Molecular analysis of exon 7 of the fibroblast growth factor receptor 2 (FGFR2) gene in an Indonesian patient with Apert syndrome: a case report

Gara Samara Brajadenta1,2*, Ariestya Indah Permata Sari1, Donny Nauphar1, Tiar Masykuroh Pratamawati1 and Vincent Thoreau2

Abstract

Background: Apert syndrome, Online Mendelian Inheritance in Man number 101200, is a rare genetic condition, with autosomal dominant inheritance, characterized by craniosynostosis, midfacial malformation, and severe symmetrical syndactyly. Apert syndrome is associated with other systemic malformations, including intellectual disability. At least seven mutations in fibroblast growth factor receptor 2 (FGFR2) gene have been found to cause Apert syndrome. Most cases of Apert syndrome are caused by one of the two most frequent mutations located in exon 7 (Ser252Trp or Pro253Arg).

Case presentation: A 27-year-old Javanese man presented borderline intellectual functioning and striking dysmorphisms. A clinical diagnosis of Apert syndrome was previously made based on these clinical features. Furthermore, POSSUM software was used before molecular analysis and the result showed suspected Apert syndrome with a cut-off point of 14. Molecular genetic analysis of FGFR2, targeting exon 7, was performed by direct sequencing. In this patient, a missense mutation c.755C>G was detected, changing a serine into a tryptophan (p.Ser252Trp).

Conclusion: We report the case of an Indonesian man with Apert syndrome with a c.755C>G (p.Ser252Trp) mutation in the FGFR2 gene. Our patient showed similar dysmorphism to previously reported cases, although cleft palate as a typical feature for p.Ser252Trp mutation was not present. In spite of the accessibility of molecular genetic testing in a few parts of the world, the acknowledgement of clinically well-defined syndromes will remain exceptionally imperative in developing countries with a lack of diagnostic facilities.

Keywords: Apert syndrome, FGFR2 mutation, Indonesian patient
1:50,000 and 1:80,000; the highest prevalence has been reported in Asian populations [10].

The fibroblast growth factor receptor 2 (FGFR2) gene, OMIM 176943, located on chromosome 10q26, is involved in AS. In 1995, Wilkie et al. identified the presence of genetic mutations in exon 7 of the FGFR2 gene in 40 unrelated patients with AS: c.755C>G (p.Ser252Trp) or c.758C>G (p.Pro253Arg) [11]. These mutations are the most frequent mutations, detected in approximately 85% and 15% of patients diagnosed as having AS, respectively [12, 13].

In this case report, we describe radiological and clinical features present in an Indonesian patient diagnosed as having AS. Moreover, we detected by molecular analysis the presence of the c.755C>G (p.Ser252Trp) mutation in the FGFR2 gene of this patient.

Case presentation
Our patient was a 27-year-old Javanese man with borderline intellectual functioning and striking dysmorphisms. Both his parents were Javanese, normal, non-consanguineous, and in their sixth decade of life. He had the third child born after a normal third pregnancy and he had two sisters who were normal. His mother had a cesarean delivery with no history of trauma, infection, or drug use during the term. No family history of similar complaints or any other congenital abnormality was reported. Our patient was born at term after an uneventful pregnancy.

He is a slow learner and attends a school for children with special needs in Cirebon, West Java, Indonesia. There he began to socialize, play with other classmates, and he likes to draw and enjoys music. The dysmorphisms found are very characteristic. On physical examination, his weight was 36 kg, height 158 cm, and occipital frontal circumference 54 cm. It was observed that he displayed hypertelorism, down-slanting palpebral fissure, strabismus, ocular proptosis, depressed nasal bridge, short philtrum, and low-set ears. In addition, acrocephaly, asymmetrical flat facies, nasal deformity, and prominent jaw were present (Fig. 1). His oral deformities showed maxilla hypoplasia with high arch palate. His V-shaped maxillary arch was filled with double rows of teeth. In addition, there was a dental fusion between maxillary premolar and first molar. Panoramic radiographs were performed for confirmation (Fig. 2). Other abnormalities found were mild scoliosis and mild pectus excavatum. Symmetrical cutaneous bilateral syndactyly involving his four fingers, his palms were spoon-shaped with an inwardly placed thumb, was present (type 2). Both feet showed type 2 symmetrical cutaneous syndactyly of the first to fifth toes. Radiographs of both hands and feet confirmed soft tissue syndactyly (Fig. 3). He had corrective surgery twice on both hands to correct for joint contractures. There was no postoperative complication. Six months after the second surgery, he could start using his fingers. A clinical diagnosis of AS was previously made based on these clinical features, as earlier mentioned in our study describing clinical manifestations of this patient [14]. Furthermore, Pictures of Standard Syndromes and Undiagnosed Malformations (POSSUM) software (https://www.possum.net.au/) was used before molecular analysis and the result showed suspected AS with a cut-off point of 14. Ethical clearance for genetic testing was obtained according to the research ethic committee of Faculty of Medicine, Swadaya Gunung Jati University, Indonesia.

