



## Clinical report

# FGFR2 mutation in a patient without typical features of Pfeiffer syndrome – The emerging role of combined NGS and phenotype based strategies



Ricarda Flöttmann<sup>a</sup>, Alexej Knaus<sup>a</sup>, Tomasz Zemojtel<sup>a</sup>, Peter N. Robinson<sup>a, b, c</sup>, Stefan Mundlos<sup>a, b, c</sup>, Denise Horn<sup>a</sup>, Malte Spielmann<sup>a, b, c, \*</sup>

<sup>a</sup> Institute for Medical Genetics and Human Genetics, Charité Universitätsmedizin Berlin, Berlin, Germany

<sup>b</sup> Max Planck Institute for Molecular Genetics, Berlin, Germany

<sup>c</sup> Berlin-Brandenburg School for Regenerative Therapies (BSRT), Berlin, Germany

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## ABSTRACT

Pfeiffer syndrome (MIM: #101600) is a rare autosomal dominant disorder classically characterized by limb and craniofacial anomalies. It is caused by heterozygous mutations in the fibroblast growth factor receptors types 1 and 2 (*FGFR1* and *FGFR2*).

We applied a next generation sequencing (NGS) panel approach comprising all 2877 genes currently known to be causative for one or more Mendelian diseases combined with the phenotype based computational tool PhenIX (Phenotypic Interpretation of eXomes).

We report on a patient presenting with multiple anomalies of hands and feet including brachydactyly and symphalangism. No clinical diagnosis could be established based on the clinical findings and testing of several genes associated with brachydactyly and symphalangism failed to identify mutations. Via next generation sequencing (NGS) panel approach we then identified a novel *de novo* missense *FGFR2* mutation affecting an amino acid reported to be mutated in Pfeiffer syndrome. Since our patient shows typical radiological findings of Pfeiffer syndrome in hands and feet but at the same time lacks several characteristic features such as clinical signs of craniosynostosis and prominent eyes we suggest introducing the term “FGFR2 associated phenotypes” for similar cases.

Our results highlight the emerging role of combined NGS and phenotype based bioinformatics strategies to establish clinical diagnoses.

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## 1. Introduction

Pfeiffer syndrome is a rare autosomal dominant disorder classically characterized by craniofacial and limb anomalies. The classic type presents with craniosynostosis, midface hypoplasia, deviated broad thumbs, broad big toes, brachydactyly and a variable degree of syndactyly in hands and feet. Pfeiffer-associated craniosynostosis displays a broad spectrum of variation, ranging from absence of synostosis to cloverleaf skull [Cohen 1993]. Premature closure of the coronal suture, resulting in brachycephaly, is most frequently observed. Pfeiffer syndrome is caused by heterozygous mutations

in the fibroblast growth factor receptors types 1 and 2 (*FGFR1* and *FGFR2*) [Johnson and Wilkie 2011]. It was suggested that *FGFR1* mutations often result in less severe craniofacial involvement [Muenke et al., 1994; Rossi et al., 2003; Hackett and Rowe 2006]. The majority of cases however are caused by gain of function mutations in *FGFR2* [Kan et al., 2002; Lajeunie et al., 2006; Chokdeemboon et al., 2013]. The *FGFR2* protein is involved in cell division and regulation of cell growth as well as differentiation [Coulier et al., 1997]. It consists of an extracellular region, composed of three immunoglobulin-like domains (Igl, IgII and IgIII), a single hydrophobic membrane-spanning segment and a cytoplasmic tyrosine kinase domain [Coulier et al., 1997]. There is a wide spectrum of mutations in *FGFR2* shown to be causative for Pfeiffer syndrome [Kan et al., 2002; Lajeunie et al., 2006; Chokdeemboon et al., 2013]. Most of them affect the third immunoglobulin-like domain (IgIII) of the *FGFR2* protein but there are some reported

\* Corresponding author. Institut für Medizinische Genetik und Humangenetik, Charité – Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany.

