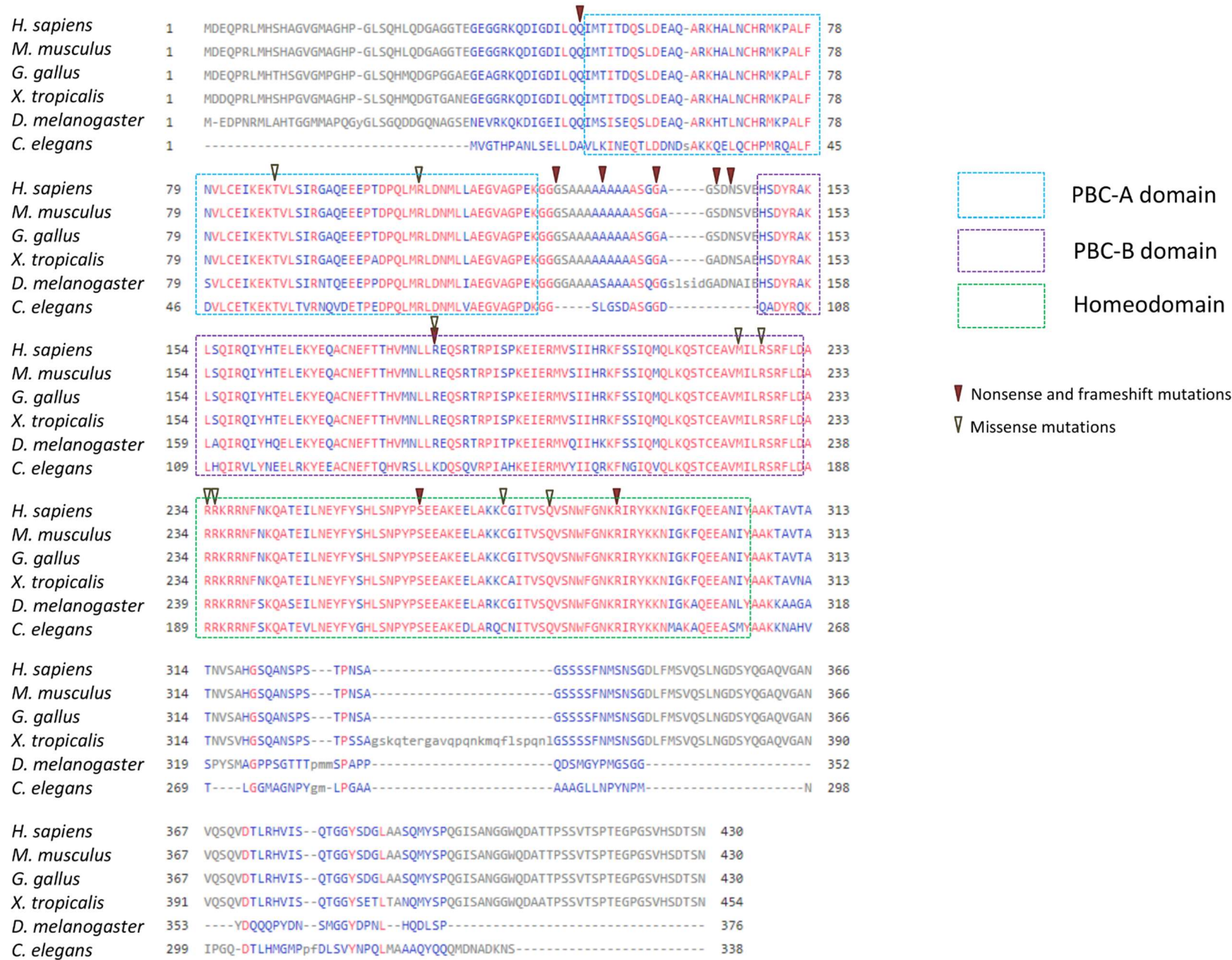


Supplementary Material



Supporting Figure S1 - Alignment of protein sequences of PBX1 homologues in different species.

