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To cite this version:

Peter Bauer, Giovanni Stevanin, Christian Beetz, Matthis Synofzik, Tanja Schmitz-Hübsch, et al.. Spinocerebellar ataxia type 11 (SCA11) is an uncommon cause of dominant ataxia among French and German kindreds. Journal of Neurology, Neurosurgery and Psychiatry, 2010, 81 (11), pp.1229. 10.1136/jnnp.2009.202150 . hal-00560316

HAL Id: hal-00560316

https://hal.science/hal-00560316

Submitted on 28 Jan 2011

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Spinocerebellar ataxia type 11 (SCA11) is an uncommon cause of dominant ataxia among French and German kindreds

Peter Bauer, MD[1,*]; Giovanni Stevanin, PhD[2,3,4,*]; Christian Beetz, PhD[5,*], Matthis Synofzik, MD[6]; Tanja Schmitz-Hübsch, MD[7]; Ullrich Wüllner, MD[7]; Eric Berthier, MD[8]; Elisabeth Ollagnon-Roman, MD, PhD[9]; Olaf Riess, MD[1]; Sylvie Forlani, PhD[2,3]; Emeline Mundwiller, BS[2,3]; Alexandra Durr, MD, PhD[2,3,4]; Ludger Schöls, MD[6,#]; Alexis Brice, MD[2,3,4]

1) Dept. of Medical Genetics, University of Tübingen, Tübingen, Germany

2) INSERM, U975 (formerly U679), Genetics physiopathology and therapy of neurodegenerative diseases of the central nervous system, Paris, France.

3) UPMC Univ. Paris6, UMR_S975, CNRS 7225, Centre de Recherche Institut du Cerveau et de la Moelle, Groupe Hospitalier Pitié-Salpêtrière, Paris, France

4) APHP, Dept. of Genetics and Cytogenetics, Groupe Hospitalier Pitié-Salpêtrière, Paris, France.

5) Institute for Clinical Chemistry and Laboratory Diagnostics, University Hospital Jena, Germany.

6) Clinical Neurogenetics, Centre of Neurology and Hertie-Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany

7) Dept. of Neurology, University of Bonn, Bonn, Germany

8) Hôpital Neurologique, Lyon, France

9) Hôpital de la Croix Rousse, Lyon, France

* These authors contributed equally to this work

Corresponding author: Ludger Schöls, Clinical Neurogenetics, Centre of Neurology and Hertie-Institute for Clinical Brain Research, University of Tübingen,

Hoppe-Seyler-Str. 3, D 72076 Tübingen, Germany; Fon: +49 7071 29 80445; Fax: +49 7071 29 4839; Email: ludger.schoels@uni-tuebingen.de

Search terms : Gait disorders/ataxias (163), spinocerebellar ataxias (298), prevalence studies (56)

Word count: Title – 111 characters; Abstract – 263 words; Manuscript – 1973 words; 22 references, 1 table, 1 figure, 1 supplementary figure.

Disclosure: The authors report no conflicts of interest.

PB received honoraria from Roche Diagnostics. Dr. Bauer received research grants of the German Research Council (BMBF) to GeNeMove (01GM0603), EUROSPA (01GM0807) and RISCA (09GM0820) as well as from the EU for EUROSCA (LSHM-CT-2004-503304), MarkMD (FP7-People PIAP-2008-230596) and TECHGENE (FP7-Health 2007-B 223143). He further received runding from the HSP-Selbsthilfegruppe Deutschland e.V.

LS served as an editorial board member of Movement Disorders and was a member of the scientific advisory board for Takeda Pharma. As participant of the MICONOS trial Dr. Schöls received fees from Santhera Pharmaceuticals. Dr. Schöls received research grants of the Deutsche Forschungsgemeinschaft (SCHO754/3-1 and SCHO754/4-1), grants of the German Research Council (BMBF) to Leukonet (01GM0644) and GeNeMove (01GM0603), funding from the EU for EUROSCA (LSHM-CT-2004-503304) and E-RARE grants to EUROSPA (01GM0807) RISCA (01GM0820) and mitoNET (01GM0864) as well as a grant of the Volkswagen Foundation (I/80711). He further received funding from the HSP-Selbsthilfegruppe Deutschland e.V.

