Exome Sequencing Identification of *EP300* Mutation in a Proband with Coloboma and Imperforate Anus: Possible Expansion of the Phenotypic Spectrum of Rubinstein-Taybi Syndrome

Koji Masuda^a Kazuhiro Akiyama^a Michiko Arakawa^d Eriko Nishi^{b, d}
Noritaka Kitazawa^e Tsukasa Higuchi^f Yuki Katou^a Katsuhiko Shirahige^{a, c}
Kosuke Izumi^{a, d}

^aInstitute for Molecular and Cellular Biosciences, Research Center for Epigenetic Disease, The University of Tokyo, Tokyo, ^bDepartment of Medical Genetics, Shinshu University Graduate School of Medicine, Matsumoto, ^cCREST, Japan Science and Technology Agency, Kawaguchi, and ^dDivision of Medical Genetics, and Departments of ^eOphthalmology and ^fGeneral Pediatrics, Nagano Children's Hospital, Azumino, Japan

Key Words

Coloboma · *EP300* mutation · Imperforate anus · Rubinstein-Taybi syndrome

Abstract

Rubinstein-Taybi syndrome (RSTS) is a multisystem developmental disorder characterized by facial dysmorphisms, broad thumbs and halluces, growth retardation, and intellectual disability. In about 8% of RSTS cases, mutations are found in *EP300*. Previously, the *EP300* mutation has been shown to cause the highly variable RSTS phenotype. Using exome sequencing, we identified a de novo *EP300* frame-shift mutation in a proband with coloboma, facial asymmetry and imperforate anus with minimal RSTS features. Previous molecular studies have demonstrated the importance of *EP300* in oculogenesis, supporting the possibility that *EP300* mutation may cause ocular coloboma. Since a wide

phenotypic spectrum is well known in *EP300*-associated RSTS cases, the atypical phenotype identified in our proband may be an example of rare manifestations of RSTS.

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Rubinstein-Taybi syndrome (RSTS; MIM 180849 and 613684) is a multisystem developmental disorder characterized by facial dysmorphisms, broad thumbs and halluces, growth retardation, and intellectual disability [Hennekam, 2006]. In about 55% of RSTS cases, mutations are found in *CREBBP* [Hennekam, 2006]. Mutations in *EP300* have been identified in up to 8% of the cases [Roelfsema et al., 2005; Negri et al., 2015]. *CREBBP* and *EP300* both encode histone acetyltransferases; therefore, RSTS is regarded as a genetic syndrome caused by altered epigenetic marks due to the loss of these histone acetyltransferases [Lopez-Atalaya et al., 2012]. Previous-

ly, 15 cases of individuals with *EP300* mutations have been reported in the literature. In general, the phenotypic features associated with *EP300* mutations are reported to be less severe and quite variable compared to typical RSTS cases with underlying *CREBBP* mutations [Roelfsema et al., 2005; Bartholdi et al., 2007; Zimmermann et al., 2007; Foley et al., 2009; Bartsch et al., 2010; Tsai et al., 2011; Woods et al., 2014]. However, such phenotypic variation has been identified in cohorts of individuals that are suspected of having RSTS features; therefore, it is possible that *EP300* mutations cause phenotypic features that are not considered typical for RSTS.

The recent introduction of exome sequencing has altered the process of defining phenotypic spectra because it has enabled the screening of genetic mutations in an unbiased fashion. Therefore, exome sequencing has expanded the phenotypic spectra of known Mendelian disorders [Izumi et al., 2014]. Here, we report an individual with an *EP300* mutation who presented with atypical manifestations for RSTS.

Case Report

The proband was born to a 33-year-old G1P1 mother after 37 5/7 weeks of gestation. At approximately 32 weeks of gestation, intrauterine growth retardation was noted. Apgar scores were 8 and 9 at 1 and 5 min, respectively. The birth weight was 1,956 g (−1.8 SD), length was 42.5 cm (−2.5 SD), and head circumference was 29.5 cm (−1.9 SD). After birth, an imperforate anus with an anocutaneous fistula beneath the vaginal opening was diagnosed. The proband was also found to have patent ductus arteriosus (PDA) and developed cardiac failure due to the increased blood flow associated with PDA. The proband underwent PDA ligation at the age of 6 days. Subsequently, because of an imperforate anus, she underwent cutback anoplasty at the age of 22 days. A systemic evaluation revealed bilateral microphthalmia and bilateral iris coloboma as well as optic disc coloboma.

