Original Article

Interstitial 8p Deletion in Two Patients with the Expansion of Phenotype and Possibility of Contribution of *PINX1* Gene and GATA4 rs3729856 Variant in Absent Nails

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ABSTRACT

Background: The short arm of chromosome 8 is subjected to recurrent genomic imbalances. We aimed to delineate the genotype/phenotype correlations of different 8p deleted segments and predict the candidate gene for absent nails (anonychia). Methods: In this study we present two patients with interstitial 8p deletion. The first is a male patient presented with failure to thrive, seizures, hypotonia, absent nails, congenital heart disease, microcephaly, and hypogenesis of corpus callosum.

Results: Array CGH showed 18.6 Mb in 8p23.2p21.3 deletion that involved *GATA4* gene. Sequencing of *GATA4* gene disclosed likely pathogenic variant rs3729856 (Ser378Gly) in the other allele. The second patient was a female presented with developmental delay, dysmorphic features, microcephaly, hypotonia, brain atrophy, and hypogenesis of corpus callosum. Array CGH showed a 25.6 Mb 8p22p12 deletion. We divided the deleted regions into three parts, and we tried to specify phenotype correlated to each region.

Conclusion: We predict that anonychia in the 1st patient may be due to deletion of one copy of *GATA4* gene and mutation in the other allele, also we suggest that *PINX1* gene may contribute for anonychia. Combined karyotype, FISH, array CGH, sequencing analysis, and in silico analysis are crucial in genotype-phenotype correlation.

Key Words: Anonychia, Corpus callosum, GATA4 gene, In silico analysis, Interstitial deletion.

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INTRODUCTION

The short arm of chromosome 8(p) is subjected to recurrent genomic rearrangement. Due to the presence of hot spots (mediated by two olfactory receptor gene – clusters within 8p23.1 region which acts as low copy repeats) leading to duplications, inversions and/or deletions of variable size either interstitial or terminal, which accounts for variable clinical spectrum. Clinical manifestations include congenital heart disease (CHD), developmental delay (DD), intellectual disability (ID), abnormalities of corpus callosum, dysmorphic features (microcephaly, hypertelorism, epicanthal folds, short nose, malformed ears, micrognathia, thin lips, upslanted palpebral fissures (**Hollox *et al.,* 2008; Páez *et al.,* 2008; Rowe *et al.,* 2009; Yu *et al.,* 2010; Ballarati *et al.,* 2011; Garcia Santiago *et al.,* 2015, Silan *et al.,* 2018, Okur *et al.,* 2021; Montenegro *et al.,* 2023**).

Patients usually demonstrates CHD including pulmonary stenosis, atrial septal defect (ASD), ventricular septal defect (VSD), complete atrioventricular canal, double outlet right ventricle and Fallot tetralogy (**Digilio *et al.,* 1998; Pehlivan *et al.,* 1999; Wat *et al.,* 2009; Li *et al.,* 2016; Kumar *et al.,* 2018**).

The 8p deletion may involve the distal region of 8p (8p31p33) and this region represent the typical 8p deletion syndrome and if involved the *GATA4* gene, it results in CHD, (**Wat *et al.,* 2009; Redaelli *et al.,* 2022**). The deletion of the proximal region 8p11.28p22.2 is reported by several authors and also have an overlap clinical manifestation with the distal region including CHD, (**Izumi *et al.,* 2011; Li *et al.,* 2016; Dai *et al.,* 2022**).

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Anonychia is the absence of finger and toe-nails. *RSPO4* gene is the 1st gene responsible for inherited anonychia. Congenital anonychia is a rare autosomal recessive disorder. Beside the *RSPO4* gene, there are several genes responsible for nail formation e.g., *FZD6, HPGD, PLCD1, COL7A1*. The *FZD6* gene is present on chromosome 8q22.3, (**Brüchle *et al.,* 2008**). There are 61 genes associated with the **anonychia** phenotype by mapping known disease genes to disease phenotypes from the HPO (HPO; www.human-phenotype-ontology.org). In this study we aimed to delineate the genotype phenotype correlations to the different 8p deleted segments and narrowing the contender regions to predict the candidate gene for anonychia and compare our findings to other authors and databases.

SUBJECTS AND METHODS:

Two patients one male and one female presented to the Clinical Genetics Clinic, Human Genetics and Genome Research Institute, National Research Center, Egypt.

