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 4
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 5
     clicense>
 6
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     International License.
     To view a copy of this license, visit http://creativecommons.org/licenses/by-sa/4.0/ or
 8
     send a letter to Creative Commons, PO Box 1866, Mountain View, CA 94042, USA.
 9
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14
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15
     <author>Nancy Green, University of N.C. Greensboro</author>
16
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18
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20
     <title>Towards Mining Scientific Discourse Using Argumentation Schemes</title>
21
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27
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     <author>van de Leemput, J., et al.</author>
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     <title>Deletion at ITPR1 Underlies Ataxia in Mice and Spinocerebellar Ataxia 15 in
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30
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39
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40
    For information on the CRAFT corpus see:
41
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42.
43
    W. A., Cohen, K. B., Verspoor, K., Blake, J. A., and Hunter, L. E. Concept Annotation
44
     in the CRAFT Corpus. BMC Bioinformatics. 2012 Jul 9;13:161. doi:
     10.1186/1471-2105-13-161.
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     М.,
49
     Palmer, M., Hunter L.E. A corpus of full-text journal articles is a robust evaluation
50
     tool for revealing differences in performance of biomedical natural language processing
51
     tools. BMC Bioinformatics. 2012 Aug 17;13(1):207. [PubMed:22901054]</citation>
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53
54
    <acknowledgments>
55
     We thank Michael Branon and Bishwa Giri for their help in analyzing this article with
```

the support of a University of North Carolina Greensboro 2016 Summer Faculty Excellence

Research Grant.

```
57
58
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59
60
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61
62
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63
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    Humans.
64
    <main-claim>Deletion at ITPR1 Underlies Ataxia in Mice and Spinocerebellar Ataxia 15 in
    Humans.</main-claim>
65
    </title>
66
67
    <section> <section-heading>Abstract</section-heading>
68
69
    We observed a severe autosomal recessive movement disorder in mice used within our
    laboratory.
70
    We pursued a series of experiments to define the genetic lesion underlying this
    disorder and to identify a cognate disease in humans with mutation at the same locus.
71
    Through linkage and sequence analysis we show here that this disorder is caused by a
    homozygous in-frame 18-bp deletion in Itprl (ItprlΔ18/Δ18), encoding inositol
    1,4,5-triphosphate receptor 1.
72
    A previously reported spontaneous Itpr1 mutation in mice causes a phenotype identical
    to that observed here.
73
    In both models in-frame deletion within Itprl leads to a decrease in the normally high
    level of Itpr1 expression in cerebellar Purkinje cells.
74
    Spinocerebellar ataxia 15 (SCA15), a human autosomal dominant disorder, maps to the
    genomic region containing ITPR1;
75
    however, to date no causal mutations had been identified.
    Because ataxia is a prominent feature in Itpr1 mutant mice, we performed a series of
76
    experiments to test the hypothesis that mutation at ITPR1 may be the cause of SCA15.
77
    We show here that heterozygous deletion of the 5' part of the ITPR1 gene, encompassing
    exons 1-10, 1-40, and 1-44 in three studied families, underlies SCA15 in
    humans.
     <main-claim>heterozygous deletion of the 5' part of the ITPR1 gene, encompassing exons
78
    1-10, 1-40, and 1-44 in three studied families, underlies SCA15 in humans.</main-claim>
79
    </section>
80
81
    <section> <section-heading>Author Summary</section-heading>
82
    <!-- This section appeared in an inset -->
83
84
    We have identified a spontaneous in-frame deletion mutation in the gene Itpr1 that
    causes a recessive movement disorder in mice.
85
    In an attempt to define whether any similar disease occurs in humans we performed a
    literature search for diseases linked to the human chromosomal region containing ITPR1.
86
    We identified the disease spinocerebellar ataxia 15 as linked to this region.
87
    High-density genomic analysis of affected members from three families revealed that
    disease in these patients was caused by deletion of a large portion of the region
    containing ITPR1.
    We show here that this mutation results in a dramatic reduction in ITPR1 in cells from
88
    these patients.
89
    These data show convincingly that ITPR1 deletion underlies spinocerebellar ataxia 15 in
    humans.
90
     <main-claim>ITPR1 deletion underlies spinocerebellar ataxia 15 in humans.
91
     </section>
92
93
    <section> <section-heading>Introduction</section-heading>
94
    <content>
    The use of forward genetics to define novel loci of interest in human disease has
95
    become increasingly viable with the implementation of large-scale mutagenesis programs.
96
    Prior to these initiatives this work was carried out in part by the investigation of
    spontaneous mutations that cause disorders in mouse breeding colonies.
