**Novel clinical criteria allow detection of SHOX haploinsufficiency caused by either gene or enhancer region defects**

**Supplementary Table 1**

Description of *SHOX* variants and their corresponding classification

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| **Nr.** | **Mutation** | **Classification** |
| 1 | P018-D1 del probes 6291-6291 ; 3'-flanking region 47.5 kb deletion [1] | Pathogenic |
| 2 | P018-B del probes 5642-6293 3'-flanking region | Pathogenic |
| 3 | Mozaic for deletion complete SHOX gene + flanking sequences | Pathogenic; mosaic |
| 4 | P018-E1 del probes 5642-9335 3'-flanking sequence | Pathogenic |
| 5 | P018-E1 del probes 5642-9335 3'-flanking sequence | Pathogenic |
| 6 | P018-D1 del probes 6291-6291 ; 47.5 kb deletion 3'-flanking sequence [1] | Pathogenic |
| 7 | 47.5 kb deletion (probes 318S and 432S) [1] | Pathogenic |
| 8 | P018-E1 del probes 5645-5646; 47.5 kb deletion 3'-flanking sequence [1] | Pathogenic |
| 9 | P018-D1 del probes 6291-6291 ; 47.5 kb deletion 3'-flanking sequence [1] | Pathogenic |
| 10 | P018-D1 del probes 6291-6291 ; 47.5 kb deletion 3'-flanking sequence [1] | Pathogenic |
| 11 | P018-E1 del 5642-10251; deletion 3'-flanking region | Pathogenic |
| 12 | P018-E1 del probes 5645-5646; 47.5 kb deletion 3'-flanking sequence [1] | Pathogenic |
| 13 | P018-D1 del probes 6291-6291 ; 47.5 kb deletion 3'-flanking sequence [1] | Pathogenic |
| 14 | P018-C del probes 5642-9334 deletion 3'-flanking sequence | Pathogenic |
| 15 | P018-D1 del probes 6291-6291 ; 47.5 kb deletion 3'-flanking sequence [1] | Pathogenic |
| 16 | P018-D1 del probes 6291-6291 ; 47.5 kb deletion 3'-flanking sequence [1] | Pathogenic |
| 17 | P018-D1 del probes 5642-6293 deletion 3'-flanking region [1] | Pathogenic |
| 18 | 47.5 kb deletion (probes 136-428) [1] | Pathogenic |
| 19 | P018-D1 del probe 9333-L10292; 5'-flanking region | VUS\* |
| 20 | P018-D1 del probe 9333-L10292; 5’-flanking region | VUS\* |
| 21 | Complete gene including 5' and3'-flanking sequences | Pathogenic |
| 22 | Complete gene including 5' and 3'-flanking sequences | Pathogenic |
| 23 | Complete gene including 5' and 3'-flanking sequences | Pathogenic |
| 24 | Complete gene including 5' and 3'-flanking sequences | Pathogenic |
| 25 | Complete gene deletion | Pathogenic |
| 26 | P018-B del probes 5642-6293 3'-flanking region | Pathogenic |
| 27 | Partial gene deletion exon 3-7; P018B 1147-9336 | Pathogenic |
| 28 | Complete gene deletion | Pathogenic |
| 29 | Complete gene deletion | Pathogenic |
| 30 | Complete gene deletion | Pathogenic |
| 31 | Complete gene deletion | Pathogenic |
| 32 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 33 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 34 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 35 | P018-B del probes 5642-6293 3'-flanking region; | Pathogenic |
| 36 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 37 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 38 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 39 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 40 | P018-F1 del 1146-9336 partial gene deletion exon 2-6 | Pathogenic |
| 41 | Complete gene deletion | Pathogenic |
| 42 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 43 | P018-F1 del 13296-1153 deletion 3'-flanking region | Pathogenic |
| 44 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 45 | Partial gene deletion exon 4-7; P018B del [probes 1148-9336 | Pathogenic |
| 46 | Complete gene deletion including 3'-flanking sequences | Pathogenic |
| 47 | Complete gene deletion including 3'-flanking sequences | Pathogenic |
| 48 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 49 | Complete gene deletion including 3'-flanking sequences | Pathogenic |
| 50 | Complete gene deletion | Pathogenic |
| 51 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 52 | Complete gene deletion | Pathogenic |
| 53 | P018-D1 del probes 6291-6291 ; 47.5 kb deletion 3'-flanking sequence [1] | Pathogenic |
| 54 | Complete gene deletion | Pathogenic |
| 55 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 56 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 57 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 58 | Complete gene deletion | Pathogenic |
| 59 | Complete gene deletion | Pathogenic |
| 60 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 61 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 62 | P018-F1 del probes 13297-1153; deletion 3'-flanking sequence | Pathogenic |
| 63 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 64 | Complete gene deletion including 5' and 3'-flanking sequences | Pathogenic |
| 65 | c.427delg, p.(Glu158fs); Frameshift mutation | Pathogenic |
| 66 | c.583C>T, p.(Arg195)\*; Nonsense mutation [2] and various publications | Pathogenic |
| 67 | c.1A>G p.(Met1); initiation codon mutation [3] | Pathogenic |
| 68 | c.503G>A, p.(Arg168Gln); Missense mutation [4] | Pathogenic |
| 69 | c.503G>A, p.(Arg168Gln); Missense mutation [4] | Pathogenic |
| 70 | c.623C>T p.(Pro208Leu); Missense mutation 1x in Esoterix (2007) SHOX locus-specific database | Pathogenic |
| 71 | P018-B del probes 5642-6293; deletion 3'-flanking region | Pathogenic |
| 72 | c.390\_c.391ins28 p.(Glu131fs); frameshift mutation | Pathogenic |
| 73 | c.399G>C p.(Glu133Asp) Missense mutation [5] | Pathogenic |
| 74 | c.461T>C p.(Leu154Pro) Missense mutation [6, 7] | Pathogenic |
| 75 | c.698C>T p.(Ala233Val) ; Missense mutation [8]; Esoterix (2007) SHOX Locus-specific database | Pathogenic |
| 76 | c.691C>T p.(His231Tyr) ; Missense mutation; not published | VUS\*\* |

