



## TSC1 intragenic deletion transmitted from a mosaic father to two siblings with cardiac rhabdomyomas: identification of two aberrant transcripts

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1 ***TSCI* intragenic deletion transmitted from a mosaic father to two siblings**  
2 **with cardiac rhabdomyomas: identification of two aberrant transcripts**

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18 **Abstract**

19 Tuberos sclerosis complex (TSC) is a rare autosomal dominant disorder characterized by  
20 non-cancerous tumors in multiple organs including the brain, kidney, lung, heart, and skin.  
21 We encountered a Japanese family consisting of two siblings (a four-year-old boy and a one-  
22 year-old girl) with multiple cardiac rhabdomyomas conveying a high risk of TSC and  
23 apparently unaffected sibling (a two-year-old girl) and parents. Whole exome sequencing and  
24 application of Integrative Genomic Viewer revealed an identical intragenic *TSC1* deletion  
25 with the breakpoints on intron 15 and exon 19 in the affected siblings, but not in the  
26 apparently unaffected sibling and parents. Subsequently, PCR-based analyses were performed  
27 using primers flanking the deletion, showing that the deletion was also present in the father  
28 and that the deletion occurred between chr9:135,777,038 (bp) and chr9:135,780,540 (bp) in  
29 association with a one bp overlap. Furthermore, RT-PCR analyses were carried out using  
30 lymphoblastoid cell lines, revealing a major in-frame insertion/deletion transcript produced by  
31 aberrant splicing using a cryptic "ag" splice acceptor motif at intron 15  
32 (r.1998\_2438delinsTTCATTAGGTGG) and a minor frameshift transcript generated by  
33 aberrant splicing between exon 15 and exon 20 (r.1998\_2502del, p.Lys666Asnfs\*15) in the  
34 affected siblings. These findings imply that the intragenic deletion producing two aberrant  
35 transcripts was generated as a somatic pathogenic variant involving the germline in the father  
36 and was transmitted to the affected siblings.

37

38 **Keywords:** *TSC1*, tuberous sclerosis complex, cardiac rhabdomyoma, germline mosaicism,  
39 intragenic deletion, aberrant transcript

40

## 41 Introduction

42 Tuberos sclerosis complex (TSC) is a rare autosomal dominant disorder characterized by  
43 non-cancerous tumors in multiple organs including the brain, kidney, lung, heart, eyes, liver,  
44 and skin (Curatolo et al., 2008). Developmental delay and seizure are also observed  
45 frequently. The prevalence is ~ one in 6,000 to 10,000 subjects, and ~ two-thirds of patients  
46 are identified as sporadic cases born to apparently healthy parents (Curatolo et al., 2008;  
47 Peron et al., 2018). TSC is caused by pathogenic variants of either *TSC1* encoding hamartin or  
48 *TSC2* encoding tuberin. Hamartin and tuberin act as tumor suppressors, and the hamartin-  
49 tuberin complex regulates cell growth and division by suppressing the function of the  
50 mammalian target of rapamycin complex 1 (mTORC1) (Curatolo et al., 2008). Thus,  
51 pathogenic variants of *TSC1* or *TSC2* result in the tumor development because of the  
52 activation of the mTORC1 function. Furthermore, since a second event including loss of  
53 heterozygosity is frequently found in tumor tissues (Rosset et al., 2017), it is likely that  
54 biallelic pathogenic variants of *TSC1/TSC2* lead to the development of non-cancerous tumors.

55 Here, we report two Japanese siblings with cardiac tumors as the sole recognizable  
56 abnormality and a *TSC1* intragenic deletion producing two aberrant transcripts which was  
57 inherited from their clinically unaffected mosaic father.