97
    Careful observation of these serendipitous events has led to the establishment and
    study of many in vivo disease models [3].
```

During the generation of a knockout line of mice we noted an early movement disorder

that was inherited independently of targeting vector transmission.

56

98

</acknowledgments>

```
We embarked on a series of experiments to identify the genetic lesion underlying this movement disorder and to identify a cognate disease and corresponding mutation in humans.
```

Here we describe this effort and the discovery of deletion at the ITPR1 locus as a cause of this disorder in mice and of spinocerebellar ataxia 15 (SCA15) in humans.

101 </content>

<main-claim>deletion at the ITPR1 locus as a cause of this disorder in mice and of
spinocerebellar ataxia 15 (SCA15) in humans./main-claim>

103 </section>

104

106 <DSEG ID="Observation">

- 107 <content>
- [para-1] During the generation of a line of mice with knockout of the gene Park7 we noted an early movement disorder that was inherited independently of targeting vector transmission.
- Our initial observations suggested the affected mice suffered from an apparently paroxysmal movement disorder, often induced by touch.
- The abnormal movements occurred predominantly below the cervical level, and the disorder appeared progressive.
- At initial examination, a human movement disorder specialist (K. G.-H.) likened the disorder to episodic intermittent ataxia or kinesiogenic paroxysmal dystonia and predicted the involvement of an ion channel mutation in the etiology.
- Affected mice presented at approximately postnatal day 14, and survival time without weaning was on average 4 wk after onset.
- [para-2] Breeding experiments suggested that the observed disorder was inherited in an autosomal recessive manner.

```
114
      </content>
115
116
      <entities-props>
117
      <entity ID="group1" paraphrase="the affected mice" />
118
      <entity ID="pheno1" paraphrase="ataxia-like movement disorder" />
119
      prop>have pheno(group1, pheno1)
120
      </entities-props>
121
      </DSEG>
122
123
      <DSEG ID="Experiment 1" >
124
      <content>
```

- To map the location of the disease-causing lesion, we performed genome-wide linkage analysis using strain-specific single nucleotide polymorphisms (SNPs) at 120 loci across the mouse genome.
- Analysis of these data showed a single genomic region with significant linkage to disease, providing a two-point LOD score of 5.13 at marker 20.MMHAP85FLG2 Chromosome 6qE1.
- The linked haplotype suggested the mutation had occurred on the 129x1/SvJ background (Figure S1).

```
128
      </content>
129
130
      <entities-props>
      <entity ID="geno1" paraphrase="homozygous mutation on chromosome 6qE1" />
131
132
      prop>have geno(group1,geno1)
133
      </entities-props>
134
135
      <argument ID="1" old-id="2.1" scheme="Agreement">
136
      oremise-list>
      <premise prop="have pheno(group1, pheno1)" />
137
138
       prop="have geno(group1,geno1)"/>
139
      mise-list>
140
      <conclusion inferred-prop="cause(geno1,pheno1,group1)"</pre>
141
        paraphrase="A homozygous mutation on chromosome 6qE1
142
        may be the cause of the ataxia-like disorder in the affected mice" />
143
      </argument>
144
145
      </DSEG>
146
147
      <DSEG ID="Experiment 2" >
```

```
148
      <content>
149
      [para-3]Literature searches revealed that among disease lines mapped to 6qE1, the
      spontaneous mutant opt mouse displays a strikingly similar presentation to that
      described here [1].
      The underlying genetic lesion causing the opt phenotype is a homozygous in-frame
150
      deletion of exons 43 and 44 of the gene Itprl (Itprlopt/opt), encoding inositol
      1,4,5-triphosphate receptor 1 (Itpr1).
151
      </content>
152
153
      <entities-props>
      <entity ID="group2" paraphrase="the spontaneous mutant opt mouse" />
154
155
      <entity ID="pheno2" paraphrase="similar presentation to that described here" />
156
      <entity ID="geno2" paraphrase="homozygous in-frame deletion of the gene Itpr1</pre>
      (Itprlopt/opt) on 6qE1" />
      <entity ID="geno2a" paraphrase="homozygous mutation of Itpr1" />
157
158
      <!-- geno2a is a generalization of geno2 that was not explicitly mentioned in text -->
159
      prop>have geno(group2, geno2)
160
      prop>have_pheno(group2, pheno2)
161
      cause (geno2,pheno2,group2)
162
      </entities-props>
163
164
      <argument ID="2" old-id="3.1" scheme="Analogy">
165
      premise-list>
166
      remise prop="have pheno(group1,pheno1)"/>
167
      conclusion-of="ARG 1" />
168
       premise prop="have pheno(group2,pheno2)" />
169
      <premise prop="similar(pheno2,pheno1)" />
170
      <premise prop="cause(geno2,pheno2,group2)" />
171
      oremise domain-prop="similar(geno2a, geno1)"
172
         paraphrase="A homozygous mutation in Itpr1 is similar to homozygous mutation on
         6qE1" />
173
      </premise-list>
174
      <conclusion inferred-prop="cause(geno2a, pheno1, group1)"</pre>
175
        paraphrase="A homozygous mutation of Itpr1 may be the cause of the disorder of the
        affected mice" />
176
      </argument>
177
178
      <content>
179
      Sequencing of all exons and intron-exon boundaries of Itpr1 in affected mice from the
      current study revealed a single mutation within Itpr1: a novel in-frame deletion of 18
      bp within exon 36 (Itpr1\Delta 18/\Delta 18).
