8. Appendices

Appendix I: Genetic tests previously performed for patients included in WES analysis and SCA36 screening.

These analyses include the most common forms of ADCA and ARCA caused by trinucleotide expansions and a panel containing genes relevant for different types of ataxia.

- SCA1, SCA2, SCA3, SCA6, SCA7, SCA12, SCA17 and DRPLA (Dentatorubral-pallidoluysian atrophy): caused by CAG triplet expansions.
- SCA8: caused by CTG triplet expansions.
- Friedreich's ataxia: caused by GAA triplet expansions.
- SureSelect Human All Exon V6Panel (Agilent Technologies, Santa Clara, CA, USA): this panel was used to study both the coding and the intronic flanking regions of ataxia related genes through NGS. Different sets of genes were used for different types of ataxia.
    - **Episodic ataxia:** *ATP1A2, ATP1A3, CACNA1A, CACNA1S, CACNB4, KCNA1, SCN1A, SCN2A, SCN4A, SLC1A3.*
    - **Dominant ataxia**: *AFG3L2, ATP1A3, CACNA1A, CACNA1G, CACNB4, CCDC88C, DNMT1, EEF2, ELOVL4, ELOVL5, FAT2, FGF12, FGF14, ITPR1,KCNA1, KCNC3, KCND3, PDYN, PRKCG, SCN2A, SLC1A3, SPG7, SPTBN2, TBP, TGM6, TMEM240, TTBK2, TUBB4A, TRPC2, ME, PLD3.*
    - **Recessive ataxia**: *ABHD12, ADCK3, ANO10, APTX, ATCAY, ATM, ATP8A2, C10orf2, CA8, CWF19L1, ADCK3, CYP27A1, DNAJC19, FXN, GRM1,KCNJ10, KIAA0226, MRE11, MTPAP, PIK3R5, PLEKHG4, PMPCA, PNKP, POLG, RNF216, SACS, SETX, SIL1, SNX14, SYNE1, SYT14, TDP1, TPP1, TTPA, VLDLR, WDR81, WWOX*
    - **Complex disorders with prominent ataxia (AR):** *AFG3L2, CLCN2, COX20, CP, DARS2, FLVCR1, HEXA, HEXB, ITPR1, LAMA1, MTTP, NCP1, NCP2, PLA2G6, PM2, PNPLA6, SPTBN2.*
    - **Complex disorders with occasional ataxia (AR):** *ACO2, AHI1, ARL13B, CC2D2A, CLN5, CLN6, EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5, GOSR2, L2HGDH, OPA1, PEX7, PHYH, POLR3A, POLR3B, PTF1A, SLC17A5, SLC25A46, SLC52A2, SLC6A19, TSFM, TXN2, WFS1.*
    - **Disorders reported with ataxia but not included in the differential diagnosis**: *EPM2A, NHLRC1, MLC1, COL18A1, HSD17B4, PEX2, WFS1, ASPA, ARSA, SLC2A1.*
    - **X-linked:** *ABCB7, ATP2B3, CASK, FMR1, OPHN1, SLC9A6.*
    - **Ataxias and paraparesias**: *ABCD1, ABHD12, AFG3L2, ARSA, ATP13A2, AUH, CYP27A1, CYP7B1, DARS2, EXOSC3, FA2H, FXN, GALC, GAN, GBA2, GFAP,GJC2, GLB1, GLRX5, GRID2, KCND3, HEXA, KIF1A, KIF1C, MARS2, MECP2, MADHC, MTPAP, NPC1, NPC2, OPA1, OPA3,PDHX, PEX16, PLA2G6, PLP1, PNPLA6, POLR3A, POLR3B, PRNP, PSAP, PSEN1, SACS, SCN8A, SDHA, SETX, SLC17A5, SLC25A15, SLC2A1, SPG11, SPG7, SPR, STUB1, SYNE1, TBP, TTC19, TTPA, TUBB4A, UCHL1, VAMP1, ZFYVE26.*

Appendix II: PCR conditions, reagents and primers used in Sanger sequencing in fATX-163, fATX-166 and fATX-173.

Supplementary Table 1: Primers for validation of mutations in fATX-167.