Drs. Brice, Dürr and Stevanin received research grants from of EU for Eurosca (LSHM-CT-2004-503304), E-RARE grant to EUROSPA (ANR-07-E-RARE-005-01/R07202DS) as well as a grant from the Agence Nationale pour la Recherche (ANR-07-NEURO-041-01/R07047DS).

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclu sive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in the Journal of Neurology, Neurosurgery & Psychiatry editions and any other BMJPGL products to exploit all subsidiary rights, as set out in our licence (http://jnnp .bmjjournals.com/ifora/licence.pdf).

Abstract

Background: At least 28 loci have been linked to autosomal dominant spinocerebellar ataxia (ADCA). Causative genes have been cloned for ten nucleotide repeat expansions (SCA1, 2, 3, 6, 7, 8, 10, 12, 17, and 31) and six genes with classical mutations (SCA5, 13, 14, 15/16, 27, adn 28). Recently, a large British pedigree linked to SCA11 has been reported to carry a mutation in the *TTBK2*-gene. In order to assess the prevalence and phenotypic spectrum of SCA11, we screened 148 index patients of predominantly German (n=69) and French (n=79) descents with ADCA tested negative for a panel of SCA mutations (SCA1, 2, 3, 6, 7, and 17), for mutations in *TTBK2*.

Methods: In the German ADCA cohort the complete coding sequence of the *TTBK2*-gene was PCR-amplified and screened for mutations by high-resolution-melting (HRM) analysis. In the French cohort, exons known to carry mutations were directly sequenced. For both cohorts, the gene-dosage alterations were assessed using a customized multiplex ligation probe amplification (MLPA) assay.

Results: In two of 148 ADCA families – one German and one French - we identified a potentially disease-causing SCA11 mutation. Interestingly, both carried an identical two basepair deletion (c.1306_1307delGA, p.D435fs448X in exon 12) leading to a premature stop codon. Gene dosage alterations were not detected in the *TTBK2*-gene. Clinically, our SCA11 patients had phenotypic characteristics as described before presenting with slowly progressive almost pure cerebellar ataxia with normal life expectancy.

Conclusion: SCA11 presented as ADCA III according to Harding's classification and is a rare cause of spinocerebellar ataxia in Caucasians accounting for less than 1% of dominant ataxias in central Europe.

Introduction

Genetic research elucidated disease-causing mutations in 18 subtypes of autosomal-dominant cerebellar ataxia (ADCA), classified as spinocerebellar ataxias (SCA) [1]. The concept of dynamic mutations leading to neurodegeneration in SCA was substantiated by repeat expansion mutations in SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12, SCA17, and SCA31 [2;3]. As one learned from *PRKCG* dysfunction (SCA14) [4], the aetiology of SCAs goes beyond polyglutamine toxicity, and recently, non-dynamic or classical disease-causing mutations have furthermore been described for SCA5 (*SPTBN2*) [5], SCA13 (*KCNC3*) [6], SCA15/SCA16 (*ITPR1*) [7], SCA20 (duplication at chromosome 11q12.2-11q12.3) [8], SCA27 (*FGF14*) [9], and SCA28 (*AFG3L2*) [10], respectively.

SCAs are rare: prevalence studies in European region to date gave estimates range between 0.9 and 3/100.000 [11]. The chance of familial cases to be diagnosed genetically depends on the ethnic origin of the family [3]. In western and central Europe 50% up to 80% of SCA patients with affected first degree relatives carry a disease causing mutation in the SCA genes identified so far [12;13].

Recently, *tau tubulin kinase 2* (*TTBK2*) has been implicated as the disease gene for SCA11 [14]. SCA11 has originally been described in a large British family and a smaller Pakistani family as rather slowly progressing, pure cerebellar ataxia. Although little is known about brain specific functions of *TTBK2*, inactivating mutations C-terminal of the serine/threonine protein kinase domain have been identified in these kindreds.