180
      </content>
181
182
      <entities-props>
183
      <entity ID="geno3" paraphrase="Itpr1\Delta18/\Delta18" />
184
      prop>have geno(group1,geno3)
185
      </entities-props>
186
187
      <argument ID="3" old-id="3.2" scheme="Agreement">
188
      remise-list>
189
      <premise prop="have pheno(group1, pheno1)" />
190
      remise prop="have geno(group1, geno3) "/>
191
      </premise-list>
192
      <conclusion inferred-prop="cause(geno3, pheno1, group1)"</pre>
193
        paraphrase="The Itpr1\(\Delta\)18 mutation may be the cause of the affected mice's
        disorder" />
194
      </argument>
195
196
      <content>
197
      To confirm the pathogenicity of this mutation we crossed heterozygous mice from the
      current study (Itpr1wt/\Delta18) with mice heterozygous for the opt mutation (Itpr1wt/opt).
198
      This resulted in two litters of mice with a total of four affected Itprlopt/Δ18 pups
      (from a total of 15) with a phenotype indistinguishable from that of the Itpr1\Delta 18/\Delta 18
      and Itprlopt/opt mice [1].
199
      Furthermore, this phenotype was similar, although less severe, to that described in a
```

mouse line with targeted deletion of Itpr1, where ataxia was described as a prominent

```
200
      </content>
201
202
      <argument old-id="3.3" scheme="Domain-specific: Proportional agreement">
203
      premise-list>
204
      premise paraphrase="The predicted proportion of Itprlopt/A18 offspring
205
      resulting from crossing Itprlwt/Δ18 mice with mice Itprlwt/opt mice is 25%" />
206
      <premise paraphrase="Approximately 25% of the offspring resulting from crossing</pre>
207
      Itprlwt/\Delta18 mice with Itprlwt/opt mice had the ataxia-like phenotype (like that of the
208
      Itpr1Δ18/Δ18 mice and Itpr1opt/opt mice) " />
      </premise-list>
209
210
      <conclusion paraphrase="The genotype of the offspring with the ataxia-like phenotype</pre>
      was Itprlopt/Δ18" />
211
      </argument>
212
213
      <arqument old-id="3.4" scheme="Domain-specific: Pathogenic effect">
214
      premise-list>
215
      conclusion-of="argument3.3" paraphrase="The genotype of the offspring with an
      ataxia-like phenotype was Itprlopt/Δ18" />
216
217
      se paraphrase="Itprlopt/opt or targeted deletion of Itprl may cause ataxia-like
218
      phenotype" />
219
      se paraphrase="If an allele has a similar effect when
220
      substituted for a known pathogenic allele (e.g. Itprlopt) it may be pathogenic" />
221
      ise-list>
222
      <conclusion paraphrase="the ItprlA18 allele is pathogenic" />
223
      </arqument>
      </DSEG>
224
225
226
      <DSEG ID="Experiment 3" >
227
      <content>
228
      [para-3 continued] As with the Itprlopt/opt mice, where the deletion of exons 43 and 44
      is also predicted to leave the translational reading frame unaffected, the in-frame
      Itpr1\Delta18/\Delta18 deletion mutation results in markedly decreased levels of Itpr1 in
      cerebellar Purkinje cells.