Gene	Primers	Sequencies 5'-3'	Amplicon length (bp)	Hybridization Tº (ºC)
MUTYH	MUTYH/E12_F	ACCTGAGTAAGATTCTGCAGAA	487	58
	MUTYH/E12_R	CAACGCTGTAGTTCCTGC		
CFL2	CFL2/E2_F	GGTACGGTGCAATTTTTGATG	560	62
	CFL2/E2_R	TAAAGGTGCACTTTCAGGAGC		
SLC38A7	SLC38A7/E8_F	CTGGCCCCACACTACCTTT	547	62
	SLC38A7/E8_R	CCAGGCATGGAGAAGTTGAG		
BOD1L1	BOD1L1/E10_F	GTGATCTGCTCAGTAACTGGAG	354	62
	BOD1L1/E10_R	GCTAGTCACAATGCCTTCATC		

Supplementary Table 2: Primers for validation of mutations in fATX-163.

Gene	Primers	Sequencies 5'-3'	Amplicon length (bp)	Hybridization Tº (ºC)
HPCAL1	HPCAL1/E3_D	CGAGATGATGCCTTAAAGCTT	381	61
	HPCAL1/E3_R	GCACCATCACTCCTGAGCAGT		
EPPK1	EPPK1/E2_D	CCAAGGAATTAGACGACAGATC	333	60
	EPPK1/E2_R	TCCTCCACCGACAGCCTCA		

Supplementary Table 3: PCR reagents

Reagent	Initial concentration	Final concentration	Volume (µL)	Origin
DNAse-RNAse free H_2O	-	-	19.5	VWR Life Science
Standard Buffer 10X with $MgCl_2$	10X	1X	2.5	Biotools
dNTPs	20mM	0.4mM	0.5	IBIANLab Technologies
Primer forward	10 µM	0.2 µM	0.5	IDT
Primer reverse	10 µM	0.2 µM	0.5	IDT
Taq Polymerase	1 U/µL	0.5 U/µL	0.5	Biotools
$MgCl_2$*	25mM	1.5mM	1.5	-
DNA			1	

*Mg^{+2}: 1.5 µL were added in *MUTYH* and *EPPK1* reactions.

Supplementary Table 4: PCR conditions.

Step	Time	Temperature (ºC)	Number of cycles
Initial	5:00min	95	1
Denaturalization	30 s	95	35
Hybridization	30 s	X*	
Extension	30 s	72	
Extension	7:00 min	72	1
Ending	∞	4	1

*X=Hybridization temperature of the primers used.

Appendix III: PCR conditions and reagents used in SCA36 screening: standard PCR and RP-PCR.

Standard PCR conditions are reagents are indicated in supplementary tables 5 and 6 respectively.

Supplementary Table 5: PCR conditions for allele amplification

Step	Time	Temperature (ºC)	Number of cycles
Initial	5:00min	95	1
Denaturalization	30 s	95	35
Hybridization	30 s	65	
Extension	30 s	72	
Extension	10:00 min	72	1
Ending	∞	10	1

Supplementary Table 6: PCR reagents for allele amplification

Reagent	Initial concentration	Final concentration	Volume (µL)	Origin
DNAse-RNAse free H_20	-	-	19	Fresenius Kabi
Standard Buffer 10X with $MgCl_2$	10X	1X	2.5	Biotools
dNTPs	5 mM	0.25 mM	1.25	Thermo Fisher
Primer forward	100 µM	0.4 µM	0.1	Roche
Primer reverse	100 µM	0.4 µM	0.1	Roche
Taq Polymerase	5 U/µl	0,06 U/µl	0.3	Biotools
$MgCl_2$	50 mM	1,5 mM	0.75	Biotools
DNA	50-150 ng/µl	2-6 ng/µl	1	-

RP-PCR conditions are reagents are indicated in supplementary tables 7 and 8 respectively.

Supplementary Table 7: PCR conditions for expansion amplification.

Step	Time	Temperature (ºC)	Number of cycles
Initial	5:00min	94	1
Denaturalization	30 s	94	45
Hybridization	30 s	56	
Extension	2:00 min	72	
Extension	10:00 min	72	1
Ending	∞	4	1

