**Supplementary Data**

**Supplementary Table 1**. Gene and phenotype MIM numbers, GenBank transcripts ID, and cytogenetic location of the analyzed genes

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| Gene | Gene MIM number | GenBank RefSeq | Cytogenetic location | Phenotype MIM number |
| *SHH* | 600725 | NM\_000193.4 | 7q36.3 | HPE3 #142945 |
| *SIX3* | 603714 | NM\_005413.4 | 2p21 | HPE2 #157170 |
| *TGIF* | 602630 | NM\_003244.4 | 18p11.31 | HPE4 #142946 |
| *ZIC2* | 603073 | NM\_007129.5 | 13q32.3 | HPE5 #609637 |
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| MIM = http:/www/omim.org; GenBank = http://www.ncbi.nlm.nih.gov/Genbank/ | | | | |

**Supplementary Table 2**. Clinical and variant data of seven patients presenting benign variants.

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| Patient | Clinical information | SNV & Population Frequency | Variant Effect | Variant versus wild type mRNA drawing by VARNA tool |
| 4 | Female, one-year-old, 35-year-old mother, presenting microcephaly, MRI showing lobar holoprosencephaly and corpus callosum agenesis, sparse anterior and posterior scalp hair, ocular hypotelorism, bilateral cleft of lip and palate, flat nose, retrognathia, and non-craniofacial defects. The variant was inherited from her father. | *SHH* sSNV (rs9333635; c.897G>C, p.Leu299=)  0 - 0,99% | HSF: Potentially causes an alteration of splicing by modification of an ESE site.  Kim et al., 2020: Alterations of codon usage, protein misfolding, and degradation; 13% reduction of SHH amount in cell assay. | Highly changed. |
| 5 | Male, six-month-old, third pregnancy of mother 33, and father 36 years old, healthy and nonconsanguineous. At birth he presented alobar HPE by trans-fontanelle ultrasound, right microphthalmia, left eyelid coloboma with ipsilateral proptosis, arrhinia, premaxillary agenesis, right microtia, left dysmorphic ear with preauricular tags, and a complex heart disease. | *SHH* sSNV (rs9333634; c.630C>T, p.Gly210=)  0 - 3.85% | HSF: Potentially causes an alteration of splicing by activation of an ECD site and creation of an ESS site.  Kim et al., 2020: Alterations of codon usage, protein misfolding, and degradation; 5% reduction of SHH amount in cell assay. | Slightly changed. |
| 7 | Male stillbirth with a prenatal diagnosis of holoprosencephaly, arrhinia, and severe bilateral microphthalmia. At birth, he also presented severe oxycephaly, ocular hypotelorism, bilateral microtia, and a rare median cleft of the lip and soft palate. | *SIX3* sSNV (rs186163123; c.219C>T, p.Pro73=)  0.01 - 0.15% | HSF: Potentially produces an alteration of an ESE site | Not changed. |
| 8 | Male, 105-days-old, 30-year-old mother, presented HPE with no further specified type, anterior narrowing of the nasal fossa, bilateral stenosis of the piriform opening, SMMCI, and lacrimal ducts stenosis. | *SIX3* sSNV c.576C> T, p.Arg192=, rs182881  0.12 - 4.87% | HSF: Potentially induces alteration of splicing by modification of an ESE site, activation of an ECD site, and creation of an ESS site. | Highly changed. |
| 9 | Male newborn, presenting cerebral CT scan with semilobar HPE, hydrocephaly, cranial asymmetry with open cranial sutures, and no facial clefts. | *ZIC2* sSNV (c.716\_718dupACC, p.His239dup, rs398124241)  0.07 - 12.70% | HSF: Potentially induces alteration of splicing by modification of an ESE site. | Not changed. |
| 10 | Male newborn presenting MRI with alobar HPE, premaxillary agenesis, absent nasal bone, ocular hypotelorism, and non-craniofacial defects. | *ZIC2* sSNV (c.716\_718dupACC, p.His239dup, rs398124241)  0.07 - 12.70% | HSF: Potentially induces alteration of splicing by modification of an ESE site. | Not changed. |
| 11 | Female newborn presenting alobar HPE, maxillary agenesis, arrhinia, ocular hypotelorism, and a sacral dimple. Parents were nonconsanguineous, 33-year-old mother, and 30-years-old, healthy father | *ZIC2* intronic SNV (rs139312964, c.1239+18G>A)  0 – 6,62% | HSV: Potential alteration of an Intronic Splicing Silencer (ISS) site and creation of an Intronic Splicing Enhancer (ISE) site, but with no impact on splicing. | -- |

SNV = Single Nucleotide Variant; sSNV = synonym SNV; HSF = Human Splicing Finding; ESE = Exonic Splicing Enhancer; ESS = Exonic Splicing Silencer; ECD = Exonic Cryptic; VARNA ([http://varna.lri.fr/)](http://varna.lri.fr/)