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A mutation in a novel ATP-dependent Lon protease gene in a kindred with mild mental retardation

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Abstract

Background—Identifying the genetic factors that contribute to memory and learning is limited by the complexity of brain development and the lack of suitable human models for mild disorders of cognition.

Methods— Previously, a disease locus was mapped for a mild type of nonsyndromic mental retardation (IQ between 50 and 70) to a 4.2-MB interval on chromosome 3p25-pter in a large kindred. The genes and transcripts within the candidate region were systematically analyzed for mutations by single-strand polymorphism analysis and DNA sequencing.

Results— A nonsense mutation causing a premature stop codon in a novel gene (*cereblon*; *CRBN*) was identified that encodes for an ATP-dependent Lon protease. The predicted protein sequence is highly conserved across species, and it belongs to a family of proteins that selectively degrade short-lived polypeptides and regulate mitochondrial replication and transcription. One member of the Lon-containing protein family is regionally expressed in the human hippocampus, an important neuroanatomic region that is involved in long-term potentiation and learning. The mutation in the *CRBN* gene described interrupts an N-myristoylation site and eliminates a casein kinase II phosphorylation site at the C terminus.

Conclusions— A gene on chromosome 3p that is associated with mild mental retardation in a large kindred is reported. This finding implicates a role for the ATP-dependent degradation of proteins in memory and learning.

The identification of specific human genetic disorders that affect critical steps in cognition has greatly facilitated our understanding of the molecular mechanisms involved in learning and memory. Research in this field is particularly important because developmental disorders of cognition or mental retardation affects approximately 1 to 3% of the general population in the United States.¹ A genetic or inherited metabolic etiology is implicated in two-thirds of mental retardation cases,² and a recessive mode of transmission accounts for nearly one-fourth of these cases.^{3–5} About one-third of mental retardation is considered severe (IQ < 50).⁶ The genetic disorders studied thus far are suboptimal models to unravel the fundamental aspects of human cognition because they are either severe⁷ or are part of a syndrome with extensive involvement of other processes besides memory and learning.⁸ Several of these syndromes affect basic cellular mechanisms such as signaling pathways,^{9–12} regulation of gene expression,^{13–17} and alterations in hippocampal dendrite morphology.¹⁸

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Previously, we described a genetic locus for autosomal recessive nonsyndromic mental retardation (ARNSMR) on chromosome 3p.¹⁹ This locus, designated as MRT2A (MIM 607417), is associated with a mild ARNSMR phenotype with IQs ranging from 50 to 70. The phenotype does not include congenital anomalies or dysmorphic features.¹⁹ A 4.2-MB physical map of the MRT2A candidate region contains five transcripts and nine genes. The nine genes with known function were excluded as a cause of ARNSMR by single-strand conformational polymorphism (SSCP) analysis followed by direct DNA sequencing of the PCR products.²⁰ In this article, we exclude four of the five transcripts as a cause for mild ARNSMR and report that a nonsense mutation in a novel gene that encodes for an ATP-dependent Lon protease is responsible for mild cognitive deficits.

Methods

Research subjects

This research study was approved by the Mid-Hudson Family Health Institute Institutional Review Board. Written informed consent was obtained from each individual before genetic analyses. High molecular weight genomic DNA was isolated by using the Puregene DNA extraction kit (Gentra Systems, Minneapolis, MN). DNA from five nuclear families with a mild type of ARNSMR and from 100 normal white control subjects (200 chromosomes) was used to identify mutations in the 11 coding regions of the human *CRBN* gene.

Genealogic information

To identify a gene that causes a mild ARNSMR, we compiled a database using seven volumes of genealogic information to establish the relationships among individuals belonging to a sectarian population. This population traces their ancestry to 12 separatists who immigrated to North America in the late 17th century to escape religious persecution in Western Europe. Their descendants have remained in the same county in the United States (1990 US census: 29,296 inhabitants) for over two centuries. In this population, 10 individuals from three branches of an extended pedigree were diagnosed with ARNSMR.¹⁹