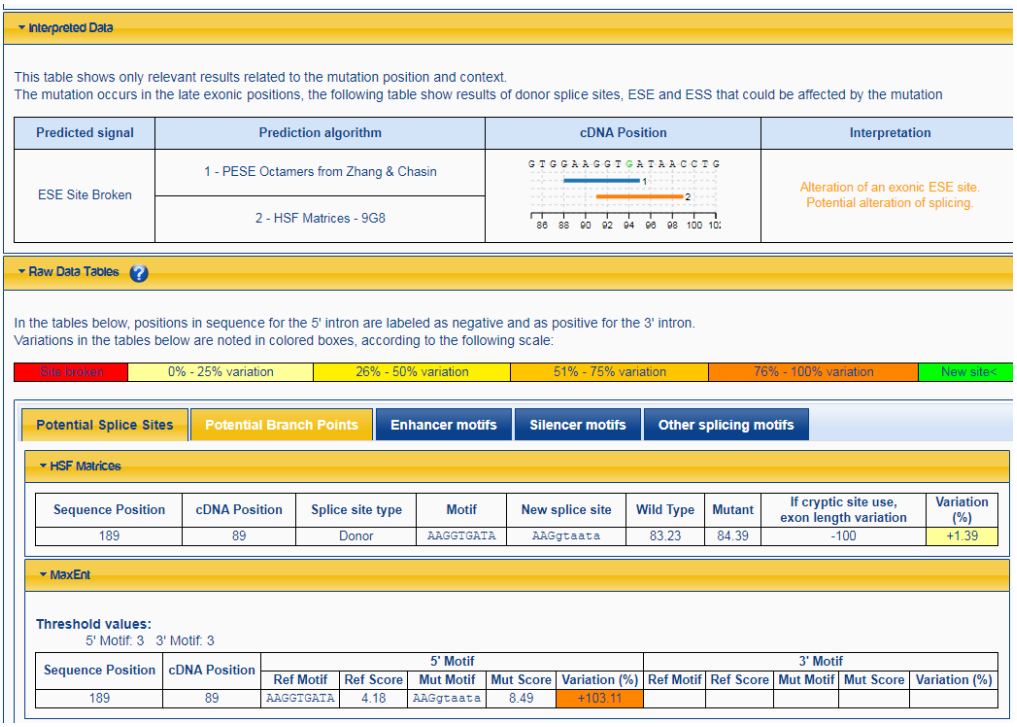
Supplementary Table 8: PCR reagents for expansion amplification.

Reagent	Initial concentration	Final concentration	Volume (µL)	Origin
DNAse-RNAse free H_20	-	-	13.5	Fresenius Kabi
Standard Buffer 10X with $MgCl_2$	10X	1X	2.5	Biotools
DMSO	100%	10%	2.5	Sigma-Aldrich
SCA36_F	5pM/µl	0.1pM/µl	0.5	Roche
SCA36_Anchor	5pM/µl	0.1pM/µl	0.5	Roche
d NTP's 7-deaza-dGTP	5 mM	0,3 mM	1.5	Thermo Fisher
$MgCl_2$	50 mM	1,5 mM	0.75	Biotools
Taq Polymerase	5 U/µl	0,2 U/µl	1	Biotools
SCA36_RV1	2.5pM/µl	0.025pM/µl	0.25	Roche
DNA	50-150 ng/µl	04-12 ng/µl	2	-

Appendix IV: AtxSPG-365 gene panel containing 365 genes related to hereditary ataxia and/or hereditary spastic paraplegia.

