|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **MISSENSE VARIATIONS** | | | | | | | | | | | | | | | | | |
| **Country** | **IDSample** | | **DoB** | | **DNA variation** | | **Biopsy** | | **Leiden database** | | **ClinVar** | | **GnomAD** | | **Bioinformatics tools: Polyphen and Human Spling Finder (HSF)** | | **Phenotype** |
| Algeria | GM 1657/17 | | 31/03/1989 | | Exon 39: c.5464G>A (p.Gly1822Ser) | | Not available | | VUS/Pat | | Uncertain significance /LB | | MAF=0.00004 | | PolyPhen: benign (score of 0.024). HSF: probably no impact on splicing. | | BMD |
| Poland | GM 2735/18 | | 14/02/2005 | | Exon 6: c.450T>G (p.Asn150Lys) | | Absence of DYS | | LB/Pat | | Uncertain | | Not reported | | PolyPhen: Benign (score=0.054). HSF: 1. Activation of an exonic cryptic donor site. 2. Activation of an exonic cryptic acceptor site, with presence of one or more cryptic branch point(s). 3. Creation of an exonic ESS site. Potential alteration of splicing. | | High CK |
| Poland | GM 3064/18 | | 26/052013 | | Exon 37: c.5192T>A (p.Val1731Glu) | | Not available | | Not reported | | Not reported | | MAF= 0.000005 | | Polyphen: possibly damaging (score=0.669). HSP: 1. Activation of an exonic cryptic acceptor site, with presence of one or more cryptic branch point(s). 2. Creation of an exonic ESS site. Potential alteration of splicing. | | High CK |
| Poland | GM 3318/18 | | 26/04/2000 | | Exon 10: c.1022T>A (p.Leu341Gln) | | Not available | | Not reported | | Not reported | | MAF= 0.000005 | | Polyphen: probably damaging (score=1.000). HSP: 1. Activation of an exonic cryptic acceptor site, with presence of one or more cryptic branch point(s). 2. Alteration of an exonic ESE site. Potential alteration of splicing. | | High CK |
| Ukraine | GM 3097/18 | | 12/04/2006 | | Exon 35: c.4979G>C (p.Trp1660Ser) | | Not available | | Not reported | | Not reported | | Not reported | | Polyphen: probably damaging, score=0.999. HSP: Alteration of an exonic ESE site. Potential alteration of splicing. | | DMD |
| **CONSENSUS SPLICE SITE VARIATIONS** | | | | | | | | | | | | | | | | | |
| **Country** | | **IDSample** | | **DoB** | | **DNA variation** | | **Biopsy** | | **Leiden database** | | **ClinVar** | | **GnomAD** | | **Bioinformatic tool: Human splicing finder (HSF)** | **Phenotype** |
| Algeria | | GM 1688/17 | | 25/03/2013 | | intron 58 c.8668+26C>A | | Not available | | VUS | | Uncertain significance | | MAF=0.0004 | | HSF: Creation of an intronic ESE site. Probably no impact on splicing. | Dystrophinopathy |
| Algeria | | GM 1693/17\* | | Not provided | | intron 38 c.5448+9G>T | | Not available | | not reported | | not reported | | not reported | | HSF: Activation of an intronic cryptic donor site. Potential alteration of splicing. | High CK |
| Algeria | | GM 1694/17\* | | 15/01/2016 | | intron 38 c.5448+9G>T | | Not available | | not reported | | not reported | | not reported | | HSF: Activation of an intronic cryptic donor site. Potential alteration of splicing. | High CK |
| Poland | | GM 1238/19 | | 03/10/2001 | | Intron 31 c.4345-3C>G | | Reduction /  Absence of DYS | | not reported | | not reported | | not reported | | HSF: 1. Activation of an intronic cryptic acceptor site. Potential alteration of splicing. 2. Alteration of the WT acceptor site, most probably affecting splicing. | High CK |
| Romania | | GM 1952/18 | | 06/12/2016 | | Intron 53 c.7873-8A>G | | Not available | | VUS | | not reported | | not reported | | HSF: 1. Activation of an intronic cryptic acceptor site. Potential alteration of splicing. 2. Alteration of the WT acceptor site, most probably affecting splicing. | High CK |

Supplementary Table 2. VUS and Phenotypes