Phenotype assignment

State-licensed school psychologists evaluated the cognitive function of the affected individuals by administering standardized tests between the ages of 6 and 12. The composite scores on the Stanford-Binet Test ranged from 50 to 70, indicating that all individuals were cognitively disabled in the mildly mentally retarded range. IQ scores were lower in males than females.¹⁹ There were no prior histories of pre- or postgestational complications in affected individuals. None of the affected individuals had peri- or postnatal infections, afebrile seizures, toxic exposures, or significant head trauma. Developmental milestones were mildly delayed since early childhood. Hearing and vision screening were normal. There was good eye contact and no autistic features. The highest reading ability achieved was at the first-grade level in a 32-year-old affected woman as assessed by the Gray Oral Reading Scale. In five affected adult individuals, writing skills were limited to signing their own names. None of the other affected individuals could write. The dysmorphicologic examination included inspection for craniofacial, thoracoabdominal, digital, and limb anomalies and standard anthropologic measurements of the head, eyes, ears, philtrum, limbs, and digits. Detailed physical and neurologic examinations in all study participants did not show microcephaly, organomegaly, phakomata, spasticity, weakness, neuropathy, visual deficits, feeding problems, or psychiatric disorders. All individuals had normal newborn screening examinations for hypothyroidism, galactosemia, maple syrup urine disease, phenylketonuria, and biotinidase. Affected individuals had normal laboratory studies on the following: MRI of the brain, high-resolution (ISCN G-banding 550 resolution) cytogenetic studies, DNA testing for a CCG repeat expansion in fragile X, fasting plasma amino acids, urine amino acids and organic acids, pyruvate, lactate, and ammonia. The

intellectual level of each unaffected person was judged to be normal by a bedside appraisal of memory, judgment, general knowledge, and calculation ability.²¹ None of the unaffected individuals had developmental delay or learning disabilities as children, and they were judged to be of normal intelligence by psychometric testing performed during grade school.

PCR SSCP

Intronic PCR primers flanking the splice sites of each coding regions of the *CRBN* gene were synthesized on an ABI DNA Synthesizer 3900 (Foster City, CA). The forward primer 5'-ACTTAGGTATGTATCAGAGG-3' and reverse primer 5'-ATATGATAGGCTTGGAGTAC-3' were used to amplify the *CRBN* exon 11 fragment. The PCR reaction contained 100 ng of genomic DNA in a total volume of 15 μ L with 0.8 mM of each primer, 0.2 mM of each dNTP, 100 mM Tris-HCl (pH 9.0), 500 mM KCl, 15mM MgCl₂, and 0.5 U of Taq polymerase (Sigma Aldrich, St. Louis, MO). The reactions were performed in a 96-well microtiter plate on a Genius thermocycler (Techne, Princeton, NJ). Amplification was carried out with an initial denaturation step of 5 minutes at 95 °C followed by 30 cycles of denaturation for 45 seconds at 95 °C, 45 seconds of annealing at varying temperatures, and 45 seconds of extension at 72 °C. The last extension step was 7 minutes at 72 °C. The PCR products of nine affected individuals were compared with 200 normal controls for differences in DNA mobility shifts by SSCP, as published previously.

DNA sequencing

When differences in SSCP were found between affected and controls, the samples were sequenced to define the nucleotide variation causing the mobility shift, as published previously.²⁰

Restriction enzyme digestion

The identification of a change in the wild-type nucleotide sequence 5'-ACGC-3' to the mutant 5'-ACGT-3' sequence (figure 1) caused an insertion of an HpyCH4IV restriction enzyme site in *CRBN* exon 11. Restriction enzyme digests were carried out using the instructions provided by the manufacturer (New England BioLabs, Beverly, MA) and electrophoresed on a 2% agarose/0.1% ethidium bromide gel. Gels were visualized on an ultraviolet light box.

Reverse transcription PCR

Reverse transcription PCR was performed using the instructions for One Step RT-PCR provided by the manufacturer (USB Co., Cleveland, OH). The oligonucleotide primers were designed in regions that overlapped the coding regions of the *CRBN* gene to prevent amplification of genomic DNA. One microgram of total RNA was used in the reaction with 0.4 μ M of the primer 5'-TTTGGGGGTAAACAGACATGGC-3' to reverse transcribe. Amplification was performed using the sense primer 5'-TTTGGGGGTAAACAGACATGGC-3' and antisense primer 5'-ATGGCACCAAGCTACCCAAGTAGATG-3'. Reverse transcription was performed at 43 °C for 30 minutes. PCR amplification was performed with an initial denaturation step of 3 minutes at 95 °C followed by 40 cycles of denaturation for 30 seconds at 95 °C, 30 seconds of annealing at 60 °C, and 120 seconds of extension at 72 °C. The last extension step was 3 minutes at 72 °C. The products were visualized with ethidium bromide on 1.2% agarose gel.