E-mail address: [malte.spielmann@charite.de](mailto:malte.spielmann@charite.de) (M. Spielmann).

mutations outside of this region [Kan et al., 2002; Lajeunie et al., 2006]. One of those mutations, outside of the IgIII-domain, was found in the original Pfeiffer family [Kan et al., 2002] and another unrelated British family [Jay et al., 2013]. It affects the IgII-domain and is characterized by the substitution of two consecutive nucleotides c.514\_515delGCinsTT encoding p.Ala172Phe. All affected individuals, a total of 11, presented with a classical Pfeiffer syndrome phenotype, including deviated broad thumbs, broad big toes, brachydactyly, and a variable degree of syndactyly as well as midface hypoplasia but with an atypical appearance in terms of cranial suture pattern. All individuals presented without craniosynostosis, thus indicating that p.Ala172Phe is associated with a milder cranial phenotype [Jay et al., 2013]. Here we report on a young girl with a novel mutation in *FGFR2* found by NGS panel approach and a newly established phenotype based computational method termed PhenIX (Phenotypic Interpretation of eXomes). PhenIX evaluates variants based on population frequency and predicted pathogenicity and ranks the genes according to variant score and a clinical relevance score [Zemojtel et al., 2014]. Our results highlight the emerging role of combined NGS and phenotype based computational strategies to establish clinical diagnoses.

## 2. Clinical report

The four year old girl was the third child of healthy, non-consanguineous German parents and presented with symphalangism and brachydactyly of both hands and feet. Family history was unremarkable and mental and motoric development was normal. Following an uneventful pregnancy, delivery was vacuum assisted at 40 weeks of gestation (birth weight 3120 g, 25th–50th centile, length 49 cm, 25th–50th centile, occipitofrontal head circumference (OFC) 36.5 cm, 75th centile). Physical examination showed a height of 109.5 cm (90th centile), weight of 16.5 kg (50th–75th centile), and OFC of 51 cm (75th centile) with no signs of skull asymmetry. The parents reported on delayed closure of the anterior fontanel. The facial anomalies included short philtrum, thin upper lip vermilion and mild midface hypoplasia (Fig. 1 A, B). Examination of the hands revealed bilateral absence of interphalangeal creases with flexion limitation in the proximal and distal interphalangeal joints and the metacarpophalangeal joints of both index fingers and in the metacarpophalangeal and proximal interphalangeal joints of the middle fingers (Fig. 1 D, G). Brachydactyly due to short middle phalanges of fingers II to V was visible as well as a short proximal phalanx of both thumbs (Fig. 1 D, G). Remarkably there was no syndactyly present in the hands (Fig. 1 A, B, E). In the feet an incomplete cutaneous syndactyly between toes II and III as well as a broad hallux was observed bilaterally (Fig. 1 H). X rays of the hands revealed hypoplastic middle phalanges of fingers II to IV bilaterally and a broad and shortened proximal phalanx of both thumbs. Intra-articular spaces of several different interphalangeal joints were reduced and cone-shaped epiphyses were present (Fig. 1 H, I). X rays of both feet showed hypoplastic middle phalanges with cone-shaped epiphyses II–IV, broad malformed first toes including the corresponding metatarsal bone, and calcaneo-cuboid fusion (Fig. 1 F, J).

Because of the brachydactyly type A1 (BDA1) phenotype (reviewed in [Mundlos, 2009]) we initially performed testing for *IHH* [Gao et al., 2001] but no mutation was detected. Subsequently *GDF5* and *NOG* were also tested because of symphalangism of finger joints. No mutation was detected. PhenIX then revealed a novel mutation in *FGFR2*: c.514\_515delGCinsAA encoding p.Ala172Asn. It was ranked first place with a gene relevant score of 1.000 as well as a variant score of 1.000. Sanger sequencing of exon 5 of *FGFR2* in this patient and her parents was performed and revealed a *de novo* status (Fig. 2).