However, pathogenic *TTBK2* mutations have not been identified in two series of ADCA patients (dHPLC mutation screening in 68 Han Chinese patients [15] and complete resequencing of all coding exons in 49 Caucasian patients [16], respectively).

In order to assess the relevance of SCA11 mutations in European ADCA patients and to better understand resulting phenotypes, we have genotyped 148 index patients of ADCA families for *TTBK2* mutations.

Material and Methods:

We selected 148 index patients from ADCA families negative for repeat expansions in SCA1, SCA2, SCA3, SCA6, SCA7 and SCA17. In addition conventional mutations in SCA5 (for 50 patients), SCA13 (for 50 patients), SCA14 (for 121 patients) and SCA28 (for 50 patients) as well as the variant in the puratrophin promoter (for 50 patients) had been excluded by direct sequencing ([13;17;18] and unpublished data). The mean age of onset was 45.3 +/- 15.7 years (range: 3-79) in n=121 index cases for whom age of onset data were available. Clinically, 86 patients (56%) had pure cerebellar ataxia (ADCA III) while 44% of index patients presented with non-cerebellar signs and symptoms like gaze palsy, epilepsy, spasticity, dystonia, Parkinsonism or peripheral neuropathy qualifying them as ADCA I according to Harding (1984) [19]. Families originated mostly from France (n=73), Germany (n=67) and other continental western European countries (n=6) while two originated from Algeria and French West Indies.

Informed consent was obtained from all patients participating in this study.

High resolution melting (HRM) curve analysis in 69 samples (Germany):

The complete coding sequence of *TTBK2* (exon 2 to 15 (ensembl 267890), and 4 additional exons in alternatively spliced transcripts (ensembl 263802 and 382403) was PCR amplified including all exon-intron-boundaries. In order to keep HRM sensitive we have designed overlapping PCR fragments for larger exons (3, 10, 11, 17, 18, and 19) giving an amplicon count of 26 for the whole gene (primers are given in supplementary table 1). The PCR and HRM were performed in a single run on a LightCycler®480 instrument (Roche Diagnostics, Mannheim, Germany) with a total reaction volume of 10 µl in a 384-well microtiter plate. The reaction mixture contained 20 ng of genomic DNA, 2.5 mM MgCl2, 10 pmol of each primer, 5 µl 2x LightCycler®480 High Resolution Master Mix (Roche Diagnostics, Mannheim,

Germany) and PCR grade water in a volume of 10 µl. A pipetting robot (Universal platform, Qiagen, Hilden, Germany) was used for PCR setup.

The PCR cycling conditions included an activation step at 95°C for 10 minutes followed by 45 cycles of 95°C for 10 seconds, a touch down of 68°C to 58°C for 15 seconds (1°C/Cycle) and 72°C for 20 seconds. The amplicons were denaturated at 95°C for 1 minute, renaturated at 40°C for 1 minute and subsequently HRM was executed over the range from 65°C to 95°C with 25 signal acquisitions per degree.

HRM curve analysis was performed using the LightCycler®480 with the gene-scanning module (version 1.5). A normal control DNA, which had been completely sequenced, served as HRM baseline sample in each fragment. To guarantee a high detection sensitivity, HRM analysis sensitivity settings were 0.7 (high sensitivity). For each resulting HRM group in each PCR fragment at least one sample was directly sequenced in order to identify or exclude variants.

Sequencing of exons 11-13 in 79 samples (France):

Exons 11 to 13 of the *TTBK2*-gene (Ensembl 267890), encoding the conserved serine rich domain of the protein in which the known mutations have been found in our German cohort and in a previously published series [14], were amplified in a ABI-9700 thermocycler (Applied Biosystems) using classical conditions with specific primers and annealing temperature as indicated in supplementary table 1. PCR products were then sequenced using the BIGDYE V3 chemistry in an ABI3730 automated sequencer (Applied Biosystems) according to the manufacturer's recommendations.

All base positions are given relative to the main transcript (ensembl 267890).