At 14 months of age, her facial dysmorphisms included a prominent forehead, facial asymmetry, bilateral epicanthus, a smaller right palpebral fissure compared to the left side, asymmetric auricle, a broad nasal bridge, and a bulbous nasal tip (fig. 1). There were no hand or feet anomalies, except for slightly broad thumbs and halluces. Her family history was unremarkable. Her development was mildly delayed; she did not begin rolling over and was unable to sit independently at 8 months of age. She started grabbing objects at 3 months. Since the proband had an imperforate anus, she underwent a sacral MRI, but no additional structural abnormalities were detected in the lumbosacral regions. A brain MRI at 9 months did not reveal any structural brain abnormalities such as corpus callosum malformations. There were no inner ear malformations.

A G-band karyotype analysis revealed 46,XX. A fluorescent in situ hybridization analysis using a TUPLE1 probe targeting the 22q11.2 region did not reveal any copy number alterations.

Fig. 1. Physical features of the proband showing facial asymmetry, broad nasal bridge and bulbous nasal tip (A), and slightly broad thumbs and halluces (B).

Materials and Methods

Exome Sequencing

Exome sequencing was performed using a protocol approved by the Institutional Review Boards at the University of Tokyo and Nagano Children's Hospital. Genomic DNA samples of the proband and her parents were isolated from peripheral blood using the NucleoSpin Blood Kit (Macherey-Nagel, Düren, Germany). Exome capture was performed with Agilent SureSelect XT Human All Exon V5 (Agilent, Santa Clara, Calif., USA). Using the captured DNA samples, 100-bp paired-end read sequencing was performed by HiSeq 2500 (Illumina, San Diego, Calif., USA).

Bioinformatics Analysis

The resulting reads were mapped to the human genome hg19 using BWA v0.7.8 with the 'bwa mem-M' option [Li and Durbin, 2009]. We used the Genome Analysis Toolkit (GATK) v3.1.1 [DePristo et al., 2011] and followed 'Best Practices' for SNV and indel discovery and genotyping. After duplicate marking, local realignment around indels, and base quality score recalibration, SNVs and indels for each sample were called independently by HaplotypeCaller. SNVs and indels from each sample were combined by GenotypeGVCFs and then filtered through a variant quality score

Fig. 2. Identification of the *EP300* mutation. **A** IGV view of the exome sequencing result. *EP300* mutation (NM_001429:exon14: c.2445delC:p.S815fs) is demonstrated by the 2 vertical lines. **B** Sanger sequencing confirmation of the mutation.

recalibration process with default parameters. SNVs and indels with a GQ above 15 were used for further analysis. The effects of variants were predicted by SnpEff v3.6b [Cingolani et al., 2012] and annotated by ANNOVAR [Wang et al., 2010]. Information and statistics regarding sequencing, mapping, coverage, and called variants are summarized in online supplementary table 1 (see www.karger.com/doi/10.1159/000375542).

Results

Exome Sequencing

After filtering, 38,490, 38,040, and 38,289 variants were confidently called from the father, mother, and proband, respectively. Among 9,197 variants with the presumed critical effect in the annotated genes, 226 de novo variants were identified from the proband. Variants >5× coverage were scrutinized. Furthermore, the variants found in only a very small proportion of total reads were eliminated. Only variants in *EP300* and *KIAA0319* were not reported in SNP database build 129 (<http://www.ncbi.nlm.nih.gov/SNP/>) and the Human Genetic Variation database (<http://www.genome.med.kyoto-u.ac.jp/SnpDB/>). Sanger sequencing verified the mutations identified by exome sequencing. The *EP300* mutation was a novel frameshift mutation (NM_001429:exon14: c.2445delC:p.H815Tfs*128; fig. 2). This mutation was only found in the proband, and not in either parent, confirming the de novo acquisition of the mutation. The *KIAA0319* variant (NM_014809:exon3:c.A127G: p.T43A) was inherited from the asymptomatic father.