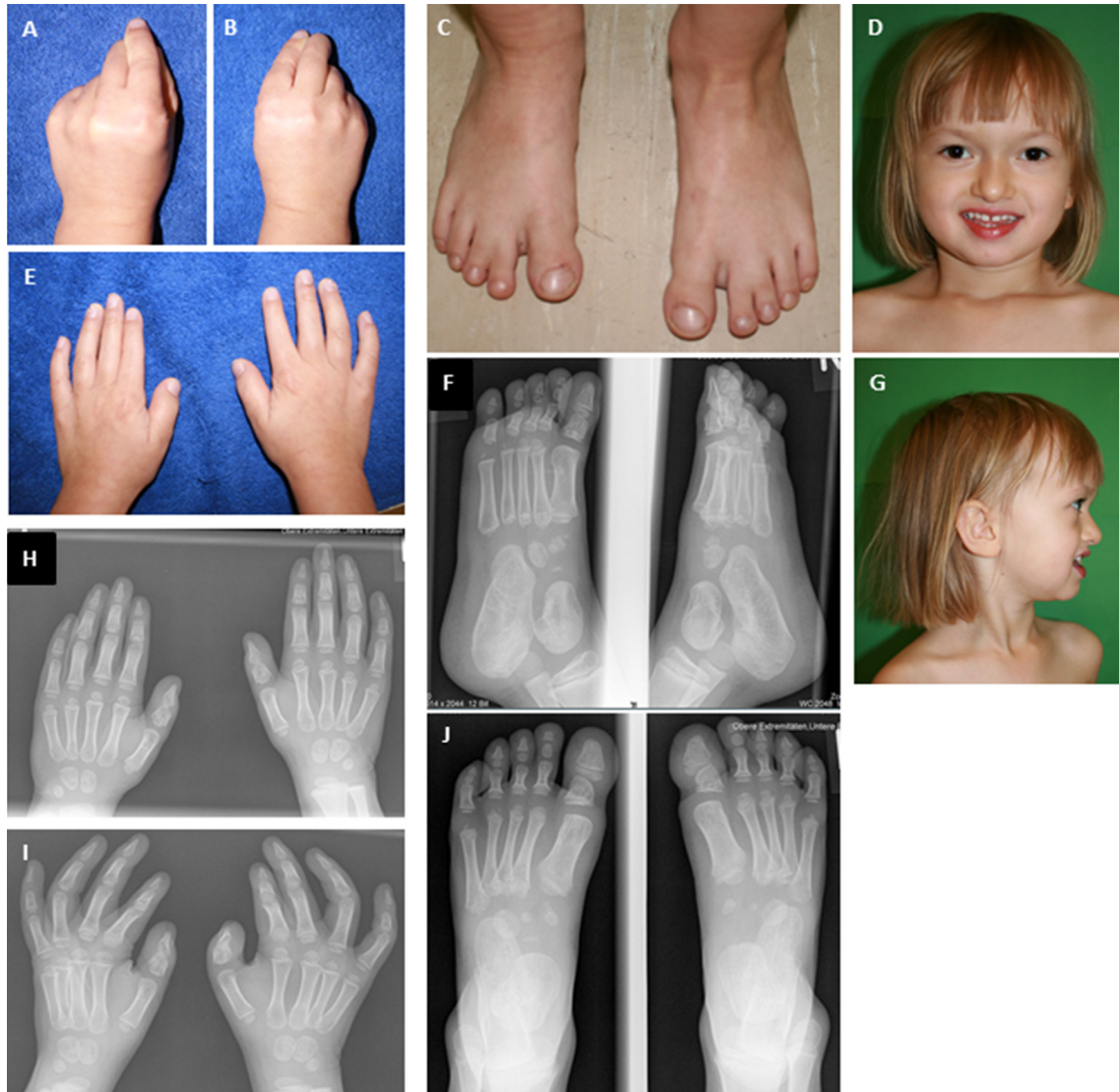
## 3. Next generation sequencing panel

Since no clinical diagnosis could be established the patient was included in a next generation sequencing (NGS) panel study comprising 2741 genes known to be causative for one or more Mendelian diseases. Therefore genomic DNA was isolated from peripheral blood samples and subjected to next generation sequencing. Subsequently a computational method termed Phenotypic Interpretation of eXomes (PhenIX), that evaluates variants based on population frequency and predicted pathogenicity and then ranks the genes according to variant score and a clinical relevance score, was used to prioritize candidate genes [Zemojtel et al., 2014]. To calculate the clinical relevance score we entered the following human phenotype ontology [Robinson et al., 2008] terms: 2–3 toe cutaneous syndactyly (HP0005709); proximal/middle symphalangism of 3rd finger (HP:0009482); proximal/middle symphalangism of 2nd finger (HP:0009579), distal/middle symphalangism of 2nd finger (HP:0009563), short proximal phalanx of 2nd finger (HP:0009597), Aplasia/Hypoplasia of the middle phalanx of the 2nd finger (HP:0009568); Aplasia/Hypoplasia of the middle phalanx of the 3rd finger (HP0009437); Aplasia/Hypoplasia of the middle phalanx of the 4th finger (HP:0009299); Aplasia/Hypoplasia of the middle phalanx of the 5th finger (HP:0009161); broad proximal phalanx of the thumb (HP:0009630); short proximal phalanx of the thumb (HP:0009638); abnormality of the philtrum (HP:0000288); thin upper lip vermilion (HP:0000219); broad toe (HP:0001837).

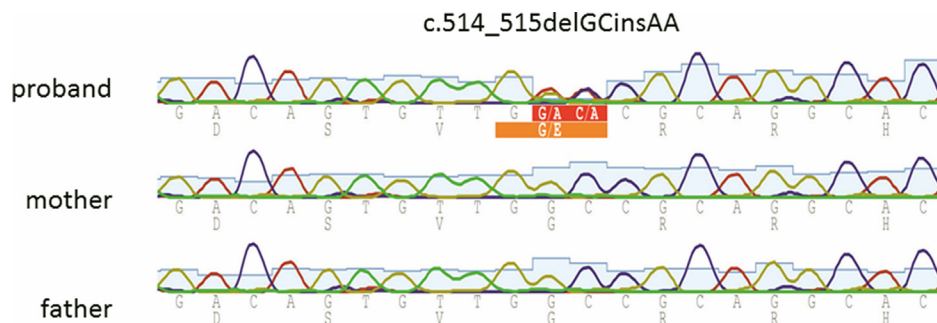
## 4. Discussion

Pfeiffer syndrome is a rare autosomal dominant disorder characterized by craniofacial and limb anomalies. The classic type presents with craniosynostosis, midface hypoplasia, deviated broad thumbs, broad great toes, brachydactyly and a variable degree of syndactyly in hands and