58

## 59 Clinical report

60 We encountered a Japanese family with two siblings who received the possible diagnosis of  
61 TSC because of the presence of multiple cardiac rhabdomyomas. The male proband (case II-1  
62 in Figure 1A) was noticed to have cardiac tumors by fetal ultrasonography at 31 weeks of  
63 gestation. He was born at 37 weeks of gestation by vaginal delivery without neonatal  
64 asphyxia. At birth, his length was 47.5 cm (~ 50 percentile), and his weight 2947 g (50–75  
65 percentile). Physical examination at birth showed no abnormalities including the skin,  
66 although a wood lamp examination was not performed (Yates, 2006). Postnatal  
67 echocardiography confirmed multiple cardiac tumors of 6.3–15.8 mm in diameter in right  
68 ventricle (RV) and left ventricle (LV). Hemodynamics remained stable without ventricular  
69 inflow or outflow obstruction by the tumors. Electrocardiogram (ECG) was normal. Since the

70 tumors were suspected as rhabdomyomas characteristic of TSC (Rosset et al., 2017), we  
71 performed brain magnetic resonance imaging (MRI), ophthalmologic examinations, and  
72 visceral ultrasound studies, detecting no other features reported in TSC (Krueger and  
73 Northrup, 2013). Echocardiography was performed at a ~ two months interval, showing  
74 spontaneous regression of the tumors, as has been reported for rhabdomyomas in TSC  
75 (Curatolo et al., 2008). On the last examination at four years of age, while he still had small  
76 cardiac tumors, he showed apparently normal growth and development with no episode of  
77 seizures.

78         The third female child (case II-3 in Figure 1A) was also found to have cardiac tumors  
79 by echocardiography performed shortly after birth because of the history of cardiac tumors in  
80 case II-1. She was born at 32 weeks of gestation, with a birth length of 43.0 cm (50–75  
81 percentile) and a birth weight of 1,820 g (50 percentile). Her cardiac tumors were 6.2–15.2  
82 mm in diameter and were identified at multiple locations including the RV free wall to  
83 interventricular septum, RV apex, LV free wall to interventricular septum, and the vicinity of  
84 anterior papillary muscle of LV (Figure 1B). There was no ventricular inflow or outflow  
85 obstruction. ECG was normal. Physical examination at birth was non-remarkable including  
86 the skin, although a wood lamp examination was not performed (Yates, 2006). She was also  
87 suspected to have cardiac rhabdomyomas characteristic of TSC, but brain MRI,  
88 ophthalmologic examinations, and visceral ultrasound studies showed no other abnormal  
89 findings described in TSC (Krueger and Northrup, 2013). The cardiac tumors gradually  
90 diminished in size. On the last examination at one year of age, she remained healthy with  
91 normal growth and development.

92         The two-year-old second child (case II-2 in Figure 1A) had no clinically discernible  
93 TSC features including cardiac masses on echocardiograms. The non-consanguineous parents  
94 had no history of cardiac, renal, brain, and skin diseases.

95         After the genetic diagnosis, we planned to perform electroencephalographic studies and  
96 the TSC-Associated-Neuropsychiatric-Disorders evaluation in the affected siblings before  
97 entering a junior school, as has been recommended (Krueger and Northrup, 2013). Although  
98 we suggested to the father to receive detailed examinations for TSC features, he refused such

99 examinations primarily because of lack of clinical symptoms. Thus, it was unknown whether  
100 the father had cryptic clinical features of TSC.

101

## 102 **Methods**

103 This study was approved by the Institutional Review Board Committee at Hamamatsu  
104 University School of Medicine, and was performed after obtaining written informed consent.  
105 We performed whole exome sequencing (WES) using leukocyte genomic DNA (gDNA)  
106 samples, to examine two possibilities: (1) the siblings had a monoallelic dominant pathogenic  
107 variant in *TSC1* or *TSC2* that was transmitted from either of the parents with germline  
108 mosaicism or intra-familial clinical variability, and (2) they had biallelic recessive pathogenic  
109 variants in a hitherto unknown gene for cardiac tumors. WES was carried out with SureSelect  
110 Human All Exon V6 (Agilent Technologies), and captured libraries were sequenced by  
111 NextSeq 500 (Illumina) with 150 bp paired-end reads. Reads were aligned to the reference  
112 genome (Human GRCh37/hg19) (UCSC Genome Browser; <http://genome.ucsc.edu/>) using  
113 BWA-MEM (Version 0.7.12) with default parameters. Data processing, variant calling,  
114 annotation, and filtering were performed as previously described (Miyado et al., 2019). Final  
115 variants were annotated with Annovar (Wang et al., 2010).