PBX1a isoform, NP\_002576.1

|                     | Protein domain or DNA localization |       |     |       |    |        |                             |     |              |
|---------------------|------------------------------------|-------|-----|-------|----|--------|-----------------------------|-----|--------------|
|                     | N-term                             | PBC-A | PBC | PBC-B | HD | C-term | Intronic and splice regions | UTR | <i>Total</i> |
| <b>Variant type</b> |                                    |       |     |       |    |        |                             |     |              |
| Frameshift          |                                    |       |     |       | 1  | 1      |                             |     | 2            |
| Inframe deletion    |                                    |       | 1   |       |    | 1      |                             |     | 2            |
| Inframe insertion   |                                    |       | 2   |       |    |        |                             |     | 2            |
| Intronic            |                                    |       |     |       |    |        | 114                         |     | 114          |
| Missense            | 6                                  | 1     | 4   | 3     | 2  | 19     |                             |     | 35           |
| Splice region       |                                    | 1     |     |       |    |        | 11                          |     | 12           |
| Synonymous          | 5                                  | 6     | 6   | 7     | 9  | 14     |                             |     | 47           |
| 3' UTR              |                                    |       |     |       |    |        |                             | 34  | 34           |
| 5' UTR              |                                    |       |     |       |    |        |                             | 10  | 10           |
| <i>Total</i>        | 11                                 | 8     | 13  | 10    | 12 | 35     | 125                         | 44  | 258          |

**Supporting Table S1 - Type and localization of exonic and intronic point variations observed in PBX1 in the general population**

N-term, C-term : N and C termini of the protein, HD: homeodomain. Only variants with an allele count > 1 are considered.