Gene dosage analysis by multiplex ligation probe amplification (MLPA) in all samples:

For *TTBK2*–gene copy number analysis we established a homemade MLPA probe set. It contained a total of 12 probes with amplicon sizes ranging from 97 to 137 nucleotides. Four probes targeting independent genomic regions serve as reference whereas eight probes target *TTBK2* exons 2, 4, 6, 8, 10, 12 14, and 15. The exon 12 probe was designed such as to have the ligation site bordered by c.1306 and c.1307, i.e. the two nucleotides deleted in our SCA11 samples (the sequences of all probes are given in supplementary table 1). Oligonucleotides were ordered at Eurofins MWG Operon (Ebersberg, Germany); MLPA reagents were from MRC-Holland (Amsterdam, The Netherlands). Reactions were performed as recommended by the manufacturer. Raw intensity of MLPA products data were obtained and analysed as described previously [20].

Results

Direct sequencing and HRM scanning identified a single heterozygous frameshift-mutation c.1306_1307delGA in exon 12, leading to a premature stop codon at amino acid 448 (p.D435Yfs448) in a French family and a second, unrelated German family. This mutation affected the three known transcripts of *TTBK2* (figure 1).

For the French family both SCA11 carrier men – father and son - have been examined at 64 and 39 years of age and presented with a slowly progressive cerebellar ataxia with dysarthria first observed at the age of 48 and 35, respectively. After 16 years from disease onset the father needed a walking aid and used a stroller. Reflexes were normal and plantar flexor in both. The father was mildly depressed. No additional neurological signs were observed. Cerebral MRI showed cerebellar atrophy affecting essentially the vermis in both cases. The grand father of the index case also complained of gait instability starting at age 58 but was not available for genetic analysis.

In the index patient of the German SCA11 family ataxia started at age 44 when he experienced mild unsteadiness in the dark. He remained able to play tennis until age 53. Examination revealed oculomotor disturbances with saccadic pursuit, gaze evoked nystagmus, dysmetric saccades and impaired optokinetic nystagmus. Cerebellar dysarthria was mild. Gait was ataxic with some problems in tandem walk and Romberg manoeuvre. There was no tremor and appendicular ataxia was more pronounced in the legs than in the arms. Despite some stiffness in walking and reduced arm swing there was no significant alteration in muscle tone or reflexes. Nerve conduction studies and sympathetic skin response were normal. MRI revealed pancerebellar atrophy. The mother developed gait ataxia between 40 and 50 years of age and died at age 76 after 5 years in a wheelchair. A brother and a sister of the mother as well as the maternal grandmother had a similar course of disease. Only the healthy 64 year old sister of the index patient was available for genetic analyses and showed to be negative for the *TTBK2*-mutation.

There was no evidence for any genealogical link between both SCA11 families with both families originating from over more than 600 km apart from each other. Moreover, haplotype analysis showed only partial genotype identity that is not arguing for a recent common ancestor (data not shown).

A non-synonymous amino acid change in exon 13 T651M was detected in one German-Polish SCA patient but not present in the similarly affected brother and thus excluded this variant as a disease-causing sequence change. Two additional silent coding variants (table 1) were found in ADCA patients and controls at similar frequencies. Gene dosage analysis using MLPA technique in all index patients (French and German cohort, n=148) was unremarkable for all samples. Patients carrying the c.1306_1307delGA, however, were readily identified by a 50% reduction of the signal intensity for exon 12 in a blinded fashion, thereby validating probe set and procedure.

Discussion

CAG expansions in SCA 1, 2, 3, 6, 7, and 17 account for 52% of French and 76% of German ADCA patients in our cohort [12;21] . In addition, an increasing number of non-repeat-expansion genes have been discovered lately.

We screened 148 index patients negative for repeat expansions for mutations in *TBBK2* and have identified an identical frameshift mutation in two unrelated families (c.1306-1307delGA). A missense variant in exon 13 (c.1952C>T, p.T651M) detected in a German-Polish family was not found in more than 150 control chromosomes but did not segregate with the phenotype and thus can not be considered to be disease-causing. Two additional coding variants in exon 13 and exon 14 (S648S and E864E) did not change the protein and mRNA processing. Of note, a focussed analysis of gene dosage for the *TTBK2*-gene in all 148 samples by MLPA was unremarkable. This renders gene dosage mutations as likely not relevant for SCA11.