229
      </content>
230
231
      <entities-props>
      <entity ID="prot1" paraphrase="markedly decreased levels of Itpr1 in cerebellar</pre>
232
      Purkinje cells" />
233
      prop>have prot(group2,prot1)
234
      prop>have prot(group1,prot1)
235
      </entities-props>
236
237
      <argument ID="4" old-id="3.5" scheme="Consistent Explanation">
238
      premise-list>
239
       prop="have geno(group2, geno2)" />
240
       premise prop="have pheno(group2,pheno2)"/>
241
      remise prop="have prot(group2,prot1)" />
242
      cause (geno2, pheno2, group2) " />
243
      cpremise prop="have geno(group1, geno3)" />
244
      <premise prop="have pheno(group1, pheno1)"/>
245
      <premise prop="have prot(group1,prot1)" />
246
      cpremise domain-prop="similar(geno2, geno3)"
247
        paraphrase="Itprlopt/opt and Itprl∆18/∆18 are similar (both are deletion mutations of
        Itpr1) " />
248
      cpremise domain-prop="similar(pheno1, pheno2)" />
249
      </premise-list>
250
      <conclusion inferred-prop="cause(geno3, pheno1, group1)"</pre>
251
        paraphrase="The mutation Itpr1\Delta18/\Delta18 may cause an ataxia-like phenotype in the
        Itpr1Δ18/Δ18 mice"
252
      </argument>
253
254
     </DSEG>
255
256
      <DSEG ID="Summary" >
257
      <content>
```

feature [4].

```
258
      [para-3 continued] In these two spontaneous mutants [1] and in the Itpr1-deficient mouse
      [4] generated by gene targeting, decreased Itpr1 expression is associated with the same
     autosomal recessive movement disorder (Figure 1).
259
      </content>
260
261
     <entities-props>
262
     <entity ID="group3s" paraphrase="these two spontaneous mutants and the Itprl-deficient</pre>
263
     generated by gene targeting"/>
264
     <entity ID="geno3s" paraphrase="homozygous deletion in Itpr1" />
265
     prop>have geno(group3s,geno3s)
266
     prop>have prot(group3s,prot1)
267
      prop>have pheno(group3s,pheno2)
268
      </entities-props>
269
270
     <argument ID="4s" old-id="3.6" scheme="Agreement">
271
     <!-- The have_prot premise does not fit Agreement's definition. -->
272
     cpremise-list>
273
      cpremise prop="have geno(group3s,geno3s)" />
274
      <premise prop="have prot(group3s,prot1)" />
275
     <premise prop="have pheno(group3s,pheno2)" />
276
     </premise-list>
277
     <conclusion inferred-prop="cause(geno3s, pheno2, group3s)"</pre>
278
       paraphrase="Homozygous deletions in Itpr1 may cause an ataxia-like phenotype in
       mice" />
279
     </arqument>
280
281
     </DSEG>
282
283
     <DSEG ID="Experiment 4">
284
     <content>
285
      [para 4] Given our interest in human neurological disease we sought to identify any
     cognate human disorders where linkage had been established to the syntenic region of
     the human genome, but where no causal mutation had been identified.
     SCA15, an adult-onset autosomal dominant progressive ataxia is linked to this locus [5].
286
287
     Although missense mutation of ITPR1 had previously been ruled out [2] and the mode of
     inheritance was inconsistent with that seen in the Itpr1A18 and Itpr1opt mice, the
     phenotypic presence of ataxia in the mice led us to reexamine this candidate gene as a
     possible cause of SCA15.
288
     </content>
289
     <entities-props>
290
     <entity ID="geno4" paraphrase="autosomal dominant mutation in ITPR1" />
291
      <entity ID="group4" paraphrase="human" />
292
     <entity ID="pheno3" paraphrase="SCA15" />
293
     prop>similar(pheno2,pheno3)
294
     cause(geno4, pheno3, group4)
295
     </entities-props>
296
297
     <argument ID="5" old-id="4.1" scheme="Analogy">
298
     remise-list>
299
     cpremise prop="have_pheno(group3s,pheno2)" />
300
     remise prop="have geno(group3s,geno3s)"/>
301
     <premise prop="similar(pheno2,pheno3)"/>
302
      conclusion-of="ARG 4s" />
      <!-- The conclusion of ARG4s is an inferred prop -->
303
304
     <premise prop="similar(geno3s,geno4)" />
305
     mise-list>
306
     <conclusion prop="cause(geno4,pheno3,group4)" />
307
     </argument>
308
309
     <content>
310
      [para 5] We obtained genomic DNA from three affected family members and one family
     member with unknown disease status from the kindred originally used to define and map
     SCA15 (family AUS1, of Australian Anglo-Celtic origin) [2].
311
     We performed two experiments concurrently in three affected members of this family:
```

sequence analysis of the coding exons of ITPR1 and high-density genome-wide SNP

genotyping.

Sequence analysis failed to show any coding alterations segregating wit

Sequence analysis failed to show any coding alterations segregating with disease or any alterations that were inconsistent with Mendelian patterns of inheritance within the family.