Mutation analysis was conducted for our patient. DNA was isolated from peripheral blood using the salt saturation method, as previously described by Miller et al. [15]. Molecular genetics analysis of FGFR2, targeting exon 7, was performed by direct sequencing at the Laboratory of Neurovascular Unit and Cognitive Impairments, University of Poitiers, France. The reference genomic DNA sequence used was NM_000141.4. Polymerase chain
reaction (PCR) amplification of exon 7 from the FGFR2 gene was performed using the primers FGFR2-F 5′-CCGGCAGTCTCCTTTGAAGT-3′ and FGFR2-R 5′-GATCTGTTAATTCCTTAGAACCCTCTCTCT-3′, resulting in a 525 bp fragment. Approximately 50 ng of DNA solution (2.5 μl) was added to 22.5 μl of PCR mixture. This PCR mixture contained 0.25 μl of 25 mM deoxyribonucleotide triphosphates (dNTPs), 3 μl of 25 mM MgCl₂, 0.25 μl of each 20 μM primer, 2.5 μl of 10× PCR buffer, 0.125 μl of 5 U/μl Diamond® high fidelity Taq DNA polymerase (Eurogentec), and 16.13 μl of H₂O. PCR was initiated with denaturation at 95 °C for 3 minutes, followed by 35 PCR cycles (at 95 °C for 30 seconds, 60 °C for 30 seconds, and 72 °C for 30 seconds) and 7 minutes final elongation at 72 °C. The amplified products were detected by electrophoresis on a 1.5% agarose gel with 0.5 mg/ml ethidium bromide and visualized under ultraviolet (UV) light. Furthermore, 5 μl of the PCR product was cleaned up with 2 μl ExoSAP reagent (ThermoFisher) according to the manufacturer’s instructions, to remove excess primers and unincorporated nucleotides enzymatically. Finally, 2 μl of the PCR product was used for the sequence reaction.

**Fig. 2** Intraoral appearance of the patient. Photograph showing V-shaped maxillary arch, crowding of teeth, prominent jaw, and high arch palate. Panoramic X-ray showing hypoplastic and retruded maxilla.

**Fig. 3** Radiological features of the hands and feet of the patient. Photographs and radiographs showing lack of digit type 2 (fused), symmetrical syndactyly of four digits in both hands (upper panels), and type 2 symmetrical syndactyly of five toes in both feet (lower panels).
(BigDye Terminator Cycle Sequencing Kit Version 3.3; Applied Biosystems), which was run on an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems), following the manufacturer’s directions. Sequencing was performed bidirectionally using the forward and reverse PCR primers. The sequence result was compared with the published reference sequence using Chromas software version 2.6.4.

In this patient, we detected a missense mutation, changing a TCG codon (coding for a serine) into a TGG (coding for a tryptophan): p.Ser252Trp (c.755C>G) (Fig. 4).

**Discussion and conclusions**

This is a case report describing an Indonesian patient with AS confirmed by molecular genetic analysis. The presence of the FGFR2 mutation, p.Ser252Trp, was consistent with the diagnosis of AS. This mutation introduces additional affinity between FGFR2 and fibroblast growth factor 2 (FGF2). Consequently, gain-of-function interaction would lead to aberrant signaling, which would explain patient manifestations [16]. Due to unavailability of parental DNA, the mutation could not be determined as de novo. However, since the parents and both his siblings had no features of AS, this mutation apparently appeared de novo. Approximately 85% of cases of AS are caused by this missense mutation. However, this frequent mutation is not generalized for all cases of AS since at least seven mutations in the FGFR2 gene have been found to cause AS [12, 13]. Subsequently, an advanced investigation of the whole gene is required with the aim of finding mutations that are possibly particular to the Indonesian population [17].