As in the British kindred [14], the French and the German SCA11 families presented with rather pure cerebellar ataxia, cerebellar atrophy and normal life expectancy (ADCA type III according to Harding). The course of disease in our families revealed rather slow progression. Patients were still able to play tennis up to 9 years with the disease and remained ambulant even 16 years after onset of ataxia. Only one patient became wheelchair dependent after more than 20 years with the disease.

Like most of the other SCA loci with point mutations SCA11 is rare [15;16]. In the SCA subtypes of ADCA III, non-dynamic SCA mutation included SCA5, 11 and 14. After excluding the probably more prevalent SCA14 [17], SCA5 and 11 screening should also be considered. The frequency of SCA11 in our highly selected study population is approximately one percent. Considering only ADCA-III patients, with pure cerebellar symptoms, increased this figure (2 SCA11 index cases in 86 ADCA-III patients).

Although unlikely, we cannot exclude however, that we may have missed mutations because of the separate approaches in the French and German cohorts using direct sequencing and HRM, respectively. Nonetheless, both methods revealed a single heterozygous frameshift mutation (c.1306-1307delGA)in exon 12. As the 148 index patients included in this study derive from a total cohort of about 450 ADCA families after exclusion of all polyglutamine mutations (SCA1, 2, 3, 6, 7, and 17), and partly exclusion of SCA5, SCA13, SCA14, and SCA28, the frequency of SCA11 in an unselected ADCA cohort would be expected to be well below 1 percent.

Our finding of a recurrent frameshift mutation suggests, in accordance with the two *TTBK2* mutations described before, that SCA11 is caused by haploinsufficiency of tau tubuline kinase 2. As up to now no experimental insights in the pathogenesis of SCA11 exit, transgenic models of SCA11 implementing the genetic evidence for pathogenic mutations in exons 11 to 13 will help to understand *TTBK2's* function and/or dysfunction in the human cerebellum.

Tables

Table 1

Mutations and rare polymorphisms in the *TTBK2*-gene. Rare variants have been detected by HRM in the German samples.

Additionally, all novel SNP positions have been genotyped in 95 control samples originating from probands without signs of neurodegeneration. Interpretation depends on frequencies in controls and in-silico modelling of potentially altered splicing signals using the MaxEntScan algorithm [22].

Position	**Frequency in ADCA patients**	**Frequency in controls**	**Interpretation**
c.-38T>C rs16957250	7 (n=69), 10,1%	16,9% AFD EUR Panel	non-coding SNP
c.1306_1307delGA	2 (n=148), 1,4%	0	SCA11 mutation
c.1944C>G, p.S648S	2 (n=69), 2,9%	0 (n=95), 0%	synonymous SNP
c.1952C>T, p.T651M	1 (n=69), 1,5% No segregation	0 (n=95), 0%	non-synonymous SNP
c.2592A>G, p.E864E rs56307230	1 (n=69), 1,5%	2 (n=95), 2,1%	synonymous SNP

Supplementary table 1

a) Primer sequences and annealing temperature for amplification of the French samples

	Forward primer	**Reverse primer**	**Amplicon length**	**Annealing temperature**
Exon 11	Aactctcttaattatgtgtggagttca	ccttacaaaagaggcccaaa	503 bp	61°C
Exon 12	Gccaaaatatcctaatcatctgaa	tggatgaaaattggagctga	503 bp	61°C
Exon 13a	Tccatcttccaaagcaggat	gatcattttctgcccaagga	599 bp	63°C
Exon 13b	Tgattgtggacaaggagcag	tctgcaaaatttaggcctgt	452 bp	50°C

b) Primer sequences and annealing temperature for amplification of the German samples