feet [Cohen 1993]. The craniosynostosis seen in Pfeiffer syndrome displays a broad spectrum of variation, ranging from absence of synostosis to cloverleaf skull. The syndrome is caused by heterozygous mutations in the fibroblast growth factor receptors types 1 and 2 (*FGFR1* and *FGFR2*) [Johnson and Wilkie 2011].

Here we report on a young girl presenting with symphalangism and brachydactyly of hands and feet. No syndactyly was present in the hands, but an incomplete cutaneous syndactyly between toes II and III could be observed bilaterally. Facial anomalies included flat philtrum and thin upper lip vermilion but no typical features of Pfeiffer syndrome such as brachycephaly or prominent eyes. No skull abnormalities or other clinical signs of craniosynostosis were detected, therefore no X-rays or CT-scans were performed in respect of radiation exposure. The girl was introduced to several clinical geneticists before but no diagnosis could be established. Since brachydactyly and symphalangism were the most prominent features we initially performed testing for *IHH*, *GDF5* and *NOG*. No mutation was detected. We included the child in a next generation sequencing (NGS) panel study comprising all 2741 genes currently known to be causative for one or more Mendelian diseases. A newly developed computational tool termed PhenIX evaluates variants that were found by an NGS approach of 2741 genes known to be causative for one or more Mendelian diseases. The evaluation is based on population frequency and predicted pathogenicity. The algorithm then ranks the genes according to variant score and a clinical relevance score [Zemojtel et al., 2014]. To calculate the clinical relevance score we entered human phenotype ontology terms of our patient. By this approach we found a novel mutation in *FGFR2* in our proband. The heterozygous missense mutation c.514\_515delGCinsAA encoding p.Ala172Asn in *FGFR2* was ranked