This case shows the common characteristics of patients with AS. There is acrocephaly, asymmetrical flat facies, and depressed nasal bridge with nasal deformity. The maxillary arch is V-shaped and sagittally narrow. Moreover, this patient displays severe dental crowding, delayed tooth eruption, and thick gingiva, which are common features of AS. However, tooth numbers are normal and not supernumerary. This patient also shows skeletal abnormalities (mild scoliosis and mild pectus excavatum), bilateral symmetrical syndactyl of both his feet and hands, and skin manifestations such as hyperhidrosis and hypopigmentation. Ocular proptosis, down-slanting of palpebral fissures, and hypertelorism are present, due to shortening of the bony orbit. It has been previously described that strabismus is more common in patients with the FGFR2 p.Ser252Trp mutation and that preaxial polydactyly is also typical of this missense mutation [7, 18]. Our patient has strabismus and preaxial polydactyly, which is consistent with the clinical manifestations of the p.Ser252Trp mutation described in AS. However, there is no cleft palate which is statistically more prevalent in patients with p.Ser252Trp mutation [19].

Moloney et al. proposed a significant correlation between PAE and p.Ser252Trp mutation, which is in the context of a CpG dinucleotide [20]. Moreover, Glaser et al. described that contributing factors to the PAE may include selection and a higher number of mutant sperms [9]. In this case, our patient’s father was 37-years old, an increased mutation frequency due to older paternal age might be possible.

Varying severity of intellectual disability has been related with AS. It has been published that 52% of patients have an intelligence quotient (IQ) lower than 70, even if individuals with normal or borderline IQ have also been reported [21]. It has been assessed that this patient has

---

**Fig. 4** Partial sequence of exon 7 in FGFR2 gene. Arrow indicates in the electropherogram the c.755G>C nucleotide change, compared with normal FGFR2 gene DNA sequence. Yellow shading indicates consecutive codon change (p.Ser252Trp)
Although borderline IQ that causes several problems, such as speech difficulties, attention deficit, and social problems. A definite diagnosis of AS should be made by molecular DNA testing. However, AS can be clinically diagnosed and managed without molecular diagnostics, especially in developing countries with limited funding and lack of diagnostic services, in order to prevent late diagnosis and to enable early intervention. Treatment of a patient with AS should begin at birth within a comprehensive multidisciplinary care unit, including craniofacial, surgical, and developmental assessment. A cranietomy was performed on this patient when he was 3-years old. However, this procedure is often performed before 6 months of age to treat craniosynostosis and this may improve intelligence. Otherwise, syndactyly or webbing of fingers causes immobility of fingers following ossification of interphalangeal joints due to segmentation of embryonic phalanges. Therefore, this patient had corrective surgery twice at the age of 4 years on both hands to correct joint contractures. Corrective surgery for syndactyly should be done in the first year of life and completed by 3 to 4 years of age [22]. Moreover, counseling for the patient should involve referral to orthodontic and orthognathic surgery for a treatment plan. Genetic counseling is very important since recurrence risk in an autosomal dominant disorder for an affected individual to have an affected offspring is 50%.

In summary, we report the case of an Indonesian man with AS with a c.755C>G (p.Ser252Trp) mutation in 

FGFR2

gene. Our patient showed similar dysmorphism to previously reported cases although a cleft palate, which is a typical feature of p.Ser252Trp mutation, was not present. In spite of the accessibility of molecular genetic testing in a few parts of the world, the acknowledgement of clinically well-defined syndromes will remain exceptionally imperative in developing countries with a lack of diagnostic facilities.

Abbreviations
AS: Apert syndrome; FGFR2: Fibroblast growth factor receptor 2; IQ: Intelligence quotient; OMIM: Online Mendelian Inheritance in Man; PAE: Paternal age effect; PCR: Polymerase chain reaction

Acknowledgements
The authors would like to thank the patient and his family for participation in this study. We thank M Rodriguez-Ballesteros for assistance with design primers. The authors also thank laboratory staff at EA-3808 Neurovascular Unit and Cognitive Impairments, University of Poitiers, France. GS Brajadenta is an awardee of Indonesia Endowment Fund for Education (LPDP) Ministry of Finance of the Republic of Indonesia.

Authors’ contributions
GSB performed the experiments, and designed and drafted the manuscript. AIS, DN, and TMP collected the blood sample, clinical details, X-ray, and photographs of this case report. VT verified the molecular analysis and reviewed the manuscript. Each of the authors participated in drafting the manuscript, and each author read and approved the final version of this paper.

Funding
We thank Indonesia Endowment Fund for Education (LPDP) Ministry of Finance of the Republic of Indonesia for financial support. This research is partly funded by The Faculty of Medicine, Swadaya Gunung Jati University, Cirebon, Indonesia.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate
Ethical approval was obtained according to the research ethic committee of Faculty of Medicine, Swadaya Gunung Jati University, Indonesia.

Consent for publication
Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests
The authors declare that they have no competing interests.