AARS2, ABCB7, ABCD1, ABHD12, ACO2, ADCK3, AFG3L2, AHI1, AIFM1, AIMP1, ALDH18A1, ALG6, ALS2, AMACR, AMPD2, AMT, ANO10, AP4B1, AP4E1, AP4M1, AP4S1, AP5Z1, APTX, ARG1, ARL13B, ARL6IP1, ARSA, ARSI, ARX, ASS1, ATAD3A, ATCAY, ATG5, ATL1, ATM, ATN1, ATP13A2, ATP1A3, ATP2B3, ATP7A, ATP8A2, ATR, ATXN1, ATXN10, ATXN2, ATXN3, ATXN7, B4GALNT1, BCKDHA, BCKDHB, BEAN1, BICD2, BSCL2, BTD, C10ORF2, C12ORF65, C19ORF12, C5ORF42, CA8, CACNA1A, CACNA1G, CACNB4, CAMTA1, CAPN1, CASK, CAV1, CC2D2A, CCDC88C, CCT5, CEP290, CEP41, CHCHD10, CHMP1A, CLCN2, CLN5, CLN6, CLP1, COG5, COL18A1, COQ2, COQ4, COQ5, COQ7, COQ9, COX20, CPT1C, CSTB, CTDP1, CTSD, CUL4B, CWF19L1, CYP27A1, CYP2U1, CYP7B1, DAB1, DARS2, DBT, DCLRE1B, DDB2, DDHD1, DDHD2, DHTKD1, DKC1, DNAJC19, DNM2, DNMT1, DSTYKD, DYRK1A, EEF2, EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5, ELOVL4, ELOVL5, EMC1, ENTPD1, EP300, EPM2A, EPT1, ERCC2, ERCC3, ERCC5, ERCC6, ERCC8, ERLIN1, ERLIN2, ETFA, ETFB, ETFDH, EXOSC3, EXOSC8, FA2H, FAAH2, FAM126A, FAM134B, FAT1, FAT2, FGF14, FIG4, FLRT1, FLVCR1, FMR1, FOLR1, FOXC1, FXN, GAD1, GALC, GAN, GBA2, GBE1, GFAP, GJB1, GJC2, GLRX5, GOSR2, GPR56, GRID2, GRM1, GRN, HACE1, HEXA, HSD17B4, HSPD1, HTRA1, IBA57, IFIH1, IFRD1, INPP5E, ITM2B, ITPR1, KCNA1, KCNA2, KCNC3, KCND3, KCNJ10, KCTD7, KDM6A, KIAA0196, KIAA0226, KIDINS220, KIF1A, KIF1C, KIF26B, KIF5A, KIF7, KLC2, KLC4, KMT2D, L1CAM, L2HGDH, LAMA1, LMNB1, LMNB2, LYST, MAG, MAN2B1, MARS, MARS2, MCEE, MECP2, MLC1, MMACHC, MMADHC, MORC2, MPZ, MRE11A, MRPL10, MTFMT, MTHFR, MTPAP, MTTP, NALCN, NHLRC1, NIPA1, NKX2-1, NKX6-2, NOL3, NOP56, NPC1, NPHP1, NT5C2, OFD1, OPA1, OPA3, OPHN1, PARN, PAX6, PC, PCNA, PDHA1, PDHB, PDSS1, PDSS2, PDYN, PEX10, PEX16, PEX6, PEX7, PGAP1, PHYH, PIK3R5, PITRM1, PITX2, PLA2G6, PLD3, PLEKHG4, PLP1, PMPCA, PNKP, PNPLA6, POLG, POLG2, POLR3A, POLR3B, PPP2R2B, PRICKLE1, PRKCG, PRNP, PRPS1, PRRT2, PSEN1, PTEN, RAB3GAP2, RAD1, RARS, RARS2, REEP1, REEP2, RIPPLY1, RPGRIP1L, RPIA, RTEL1, RTN2, SACS, SAMD9L, SARS2S, SCARB2, SCN1A, SCN2A, SCN8A, SCYL1, SERAC1, SETX, SIL1, SLC16A2, SLC17A5, SLC1A3, SLC25A15, SLC25A46, SLC2A1, SLC33A1, SLC46A1, SLC52A2, SLC52A3, SLC6A19, SLC9A1, SLC9A6, SNAP25, SNX14, SPAST, SPG11, SPG20, SPG21, SPG7, SPTAN1, SPTBN2, SQSTM1, STUB1, SURF1, SYNE1, SYT14, TARDBP, TBP, TCTN1, TCTN2, TCTN3, TDP1, TDP2, TECPR2, TERT, TFG, TGFB1, TGM6, THG1L, TINF2, TMEM138, TMEM216, TMEM231, TMEM237, TMEM240, TMEM67, TPP1, TRAPPC11, TRMT5, TRPC3, TSEN54, TTBK2, TTC19, TTPA, TTR, TUBB4A, UBA5, UBQLN2, UCHL1, USP8, VAMP1, VCP, VHL, VLDLR, VPS37A, VRK1, VWA3B, WDR48, WDR73, WFS1, WWOX, XPA, XPC, ZFR, ZFYVE26, ZFYVE27.

Appendix V: Splicing *in silico* results for *POLR3A* c.3688G>A variant.



▾ Interpreted Data

This table shows only relevant results related to the mutation position and context.
The mutation occurs in the late exonic positions, the following table show results of donor splice sites, ESE and ESS that could be affected by the mutation

Predicted signal	Prediction algorithm	cDNA Position	Interpretation
ESE Site Broken	1 - PESE Octamers from Zhang & Chasin	G T G G A A G G T G A T A A C C T G 1 2 86 88 90 92 94 96 98 100	Alteration of an exonic ESE site. Potential alteration of splicing.
	2 - HSF Matrices - 9G8		

▾ Raw Data Tables

In the tables below, positions in sequence for the 5' intron are labeled as negative and as positive for the 3' intron.
Variations in the tables below are noted in colored boxes, according to the following scale:

Site broken	0% - 25% variation	26% - 50% variation	51% - 75% variation	76% - 100% variation	New site<

Potential Splice Sites | Potential Branch Points | Enhancer motifs | Silencer motifs | Other splicing motifs

▾ HSF Matrices

Sequence Position	cDNA Position	Splice site type	Motif	New splice site	Wild Type	Mutant	If cryptic site use, exon length variation	Variation (%)
189	89	Donor	AAGGTGATA	AAGgtaata	83.23	84.39	-100	+1.39

▾ MaxEnt

Threshold values:
5' Motif: 3 3' Motif: 3

Sequence Position	cDNA Position	5' Motif					3' Motif				
		Ref Motif	Ref Score	Mut Motif	Mut Score	Variation (%)	Ref Motif	Ref Score	Mut Motif	Mut Score	Variation (%)
189	89	AAGGTGATA	4.18	AAGgtaata	8.49	+103.11					

Supplementary Figure 1: Results obtained from HSF (Human Splicing Finder), version 3.1 for *POLR3A* c.3688G>A.

A

```
********** NetGene2 v. 2.4 **********


The sequence: sequence1 has the following composition:

Length: 240 nucleotides.
23.3% A, 27.1% C, 29.2% G, 20.4% T, 0.0% X, 56.2% G+C

Donor splice sites, direct strand
---------------------------------
   No donor site predictions above threshold.

Donor splice sites, complement strand
-------------------------------------
   No donor site predictions above threshold.

Acceptor splice sites, direct strand
------------------------------------
               pos 5'->3'  phase strand  confidence  5'    intron exon      3'
                    34       0     +        0.00       CTGAATCCAG^GTGGTGGTGC

Acceptor splice sites, complement strand
----------------------------------------
   pos 3'->5'  pos 5'->3'  phase strand  confidence  5'    intron exon      3'
       134         107       0     -        0.32       CTGCCCGCAG^GTTATCACCT
       116         125       0     -        0.19       CTTCCACCAG^AAGCTTGTAC
       113         128       0     -        0.18       CCACCAGAAG^CTTGTACTTC

-------------------------------------------------------------------------------

CUTOFF values used for confidence:

Highly confident donor sites (H):     95.0 %
Nearly all true donor sites:          50.0 %

Highly confident acceptor sites (H):  95.0 %
Nearly all true acceptor sites:       20.0 %
```

B

```
********** NetGene2 v. 2.4 **********


The sequence: sequence1 has the following composition:

Length: 240 nucleotides.
23.8% A, 27.1% C, 28.8% G, 20.4% T, 0.0% X, 55.8% G+C

Donor splice sites, direct strand
---------------------------------
               pos 5'->3'  phase strand  confidence  5'      exon intron    3'
                   126       1     +        0.65       CTGGTGGAAG^GTAATAACCT

Donor splice sites, complement strand
-------------------------------------
   No donor site predictions above threshold.

Acceptor splice sites, direct strand
------------------------------------
               pos 5'->3'  phase strand  confidence  5'    intron exon      3'
                    34       0     +        0.00       CTGAATCCAG^GTGGTGGTGC

Acceptor splice sites, complement strand
----------------------------------------
   pos 3'->5'  pos 5'->3'  phase strand  confidence  5'    intron exon      3'
       134         107       0     -        0.18       CTGCCCGCAG^GTTATTACCT
       116         125       0     -        0.19       CTTCCACCAG^AAGCTTGTAC
       113         128       0     -        0.19       CCACCAGAAG^CTTGTACTTC

-------------------------------------------------------------------------------

CUTOFF values used for confidence:

Highly confident donor sites (H):     95.0 %
Nearly all true donor sites:          50.0 %

Highly confident acceptor sites (H):  95.0 %
Nearly all true acceptor sites:       20.0 %
```

Supplementary Figure 2: Predicted results for the *POLR3A* wild-type sequence (A) and for the c.3688G>A variant (B) using the NetGene2 software.