| Article or accession number   | Heterozygous variant (NM_002585.4) | Protein change (NP_002576.1) | Chromosomal sex | Face and head anlies (HP:0000271, HP:0000234) | Renal and urinary anlies (HP:0000077, HP:0000079)        | Abnl external genitalia (HP:0000811)                         | Gonadal dysgenesis/uterine anlies (HP:0000133, HP:0000130)              | Abnl skeletal morphology (HP:0011842) | Cardiac anlies/arrhythmia (HP:0001627, HP:0011675) | Lung hypoplasia (HP:0002089) | Deafness (HP:0000365) | Morphological NCS anlies (HP:0002011) | ID (HP:0001249) | Other   | Note |
|---|------------------------------------|------------------------------|-----------------|---|--|--|---|---------------------------------------|--|------------------------------|-----------------------|---------------------------------------|-----------------|---|------|
| ClinVar VCV000666556  | c.145C>T                           | p.(Gln49Ter)                 | XY              | NP  | CAKUT  | NP   | NP  | NP                                    | NP   | NP                           | NP                    | NP                                    | NP              | NP  |      |
| (Fromer et al., 2014)   | c.264C>A                           | p.(Thr88Ile)                 | XY              | NP  | NP   | NP   | NP  | NP                                    | NP   | NP                           | NP                    | NP                                    | NP              | Schizophrenia   |      |
| (Arts et al., 2020)   | c.319C>T                           | p.(Arg107Trp)                | XX              | Facial gestalt                                | Dilated bilateral renal pelves, horseshoe kidney         | No   | Bicornuate uterus   | No                                    | No   | Yes                          | Not evaluable         | No                                    | Not evaluable   | Polyhydramnios<br>Increased nuchal translucency<br>Diaphragmatic defect<br>Asplenia | Sibs |
| (Arts et al., 2020)   | c.319C>T                           | p.(Arg107Trp)                | XY              | Yes   | Bilateral renal pelvic dilatation<br>Hypoplastic kidneys | Normal-appearing female genitalia                            | Normal-appearing female gonads and uterus                               | No                                    | Ventricular septal defect<br>Overriding aorta      | Yes                          | Not evaluable         | No                                    | Not evaluable   | Diaphragmatic defect<br>Supernumerary spleen  | Sibs |
| ClinVar VCV000817312.1  | c.370_371dup                       | p.(Gly125fs)                 | NP              | NP  | NP   | NP   | NP  | NP                                    | NP   | NP                           | NP                    | NP                                    | NP              | NP  |      |
| ClinVar RCV001008581.1  | c.392del                           | p.(Ala131fs)                 | NP              | NP  | NP   | NP   | NP  | NP                                    | NP   | NP                           | NP                    | NP                                    | NP              | NP  |      |
| (Riedhammer et al., 2017)   | c.413_419del                       | p.(Gly138fs)                 | XY              | Facial gestalt                                | Bilateral dysplastic kidneys                             | Cryptorchidism   | No  | Hypoplasia of the clavicles           | No   | No                           | No                    | No                                    | Yes             | No  |      |
| (Heidet et al., 2017)   | c.422C>G                           | p.(Ser141Ter)                | NP              | NP  | NP   | NP   | NP  | NP                                    | NP   | NP                           | NP                    | NP                                    | NP              | NP  |      |
|   | c.428del                           | p.(Asn143fs)                 | XX              | No  | Bilateral renal hypoplasia                               | No   | No  | Scoliosis                             | No   | No                           | Yes                   | No                                    | No              | No  |      |
|   | NG_028246.2 (PBX1):c.511-2A>G      | Splicing alteration          | XY              | No  | Bilateral renal hypoplasia with oligonephronia           | No   | No  | No                                    | No   | No                           | No                    | No                                    | No              | Oligohydramnios   |      |
|   | c.550C>T                           | p.(Arg184Ter)                | XX              | Facial gestalt                                | Bilateral multicystic dysplastic kidneys                 | No   | No  | No                                    | No   | No                           | No                    | No                                    | Yes             | No  |      |
| (Alankarage et al., 2020)   | NP                                 | p.(Arg184Pro)                | XY              | Facial gestalt                                | Hyperechogenic kidneys                                   | No   | No  | Clinodactyly                          | Tetralogy of Fallot with absent pulmonary valve    | No                           | No                    | No                                    | Yes             | Recurrent bronchiolitis   |      |
| (Slavotinek et al., 2017)   | c.671T>A                           | p.(Met224Lys)                | XY              | Facial gestalt<br>Micrognathia                | No   | Cryptorchidism<br>Microphallus                               | Intra-abdominal testicular tissue<br>Persistence of the Mullerian ducts | No                                    | Patent ductus arteriosus                           | Yes                          | No                    | No                                    | Yes             | Diaphragmatic defect  |      |
|   | c.680G>C                           | p.(Arg227Pro)                | XY              | Branchial cartilaginous rests                 | No   | Cryptorchidism   | No  | No                                    | No   | No                           | No                    | No                                    | Yes             | No  |      |
| ClinVar VCV000559855  | c.701G>A                           | p.(Arg234Gln)                | XX              | NP  | CAKUTHD  | NP   | NP  | NP                                    | NP   | NP                           | NP                    | NP                                    | NP              | NP  |      |
| (Slavotinek et al., 2017)   | c.701G>C                           | p.(Arg234Pro)                | NP              | No  | Unilateral pyelectasis<br>Hyperechogenic kidneys         | No   | No  | No                                    | Patent ductus arteriosus                           | No                           | No                    | No                                    | Yes             | No  |      |
| (Kia, Sarafoglou, Mooganayakanakote Siddappa, & Roberts, 2019; Slavotinek et al., 2017) | c.704G>A                           | p.(Arg235Gln)                | XY              | Macrocephaly                                  | No   | Small genital tubercule<br>Labial folds<br>Vaginal introitus | Uterus didelphys<br>Right gonad: ovotestis<br>Left gonad: agenesis      | No                                    | No   | No                           | No                    | No                                    | Yes             | Polyhydramnios<br>Respiratory distress<br>Hypotonia                                 |      |