Received: 6 March 2018 Accepted: 24 June 2019
Published online: 07 August 2019

References
1. Apert ME. De l'acrocéphalosyndactylie. Bull Mem Soc Med Hôp. 1906;23:1310–30.
2. Cohen MM, Kreiborg S. The central nervous system in the Apert syndrome. Am J Med Genet. 1990;35:36–45.
3. Blank CE. Apert's syndrome (a type of acrocephalo-syndactyly)-observations on a British series of thirty-nine cases. Ann Hum Genet. 1960;24:151–64.
4. Upton J, Zuker RM. Apert syndrome. Clin Plast Surg. 1991;18:413–5.
5. Cohen MM, Kreiborg S. Visceral anomalies in the Apert syndrome. Am J Med Genet. 1993;45:758–60.
6. Cohen MM, Kreiborg S. Skeletal abnormalities in the Apert syndrome. Am J Med Genet. 1993;46:624–32.
7. Mantilla-Capacho JM, Arnaud L, Diaz-Rodriguez M, Barros-Nunez P. Apert syndrome with preaxial polydactyly showing the typical mutation Ser252Trp in the FGFR2 gene. Genet Couns. 2005;16:403–6.
8. Hall RK. Facial dysmorphism and syndrome diagnosis. In: Pediatric orofacial medicine and pathology. 1st ed. London: Chapman & Hall Inc; 1994. p. 53.
9. Glaser RL, Bromm KW, Schulman RL, Eskeniati B, Wroblewski AJ, Labs EW. The paternal-age effect in Apert syndrome is due, in part, to the increased frequency of mutations in sperm. Am J Hum Genet. 2003;73:939–47.
10. TolaroVA MW, Harris JA, Orwadey DE, Vargervik K. Birth prevalence, mutation rate, sex ratio, parent’s age, and ethnicity in Apert syndrome: Am J Med Genet. 1997;72:394–8.
11. Wilkie AO, Slaney SF, Oldridge M, Poole MD, Ashworth GJ, Hockley AD, et al. Apert syndrome results from localized mutations of FGFR2 and is allelic with Crouzon syndrome. Nat Genet. 1995;9:165–72.
12. Bochukova EG, Roscioli T, Hedges DJ, Taylor IB, Johnson D, David DJ, et al. Rare mutations of FGFR2 causing apert syndrome: identification of the first partial gene deletion, and an Alu element insertion from a new subfamily. Hum Mutat. 2009;30:204–11.
13. Oldridge M, Zackai EH, McDonald-McGinn DM, Iseki S, Morris-Kay GM, Twigg SR, et al. De novo alu-element insertion in FGFR2 identify a distinct pathological basis for Apert syndrome. Am J Hum Genet. 1999;64:44–61.
14. Sari AI, Nauphar D, Pratamatwati TM, Soeroeso VM. Oral findings of Apert syndrome case found in Cirebon. Ann Transl Med. 2017;5(suppl 2):AB108. https://doi.org/10.21037/atm.2017.s108.
15. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nuclic Acids Res. 1988;16:1215.
16. Ibrahimi OA, Eliseenkova AV, Piotnikov AN, Yu K, Omitz DM, Mohammadi M. Structural basis for fibroblast growth factor receptor 2 activation in Apert syndrome. Proc Natl Acad Sci. 2011;108:7182–7.
17. Mundhoffer FE, Sierzanska EA, Faradze SM, Hamel BC. p.Ser252Trp and p. Pro253Arg mutations in FGFR2 gene causing Apert syndrome: the first clinical and molecular report of Indonesian patients. Singap Med J. 2013;54:e72–5.
18. Khong JJ, Anderson PJ, Hammerton M, Roscioli T, Selva D, David DJ. Differential effects of FGFR2 mutation in ophthalmic findings in Apert syndrome. J Craniofac Surg. 2007;8:39–42.

19. Lajeunie E, Cameron R, El Ghouzzi V, de Parseval N, Journeau P, Gonzales M, et al. Clinical variability in patients with Apert’s syndrome. J Neurosurg. 1999;90(3):443–7.

20. Moloney DM, Slaney SF, Oldridge M, Wall SA, Sahlin P, Stenman G, Wilkie AOM. Exclusive paternal origin of new mutations in Apert syndrome. Nature Genet. 1996;13:48–53.

21. Alp E, Alp H, Koc H, Ucar C, Cimen D. Apert syndrome. Turkiye Klinikleri J Pediatr. 2007;16:264–8.

22. Khan S, Chatra L, Shenai P, Veena KM. Apert syndrome: A case report. Int J Clin Pediatr Dent. 2012;5:203–6.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.