GenBank accession nos

The DNA and protein sequences are as follows: *CRBN*, NM_016302; *PRSSI5*, NM_004793; *Homo sapiens CRBN*, NP_057386; *Mus musculus CRBN*, NP_780566; and *Rattus norvegicus CRBN*, XP_216255.

Results

Mutational analysis of candidate transcripts and genes

A genome-wide search established genetic linkage between loci on chromosome 3p24-pter and a ARNSMR locus in the population isolate.¹⁹ Homozygosity mapping narrowed the genetic interval to a 4.2-million-base-pair region between loci D3S3630 and D3S1304.²⁰ This interval contained five transcripts (LOC51185, LOC375321, LOC132049, LOC377019, and FLJ10702) and nine genes (*IL5RA*, *TRNT1*, *LRRN1*, *SETMAR*, *SUMF1*, *ITPR1*, *BHLHB2*, *EDEM*, and *MRPS36P1*). The coding regions and splice sites of these genes and transcripts were analyzed systematically for mutations by SSCP analysis followed by direct DNA sequencing of the PCR products. The nine genes with known function were excluded by these methods as a cause of mental retardation in our sample. SSCP analysis of the transcripts revealed a variation in LOC51185 that segregated with the disease phenotype (see figure 1A). The other transcripts did not have any DNA sequence variations by SSCP and DNA sequencing.

Identification of truncating mutation in mild ARNSMR

DNA sequencing of the LOC51185 PCR product in affected individuals detected a homozygous C→T nonsense mutation at nucleotide position 1,274 of the LOC51185 cDNA (1,274C→T). This substitution changed an arginine residue to a stop codon (see figure 1B) in exon 11 (R419X). Restriction enzyme analysis with HpyCH4IV confirmed the finding and permitted a rapid analysis of samples (see figure 1C). The 1,274C→T mutation was absent on 400 chromosomes from 200 unrelated control individuals. There were no carriers of the mutation identified in the control samples. None of the unaffected individuals in the family was homozygous for the mutation. Obligate carriers and individuals identified as heterozygotes by linkage analysis¹⁹ were also heterozygous for the mutation. Only affected individuals in the family were homozygous for the 1,274C→T mutation (figure 2).

Description of gene (cereblon) for mild NSMR.

The LOC51185 transcript was previously identified as part of a large-scale research effort to sequence human cDNA clones containing open reading frames.²² The LOC51185 transcript contains 11 predicted exons and a putative protein product of 442 amino acids. There is a 237-amino acid ATP-dependent Lon protease domain and other motifs that include casein kinase II (CK2) phosphorylation, *N*-myristoylation, protein kinase C phosphorylation, and *N*-glycosylation sites (see figure 2). The LOC51185 transcript was assigned the gene name *cereblon* (HUGO symbol *CRBN*) based on its putative role in cerebral development and the presence of its large, highly conserved Lon domain. The truncating mutation in the *CRBN* gene that causes a mild type of ARNSMR spares the Lon domain, interrupts an *N*-myristoylation site, and eliminates a CK2 phosphorylation site at the C terminus (figure 3). Reverse transcription PCR demonstrated abundant expression of *CRBN* in the human brain (data not shown).

Discussion

Three lines of evidence strongly suggest that the mutation in the *CRBN* gene causes mild mental retardation. First, a small interstitial deletion of one allele involving the candidate interval for ARNSMR between loci D3S3630 and D3S1304 on chromosome 3p25-pter results in a viable human phenotype of ptosis, microcephaly, growth retardation, and mental retardation (3p syndrome).^{23,24} The extensive impairment in the 3p syndrome as compared with the mild mental retardation in our cases suggests that a loss of function of the *CRBN* gene and other genes within the interval has neurologic and cognitive consequences. The deletion of one copy of the *CRBN* gene in the 3p syndrome also indicates that haploinsufficiency of the *CRBN* gene

is not lethal. Second, the nonsense mutation in the *CRBN* gene causes a severe alteration of the predicted protein structure by causing a stop codon that prematurely truncates the protein. As shown in figure 3, the mutation disrupts an *N*-myristoylation site and deletes a CK2 phosphorylation motif. These disruptions may prevent proper subcellular targeting and alter long-term potentiation in the hippocampus.²⁵ Third, both the *CRBN* and another gene, *PRSS15*, encode for ATP-dependent Lon proteases that are highly expressed in the human brain. Although the *PRSS15* gene has not been directly associated with human disease, its presence in the hippocampus²⁶ and its location in the candidate region for a severe form of ARNSMR²⁷ suggest a role in memory and learning. Most, if not all, human Lon-containing proteins are localized to the mitochondria where they selectively degrade short-lived polypeptides^{28,29} and regulate mitochondrial replication and transcription by specific binding to GT-rich mitochondrial DNA sequences.³⁰ Therefore, we hypothesize that a defect in the *CRBN* gene may perturb nuclear regulation of mitochondrial energy metabolism and cause memory and learning difficulties.