	Forward primer	**Reverse primer**	**Amplicon length**	**Annealing temperature**
Exon 2	Taaatggaaaccacccttgg	ttttatcaggataccaaaaattaccc	228 bp	touchdown
Exon 3a	Agcaacttccattttcagaact	gggctttggagaaatttacg	109 bp	touchdown
Exon 3b	Aagagatcaattgctttcaagttaga	tgtaattaaggttcaggaagatacaaa	300 bp	touchdown
Exon 4	gagaaaccatagtatattcttttccactg	ttgttgttactttttattcatattcactg	209 bp	touchdown
Exon 5	Tggtaagtgagtagtttcccagtc	tcaaactcattctgtgctctgg	261 bp	touchdown
Exon 6	aaaatgtaagaatgataaaatgtaagga	ttagcattgtttaaaaagtgtgatacta	258 bp	touchdown
Exon 7	Tcatggttcaagtgtgctga	aaaaagaagtcaatctgatttaagcta	207 bp	touchdown
Exon 8	Tgaaacaaaatggttagaccttga	gcagaaatttttatttcttctaccaca	231 bp	touchdown
Exon 9	Gcctaaccaccaagagaaattg	tgagtgaaagcatagactaagagagaa	240 bp	touchdown
Exon 10a	Ctccttctgggattccgtct	tccaggaaccgtaccttgtc	375 bp	touchdown
Exon 10b	Caccaccatcactgtca	tgagcacaaattcattctccat	398 bp	touchdown
Exon 10c	Tggacaacatgttcgagagc	cagcgtctgcagctccttat	469 bp	touchdown
Exon 11a	Gaacatcagctggctccaag	acctgcatagctgctggtg	327 bp	touchdown
Exon 11b	Ctggctccaagctgagact	gcctccctcctaggctct	560 bp	touchdown
Exon 12	Tgactgattgccataaagaa	aatgaccacatattacttttaataac	157 bp	touchdown
Exon 13/14	Tcagtttttaaagaaaatcacattaca	atccaggaaagacaccacca	319 bp	touchdown
Exon 15	agatagtctcatagtaatttttgattcttg	ccagccttctgaactgtaagaaata	327 bp	touchdown
Exon 16	Ttgcctagaatgtttttcatgattt	tggtaccttagaagctttaaaaagaaa	300 bp	touchdown
Exon 17a	Aaatgtttccttaggacagttgagt	aagtacttcaggctcctcatcaga	396 bp	touchdown
Exon 17b	Gagcaggaccttcaggatttt	atagcaccttgagtggataacagag	378 bp	touchdown
Exon 18	Tctttgttgatattttacataattgcat	agtttccccagggagattttc	387 bp	touchdown

c)) Oligonucleotides in *TTBK2*-specific MLPA probeset

target	**5' half-probe[1]**	**3' half-probe[2]**	**Length of MLPA product[3]**
TTBK2 Exon 2	Cctgtgtagctgttctaccatatcagtgtattgca	Atgagtgggggaggagagcagctggatatcctgagtgttgg	118 bp
TTBK2 Exon 4	Ccagggaaagaccatgtttgtagatttattggctgtgggagga	atgatcgattcaactatgtggtcatgcagttgcagg	121 bp
TTBK2 Exon 6	Cgaacttcgctatgggtcgctttcctagtacatgtaggaaatgttaca	Tgcttgattttggcttggctcgacaatttaccaattcctgtgg	133 bp
TTBK2 Exon 8	Cctttggtccttattctacatgttggtggagt	Ttgtggttggtcagctgccctgg	97 bp
TTBK2 Exon 10	Ccgtgtttgacaatagcatcaagacttttggagta	Attgagagtgaccctttgactgggagaagactgg	112 bp
TTBK2 Exon 12[4]	Ccaattcgtgtccgctcagagattactcagccagacagag	Atattccactggtgcgaaagttacgttccattcacagctttgagctgg	130 bp
TTBK2 Exon 14	Ccatcatctcccagtctagaaagagcaaaattcca	Aggccagtttcatgggtcaacacagatcagg	108 bp
TTBK2 Exon 15	Ccatgaccagaggagttcgtccccacatctggggaga	Agcaagtcacctcccagccactcagg	105 bp
Reference 1 (CYP7B1)	Ccaggccgatttttgcacttatggaaataaaacaattg	Ttggttatacttttaacttattttgatttagaaataattgatgataagcccatagg	137 bp
Reference 2 (DACH)	Gtttcagttctagacctggaaggcctcctaagaggactcaaa	Gtgtcacctccccagagaactctcacatcatgccgcattctg	126 bp
Reference 3 (SERPINB2)	Ccagagaactttaccagctgtgggttcatgca	Gcagatccagaagggtagttatcctgatgcgattttgcagg	115 bp
Reference 4 (SLC33A1)	Cagctttgcaaaaaactgggtggctcat	gtgttacagccctggatggttattatgtgg	100 bp