116 To examine the structure of transcripts and the occurrence of nonsense mediated mRNA  
117 decay (NMD) (Kuzmiak and Maquat, 2006), we performed reverse-transcriptase (RT)-PCR  
118 for mRNAs extracted from lymphoblastoid cell lines (LCLs) incubated for 8 hours with NMD  
119 inhibitor cycloheximide (CHX) (Sigma) or with dimethyl sulfoxide as a control material.  
120 Subsequently, RT-PCR products were subjected to electrophoresis and direct sequencing.

121

## 122 **Results**

123 WES was performed for the affected siblings (II-1 and II-3) and the parents, identifying  
124 apparently *de novo* sequence variants at exon 19 of *TSC1* which were initially evaluated as  
125 three successive 4–6 bp insertions, *i.e.*, c.2433\_2434insTATC, c.2437\_2438insTTCATT, and  
126 c.2438\_2439insGGTGG, in both the affected siblings (reference sequence: *TSC1* transcript  
127 variant 1, Genbank NM\_000368.4) (<http://www.ncbi.nlm.nih.gov/>). Since such a complicated

128 finding indicated genomic alteration affecting exon 19 of *TSC1*, we performed manual  
129 inspection of aligned sequences around exon 19 by Integrative Genomic Viewer (IGV)  
130 (<https://www.igv.org>). Consequently, clipped and discordant read pairs which aligned to the  
131 sequence of exon 19 and that of intron 15 were identified in the affected siblings, whereas  
132 such aberrant reads were undetected in the parents (Figure 2A). Furthermore, genotyping for  
133 rs75802666 revealed that the siblings were heterozygous for the paternally inherited "T" allele  
134 and maternally derived "C" allele, and that the clipped and discordant reads were associated  
135 with the "T" allele. These findings indicated the presence of an identical deletion spanning  
136 from 3'-region of intron 15 to 5'-region of exon 19 on the paternally inherited *TSC1* of the  
137 affected siblings. We next performed PCR amplification with primers flanking the deletion  
138 and those for non-deleted wildtype allele, detecting the deletion in the father as well as in the  
139 affected siblings, but not in the mother and the unaffected sibling (II-2) (Figure 2B). This  
140 showed that the deletion took place as a somatic mosaicism involving germline in the father.  
141 In addition, Sanger sequencing of the PCR products showed that the deletion occurred  
142 between chr9:135,777,038 (bp) and chr9:135,780,540 (bp) in association with a one bp  
143 overlap, and that the sequence of intron 15 contained the three 4–6 bp segments incorrectly  
144 evaluated as insertions. Assuming that the overlapping one bp is g.135,777,039 (bp) at exon  
145 19, this deletion is described as NC\_000009.11:g.135777040\_135780539del  
146 (NM\_000368.4:c.1997+429\_2438del). The breakpoints were found to reside on non-repeat  
147 sequences by Repeatmasker (<http://www.repeatmasker.org>). This intragenic deletion was  
148 absent from gnomAD database (<http://gnomad.broadinstitute.org/>), Human Genetic Variation  
149 database (<http://www.hgvd.genome.med.kyoto-u.ac.jp/>), and allele frequency data of 4,773  
150 Japanese individuals (4.7KJPN) (<https://jmorp.megabank.tohoku.ac.jp/>). We deposited this  
151 deletion in the Leiden Open Variation Database (LOVD)  
152 (<http://databases.lovd.nl/shared/genes>) (ID: 0000674869).

153 We further searched for other variant(s) which satisfied the following criteria: (1)  
154 segregation pattern consistent with Mendelian inheritance with complete penetrance; (2)  
155 minor allele frequency of  $\leq 0.01$  in the above three public databases; and (3) high  
156 pathogenicity predicted by Combined Annotation-Dependent Depletion

157 (<http://cadd.gs.washington.edu/snv>) and Polyphen-2\_HumVar  
158 (<http://genetics.bwh.harvard.edu/pph2/>). However, there was no such a variant in this family.