A truncating mutation in another gene, *neurotrypsin*, which is highly expressed in the hippocampus, causes an atypical type of severe ARNSMR in Algerians by altering proteolysis at the synaptic level.^{7,31} The phenotype in individuals with *neurotrypsin* mutations is characterized by normal psychomotor development during the first 2 years of life followed by a progressive cognitive deterioration leading to severe mental retardation with IQs of <50.⁷ This phenotype is distinctly different from the mild mental retardation that we describe in individuals with a truncating mutation in the *CRBN* gene.

The discovery of *CRBN* as a gene that is involved in mild mental retardation will enable the development of a test for early diagnosis and prenatal counseling in the large population isolate that we have studied.¹⁹ It will also focus research efforts to search for other mutations in the ATP-dependent Lon protease gene family as causes for ARNSMR. Although the pathogenic consequences caused by the truncating mutation in the *CRBN* gene are not clear, a partial loss of function of *CRBN* could alter long-term potentiation by affecting physiologic changes in the mitochondria. Confirming this hypothesis may provide opportunities for therapy and contribute to a better understanding of mitochondrial function and failure in other human neurologic disorders such as Friedreich ataxia,³² Parkinson, and Alzheimer diseases.³³ More specifically, this discovery will clarify the basic mechanisms that are involved in mental retardation and facilitate research on the functional role of the *CRBN* gene in memory and learning.

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References

1. Massey PS, McDermott S. State-specific rates of mental retardation—United States, 1993. MMWR 1996;45:61–65. [PubMed: 8544809]
2. Curry CJ, Stevenson RE, Aughton D, et al. Evaluation of mental retardation: recommendations of a Consensus Conference: American College of Medical Genetics. Am J Med Genet 1997;72:468–477. [PubMed: 9375733]
3. Wright SW, Tarjan G, Eyer L. Investigation of families with two or more mentally defective siblings; clinical observations. Am J Dis Child 1959;97:445–463.
4. Priest JH, Thuline HC, Laveck GD, Jarvis DB. An approach to genetic factors in mental retardation. Studies of families containing at least two siblings admitted to a state institution for the retarded. Am J Ment Defic 1961;66:42–50. [PubMed: 13738004]