|  |   |                             |    |   |  |                               |    |    |                            |   |     |     |          |     |  |      |
|--|---|-----------------------------|----|---|--|-------------------------------|----|----|----------------------------|---|-----|-----|----------|-----|--|------|
| (Slavotinek et al., 2017)  | c.704G>A                                | p.(Arg235Gln)               | XY | Blue sclerae<br>Redundant nuchal skin<br>Microtia<br>Micrognathia                 | Dilated fetal ureters  | Cryptorchidism                | No |    | Brachydactyly              | Patent ductus arteriosus                              | Yes | No  | No       | No  | No   |      |
| (Eozenou et al., 2019)   | c.704 G>T                               | p.(Arg235Gln)               | XY |   |  |                               |    |    |                            |   |     |     |          |     |  |      |
| (Slavotinek et al., 2017)  | c.783dup                                | p.(Ser262fs)                | NP | Dysplastic ears<br>External auditory canal stenosis<br>Microtia<br>Facial gestalt | No   | No                            | No |    | No                         | No  | No  | Yes | No       | Yes | No   |      |
| ClinVar VCV000598775   | c.818G>A                                | p.(Cys273Tyr)               | NP | NP  | CAKUTED  | NP                            | NP | NP | NP                         | NP  | NP  | NP  | NP       | NP  | NP   |      |
| ClinVar RCV001251145.1   | c.836A>G                                | p.(Gln279Arg)               | NP | NP  | CAKUTED  | NP                            | NP | NP | NP                         | NP  | NP  | NP  | NP       | NP  | NP   |      |
| (Eozenou et al., 2019)   | c.862C>T                                | p.(Arg288Ter)               | NP | Dysplastic ears<br>External auditory canal stenosis<br>Facial gestalt             | Bilateral renal hypoplasia   | No                            | No |    | Brachydactyly              | No  | No  | No  | No       | Yes | No   |      |
| (Fitzgerald, Powell-Hamilton, Shillingford, Robinson, & Gripp, 2021) | arr[hg19] 1q23.3(164749027_164786500)x1 | p.?<br>Deletion of exon 3-6 | XY | Abnormality of the pinna<br>Prominent forehead                                    | Bilateral renal hypoplasia   | Hypospadias<br>Cryptorchidism | No |    | Unilateral hip dysplasia   | Tetralogy of Fallot                                   | No  | No  | No       | Yes | Failure to thrive<br>Single left palmar crease | Sibs |
|  | arr[hg19] 1q23.3(164749027_164786500)x1 | p.?<br>Deletion of exon 3-6 | XX | Abnormality of the pinna<br>Bifid uvula   | Hyperechogenic kidneys   | No                            | No |    | Scoliosis<br>Short stature | No  | No  | No  | Seizures | No  | No   | Sibs |
|  | arr[hg19] 1q23.3(164749027_164786500)x1 | p.?<br>Deletion of exon 3-6 | XX | Abnormality of the pinna  | Bilateral renal hypoplasia   | No                            | No |    | Short stature              | No  | No  | No  | No       | No  | Failure to thrive                              | Sibs |
| (Le Tanno et al., 2017)  | Whole gene deletion                     |                             | XY | Facial gestalt  | Bifid right ureter<br>Bilateral renal pelvic dilatation<br>Urethral valves                 | No                            | No |    | No                         | No  | No  | No  | Seizures | Yes | Sacral dimple                                  |      |
|  | Whole gene deletion                     |                             | XX | Microcephaly<br>Facial gestalt  | Bilateral renal hypoplasia<br>Nephrocalcinosis   | No                            | No |    | Scoliosis                  | Ventricular septal defect<br>Patent ductus arteriosus | No  | Yes | No       | Yes | No   |      |
|  | Whole gene deletion                     |                             | XX | Facial gestalt  | Bilateral renal hypoplasia and dysplasia<br>Right ectopic kidney<br>Hyperechogenic kidneys | No                            | No |    | No                         | Ventricular septal defect<br>Patent ductus arteriosus | No  | No  | No       | Yes | Sacral dimple                                  |      |



|                 |                     |  |    |                |    |                                |    |                 |                   |    |    |    |     |   |
|-----------------|---------------------|--|----|----------------|----|--------------------------------|----|-----------------|-------------------|----|----|----|-----|---|
| DECIPHER 262739 | Whole gene deletion |  | XY | Facial gestalt | No | Cryptorchidism<br>Microphallus | No | Hip dislocation | Cardiac arrythmia | No | No | No | Yes | Ectopic anus<br>Prune belly<br>Anterior hypopituitarism |
|-----------------|---------------------|--|----|----------------|----|--------------------------------|----|-----------------|-------------------|----|----|----|-----|---|

**Supporting Table S2: Phenotype of previously published PBX1-mutated patients**

NMD: Nonsense-Mediated Decay, LoF: Loss of Function, Anlies: anomalies, Abnl: abnormal, CNS: Central Nervous System, ID: Intellectual Disability, NP: not provided, Sibs : siblings