A

Donor site predictions for 195.77.18.22.320.0 :

Start	End	Score	Exon	Intron
193	207	0.82	ctatgag	gtaccact
223	237	0.40	ccttagg	gttaggct

Acceptor site predictions for 195.77.18.22.320.0 :

Start	End	Score	Intron	Exon
14	54	0.81	tttctctgtctctgaatcc	aggtggtggtgcagggcattcc

B

Donor site predictions for 195.77.18.22.332.0 :

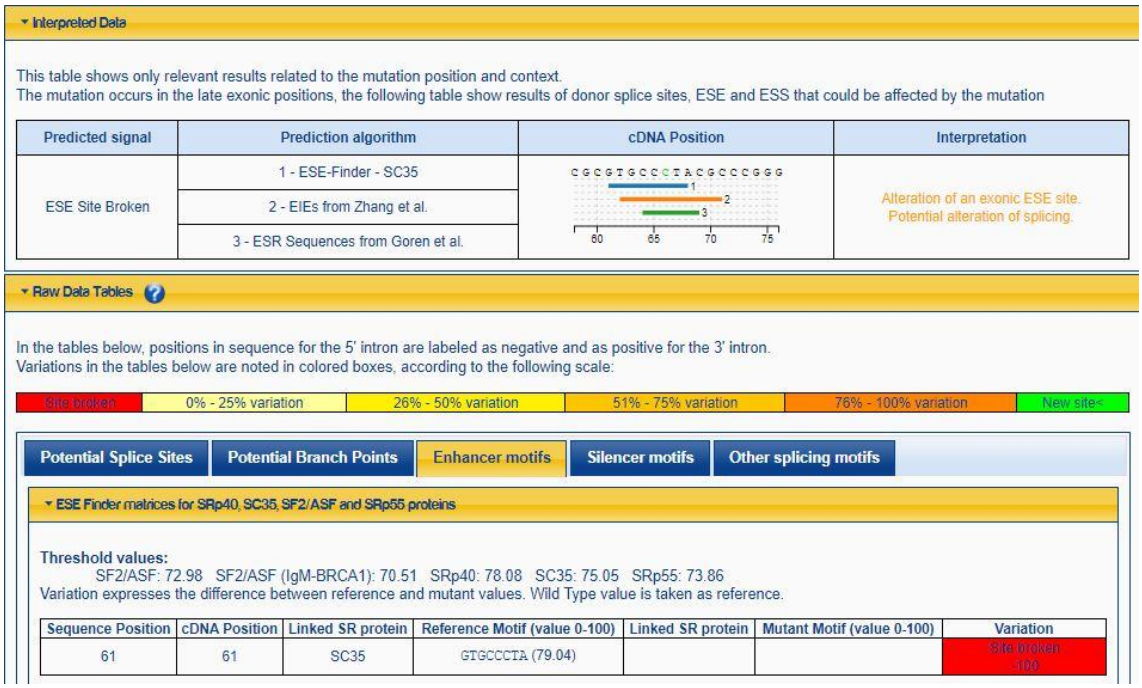
Start	End	Score	Exon	Intron
119	133	0.71	gtggaag	gtaataac
193	207	0.82	ctatgag	gtaccact
223	237	0.40	ccttagg	gttaggct

Acceptor site predictions for 195.77.18.22.332.0 :

Start	End	Score	Intron	Exon
14	54	0.81	tttctctgtctctgaatcc	aggtggtggtgcagggcattcc

Supplementary Figure 3: Predicted results for the POLR3A wild-type sequence (A) and for the c.3688G>A variant (B) using the NNSplice software.

Appendix VI: Splicing *in silico* results for *SHQ1* c.66C>T variant for family fATX-166 in WES analysis.



Supplementary Figure 4: Results obtained from HSF (Human Splicing Finder), version 3.1 for *SHQ1* c.66T>C.

A.

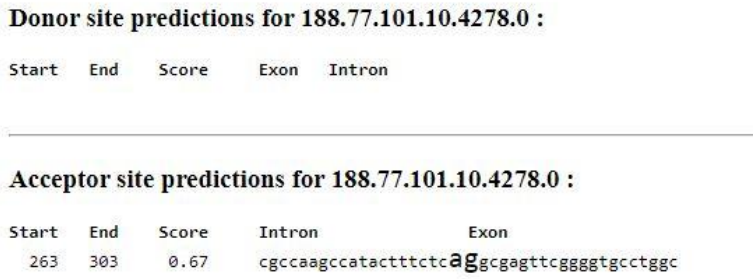
```
The sequence: sequence1 has the following composition:

Length: 303 nucleotides.
14.2% A, 33.0% C, 33.3% G, 19.5% T, 0.0% X, 66.3% G+C

Donor splice sites, direct strand
---------------------------------
   No donor site predictions above threshold.

Donor splice sites, complement strand
-------------------------------------
   pos 3'->5'  pos 5'->3'  phase strand  confidence  5'      exon intron    3'
       148         156       1     -        0.52       GAACGCCGGG^GTCAGCATCG

Acceptor splice sites, direct strand
------------------------------------
               pos 5'->3'  phase strand  confidence  5'    intron exon      3'
                   107       0     +        0.07       GCAGTGAGAG^CGAGCGGCGC
                   166       2     +        0.19       TCGACCTCAG^CCAGGATCCG
                   170       0     +        0.18       CCTCAGCCAG^GATCCGGACT

Acceptor splice sites, complement strand
----------------------------------------
   No acceptor site predictions above threshold.

-------------------------------------------------------------------------------

CUTOFF values used for confidence:

Highly confident donor sites (H):     95.0 %
Nearly all true donor sites:          50.0 %

Highly confident acceptor sites (H):  95.0 %
Nearly all true acceptor sites:       20.0 %
```