[1]in addition to the specific sequence given in the table, each 5' half probe carries gggttccctaagggttgga at its 5' end

[2]in addition to the specific sequence given in the table, each 3' half probe carries tctagattggatcttgctggcac at its 3' end

[3]including the sequences which are identical for all 3' and 5' halfprobes (see footnotes 1 and 2)

[4]the 2bp deletion identified twice in the present study is targeted by the last nucleotide of the *TTBK2* exon 12 5' half probe and the first nucleotide of the corresponding 3' half probe

Acknowledgments:

We are grateful to the family members for their participation, to Drs N. Wood, A. Houlden and P. Giunti for information on SCA11 sequence, to Is Mäckle-Jentsch (Tübingen) and the DNA and Cell bank of the Centre de Recherche de l' Institut du Cerveau et de la Moelle Epiniere (Paris) for technical assistance and to the clinicians who referred to us some of the patients.

This work was funded by INSERM, the European Union (to the EUROSCA consortium) and the Programme Hospitalier de Recherche Clinique (to A Durr).

Figure 1:

Pedigrees of the French and German SCA11 families are shown. The chromatogram shows the identified mutation in exon 12.

Square symbols are men, the circles are women. The filled symbols are affected individuals. The numbers are an internal reference for each sampled individual. Stars indicate sampled subjects.

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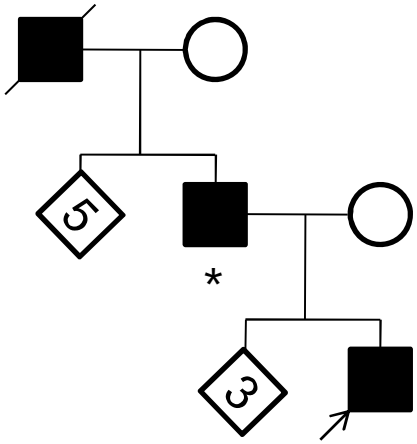
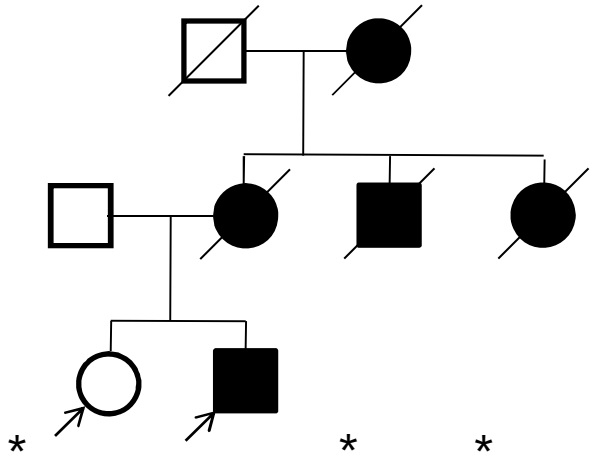
French family