159 We next performed RT-PCR with primers for exon 15 and exon 20, revealing two  
160 aberrant mRNAs (transcript-1 and transcript-2) and wildtype mRNA in the affected siblings,  
161 and wildtype mRNA alone in the parents and the unaffected sibling (Figure 3A). The bands  
162 for transcript-1 were clearly identified with and without CHX treatment, whereas those for  
163 transcript-2 were quite faint without CHX treatment and weakly detected with CHX  
164 treatment. This indicated that transcript-1 escaped NMD, whereas transcript-2 grossly  
165 underwent NMD. Sequencing analysis showed that transcript-1 was produced by aberrant  
166 splicing using a cryptic "ag" splice acceptor motif at intron 15  
167 (r.1998\_2438delinsTTCATTAGGTGG) (Figure 3B). Indeed, the cryptic splice acceptor motif  
168 was found to be accompanied by a pyrimidine (Y)-rich sequence and a candidate branch site  
169 required for splicing (Strachan and Read, 2011) by SVM-BP finder  
170 ([http://regulatorygenomics.upf.edu/Software/SVM\\_BP](http://regulatorygenomics.upf.edu/Software/SVM_BP)). Since transcript-1 was associated  
171 with in-frame insertion and deletion, this was consistent with transcript-1 escaping NMD.  
172 Sequencing analysis also revealed that transcript-2 was generated by aberrant splicing  
173 between exon 15 and exon 20 (r.1998\_2502del, p.Lys666Asnfs\*15) (Figure 3C). Since  
174 transcript-2 harbored a premature stop codon on exon 20 because of the frameshift, this was  
175 compatible with transcript-2 being subject to NMD.

176

## 177 Discussion

178 Molecular studies identified a heterozygous intragenic deletion of *TSC1* in two Japanese  
179 siblings with cardiac rhabdomyomas. Unpredictably, RT-PCR analyses revealed a major  
180 transcript-1 produced with a cryptic splice acceptor motif at intron 15, as well as a minor  
181 transcript-2 generated with a wildtype splice acceptor motif at intron 19. Both transcript-1 and  
182 transcript-2 were missing 5'-region of the coiled coil domain and tuberlin interaction domain  
183 (Peron et al., 2018), and transcript-2 was primarily subject to NMD. According to the ACMG  
184 Standards and Guidelines (Richards et al., 2015), the intragenic deletion  
185 (NC\_000009.11:g.135777040\_135780539del) is regarded as "pathogenic", because they are

186 positive for PVS1 (multiexon deletion), PS3 (confirmation by RNA analysis), PM2 (absence  
187 from controls), and PP4 (patient's phenotype specific for a disease with a single genetic  
188 etiology). In addition, since this deletion occurred between non-homologous regions and was  
189 mediated by one bp microhomology, it is likely that the deletion was generated by replication-  
190 based mechanisms such as fork stalling and template switching and microhomology-mediated  
191 break-induced replication (Nilsson et al., 2017). To our knowledge, this intragenic deletion  
192 has not been reported, although a number of variants including a similar intragenic deletion  
193 involving *TSC1* exons 15–19 has been registered in the LOVD database.