5. Bartley JA, Hall BD. Mental retardation and multiple congenital anomalies of unknown etiology: frequency of occurrence in similarly affected sibs of the proband. *Birth Defects* 1978;14:127–137. [PubMed: 728557]
6. Boyle CA, Yeargin-Allsopp M, Doernberg NS, Holmgren P, Murphy CC, Schendel DE. Prevalence of selected developmental disabilities in children 3–10 years of age: the Metropolitan Atlanta Developmental Disabilities Surveillance Program, 1991. *MMWR CDC Surveill Summ* 1996;45:1–14. [PubMed: 8602136]
7. Molinari F, Rio M, Meskenaite V, et al. Truncating neurotrypsin mutation in autosomal recessive nonsyndromic mental retardation. *Science* 2002;298:1779–1781. [PubMed: 12459588]
8. Weeber EJ, Levenson JM, Sweatt JD. Molecular genetics of human cognition. *Mol Intervent* 2002;2:376–391.
9. Matsuura T, Sutcliffe JS, Fang P, et al. De novo truncating mutations in E6-AP ubiquitin–protein ligase gene (UBE3A) in Angelman syndrome. *Nat Genet* 1997;15:74–77. [PubMed: 8988172]
10. Albrecht U, Sutcliffe JS, Cattanaach BM, et al. Imprinted expression of the murine Angelman syndrome gene, Ube3a, in hippocampal and Purkinje neurons. *Nat Genet* 1997;17:75–78. [PubMed: 9288101]
11. Costa RM, Federov NB, Kogan JH, et al. Mechanism for the learning deficits in a mouse model of neurofibromatosis type 1. *Nature* 2002;415:526–530. [PubMed: 11793011]
12. Xing J, Ginty DD, Greenberg ME. Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. *Science* 1996;273:959–963. [PubMed: 8688081]
13. Galdzicki Z, Siarey R, Pearce R, Stoll J, Rapoport SI. On the cause of mental retardation in Down syndrome: extrapolation from full and segmental trisomy 16 mouse models. *Brain Res Brain Res Rev* 2001;35:115–145. [PubMed: 11336779]
14. Shahbazian M, Young J, Yuva-Paylor L, et al. Mice with truncated MeCP2 recapitulate many Rett syndrome features and display hyperacetylation of histone H3. *Neuron* 2002;35:243–254. [PubMed: 12160743]
15. Petrij F, Giles RH, Dauwerse HG, et al. Rubinstein–Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature* 1995;376:348–351. [PubMed: 7630403]
16. Wang YH, Amirhaeri S, Kang S, Wells RD, Griffith JD. Preferential nucleosome assembly at DNA triplet repeats from the myotonic dystrophy gene. *Science* 1994;265:669–671. [PubMed: 8036515]
17. Darnell JC, Jensen KB, Jin P, Brown V, Warren ST, Darnell RB. Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell* 2001;107:489–499. [PubMed: 11719189]
18. Yang EJ, Yoon JH, Min do S, Chung KC. LIM kinase 1 activates cAMP-responsive element-binding protein during the neuronal differentiation of immortalized hippocampal progenitor cells. *J Biol Chem* 2004;279:8903–8910. [PubMed: 14684741]
19. Higgins JJ, Rosen DR, Loveless JM, Clyman JC, Grau MJ. A gene for nonsyndromic mental retardation maps to chromosome 3p25-pter. *Neurology* 2000;55:335–340. [PubMed: 10932263]
20. Higgins JJ, Pucilowska J, Lombardi RQ, Rooney JP. Candidate genes for recessive non-syndromic mental retardation on chromosome 3p (MRT2A). *Clin Genet* 2004;65:496–500. [PubMed: 15151510]
21. Strub R, Black F. Higher cognitive functions. In: *The mental status examination in neurology*. 2nd ed. Philadelphia: Davis, 1986:125–137.
22. Strausberg RL, Feingold EA, Grouse LH, et al. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. *Proc Natl Acad Sci USA* 2002;99:16899–16903. [PubMed: 12477932]
23. Narahara K, Kikkawa K, Murakami M, et al. Loss of the 3p25.3 band is critical in the manifestation of del(3p) syndrome: karyotype–phenotype correlation in cases with deficiency of the distal portion of the short arm of chromosome 3. *Am J Med Genet* 1990;35:269–273. [PubMed: 2178418]
24. Cargile CB, Goh DL, Goodman BK, et al. Molecular cytogenetic characterization of a subtle interstitial del(3)(p25.3p26.2) in a patient with deletion 3p syndrome. *Am J Med Genet* 2002;109:133–138. [PubMed: 11977162]
25. Charriaud-Marlangue C, Otani S, Creuzet C, Ben-Ari Y, Loeb J. Rapid activation of hippocampal casein kinase II during long-term potentiation. *Proc Natl Acad Sci USA* 1991;88:10232–10236. [PubMed: 1946443]

26. Wang N, Gottesman S, Willingham MC, Gottesman MM, Maurizi MR. A human mitochondrial ATP-dependent protease that is highly homologous to bacterial Lon protease. *Proc Natl Acad Sci USA* 1993;90:11247–11251. [PubMed: 8248235]
27. Basel-Vanagaite L, Alkelai A, Straussberg R, et al. Mapping of a new locus for autosomal recessive non-syndromic mental retardation in the chromosomal region 19p13.12-p13.2: further genetic heterogeneity. *J Med Genet* 2003;40:729–732. [PubMed: 14569116]
28. Maurizi MR, Trisler P, Gottesman S. Insertional mutagenesis of the lon gene in *Escherichia coli*: lon is dispensable. *J Bacteriol* 1985;164:1124–1135. [PubMed: 2999072]
29. Goff SA, Goldberg AL. An increased content of protease La, the lon gene product, increases protein degradation and blocks growth in *Escherichia coli*. *J Biol Chem* 1987;262:4508–4515. [PubMed: 3549709]
30. Lu B, Liu T, Crosby JA, Thomas-Wohlever J, Lee I, Suzuki CK. The ATP-dependent Lon protease of *Mus musculus* is a DNA-binding protein that is functionally conserved between yeast and mammals. *Gene* 2003;306:45–55. [PubMed: 12657466]
31. Gschwend TP, Krueger SR, Kozlov SV, Wolfer DP, Sonderegger P. Neurotrypsin, a novel multidomain serine protease expressed in the nervous system. *Mol Cell Neurosci* 1997;9:207–219. [PubMed: 9245503]
32. Pandolfo M. Friedreich's ataxia: clinical aspects and pathogenesis. *Semin Neurol* 1999;19:311–321. [PubMed: 12194387]
33. Hashimoto M, Rockenstein E, Crews L, Masliah E. Role of protein aggregation in mitochondrial dysfunction and neurodegeneration in Alzheimer's and Parkinson's diseases. *Neuromol Med* 2003;4:21–36.
34. Wu CH, Yeh LS, Huang H, et al. The Protein Information Resource. *Nucleic Acids Res* 2003;31:345–347. [PubMed: 12520019]