- However, visualization of log R ratio and B allele frequency metrics from the genome-wide SNP genotyping experiments clearly showed data consistent with a heterozygous genomic deletion across the first one-third of ITPR1 and across the first half of a neighboring gene, SUMF1 (Figure 2).
- This deletion was apparent in all three affected family members studied and absent from the family member with unknown affection status (Figure 3).
- 315 The SNP data showed a deletion of between 188 kb and 210 kb in size;
- examination of SNPs at the flanking unknown regions of this deletion allowed us to delimit the borders of the deletion to 7.5 kb on the telomeric side of the deletion (between rs12634249 and rs793396) and ~14.4 kb on the centromeric side of the deletion (between rs4073665 and rs17709863).

```
317
      </content>
318
319
      <entities-props>
320
      <entity ID="group5" paraphrase="three affected family members ... from the kindred</pre>
      originally used to define and map SCA15 (family AUS1, of Australian Anglo-Celtic
      origin) " />
321
      <entity ID="geno5" paraphrase="heterozygous genomic deletion across the first one-third</pre>
      of ITPR1 and across the first half of a neighboring gene, SUMF1" />
322
      prop>have pheno(group5, pheno3)
323
      >have geno(group5, geno5)
324
      </entities-props>
325
326
      <argument ID="6" old-id="5.1" scheme="Agreement">
327
      remise-list>
328
      remise prop="have_pheno(group5, pheno3)" />
329
      <premise prop="have_geno(group5, geno5)" />
330
      </premise-list>
331
      <conclusion inferred-prop="cause(geno5, pheno3, group5)"</pre>
332
        paraphrase="The ITPR1-SUMF1 deletion may be the cause of the family members SCA15" />
333
      </argument>
334
335
      </DSEG>
336
337
      <DSEG ID="Experiment 5">
338
      <content>
339
      In an attempt to define whether this variation was a benign polymorphism we analyzed
```

- In an attempt to define whether this variation was a benign polymorphism we analyzed genome-wide SNP data at this locus, produced using the same genotyping chip, from 577 individuals of European descent who were either controls or individuals with an unrelated neurological disorder.
- We failed to find any deletions affecting the coding sequence of either gene, ITPR1 or SUMF1;
- we did, however, identify a single individual with a possible heterozygous deletion approximately 6 kb in size within intron 40-41 of ITPR1, at least 5 kb away from exon 40.
- 342 Given the location of this alteration it is unlikely to effect the expression or splicing of ITPR1.

```
343
     </content>
344
345
     <entities-props>
346
     <entity ID="group6" paraphrase="controls or individuals with an unrelated neurological</pre>
347
     >not(have pheno(group6, pheno3))
348
     op>not(have geno(group6, geno5))
349
     </entities-props>
350
351
     <argument ID="7" old-id="5.2-5.3" scheme="Difference">
352
     premise-list>
     cpremise prop="have_pheno(group5, pheno3)" />
353
354
      prop="have geno(group5, geno5)" />
355
     cpremise prop="not(have pheno(group6, pheno3))" />
356
     cpremise prop="not(have_geno(group6, geno5))"/>
```

```
357
      </premise-list>
358
      <conclusion inferred-prop="cause(geno5, pheno3, group5)"</pre>
359
        paraphrase="The ITPR1-SUMF1 deletion may be the cause of the family members SCA15" />
360
      <!-- Could be analyzed as two arguments, one for control group and another for
      individuals with unrelated neurological disorder.
      Domain expert says that evidence from individuals with an unrelated neurological
361
      disorder is stronger than evidence from control group. -->
362
      </argument>
363
364
      </DSEG>
365
366
      <DSEG ID="Experiment 6">
367
      <content>
368
      [para 6] In an attempt to fine-map the breakpoints of the disease-causing deletion we
      performed a series of experiments designed to refine the unknown intervals at the edges
```

- between definite deleted and definite diploid sequences.
- These data narrowed the unknown borders to ~4 kb on the telomeric side and ~7 kb on the 369 centromeric side.
- We used all possible combinations of forward orientation primers designed within the 370 newly defined telomeric boundary and of reverse orientation primers designed within the newly defined centromeric boundary in PCR assays in an attempt to amplify across the deletion in affected family members.
- 371 Using PCR primers T3F and C11R, which should be more than 200 kb apart, we were able to amplify a fragment 953 bp in size using DNA from each of the three affected family members as template.
- 372 Sequencing of this fragment revealed a deletion of 201,509 bp (Figure S3), removing the first three of the nine exons of SUMF1 and the first ten of the 58 exons of ITPR1.
- 373 We were unable to amplify the deletion-specific fragment in the family member of unknown affection status, or in 275 neurologically normal controls.