**Fig. 1. Images of the proband.** [A,B,C,E]: photograph of hands and feet: note limited joint flexion and brachydactyly in the hands with absence of syndactyly and the partial cutaneous 2/3 syndactyly in the feet as well as a bilateral broad thumb. [D,G]: AP and right lateral photograph: facial anomalies included short philtrum and thin upper lip vermilion. No clinical signs of skull or facial asymmetry. Note only mild midface hypoplasia and no dystopic helices. [H,I]: X rays of hands: Hypoplastic middle phalanges of fingers 2 to 5, a short proximal phalanx of both thumbs and cone shaped epiphyses. Intra-articular space of some interphalangeal joints was reduced. Index fingers and middle fingers: symphalangism of proximal interphalangeal joints. [F,J]: X-rays of feet: hypoplastic middle phalanges with cone-shaped epiphyses II–IV, broad malformed first toes including the corresponding metatarsal bone, and calcaneo-cuboid fusion.



**Fig. 2.** DNA sequencing of our proband and her parents showing heterozygosity of two adjacent nucleotides in the proband. *FGFR2*: c.514\_515delGCinsAA encoding p.Ala172Asn (NM\_001144914).

first place using the PhenIX software. Sanger sequencing of exon 5 of *FGFR2* in the patient and the parents confirmed that the mutation had occurred *de novo* (Fig. 2). Interestingly our patient showed a normal OFC with neither signs of skull or facial asymmetry nor any other dysmorphic facial features associated with Pfeiffer syndrome, thereby giving no hint for Pfeiffer syndrome on clinical examination. The radiological findings, including hypoplastic middle phalanges of fingers, a broad and shortened proximal phalanx of both thumbs, reduced intra-articular spaces of different interphalangeal joints, cone-shaped epiphyses in hands and feet, broad malformed first toes and the fusion of os calcaneus and os cuboideum however correspond to other reported Pfeiffer syndrome cases [Saldino et al., 1972].

Interestingly we found two other families in the literature with a mutation affecting the same amino acid position and who were reported to have a „milder“ form of Pfeiffer syndrome marked by absence of craniosynostosis. Those two families, Pfeiffer's original family and another unrelated British family, carried a c.514\_515delGCinsTT encoding p.Ala172Phe mutation [Kan et al., 2002; Jay et al., 2013]. In Pfeiffer's original family all affected members showed broad radially deviated thumbs and syndactyly of hands and feet were seen in four affected patients. Midface hypoplasia was present in four patients [Pfeiffer 1964]. In the second British family, harboring the same mutation as the original Pfeiffer family, midface hypoplasia, bulging eyes, broad radially deviated thumbs, and a variable pattern of syndactyly was present in all generations. In both families the absence of toe middle phalanges was noted [Jay et al., 2013]. Comparison of the phenotypic characteristics of our patient and the two other families is given in Table 1.

Although the sequence change we found in our patient hasn't been described until now it affects the same position that has been detected in the two previously mentioned families and has been studied in an extensive biochemical analysis by Ibrahim et al. [Ibrahim et al., 2005]. Fibroblast growth factor receptors (FGFRs) are tyrosin kinases which dimerize upon activation by fibroblast growth factors (FGFs) and heparin. The amino acid residue on position 172 has been shown to be involved in receptor–receptor connection in the symmetric two-end model [Ibrahim et al., 2005].