194 Genetic studies were useful to confirm the diagnosis of TSC. In this regard, targeted  
195 *TSC1/TSC2* sequencing by next generation sequencer (NGS) (or classic Sanger method),  
196 followed by deletion/duplication analysis for the two genes by several methods (*e.g.*,  
197 quantitative PCR, multiplex ligation-dependent probe amplification, and array comparative  
198 hybridization) in the absence of a sequence variant, has been regarded as the gold standard  
199 genetic test for TSC, and a multigene NGS panel and WES are considered when clinical  
200 features are mild or obscure (Peron et al., 2018). This current genetic approach is rational and  
201 useful. Indeed, the intragenic deletion identified in this study would be detected by  
202 deletion/duplication analysis. However, WES can identify sequence variants not only in  
203 previously known genes but also in hitherto unknown genes. Furthermore, because of the  
204 development of applications, WES can reveal sequence disruption by employing IGV (as  
205 shown here), various sizes of deletions/duplications by utilizing WES-based copy-number  
206 variant calling (Nord et al., 2011) and eXome Hidden Markov Model (Fromer and Purcell,  
207 2014), and full or segmental disomies by estimating B-allele frequencies for single nucleotide  
208 polymorphisms (Yaouy et al., 2020). Considering such increasing utility and improved cost-  
209 performance, we performed WES from the outset in this study.

210 The affected siblings had cardiac rhabdomyomas as the sole recognizable abnormality.  
211 This may be due to the young ages of the siblings (four years and one year), because *TSC1*  
212 pathogenic variants are associated with multiple features in an age-dependent manner. For  
213 example, TSC is associated with cardiac rhabdomyomas in 30–50% of patients from the late  
214 fetal period to early infancy, intellectual disability in 30–50% of patients from infancy,

215 epilepsy in 60–70% of patients from infancy, retinal hamartomas in ~50% of patients from  
216 infancy, hypomelanotic macules in ~90% of patients from infancy to childhood, facial  
217 angiofibromas in ~75% of patients from childhood, and renal angiomyolipomas in ~80% of  
218 patients from childhood to puberty (Northrup and Krueger, 2013; Curatolo et al., 2008; Rosset  
219 et al., 2017; Wataya-Kaneda et al., 2013). Thus, the siblings may exhibit additional major  
220 and/or minor clinical features of TSC in later ages. Importantly, since cardiac rhabdomyoma  
221 is regarded as an indication for possible TSC (Northrup and Krueger, 2013), it is  
222 recommended to perform genetic diagnosis and clinical workup in patients with cardiac  
223 rhabdomyoma (Krueger and Northrup, 2013).

224         The father was found to have somatic mosaicism involving germline, although we could  
225 not examine the frequency of *TSC1* mosaicism in his sperms. In this regard, two matters are  
226 notable. First, germline mosaicism may be not so rare in TSC. Indeed, several families  
227 harboring  $\geq$  two affected children born to apparently unaffected parents have been reported  
228 (Rose et al., 1999). Furthermore, because of the possibility of latent mosaicism, the recurrent  
229 risk has been estimated to be up to 2% in families with apparently sporadic TSC (Peron et al.,  
230 2018). Second, even individuals with low-level (<10%) mosaicism almost invariably have  $\geq$   
231 two TSC clinical features especially facial angiofibromas and renal angiomyolipomas,  
232 although the phenotypic severity remain milder than that of non-mosaic patients (Giannikou  
233 et al., 2019). Thus, it is possible that some clinical feature remained undetected in the father  
234 and/or that he had some clinical feature such as cardiac rhabdomyomas at a younger age.

235         In summary, we identified *TSC1* intragenic deletion producing two aberrant transcripts  
236 which was transmitted from mosaic father to two siblings with cardiac rhabdomyomas. The  
237 results argue for the value of molecular studies in the identification of underlying genetic  
238 cause.  
239