374

</content>

```
375
376
      <argument ID="8" old-id="6.1" scheme="Difference">
377
      <!-- Domain expert says this argument uses different evidence than previous
378
      arguments, so provides additional evidence for conclusion -->
379
      cpremise-list>
380
       premise prop="have pheno(group5, pheno3)" />
381
      cpremise prop="not(have pheno(group6, pheno3))" />
382
      remise prop="have geno(group5, geno5)" />
383
      remise prop="not(have geno(group6, geno5))" />
384
      </premise-list>
385
      <conclusion inferred-prop="cause(geno5, pheno3, group5)"</pre>
386
        paraphrase="A deletion in SUMF1-ITPR1 may be the cause of SCA15 in the affected
        group." />
387
      </argument>
388
389
      </DSEG>
390
391
      <DSEG ID="Experiment 7">
392
      <content>
```

- 393 [para 7] To further establish genetic deletion at ITPR1 as the cause of SCA15 we analyzed two additional families with an inherited cerebellar ataxia similar to that described in the AUS1 family, ascertained through neurology clinics in London, United Kingdom.
- 394 DNA extracted from probands from these two families (family H33 and family H27) were also analyzed using Illumina Infinium HumanHap550 genotyping chips.
- These experiments showed deletion at the SCA15 locus in all affected members assayed, 395 from SUMF1 through ITPR1.
- 396 These mutations segregated with disease in these two families (Figure S3).
- 397 A strategy similar to the one outlined above enabled us to sequence over the breakpoint in family H27 but not family H33.
- In the former, the deletion spans 344,408 bp, removing exons 1-3 of SUMF1 and 1-44 of 398 ITPR1:
- in the latter, we estimate that the deletion is 310 kb in length and that it removes 399 exons 1-3 of SUMF1 and exons 1-40 of ITPR1.
- 400 The site of mutation is of interest, particularly the fact that in each of the three families the telomeric end of the deletion is anchored between exons 3 and 4 of SUMF1;

```
401
     sequence searches failed to identify any repeat sequences that might explain this
     phenomenon.
402
     </content>
403
404
     <entities-props>
405
     <entity ID="group7" paraphrase="family H33 and family H27 affected members" />
406
     <entity ID="group8" paraphrase="family H33 and family H27 unaffected members" />
      <entity ID="pheno4" paraphrase="inherited cerebellar ataxia" />
407
408
     prop>have pheno(group7, pheno4)
409
     >similar(pheno4, pheno3)
410
     prop>have geno(group7, geno5)
411
     >not(have pheno(group8, pheno4))
412
     >not(have geno(group8, geno5))
413
     </entities-props>
414
415
     <argument ID="9" old-id="7.1" scheme="Difference">
416
     remise-list>
417
     cpremise prop="have phenotype(group7, pheno4)" />
418
     <premise prop="have geno(group7, geno5)" />
419
     <premise prop="not(have pheno(group8, pheno4))" />
420
     remise prop="not(have geno(group8, geno5))" />
421
     </premise-list>
422
     <conclusion inferred-prop="cause(geno5, pheno4, group7)"</pre>
423
       paraphrase="The ITPR1-SUMF1 deletion may be the cause of the H33 and H27
       family members' cerebellar ataxia similar to AUS1's ataxia (SCA15)" />
424
425
     </arqument>
426
427
     </DSEG>
428
429
     <DSEG ID="Summary" >
430
     <content>
431
     With three cerebellar ataxia families segregating a SUMF1-ITPR1 deletion, and this
     deletion not observed in a control population, we may reasonably conclude that the
     association is causal, and that the deletion is indeed the genetic basis of the
     disease, with SCA15 the diagnosis in the two British families as well as the original
     Australian family.
432
     </content>
433
434
     <entities-props>
435
     <entity ID="group9" paraphrase="affected members of AUS1, H33, and H27" />
     <entity ID="group10" paraphrase="unaffected members of AUS1, H33, and H27 and controls"</pre>
     />
437
     >have geno(group9, geno5)
438
     prop>have pheno(group9, pheno4)
439
     >not(have geno(group10, geno5))
440
     >not(have pheno(group10, pheno4))
441
     cause(geno5, pheno3, group9)
442
     </entities-props>
443
444
     <argument ID="10" old-id="7.2-7.3" scheme="Difference">
445
     remise-list>
446
     remise prop="have geno(group9, geno5)" />
447
     <premise prop="have pheno(group9, pheno4)" />
448
     remise prop="not(have geno(group10, geno5))" />
449
     <premise prop="not(have pheno(group10, pheno4))" />
450
     </premise-list>
451
     <conclusion prop="cause(geno5, pheno3, group9)"</pre>
452
       paraphrase="The ITPR1-SUMF1 deletion may be the cause of SCA15 in AUS1, H33 and H27"
453
     <!-- Strictly speaking the conclusion should be cause(geno5, pheno4, group9) but the
     text specifies SCA15 (pheno3). -->
454
     </arqument>
455
     </DSEG>
456
457
     <DSEG ID="Discussion" >
458
     <content>
```

```
459
      [para 8] It is improbable that heterozygosity for the deletion of SUMF1, encoding
     sulfatase modifying factor 1, of itself causes or contributes to SCA15.