MRC-Holland kit (P018) version and probe numbers are provided if necessary for the classification of the deletion [9]. Mutations detected by Sanger sequencing are described using HGVS for RefSeq NM\_000451.3.

VUS: variant of uncertain significance.

\*This upstream enhancer deletion was *in silico* classified as a class 3 variant of uncertain significance (VUS), and was observed in patients 19 and 20 from unrelated families. In patient 20, single nucleotide polymorphism (SNP) array analysis confirmed the presence of a deletion in this region (arr Xp22.33(1-416,314)x1 mat).

Auxological data of patient 19 are: height -1.9 SDS, sitting height to standing height (SH/H) ratio 2.6 SDS, and her arm span is unknown. Her father also carries the mutation, and his height is -1.5 SDS and SH/H ratio 2.0 SDS.

Auxological data of patient 20 are: height -2.5 SDS, SH/H ratio 0.8 SDS, arm span minus height -3.1 cm, and she shows bowing of the forearm and muscular hypertrophy. She was treated with recombinant human growth hormone. Her mother also carries the mutation, and her height is -0.6 SDS and SH/H ratio 0.8 SDS.

Given the typical phenotype in multiple subjects with this class of enhancer deletions, we consider this variant as likely pathogenic.

\*\*This intragenic *SHOX* mutation was *in silico* classified as a variant of uncertain significance according to the ACMG/AMP classification (PM2, PP2, BP4). The variant is reported only once in the gnomAD reference database of control individuals (heterozygous in a European (non-Finnish) individual in 121016 alleles (MAF 0.00083%)) supporting the pathogenicity as described by Nykamp et al. [10].

The patient has a height of -2.8 SDS and SH/H ratio of 2.7 SDS. Her arm span is unknown. Her father also carries the mutation, and his height is -2.4 SDS and his SH/H ratio 1.5 SDS, thus fitting *SHOX* haploinsufficiency. Based on these considerations, we classified this variant to be possibly pathogenic.

**References**

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