In the known mutation (c.514\_515delGCinsTT, p.Ala172Phe), the alanine residue at amino acid position 172 is changed to phenylalanine, resulting in mild features of Pfeiffer syndrome. This mutation affects the IgII-domain and is characterized by the substitution of a small alanine to a bulkier non-polar phenylalanine at position 172 [Jay et al., 2013]. This substitution increases the hydrophobic interaction and stabilizes the dimer. Crystallographic analysis showed

that the aromatic benzyl groups stack optimally against each other and provided evidence that the p.Ala172Phe results in receptor gain of function – a mechanism predominantly observed in *FGFR2* mutations [Ibrahim et al., 2005]. In the novel mutation that was found in our patient (c.514\_515delGCinsAA, p.Ala172Asn) the same alanine residue is changed to asparagine, a bulkier and notably polar amino acid, but still resulting in a mild phenotype. To evaluate if the mutation p.Ala172Asn might induce *FGFR2* dimer-stabilizing effect that would support the gain-of-function hypothesis, a possible dimer configuration was modeled based on the ternary complex of *FGFR1*, *FGF2* and heparin (crystal structure with PDB ID 1FQ9), following the symmetric two-end model of dimerization that was suggested by Ibrahim et al. for the *FGFR2* p.Ala172Phe mutation [Ibrahim et al., 2005]. As the template molecule for the *FGFR2* p.Ala172Phe mutant, we used a crystal structure of the *FGFR2* mutant p.Ala172Phe (PDB ID 3OJ2). The sequence homology between the two *FGFR* variants is approximately 71%, so that the two chains can be superimposed reasonably well based on a sequence alignment (Fig. 3A). Exchange of residue 172 from phenylalanine to asparagine in the *FGFR2* chains of the modeled dimer, followed by energy minimization with the CHARMM forcefield and the GBSW implicit water model leads to configuration that allows for hydrogen bonding mediated by the carboxamide moieties of the asparagine side chains (Fig. 3B) [Im et al., 2003; Brooks et al., 2009]. This might lead to a *FGFR2* dimer-stabilizing effect similar to that proposed for p.Ala172Phe mutation, albeit based on a different chemical mechanism (hydrogen bonding instead of hydrophobic  $\pi$ -stacking interaction). Hydrogen bonding is known to induce relatively stable interactions on buried (not directly solvent-exposed) protein interfaces. However, it remains unclear why this amino acid change leads to such a mild phenotype without any signs of cranial involvement. It could be speculated that the gain of function in the p.Ala172Asn mutant might be more likely to determine a prevalent hand phenotype rather than a cranial premature fusion as described by Jay et al. [Jay et al., 2013].

Since our patient has features that are consistent with those associated with the *FGFR1* p.P252R mutation, especially the shape of the halluces and the radiological appearance of the feet, we specifically looked for that mutation in the data [Bessenyey et al., 2014]. The *FGFR1* p.P252R mutation was not present.