240 **Declaration of competing interest**

241 The authors declare no conflict of interest.

242

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246

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250

- 251 **References**
- 252 Curatolo, P., Bombardieri, R., Jozwiak, S., 2008. Tuberous sclerosis. *Lancet*. 372, 657-668.
- 253 [https://doi.org/10.1016/S0140-6736\(08\)61279-9](https://doi.org/10.1016/S0140-6736(08)61279-9).
- 254 Fromer, M., Purcell, S.M., 2014. Using XHMM software to detect copy number variation in
- 255 whole-exome sequencing data. *Curr. Protoc. Hum. Genet.* 81, 7.23.1-21.
- 256 <http://doi.org/10.1002/0471142905.hg0723s81>.
- 257 Giannikou, K., Lasseter, K.D., Grevelink, J.M., Tyburczy, M.E., Dies, K.A., Zhu, Z., Hamieh,
- 258 L., Wollison, B.M., Thorner, A.R., Ruoss, S.J., Thiele, E.A., Sahin, M., Kwiatkowski,
- 259 D.J., 2019. Low-level mosaicism in tuberous sclerosis complex: prevalence, clinical
- 260 features, and risk of disease transmission. *Genet. Med.* 21, 2639-2643.
- 261 <https://doi.org/10.1038/s41436-019-0562-6>.
- 262 Krueger, D.A., Northrup, H., 2013. Tuberous sclerosis complex surveillance and
- 263 management: Recommendations of the 2012 international tuberous sclerosis complex
- 264 consensus conference. *Pediatr. Neurol.* 49, 255-265.
- 265 <https://doi.org/10.1016/j.pediatrneurol.2013.08.002>.
- 266 Kuzmiak, H.A., Maquat, L.E., 2006. Applying nonsense-mediated mRNA decay research to
- 267 the clinic: progress and challenges. *Trends. Mol. Med.* 12, 306–316.
- 268 <https://doi.org/10.1016/j.molmed.2006.05.005>.
- 269 Miyado ,M., Fukami, M., Takada, S., Terao, M., Nakabayashi, K., Hata, K., Matsubara, Y.,
- 270 Tanaka, Y., Sasaki, G., Nagasaki, K., Shiina, M., Ogata, K., Masunaga, Y., Saito, H.,
- 271 Ogata, T., 2019. Germline-derived gain-of-function variants of Gs $\alpha$ -coding *GNAS* gene
- 272 identified in nephrogenic syndrome of inappropriate antidiuresis. *J. Am. Soc. Nephrol.*
- 273 30, 877–889. <https://dx.doi.org/10.1681%2FASN.2018121268>.
- 274 Nilsson, D., Pettersson, M., Gustavsson, P., Förster, A., Hofmeister, W., Wincent, J.,
- 275 Zachariadis, V., Anderlid, B.M., Nordgen, A., Mäkitie, O., Wirta, V., Käller, M., Vezzi,
- 276 F., Lupski, J.R., Nordenskjöld, M., Lundberg, E.S., Carvalho, C.M.B., Lindstrand, A.,
- 277 2017. Whole-genome sequencing of cytogenetically balanced chromosome translocations
- 278 identifies potentially pathological gene disruptions and highlights the importance of

- 279 microhomology in the mechanism of formation. *Hum. Mutat.* 38, 180–192.  
280 <https://doi.org/10.1016/j.molmed.2006.05.005>.
- 281 Nord, A.S., Lee, M., King, M.C., Walsh, T., 2011. Accurate and exact CNV identification  
282 from targeted high-throughput sequence data. *BMC. Genomics.* 12:184.  
283 <https://doi.org/10.1186/1471-2164-12-184>.
- 284 Northrup, H., Krueger, D.A., 2013. Tuberous sclerosis complex diagnostic criteria update:  
285 Recommendations of the 2012 international tuberous sclerosis complex consensus  
286 conference. *Pediatr. Neurol.* 49, 243-254.  
287 <http://doi.org/10.1016/j.pediatrneurol.2013.08.001>.
- 288 Peron, A., Au, K.S., Northrup, H., 2018. Genetics, genomics, and genotype-phenotype  
289 correlations of TSC: Insights for clinical practice. *Am. J. Med. Genet.* 178, 281-290.  
290 <https://doi.org/10.1002/ajmg.c.31651>.
- 291 Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W.W., Hegde,  
292 M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H.L., ACMG Laboratory Quality  
293 Assurance Committee, 2015. Standards and guidelines for the interpretation of  
294 sequence variants: a joint consensus recommendation of the American College of  
295 Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet.*  
296 *Med.* 17, 405-424. <http://doi.org/10.1038/gim.2015.30>.
- 297 Rose, V.M., Au, K.S., Pollom, G., Roach, E.S., Prashner, H.R., Northrup, H., 1999. Germ-  
298 line mosaicism in tuberous sclerosis: How common? *Am. J. Hum. Genet.* 64, 986-992.  
299 <https://doi.org/10.1086/302322>.
- 300 Rosset, C., Netto, C.B.O., Ashton-Prolla, P., 2017. TSC1 and TSC2 gene mutations and their  
301 implications for treatment in tuberous sclerosis complex: a review. *Genet. Mol. Biol.*  
302 40, 69-79. <http://dx.doi.org/10.1590/1678-4685-GMB-2015-0321>.
- 303 Strachan, T., Read, A.P., 2011. Nucleic acid structure and gene expression, In: Strachan, T.,  
304 Read, A.P. (Eds.), *Human molecular genetics*, fourth ed., New York: Garland Science,  
305 pp. 1-27.

