Not Apert syndrome: A critique of a recent case report by Pan and Yang

Andrew O. M. Wilkie

MRC Weatherall Institute of Molecular Medicine

University of Oxford

John Radcliffe Hospital

Oxford OX3 9DS

United Kingdom

ORCID: 0000-0002-2972-5481

To the Editors:

Apert syndrome (AS) is diagnosed based on a characteristic combination of craniosynostosis involving multiple sutures of the skull vault and facial bones, and symmetrical cutaneous and bony syndactyly of the hands and feet. It is caused by a limited spectrum of mutations in the gene *FGFR2*, encoding fibroblast growth factor (FGF) receptor type 2, a membrane-spanning receptor tyrosine kinase involved in the transduction of extracellular FGF signals into the cell. Over 98% of AS mutations comprise one or other of two "canonical" missense changes, p.(Ser252Trp) and p.(Pro253Arg), located at adjacent amino acids in the linker between the second and third extracellular immunoglobulin-like domains (Bochukova et al., 2009; Wilkie et al., 1995). AS is distinguished phenotypically from two allelic craniosynostosis syndromes, Pfeiffer syndrome (PS) and Crouzon syndrome (CS), based on a graded severity of limb manifestations, where AS>PS>CS.

In a recent report in *American Journal of Medical Genetics A* (*AJMG*), Pan & Yang (2025) presented the clinical features and molecular analysis of a child whom they diagnosed as having AS, and who harboured a previously undescribed heterozygous *de novo* mutation in *FGFR2*, c.514_515delinsCT encoding p.(Ala172Leu). There are various aspects of this case report that, if left unchallenged, could magnify existing confusion about genotype-phenotype correlations and pathogenic mechanisms in *FGFR2*-related craniosynostosis syndromes.

The key issues raised by the (Pan & Yang, 2025) report can be summarised as follows, and will be addressed in turn:

(1) The clinical diagnosis in their patient is not AS, but PS.
(2) The authors did not reference the existing literature on the particular significance of substitutions at the p.Ala172 codon of FGFR2.
(3) The authors' tabulation of *FGFR2* mutations causing AS contains errors that obscure true genotype-phenotype correlations.

(1) The clinical diagnosis in their patient is not AS, but PS.

In a seminal series of articles published in *AJMG* during the 1990s, M. Michael Cohen Jr and Sven Kreiborg reviewed the pathological features of AS as they affected multiple different organ systems. In their article on the hands and feet (Cohen & Kreiborg, 1995), they classified the AS syndactyly according to whether digits 2-4 (type 1), digits 2-5 (type 2), or all five digits (type 3) were included in the central syndactylous mass. In the case presented by Pan and Yang, only digits 3 and 4 of the hands, and digits 2 and 3 of the feet, were affected by (partial cutaneous) syndactyly. Clearly the syndactyly in their patient was milder than is recognised in AS.

If not AS, what is the diagnosis? The condition to which the label PS is now bestowed was first described by Rudolf A. Pfeiffer, based on the clinical findings in a three-generation family (Pfeiffer, 1964). Remarkably, this family was subsequently shown (Kan et al., 2002) to harbour the heterozygous *FGFR2* mutation c.514_515delinsTT encoding p.(Ala172Phe) – in other words, a double

nucleotide change at the identical position to that in the (Pan & Yang, 2025) case, but encoding a substitution to phenylalanine instead of leucine. In Pfeiffer's family, both the hands and feet of affected individuals manifested a pattern of involvement (incomplete cutaneous syndactylies, accompanied by broad and medially deviated thumbs and big toes), very similar to the (Pan & Yang, 2025) patient. Clearly therefore, their patient has PS, not AS.

(2) The authors did not reference the existing literature on the particular significance of substitutions at the Ala172 codon of FGFR2.

As well as Pfeiffer's original family just mentioned, three other patients or families have subsequently been reported who harbour heterozygous variants at the FGFR2 Ala172 codon. In two families (Cheng, Lo, & Luk, 2018; Jay et al., 2013), the variant present is identical to that in Pfeiffer's original family; based on examination of haplotypes or ancestral background, each is likely to represent an independent double mutational event. In a sporadically affected individual, yet another double nucleotide variant at the same codon, c.514_515delinsAA encoding p.Ala172Asn, was reported (Flottmann et al., 2015); this patient was considered to have the typical hands and feet of PS, but the craniofacial features associated with PS were absent.