Figure 1.

Identification of the 1,274C→T mutation in CRBN exon 11. (A) Representative sample of the single-strand conformational polymorphism (SSCP) mobility shifts in one family branch with mild autosomal recessive nonsyndromic mental retardation. SSCP was performed on the 331-bp PCR products of exon 11, including its 5' and 3' splice sites. The mobility shift patterns co-segregated with the affection status and correlated with the results of linkage analysis reported previously.¹⁹ Affected individuals (filled symbols) have two upper bands and one lower band. Carriers (partially filled symbols) have six bands including two upper bands, two middle bands, and two lower bands. Noncarriers (open symbols) have one upper band, two middle bands, and two lower bands. The SSCP pattern of the noncarrier was the same as the patterns in 200 normal individuals (400 chromosomes). (B) Direct sequencing of PCR products. (Top) The CRBN sequence between nucleotide (nt) 1,263 and 1,284 from a normal individual (noncarrier). (Bottom) A 1,274C→T substitution in an affected individual in the family. Below the nucleotide sequence is the amino acid sequence. The mutation (arrow) results in a stop codon at the arginine (R) residue at codon 419 and truncates the CRBN protein (R419X). (C) A 2% agarose/0.1% ethidium bromide gel showing the HpyCH4IV restriction enzyme analysis of CRBN exon 11. Lane 1 is a 100-bp ladder. The PCR product of a normal individual (lane 2) is not cleaved by HpyCH4IV and shows a single band representing the 331-bp wild-type alleles. Carriers show two bands representing the 331-bp wild-type allele and the cleaved mutant allele (164-bp, 167-bp) (lanes 3 to 6). The mutation creates a restriction site so that the amplified 331-bp fragment is cleaved in half (164-bp, 167-bp) (lanes 7 to 10).

Figure 2.

Segregation of the CRBN mutation using HpyCH4IV restriction enzyme digestion of exon 11 in an abridged family pedigree. As in figure 1C, a single 331-bp band represents the wild-type allele and is homozygous in Individuals 2, 8, 10, 19, 24, 26, 30, and 31. Carriers (Individuals 1, 3, 4, 5, 6, 7, 9, 12, 15, 16, 20, 22, 27, 28, and 32) show two bands representing the 331-bp wild-type allele and the cleaved mutant allele (164-bp, 167-bp). The mutation creates a restriction site so that the amplified 331-bp fragment is cleaved in half (164-bp, 167-bp) and is shown as a single lower band in affected Individuals 11, 13, 14, 17, 18, 21, 23, 25, and 29. Filled circles (females) and squares (males) represent affected individuals with mental retardation. Unaffected individuals are indicated by open symbols. Consanguineous marriages are indicated by double bars joining the individuals. For confidentiality reasons, the order and the sex of at-risk individuals are changed.

Figure 3.

Alignment of the amino acid sequences from different species with similarities to the CRBN protein. Low consensus alignments are in gray and neutral consensus alignments are in black. The underlined sequences show the Lon domain from amino acid residues 81 to 317 of the predicted protein for human CRBN and the homologous proteins for mice and rats. Prosites predictions³⁴ for the casein kinase II phosphorylation site (filled circles), myristoylation sites (open circles), protein kinase C phosphorylation site (filled squares), and a glycosylation site (open squares) are shown. The arrow shows the position of the stop codon (R419X) that interrupts an N-myristoylation site and eliminates a casein kinase II phosphorylation site.