- 306 Wang, K., Li, M., Hakonarson, H., 2010. ANNOVAR: functional annotation of genetic  
307 variants from high-throughput sequencing data. *Nucleic. Acids. Res.* 38, e164.  
308 <https://doi.org/10.1093/nar/gkq603>.
- 309 Wataya-Kaneda, M., Tanaka, M., Hamasaki, T., Katayama, I., 2013. Trends in the prevalence  
310 of tuberous sclerosis complex manifestations: An epidemiological study of 166 Japanese  
311 patients. *PLoS. One.* 8, e63910. <https://doi.org/10.1371/journal.pone.0063910>.
- 312 Yates, J.R.W., 2006. Tuberous sclerosis. *Eur. J. Hum. Genet.* 14, 1065-1073.  
313 <https://doi.org/10.1038/sj.ejhg.5201625>.
- 314 Yauy, K., Leeuw, N., Yntema, H.G., Pfundt, R., Gilissen, C., 2020. Accurate detection of  
315 clinically relevant uniparental disomy from exome sequencing data. *Genet. Med.* 22, 803-  
316 808. <https://doi.org/10.1038/s41436-019-0704-x>.
- 317

**Figure Legends**

318

319 **Figure 1.** Clinical findings.320 **A.** The pedigree of this family.

321 **B.** Echocardiographic images of case II-3 (left, parasternal short axis view; and right, apical  
322 4-chamber view). RV, right ventricle; LV, left ventricle; APM, anterior papillary muscle;  
323 PPM, posterior papillary muscle; RA, right atrium; and LA, left atrium.

324

325 **Figure 2.** Summary of molecular studies using gDNA.326 **A.** IVG screen-shot of WES pair-end reads.

327 **B.** PCR-based analyses of the deletions. *TSC1* consists of 23 exons and encodes 1,164 amino  
328 acids. P1–P3 indicate the genomic positions of the utilized primers (P1, 5'-  
329 TGTGTAGCCTCAGGGCTCTT-3'; P2, 5'-GGCCATTTTATGCCTCTGTT-3'; and P3,  
330 5'-GATACCAGCAGCACCAAAAAC-3').

331

332 **Figure 3.** Summary of molecular studies using mRNA.333 **A.** RT-PCR products obtained with P4 and P5 primers (P4, 5'-

334 CCAAAGCAAGCCTTTACTCC-3'; and P5, 5'-TGAGTGCTTGTCTGCAGTTG-3').

335 **B.** Genomic sequence around the fusion point between retained intron 15 and exon 19 and  
336 partial electrochromatogram of transcript-1, indicating aberrant splicing using an  
337 alternative "ag" splice acceptor motif accompanied by a pyrimidine (Y)-rich sequence and  
338 a putative branch site at intron 15; the "a" nucleotide highlighted with red at the putative  
339 branch site is highly conserved among branch sites. On the electrochromatogram, exonic  
340 and transcribed intronic nucleotides are written with large and small letters, respectively;  
341 the "A" nucleotide highlighted with yellow is provisionally assigned to exon 19, although  
342 it is shared by intron 15 and exon 19 (see Figure 2B).

343 **C.** Partial electrochromatogram of transcript-2, showing aberrant splicing between exon 15  
344 and exon 20.