Although Pan & Yang (2025) modelled the effect of the p.Ala172Leu variant on FGFR2 structure, they did not acknowledge that an experimental analysis of the impact of the p.Ala172Phe substitution on FGF ligand binding, *and* a corresponding crystal structure, were reported over 20 years ago (Ibrahimi, Yeh, et al., 2005). Crucially, the pathogenic nature of this substitution helped to resolve a long-standing controversy about whether the formation of productive FGF:FGFR signalling complexes involved a symmetric or asymmetric interaction (Pellegrini, Burke, von Delft, Mulloy, & Blundell, 2000; Plotnikov, Schlessinger, Hubbard, & Mohammadi, 1999). In the symmetric model, the p.Ala172 residues locate to a mutually interacting interface of the IgII domains, and substitution to Phe was shown to strengthen the van der Waals interaction, leading to gain-of-function. Along similar lines, the substitution to large, non-polar leucine described by (Pan & Yang, 2025) is also

likely to strengthen this hydrophobic interface, a totally different mechanism than was suggested by their modelling.

(3) The authors' tabulation of *FGFR2* mutations causing AS contains errors that obscure true genotype-phenotype correlations.

In an attempt to be comprehensive, Pan & Yang (2025) listed (see Table 1 of their publication) various atypical mutations that have been reported in AS, but unfortunately their tabulation of variant cases contained six examples (which they labelled as 1a, 1b, 1c, 1e, 1i and 1j) that are likely to be errors. Taken at face value, these erroneous inclusions obscure the truly exquisite pattern of genotype-phenotype correlations for *FGFR2* mutations that exist in AS. Aside from the canonical mutations referred to above, the three other types of rare variants that do occur in AS comprise one of (i) different amino acid substitutions at the canonical sites; (ii) variants such as *Alu* insertions that disrupt the normal splice preference for the IgIIIc isoform in mesenchymal cells; or (iii) deletions that include the IIIc exon and are therefore also predicted to disturb this splicing balance. Table 1 summarises the reasons for rejecting six of the AS variants presented by (Pan & Yang, 2025) and includes two genuine AS-associated non-canonical variants that were not cited by these authors. The key mechanism shared between these apparently disparate AS-associated mutation types involves an alteration of normal FGF-FGFR2 ligand binding dependencies (Ibrahimi, Chiu, McCarthy, & Mohammadi, 2005; Ibrahimi et al., 2001; Ibrahimi et al., 2004; Oldridge et al., 1999; Yu & Ornitz, 2001)).

In summary, the case reported by (Pan & Yang, 2025) represents an interesting addition to the literature, but the authors' interpretation must be adjusted in several important aspects to take account of existing knowledge of the subject.

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Table 1 Non-canonical *FGFR2* mutations reported in Apert syndrome.

	Annotation in Table 1 of (Pan & Yang, 2025)	Reference	*FGFR2* variant (DNA; NM_000141.5, ENST00000358487.10)	FGFR2 variant (protein)	Comment
Incorrectly classified	1a	(Tonni et al., 2022)	c.758C>G	p.(Pro253Arg)	Typical AS mutation, incorrectly annotated in original publication as p.(Pro252Arg)
	1b	(Shi et al., 2020)	c.755C>G	p.(Ser252Trp)	Typical AS mutation, incorrectly annotated in original publication as p.(Ser137Trp)
	1c	Gou (2018)	c.870G>T	p.(Trp290Cys)	No phenotypic information accessible, variant is typically associated with severe PS
	1e	(Torres, Hernandez, Barrera, Ospina, & Prada, 2015)	c.939+42T>A	-	Experimental artefact, variant contained within E8-forward PCR primer
	1i	Song et al. (2022)	c.544G>T	p.(Gly182Trp)	Rare variant in gnomAD v4.1.0 (10/1614212); no syndactyly noted
	1j	Song et al. (2022)	c.1852G>C	p.(Ala618Pro)	No syndactyly noted
Non-canonical amino acid substitution at Ser252-Pro253	1g	(Oldridge et al., 1997)	c.755_756delinsTT	p.(Ser252Phe)	Multinucleotide mutations create bulky side chains, analogous to Ser252Trp and Pro253Arg
	1h	(Lajeunie et al., 1999)	c.755_756delinsTT	p.(Ser252Phe)	
	-	(Goriely et al., 2005)	c.755_756delinsTC	p.(Ser252Phe)	
	2d	(Lumaka et al., 2014)	c.756_758delinsCTT	p.(Pro253Phe)	
Altered splicing (mostly *Alu* insertions)	2m	(Oldridge et al., 1999)	c.940-4_940-3insAlu	-	Disrupted exon IIIc acceptor site drives ectopic IIIb isoform expression (experimentally demonstrated)
	2m	(Oldridge et al., 1999)	c.1041_1042insAlu	-	Likely similar mechanism to above *Alu* insertion
	1l	(Bochukova et al., 2009)	c.1002_1003insAlu	-	

	-	(Topa et al., 2023)	c.940-19_940-18insAlu	-	
Exon IIIc deletion	1k	(Bochukova et al., 2009)	c.940-890_1084+895del	-	Deletes IIIc exon
	1f	(Fenwick, Bowdin, Klatt, & Wilkie, 2011)	c.940-1290_1021del	-	Creates chimeric IIIb/IIIc exon