460
     Homozygous mutation of SUMF1 results in autosomal recessive multiple sulfatase
     deficiency, a metabolic disorder characterized by hepatosplenomegaly, deafness, and
     developmental delay [6,7].
461
     No co-occurrence of ataxia has been described in (heterozygous) parents of patients
     with multiple sulfatase deficiency.
462
     </content>
463
464
     <entities-props>
465
     <entity ID="group11" paraphrase="individuals heterozygous for deletion of SUMF1" />
466
     <entity ID="geno6" paraphrase="heterozygous deletion of SUMF1" />
467
     prop>have geno(group11, geno6)
468
     >not(have pheno(group11,pheno4))
469
     >not(cause(geno6, pheno4, group4))
470
     </entities-props>
471
472
     <argument ID="11" old-id="8.1" scheme="Failed Method of Agreement (no effect)">
473
     remise-list>
474
      prop="have geno(group11, geno6)"
475
       paraphrase="Parents of children affected by multiple sulfatase deficiency
476
     who do not themselves have the deficiency are heterozygous for SUMF1 mutation
477
     (according to Mendelian inheritance principles and since homozygous mutation
478
     of SUMF1 results in that deficiency) " />
479
     <premise prop="not(have pheno(group11,pheno4))" />
480
     </premise-list>
481
     <conclusion prop="not(cause(geno6, pheno4, group4))" />
482
     <!-- The conclusion generalizes scope from group11 to group4 (humans). -->
483
     </argument>
484
485
     <content>
486
     Conversely, mutation of ITPR1 is biologically plausible as a cause of ataxia: the
     protein is highly expressed in Purkinje cells;
487
     as we have shown here, mice with mutation at this locus present with ataxia;
488
     and perturbed Ca2+ signaling has previously been implicated in the etiology of ataxia,
     notably in episodic ataxia type 2 and SCA6 [8].
489
     </content>
490
491
     <entities-props>
492
     <entity ID="prot2" paraphrase="perturbed Ca2+ signaling" />
493
     <entity ID="geno7" paraphrase="mutation of ITPR1" />
494
     cause(geno3s, prot1, group3s)
495
     cause(prot1, prot2, geno3s)
496
     >have geno(group3s, geno3s)
497
     prop>have pheno(group3s, pheno2)
498
     cause(geno7, prot1, group4)
499
     cause(prot1,prot2, group4)
500
     cause(prot2, pheno4, group4)
501
     cause(geno7, pheno4, group4)
502
     </entities-props>
503
504
     <arqument ID="12" old-id="8.2" scheme="Eliminate Difference">
505
     remise-list>
506
     oremise prop="not(cause(geno6, pheno4, group4))" conclusion-of="Arg 11" />
507
     oremise prop="cause(geno5, pheno3, group9)" conclusion-of="Arg 10"
508
         paraphrase="The ITPR1-SUMF1 deletion may be the cause of cerebellar ataxia in AUS1,
         H33 and H27" />
509
     mise-list>
510
     <conclusion prop="cause(geno7, pheno4, group4)"</pre>
511
       paraphrase="ITPR1 mutation may be the cause of cerebellar ataxia in humans" />
512
513
514
     <argument ID="13" old-id="8.3" scheme="Consistent Explanation" >
515
     <!-- This argument is based on in-vivo mouse and in-vivo human data. -->
516
     remise-list>
517
     cause (geno3s, prot1, group3s)" />
```

```
518
      cause (prot1, prot2, geno3s)"
       paraphrase="Decreased levels of Itpr1 protein in cerebellar Purkinje cells in mice
519
        results in perturbed Ca2+ signaling" />
520
     <premise prop="have geno(group3s, geno3s)" />
521
       premise prop="have pheno(group3s, pheno2)" />
522
      cause (geno7, prot1, group4)"
523
        paraphrase="Mutation of ITPR1 in humans results in decreased levels of ITPR1 protein
        in cerebellar Purkinje cells in humans" />
524
      cause (prot1, prot2, group4)"
525
       paraphrase="Decreased levels of ITPR1 protein in cerebellar Purkinje cells in humans
        results in perturbed Ca2+ signaling" />
526
     <premise prop="cause(prot2, pheno4, group4)" />
      </premise-list>
527
528
      <conclusion prop="cause(geno7, pheno4, group4)"</pre>
       paraphrase="Mutation of ITPR1 causes ataxia in humans" />
529
530
     </argument>
531
532
     <content>
533
     In further support of this conclusion, analysis of protein levels of ITPR1 in
     Epstein-Barr virus (EBV) immortalized lymphocytes from affected and unaffected AUS1
     family members revealed that all affected members showed a dramatic decrease in ITPR1
     levels when compared with the family member without the deletion (Figure 4). </content>
534
535
     <entities-props>
536
      <entity ID="group12" paraphrase="AUS1 family members without deletion in ITPR1" />
537
     prop>have prot(group5, prot1)
538
     >not(have_prot(group12, prot1))
539
     cause (prot2, pheno4, group4) 
540
     </entities-props>
541
542
     <!-- This argument is stronger than 12 because it is based on human data.