B.

```
The sequence: sequence1 has the following composition:

Length: 303 nucleotides.
14.2% A, 32.7% C, 33.3% G, 19.8% T, 0.0% X, 66.0% G+C

Donor splice sites, direct strand
---------------------------------
   No donor site predictions above threshold.

Donor splice sites, complement strand
-------------------------------------
   pos 3'->5'  pos 5'->3'  phase strand  confidence  5'      exon intron    3'
       148         156       1     -        0.55       GAACGCCGGG^GTCAGCATCG

Acceptor splice sites, direct strand
------------------------------------
              pos 5'->3'  phase strand  confidence  5'    intron exon      3'
                  107       0     +        0.07       GCAGTGAGAG^CGAGCGGCGC
                  166       2     +        0.19       TCGACCTCAG^CCAGGATCCG
                  170       0     +        0.18       CCTCAGCCAG^GATCCGGACT

Acceptor splice sites, complement strand
----------------------------------------
   No acceptor site predictions above threshold.

-------------------------------------------------------------------------------

CUTOFF values used for confidence:

Highly confident donor sites (H):     95.0 %
Nearly all true donor sites:          50.0 %

Highly confident acceptor sites (H):  95.0 %
Nearly all true acceptor sites:       20.0 %
```

Supplementary Figure 5: Predicted results for the *SHQ1* wild-type sequence (A) and for the c.66C>T variant (B) using the NetGene2 software.

A



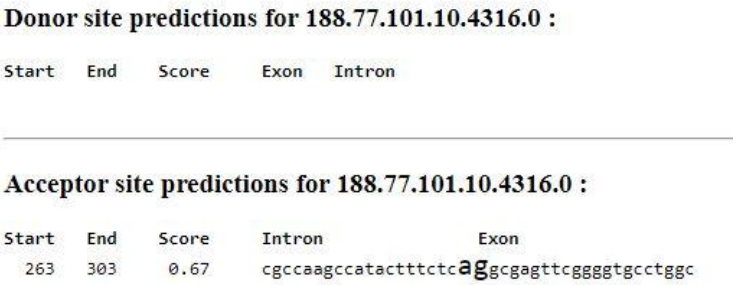
Donor site predictions for 188.77.101.10.4278.0 :

Start	End	Score	Exon	Intron

Acceptor site predictions for 188.77.101.10.4278.0 :

Start	End	Score	Intron	Exon
263	303	0.67	cgccaagccatactttctc**ag**	gcgagttcggggtgcctggc

B



Donor site predictions for 188.77.101.10.4316.0 :

Start	End	Score	Exon	Intron

Acceptor site predictions for 188.77.101.10.4316.0 :

Start	End	Score	Intron	Exon
263	303	0.67	cgccaagccatactttctc**ag**	gcgagttcggggtgcctggc

Supplementary Figure 6: Predicted results for the *SHQ1* wild-type sequence (A) and for the c.66C>T variant (B) using the NNSplice software.

Appendix VII: Genes presenting compound heterozygous mutations in fATX-166.

Supplementary Table 9: Genes carrying compound heterozygous mutations in fATX-166.