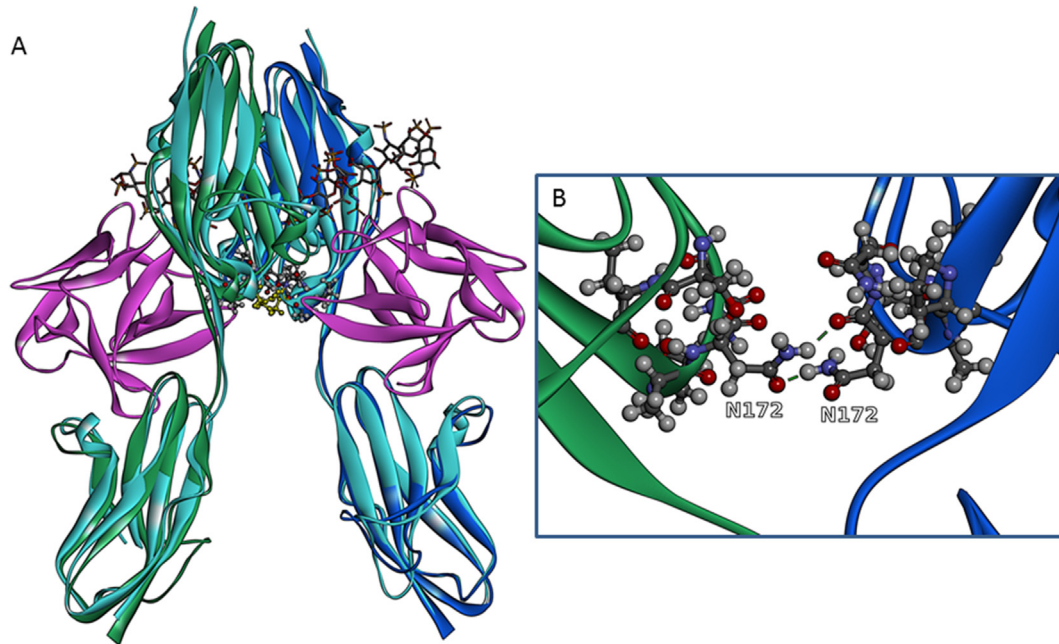
We describe a patient who shows typical radiological findings of Pfeiffer syndrome in hands and feet but at the same time is missing some of the characteristic features such as clinical signs of craniosynostosis and prominent eyes. Diagnosis could not be made based on clinical signs. Via next generation sequencing (NGS) panel approach we then identified a novel *de novo* missense *FGFR2*

**Table 1**  
Comparison of the phenotypic characteristics.

	Our patient	Jay et al.			Pfeiffer's original family					
		Proband	Mother	Maternal grandfather	Proband	Brother	Father	Paternal uncle I	Paternal uncle II	
<b>Head</b>										
Craniosynostosis	–	–	NA	NA	–	–	NA	NA	NA	
Brachycephaly	–	–	–	–	+	+	+	+	+	
Midface hypoplasia	+	–	+	+	+	NA	+	+	+	
<b>Hands</b>										
Broad thumb	–	+	+	+	+	+	+	+	+	
Syndactyly	–	+	+	+	+	+	+	–	+	
Symphalangism	+	–	NA	NA	NA	NA	+	NA	–	
<b>Feet</b>										
Broad great toe	+	+	+	+	+	+	+	+	+	
Syndactyly	+	+	+	+	+	+	+	–	+	
Fused tarsal bones	+	NA	NA	NA	NA	NA	NA	NA	+	
Absent/hypoplastic middle phalanges	+	+	NA	NA	+	–	+	+	+	

+ feature identified in proband, – feature not identified in proband, NA: information not available, Individuals of Pfeiffer's original family that are listed here are reported in detail in the original article [Pfeiffer 1964].





**Fig. 3. Model of the p.Ala172Asn mutation.** [A]: Two FGFR2 chains with a model of the p.Ala172Asn (A172N) mutation (red and blue, based on crystal structure with PDB ID 3J02) superimposed on the ternary complex of FGFR1 (cyan), FGF2 (purple) and heparin (sticks representation) (PDB ID 1FQ9). The p.Ala172Asn mutation site is indicated in yellow. [B] Model of the p.Ala172Asn mutation in the symmetric two-end model of FGFR dimerization. The lack of the hydrophobic  $\pi$ -stacking interaction of the known p.Ala172Phe mutation is compensated by the formation of hydrogen bonds (indicated as green sticks) between the asparagines at position 172. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mutation affecting an amino acid reported to be mutated in Pfeiffer syndrome. Therefore we suggest introducing the term “FGFR2 associated phenotypes” for similar cases. Our results highlight the emerging role of combined NGS and phenotype based bioinformatics strategies to establish clinical diagnoses.

### Conflicts of interest

The authors declare no conflict of interest.

### Acknowledgments

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