543
     Domain expert says human data is stronger than mouse data, but in-vivo data is stronger
     than
544
     in-vitro data for the same species. -->
545
546
     <argument ID="14" old-id="8.4" scheme="Difference Consistent Explanation">
547
      oremise-list>
548
      prop="have prot(group5, prot1)" />
549
     remise prop="not(have prot(group12, prot1))" />
550
     cause (prot1, prot2, group4) "
551
       paraphrase="Decreased levels of ITPR1 protein in cerebellar Purkinje
552
        cells in humans results in perturbed Ca2+ signaling" />
553
      cause (prot2, pheno4, group4)" />
554
     </premise-list>
     <conclusion prop="cause(geno7, pheno4, group4)" />
555
556
     <!-- conclusion generalizes to group4 (humans). -->
557
     </argument>
558
559
      <content>
      [para 9] Itpr1 contains three domains, an N-terminal inositol triphosphate binding
560
     domain, a coupling domain, and a C-terminal transmembrane domain;
561
     it also contains two protein kinase A phosphorylation sites and an ATP-binding site.
562
     Itpr1 is coupled to Ca2+ channels and facilitates Ca2+ release from the endoplasmic
     reticulum after binding by the intracellular second messenger inositol
     1,4,5-triphosphate [9].
563
     Itpr1 is enriched in the Purkinje cells of the cerebellum [4].
564
     ITPR1 mutations have more than one potential pathogenic mechanism.
565
     First, the disease may be a result of haploinsufficiency at ITPR1;
566
     this concept is consistent with the observation that heterozygous deletion leads to a
     later onset disorder in humans, whereas homozygous deletion in mice leads to an early
     onset disorder, able to be expressed within the much shorter life span of the mouse.
567
     Second, we cannot rule out the existence of an alternate start site for ITPR1 that may
     result in a product that confers a pathogenic gain of function to the protein;
568
     however, Western blot analysis of cells derived from affected AUS1 family members,
     which was performed using an antibody raised against the C-terminal portion of ITPR1,
```

failed to identify any disease-specific truncated protein products.

Clearly, the identification of distinct ITPR1 mutations underlying SCA15 will help 569 elucidate the pathogenic mechanism of this disorder. 570 </content> 571 572 <content> 573 [para 10] 574 We show here the utility of investigating spontaneous mouse mutations in understanding Currently, the small number of aged Itpr1wt/ Δ 18 animals precludes us from examining 575 these mice for subtle signs and symptoms similar to those seen in SCA15 patients; 576 however, these mice are clearly of interest to us as a potential model of SCA15. These data also demonstrate that genome-wide SNP assay can facilitate rapid detection 577 of structural genomic mutations that may underlie disease. 578 The data provided by these approaches provide compelling evidence that heterozygous deletion of ITPR1 causes SCA15.

Clearly, sequence analysis of ITPR1 in potential SCA15 cases may provide additional insight into the disease, particularly if a stop mutation were to be identified;

however, the mutational mechanism noted here means that standard sequencing approaches alone are insufficient to confidently rule out ITPR1 mutation as a cause of disease: a comprehensive gene dosage approach is also required.

Given that SCA16 and autosomal dominant congenital nonprogressive ataxia have both recently been mapped to regions overlapping with the SCA15 locus [10,11], ITPR1 is a gene of importance for screening in these families.

These data add weight to a role for aberrant intracellular Ca2+ signaling in Purkinje cells in the pathogenesis of spinocerebellar ataxia.

583 </content>

586 </DSEG>

584

588

587 </section>

589 </article>

590 </document>