Discussion

Here, we report a proband with ocular coloboma, facial asymmetry, imperforate anus, and mild developmental delay due to a frameshift mutation in *EP300*, which was discovered by exome sequencing. Hitherto, 15 individuals with *EP300* mutations have been reported in the literature. Although *EP300* was initially identified as a second gene underlying RSTS, the characteristic dysmorphic or physical features of RSTS are often mild [Zimmermann et al., 2007]. Therefore, it remains possible that there has been an ascertainment bias against individuals with *EP300* mutations. Since our case represents the first proband with minimal RSTS and ocular coloboma in whom a *EP300* mutation was identified, it remains unclear whether ocular coloboma is caused by *EP300* mutation because there may be other mutations undetected in the proband's genome that could explain her atypical features. Before reaching the conclusion that *EP300* mutations cause ocular coloboma, more cases with similar phenotype and *EP300* mutations need to be identified. However, clinical studies characterizing a cohort of RSTS patients and basic research findings support the hypothesis that *EP300* mutation may cause ocular coloboma.

Although ophthalmologic manifestations are not regarded as cardinal features of RSTS, the ocular abnormalities are often identified in individuals with RSTS. Frequently observed ocular manifestations are lacrimal duct obstruction, ptosis, strabismus, and refractive errors [van Genderen et al., 2000]. Coloboma is also reported as occasional ocular manifestation of RSTS [van Genderen et al., 2000].

The cardinal function of *EP300* in oculogenesis can be inferred from previous studies. In *EP300* knockout mice, ocular expression of *Pax6* and *Sox2* is reduced [Wolf et al., 2013]. Mutations in *PAX6* and *SOX2* are known etiologies of ocular coloboma in humans, suggesting the importance of proper *EP300* expression in ocular embryogenesis [Azuma et al., 2003; Wang et al., 2008]. Furthermore, *EP300* was shown to activate *PAX2* [Hoffmeister et al., 2002]. *PAX2* mutation is associated with renal coloboma syndrome [Sanyanusin et al., 1995]. Therefore, it is reasonable to speculate on the essential role of *EP300* in ocular embryogenesis.

Clinically, one of the differential diagnoses was CHARGE syndrome (MIM 214800), based on ocular coloboma, facial asymmetry, and congenital heart defect. CHARGE syndrome is caused by mutations in *CHD7* [Visser et al., 2004]. It is very intriguing to note

that a previous chromatin immunoprecipitation-sequencing study revealed genome-wide colocalization between *CHD7* and *EP300* at gene enhancer elements, suggesting that *CHD7* and *EP300* may control similar downstream target genes [Schnetz et al., 2010]. In fact, recent articles have revealed a common molecular target of *EP300* and *CHD7*. The molecular interaction between *EP300* and *p53* is well known, and *EP300* protein facilitates the function of *p53* [Avantaggiati et al., 1997; Lill et al., 1997]. Recently, *p53* was found to be a downstream target of *CHD7*, and *p53* heterozygosity partially rescues the CHARGE syndrome phenotype in *Chd7* null mice [Van Nostrand et al., 2014]. Therefore, *p53*-mediated gene expression alteration may underlay the shared molecular phenomenon explaining the phenotypic similarities between RSTS and CHARGE syndrome.

Previously, the *EP300* mutation has been shown to cause the highly variable RSTS phenotype. The recent discovery of an *EP300* mutation in a proband with a Cornelia de Lange syndrome-like phenotype using exome sequencing further suggests the pleiotropic effect of *EP300* mutations [Woods et al., 2014]. However, the phenotypic spectrum of *EP300* mutations has yet to be fully uncovered, and in order to understand its full spectrum, unbiased genetic screening achieved by exome sequencing would be important.

In summary, we report a proband with ocular coloboma, mild intellectual disability, imperforate anus, and minimal RSTS features, in whom an *EP300* mutation was identified. Given the basic and clinical research findings, *EP300* represents a potential candidate gene for ocular coloboma. Since a wide phenotypic spectrum is well known in *EP300*-associated RSTS cases, the atypical phenotype identified in our proband may be an example of rare manifestations of RSTS.

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