German family



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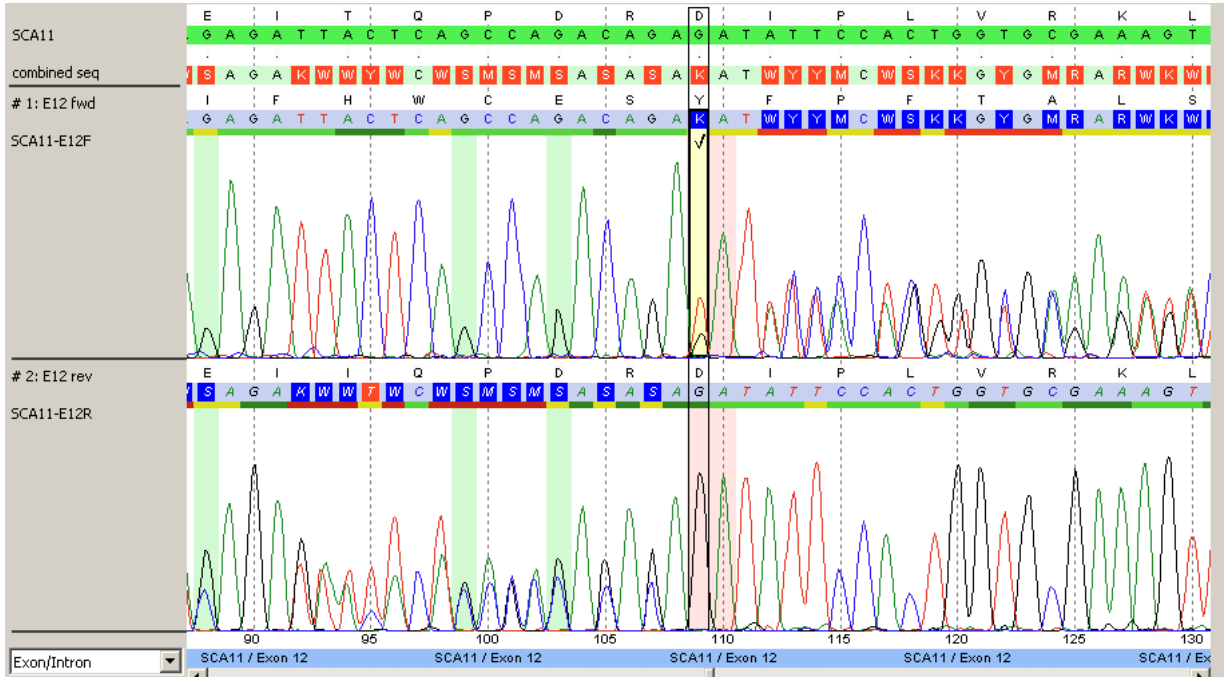
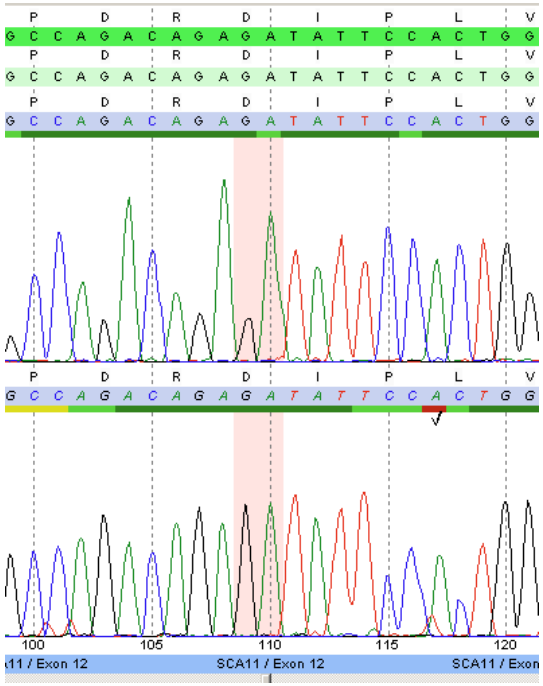
(exon 12 of TTBK2,
transcript Ensembl 267890 TTBK2-203)

c.1096_1097delGA, p.D366YfsX
(exon 9 of TTBK2,
transcript Ensembl 399479 TTBK2-201)

c.2521_2522delGA, p.D841fsX
(exon 13 of TTBK2,
transcript Ensembl 263802)

Chromatogram of the German SCA11 patient



Control chromatogram for exon 12