Mutations in the *FGFR2* gene in Mexican patients with Apert syndrome

**A. Ibarra-Arce[1], G. Ortiz de Zárate-Alarcón[1], L.G. Flores-Peña[1],
F. Mart**í**nez-Hernández[2], M. Romero-Valdovinos[3] and A. Olivo-Díaz[3]**

[1]División de Genética, Hospital General Doctor Manuel Gea González,
D.F., México
[2]Departamento de Ecología de Agentes Patógenos,
Hospital General Doctor Manuel Gea González, D.F., México
[3]Departamento de Biología Molecular e Histocompatibilidad,
Hospital General Doctor Manuel Gea González, D.F., México

Corresponding author: A. Olivo-Díaz
E-mail: aolivod@yahoo.com

ABSTRACT. Apert syndrome (AS) is a frequent acrocephalosyndactyly, with autosomal dominant inheritance. AS has been associated with mutations in fibroblast growth factor receptor 2 (*FGFR2*), and approximately 99% of cases show 2 of the frequent mutations located in exon IIIa (Ser252Trp or Pro253Arg). The purpose of the present study was to describe the mutations in exon IIIa of *FGFR2* in Mexican AS patients and the relationships with clinical features. Exon IIIa of *FGFR2* from 6 AS patients was amplified by polymerase chain reaction. Mutations in exon IIIa of the *FGFR2* gene were identified by digestion with the restriction endonuclease Bstx1 and polyacrylamide gel electrophoresis. PCR fragments were cloned into the PCR 2.1 vector, and both DNA strands were sequenced using the T7 promoter and

M13 universal cloning region oligonucleotides. Sequence alignment was performed using the MEGA software version 5. The patients' major clinical features included craniosynostosis, hypertelorism, proptosis, otitis media, midfacial hypoplasia, rhizomelic shortening, and hyperhidrosis. Mutation S252W was present in 4 patients, while the other 2 patients had P253R. In conclusion, either S252W or P253R mutations were present independently in AS patients; however, the 2 mutations were not found together. None of the clinical features were associated with any of the mutations, suggesting that other mutations may be involved in the development of this syndrome.

Key words: Apert syndrome; Craniosynostosis; *FGFR2* mutations

INTRODUCTION

Apert syndrome (AS, OMIM 101200), also known as acrocephalosyndactylia, is a rare autosomal dominant malformation characterized by craniosynostosis, severe sindactyly, and skin, skeletal, brain, and visceral abnormalities (Freiman et al., 2006). Patients exhibit varying degrees of mental deficit and premature fusion of the cervical vertebrae (C5-C6). The prevalence is estimated to be 1/160,000 newborns, representing 4.5% of all syndromic craniosynostosis cases (Kawa-Karasik et al., 2003). Fibroblast growth factor receptors (FGFRs) are members of the intrinsic tyrosine kinase class of transmembrane growth factor receptors and play a role in embryonic development through their association with fibroblast growth factors and participation in the formation of bones and cartilage (Zhang et al., 1999). Identification of *FGFR* mutations in craniosynostosis indicates their crucial function as signaling transducer molecules during the normal development of cranial sutures.

AS frequently occurs as a new mutation and is related to advanced age of the father (Yoon et al., 2009). These mutations are typically present in *FGFR2* in chromosome 10q25-q26 (Wilkie et al., 1995). Recently, frameshift mutations and alternative splicing have been detected in the *FGFR* gene, mainly in exon IIIa, which encodes an immunoglobulin-like domain with autosomal dominant inheritance. FGFRs are involved in a number of genetic disorders related to bone development, such as Crouzon, Pfeiffer, Apert, and Jackson Weiss syndromes. They have phenotypic differences such as syndactyly, hypertelorism, and midfacial hypoplasia, suggesting their association with other genes or different penetrance of the gene (Zhang et al., 1999).

Park et al. (1995) identified 2 mutations in exon IIIa of the *FGFR2* gene that were related to AS, including Ser252Trp (c.755C > G) and Pro253Arg (c.758C > G). These mutations are located in the linker region between immunoglobulin-like loops II and III of FGFR2: the former is present in approximately 2/3 of patients and is associated with a more severe craniofacial phenotype, while the latter is found in the remaining 1/3 of patients and is associated with more severe syndactyly (Slaney et al., 1996).

In this study, we examined the mutations and clinical features present in Mexican patients diagnosed with AS.

MATERIAL AND METHODS

Six patients with AS diagnosed through genetic analysis were included in the study. The study complied with the current health laws of Mexico and was approved by the Ethics and Research Committees of the Hospital General Dr. Manuel Gea González. Written informed consent was obtained from all patients or their parents.

DNA was obtained from 20 mL ethylenediamine tetraacetic acid-peripheral blood using the proteinase K and phenol/chloroform extraction methods (Sambrook et al., 2001). Polymerase chain reaction (PCR) of exon IIIa from the *FGFR2* gene was performed using the primers *FGFR2*-F 5'-TGACAGCCTCTGACAACACAAC-3' and *FGFR2*-R 5'-GGAAATCA AAGAACCTGTGGC-3', generating a 350-bp fragment (Meyers et al., 1996). The PCR mixture contained 10 mM Tris, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 mM of each primer, 2 U *Taq* polymerase (Epicentre Biotechnologies, Madison, WI, USA), and approximately 200 ng DNA in a final reaction volume of 50 μL. The temperature profile was: 95°C for 30 s, 62°C for 1 min, and 72°C for 1 min for 35 cycles. The amplified products were detected by electrophoresis on a 2% agarose gel with 0.5 mg/mL ethidium bromide and visualized under ultraviolet (UV) light. Mutations in exon IIIa of the *FGFR2* gene were identified by digestion with the restriction endonuclease *Bst*XI (Promega, Madison, WI, USA). When the mutation was present, 2 fragments of 141 and 212 base pairs were observed. Digested products were separated on a 6% acrylamide/bisacrylamide gel and bands were visualized using ethidium bromide staining under UV light.