Family	Gene	cDNA change /Protein change	Frequency	Type of change	*In silico* predictors	
					SIFT	Polyphen-2
fATX-166	*CELA3A* (NM_005747)	c.314A>G/p.E105G	24 (rs75527968)	missense	T	B
		c.321G>C/p.L107	30 (rs786742649	synonymous	-	-
	ALG6 (NM_013339)	-	0	splice region variant	-	-
		-	0	splice acceptor variant	-	-
		c.1326-1327G>TTTTTT/p.-442-443FF	0	inframe insertion	-	-
	REG3A (NM_138938)	c.186A>G/p.T62T	8378	synonymous	-	-
		c.149A>C/p.H50P	412 (rs201139260)	missense	D	PD
		c.115C>A/p.R39S	2 (rs141740162)	missense	T	B
		c.106G>C/p.A36P	0 (rs879246680)	missense	T	B
		c.92G>A/p.R31K	6 (rs757491659)	missense	T	B
		c.85C>A/p.31T	3 (rs558734956)	missense	T	B
		-	0 (rs879047568)	splice region variant	-	-
	MMP16 (NM_0059419)	c.1649A>G/p.D550G	0	missense	T	B
		-	0	splice region variant	-	-
	DAB2IP (NM_032552)	c.1017C>T/p.L339L	295 (rs1504289269	synonymous	-	-
		c.1224C>T/p.D408D	40 (rs149420814)	synonymous	-	-
	OR8G1 (NM_001002905)	c.853C>T/p.L285L	7 (rs943179881)	synonymous	-	-
		c.861C>T/p.P287P	10 (rs202064362)	synonymous	-	-
		c.867C>T/p.I289I	13 (rs201086595)	synonymous	-	-
		c.870C>T/p.Y290Y	17 (rs199587033)	synonymous	-	-
	ZNF676 (NM_001001411)	c.658A>G/p.T220A	189 (rs7253392)	missense	T	B
		c.650T>G/p.V219G	8 (rs769830637)	missense	T	B
		c.649G>A/p.V217I	335 (rs201994000)	missense	T	B
		c.645T>C7p.H645H	0 (rs767498279)	synonymous	-	-
		c.640A>G/p.K214E	0 (rs7253403)	missense	T	B

T: tolerated, B: benign, D: deleterious, PD: probably damaging. NM_X: Indicates the reference transcript variant used for each of the genes. RS represents the mutation identifier in Ensembl Genome Browser 37.

Appendix VIII: SCA36 screening results from a cohort of 52 patients.

Supplementary Table 10: SCA36 screening results for 10 patients belonging to families fATX-163, fATX-166 and fATX-167.

Family	Patient Identification	Standard PCR: Non-expanded alleles length (bp)	RP-PCR
fATX-167	SGT-1494	167-183	-
	SGT-1493	166-183	-
fATX-166	SGT-85	167-195	-
	SGT-1457	166-195	-
fATX-163	**SGT-1433**	**172**	+
	SGT-1432	**183**	+
	SGT-1459	**183**	+
	53	**171**	+
	54	**183**	+

Patients previously included in WES analysis are identified by SGT-X. **Standard PCR results:** Non-expanded alleles length determined by capillary electrophoresis and expressed in bp. Heterozygote individuals present two alleles of different lengths. Homozygote individuals present two alleles of the same length and a single number is indicated. **RP-PCR results:** - indicates SCA36 negative and + indicated SCA36 positive and are highlighted in red.

Supplementary Table 11: SCA36 screening results for 43 patients included in the cohort and not belonging to families fATX-163, f-ATX-166 or fATX-167.

Patient Identification	Standard PCR: Non-expanded alleles length (bp)	RP-PCR
1	183	-
2	166-183	-
4	172-183	-
5	167-172	-
6	167-183	-
7	172-189	-
8	172-183	-
9	172	-
10	167-194	-
11	172-183	-
12	172-183	-
13	183	-
14	172-183	-
15	166	-
17	172-183	-
18	195-200	-
19	167-183	-
20	183	-
21	167	-
22	183-212	-
24	166	-
25	**183**	+
26	166-183	-
27	171-183	-
28	**199**	+
29	166-194	-
30	166-171	-
31	166-200	-
32	166-194	-
37	166-194	-
33	166-183	-
34	166-183	-
35	166-189	-
36	183-194	-
38	183-194	-
39	183	-
40	167-183	-
41	167-183	-
42	167-183	-
43	183	-
44	172-183	-
45	172-183	-
48	183	-

Standard PCR results: Non-expanded alleles length determined by capillary electrophoresis and expressed in base pairs (bp). Heterozygote individuals present two alleles of different lengths. Homozygote individuals present two alleles of the same length and a single number is indicated. **RP-PCR results:** - indicates SCA36 negative and + indicated SCA36 positive and are highlighted in red. Two patients, from different families, are SCA36 positive.