>            |
| Calvarial mineralization defects  | +                                 |                |                | +                         |
| Hydrocephalus                     |                                   |                |                | +                         |
| Agenesis of corpus callosum       |                                   |                |                | +                         |
| Microtia                          |                                   |                |                | +                         |
| Narrow thorax                     | +                                 |                |                | +                         |
| Short limbs                       | +                                 | +              | +              | +                         |
| Limited elbow extension           | +                                 |                |                | +                         |
| Aracnodactyly appearance          | +                                 |                |                | +                         |
| Oligodactyly                      | + <sup>c</sup>                    |                |                | + <sup>d</sup>            |
| Distal aphalangia of thumbs       | +                                 |                |                | +                         |
| Radio-ulnar-humeral fusion        | +                                 |                |                | +                         |
| Hypoplasia of scapulae            | +                                 |                |                | +                         |
| Advanced carpal maturation        | +                                 |                |                | +                         |
| Skeletal mineralization decreased |                                   |                |                | +                         |
| Absence of clavicles              |                                   |                |                | +                         |
| Thin ribs                         |                                   |                |                | +                         |
| Torax kyphosis                    |                                   |                |                | +                         |
| Spina bifida occulta              |                                   |                |                | +                         |
| Hypoplasia of pelvis              | +                                 |                |                | ?                         |
| Angulated bones in lower limbs    | + <sup>e</sup>                    | + <sup>e</sup> | + <sup>e</sup> | + <sup>f</sup>            |
| Elongated toes                    | +                                 |                |                | +                         |
| Absence of halux                  | +                                 | +              | +              | +                         |

<sup>a</sup> Encephalocele. <sup>b</sup> Generalized spina bifida occulta. <sup>c</sup> Only the hallux is absent. <sup>d</sup> Hallux and other fingers and toes are absent. <sup>e</sup> Angulated femur. <sup>f</sup> Angulated tibia.

**Table S4:** BioID results (Excel file)



Table S5: HPO terms described in figure 6.

| Phenotype                             | HPO        |                   |                   |
|---------------------------------------|------------|-------------------|-------------------|
| Absent hallux                         | HP:0012386 |                   |                   |
| Accelerated skeletal maturation       | HP:0005616 |                   |                   |
| Advanced ossification of carpal bones | HP:0004233 |                   |                   |
| Agenesis of corpus callosum           | HP:0001274 |                   |                   |
| Arachnodactyly                        | HP:0001166 |                   |                   |
| Broad fingertip                       | HP:0011300 |                   |                   |
| Calvarial mineralization defects      |            |                   |                   |
| Camptodactyly                         | HP:0012385 |                   |                   |
| Conductive hearing loss               | HP:0000405 |                   |                   |
| Convex nasal bridge                   | HP:0000426 |                   |                   |
| Cranial lacunae                       |            |                   |                   |
| Craniosynostosis                      | HP:0001363 |                   |                   |
| Cupped ear                            | HP:0000378 |                   |                   |
| Decreased skull ossification          | HP:0004331 |                   |                   |
| Development delay                     | HP:0012758 |                   |                   |
| Distal phalanga of thumbs             |            |                   |                   |
| Down slanted palpebral fissures       | HP:0000494 |                   |                   |
| Elongated toes                        | HP:0010511 |                   |                   |
| Exophthalmos                          | HP:0000520 |                   |                   |
| Exotropia                             | HP:0000577 |                   |                   |
| Exotropia                             | HP:0000577 |                   |                   |
| Gracile bones                         | HP:0003100 |                   |                   |
| Hydrocephalus                         | HP:0000238 |                   |                   |
| Hypoplastic pelvis                    | HP:0008839 |                   |                   |
| Hypoplastic scapulae                  | HP:0000882 |                   |                   |
| Hypoplastic scrotum                   | HP:0000046 |                   |                   |
| Limited elbow extension               | HP:0001377 |                   |                   |
| Microtia                              | HP:0008551 |                   |                   |
| Midface retrusion                     | HP:0011800 |                   |                   |
| Myopia                                | HP:0000545 |                   |                   |
| Narrow thorax                         | HP:0000774 |                   |                   |
| Occipital encephalocele               | HP:0002085 |                   |                   |
| Oligodactyly                          | HP:0012165 |                   |                   |
| Prominent nose                        | HP:0000448 |                   |                   |
| Recurrent globe subluxation           |            |                   |                   |
| Respiratory failure                   |            |                   |                   |
| Rocker bottom foot                    | HP:0001838 |                   |                   |
| Scoliosis                             | HP:0002650 |                   |                   |
| Short angulated femur                 |            |                   |                   |
| Short thumbs / hallux                 | HP:0009778 | HP:0010109        |                   |
| Spina bifida occulta                  | HP:0003298 |                   |                   |
| Synostosis                            | HP:0100240 | <u>HP:0004278</u> | <u>HP:0100238</u> |
| Thin ribs                             | HP:0000883 |                   |                   |
| Thoracic kyphosis                     | HP:0002942 |                   |                   |
| Underdeveloped genitalia              | HP:0000050 | HP:0012815        |                   |
| Ventral chordee                       | HP:0000041 |                   |                   |

## References

1. Morton, J.E.V., Frentz, S., Morgan, T., Sutherland-Smith, A.J. and Robertson, S.P. (2016) Biallelic mutations in CYP26B1: A differential diagnosis for Pfeiffer and Antley–Bixler syndromes. *Am. J. Med. Genet. Part A*, **170**, 2706–2710.
2. Laue, K., Pogoda, H.M., Daniel, P.B., Van Haeringen, A., Alanay, Y., Von Ameln, S., Rachwalski, M., Morgan, T., Gray, M.J., Breuning, M.H., *et al.* (2011) Craniosynostosis and multiple skeletal anomalies in humans and zebrafish result from a defect in the localized degradation of retinoic acid. *Am. J. Hum. Genet.*, **89**, 595–606.