PCR fragments were cloned in the PCR 2.1 vector (Invitrogen, Carlsbad, CA, USA) according to the manufacturer recommendation. After plasmid purification, the fragments were sequenced using specific oligonucleotides for the plasmid T7 promoter and M13 universal cloning region. Both DNA strands were sequenced using an ABI Prism 310 system (Applied Biosystems, Foster City, CA, USA). Multiple alignment of the sequences was conducted using the MEGA software version 5.

RESULTS

All patients presented craniosynostosis, hyperterolism, eye proptosis, otitis media, mid-facial hypoplasia, hyperhidrosis, and hand and foot anomalies. They were differentiated from the Crouzon, Pfeiffer, Seathre-Chotzen, Muenke, and Jackson-Weiss syndromes in accordance with clinical criteria. Four patients were children aged 1-8 years and 2 were adults from 26-40 years; their main features are shown in Table 1. In 33.3% of patients, strabismus, hypoacousia, and choana stenosis or atresia was present, and no patient presented cleft palate. Regarding extremities, 88.3% presented rhizomelic shortening, 50% presented shoulder shortening and elbow alterations, 33.3% presented syndactyly type I in the hands, and 16.7% presented syndactyly type II and type III, respectively. Central nervous system malformations included agenesis of the corpus callosum (50%) and hydrocephalus (33.3%). Fifty percent of patients presented heart diseases and 16.7% had urogenital abnormalities. Sequence analysis revealed that 66.6% of cases had the mutation S252W, in contrast with mutation P253R, which was present in only 33.3% of cases. No patients presented both mutations simultaneously. Figure 1 shows the alignments of nucleotides (A), the electropherogram with the sequences of each patient (B), and the amino acid sequence, indicating the position of the fragment studied in the FGFR2 protein (C).

Table 1. Clinical analysis and mutations in exon IIIa of *FGFR2* in Apert syndrome patients.

Clinical features	Patients				
	1	2	3	4	5
Age (years)	8	1	26	4	2
Craniosynostosis	+	+	+	+	+
Hypertelorism	+	+	+	+	+
Eye proptosis	+	+	+	+	+
Strabismus	--	+	+	--	--
Hearing loss	--	ND	+	--	--
Otitis media	+	+	+	+	+
Midfacial hypoplasia	+	+	+	+	+
Choana/stenosis or atresia	+	--	+	--	--
Rhizomelic shortening	+	+	+	+	+
Shoulder shortening	+	+	+	--	--
Elbow alterations	+	+	+	--	--
Syndactyly type I in the hands	+	+	--	--	+
Syndactyly type II in the hands	--	--	+	--	--
Syndactyly type III in the hands	--	--	--	+	--
Hydrocephalus	+	--	+	--	--
Agenesis of the corpus callosum	+	ND	+	--	+
Heart disease	+	+	+	--	--
Urogenital abnormalities	+	ND	--	--	--
Hyperhidrosis	+	+	+	+	+
Mutation in amino acids	**S252W**	**S252W**	**P253R**	**S252W**	**S252W**
Mutation in nucleotides	**C934G**	**C934G**	**C937G**	**C934G**	**C934G**

ND = not determined.

Figure 1. Molecular features of Mexican patients with Apert syndrome. **A.** Mutations in nucleotide sequence of patients with Apert syndrome. **B.** Electropherogram of each sequence. **C.** Mutations in amino acid sequences. Numbers in each figure show the GenBank accession No. H1 = Haplotype 1, H2 = Haplotype 2.

DISCUSSION

The mutations identified in this study differed from those reported by Meyers et al. (1996) who used a similar method to analyze *FGFR2* exon IIIa. They reported the insertion insTGG after nucleotide 982, resulting in addition of the amino acid glycine after threonine at the position 268 mutation, which was observed when the PCR product was digested with the *Bst*XI enzyme; the mutation was present in individuals with Crouzon, Jackson Weiss, and Pfeiffer syndromes, but not in those with AS.

The S252W and P253R mutations were described by Wilkie et al. (1995) as associated to AS, presenting more severe syndactyly in patients with the Pro253Arg mutation compared to Ser252Trp. However, patients analyzed in this study showed no association between these mutations and syndactyly.

The lack of phenotypic differences in both subgroups of patients with AS is not surprising, as described by Park et al. (1995), considering that these mutations are adjacent and are in the same functional domain, which is the linker region between immunoglobulin-like domains II and III. Most *FGFR* mutations for the identified syndromic conditions occur in the linker of the immunoglobulin-like III domain. These mutations have been suggested to modify ligand-binding specificity and subsequently alter the ability of the receptors to be activated by dimerization and proceed along their signaling pathways (Park et al., 1995). Mutation S252W is reportedly associated with cleft palate, while urogenital abnormalities, hydronephrosis, hernia inguinal, and severe syndactyly are often present in patients with mutation P253R. In our patients, none of these features were associated with any of the mutations. This lack of association may be because of the small sample size or because other genes, in combination with the *FGFRs*, participate to different degrees in the development of this syndrome. Further investigation is necessary to determine the mechanisms involved in craniosynostosis.

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