Clinical report

Patient No.1: A male patient aged 30 days at the time of examination presenting with growth retardation, recurrent chest infection and intractable seizures. He was a full-term offspring first child of a consanguineous parents, pregnancy history demonstrated intrauterine growth retardation (IUGR) and delivery was uneventful with 1.7kg birth weight. Admitted to NICU due to respiratory distress, poor suckling, and low birth weight for 1 week with a history of a male uncle aged 13 years had seizures.

On examination, the patient was lethargic with frequent myoclonic seizures. Anthropometric measurements: Weight was 2kg (-4.0SD underweight), head circumference 31cm (-3.1SD microcephalic), and length 45cm (-3.2SD short stature). Facial dysmorphism in the form of microcephaly, hairy forehead, mild synophyrous, squint, bilateral epicanthic folds, blue sclera, depressed nasal root, broad bulbous nose, long flat philtrum, thin lips, retromicrognathia, bilateral malformed posteriorly rotated low set ears with absent tragus and prominent antitragus (Figure 1a, b). Hands and feet showed complete absence of nails (total anonychia), hypoplastic 5th toe with bilateral deep plantar creases (Figure 1c, d). Cardiac examination showed pansystolic murmur over the pericardium. Neurological examination revealed depressed Moro reflex, marked axial hypotonia with prominent head lag. Thyroid profile, serum calcium, phosphorus and magnesium were normal.

Pelviabdominal ultrasound was normal, Echocardiography expressed a premembranous ventricular septal defect (7mm), two atrial septal defects (secondum) (6.5mm) each, with mild valvular pulmonary stenosis and dilated right ventricle. Neuroimaging revealed hypogenesis of corpus callosum, defective myelination and mild reduced cerebellar size for age. Depressed waves with subcortical focus were detected by EEG.

Patient No.2: A female child aged 3 years and 8 months presented with global developmental delay, hyperactivity and delayed speech. She was the only child of non-consanguineous parents. She was delivered at full term with a birth weight of 2.500kg and had a history of growth retardation in the first year of life.

On examination: Dysmorphic features were prominent (narrow forehead, bilateral epicanthic folds, hypertelorism, squint, full cheeks, depressed nasal bridge, bulbous broad nose, long flat philtrum, retromicrognathia, bilateral low set small malformed ears with abnormal thick folding (Figure 1e, f). Short neck, wide spaced nipples, incomplete transverse palmar crease on left hand, talipes valgus, bilateral syndactyly between 3rd and 4th toes, and bilateral flat foot were noticed (Figure 1g, h). Anthropometric measurements showed: Head circumference 44cm (-4.6SD; microcephalic), height and weight were on mean for age and sex. Neurological examination revealed Hypotonia and hyporeflexia, Echo and pelviabdomial U/S were Normal, EEG was normal while MRI brain showed atrophic brain changes and hypogenesis of corpus callosum (Figure 1i, j).

Cytogenomic studies:

G-banded chromosomal analysis of peripheral blood lymphocytes was performed for patients and their parents according to **Verma and Babu, (1995)**. A minimum of 20 metaphases were analyzed and karyotyped according to the International System for Human Cytogenomic Nomenclature (**McGowan Jordan *et al.,* 2020**).

Fluorescence in situ hybridization (FISH) studies performed according to **Pinkel *et al.,* (1986)** and manufacturer instructions using ToTel Vysion probe mixtures and chromosome 8 Locus Specific Identifier LSI (8p22) (spectrum Orange) and (8q24.12-q24.13) (spectrum Green) (Abbot Laboratories, Illinois, USA).

Array CGH: the technique was performed according to the manufacturer's instructions for each used equipment.

GATA4 sequencing:

Exons and exon-intron boundaries corresponding to human *GATA4* gene (GeneBank Accession No.: NG_008177; Version NG_008177.1) were amplified using specific primers as reported by **Okubo *et al.,* (2004)**. Purified samples were subjected to cycle sequencing using a Big Dye Terminator v3.1 Kit and injected into a ABI3730XL sequencer.

Using Phenomizer - Human Phenotype Ontology (HPO) server and UCSC database (GRCH37/hg19], the patients' phenotype and corresponding gene list across the deleted regions were extracted.

Table 1: Represent the clinical manifestations of our patients 1 and 2 in comparison to other authors:

	Klopocki *et al.*, 2006	Wat *et al.*, 2009	Willemsen *et al.*, 2009	Ballarati *et al.*, 2011	Fisch *et al.*, 2011	Izumi *et al.*, 2011	Khelifa *et al.*, 2015	Li *et al.*, 2016	Shi *et al.*, 2017	Wagner-Mahler *et al.*, 2019	Lo Bianco *et al.*, 2020	Catusi *et al.*, 2021	Dai *et al.*, 2022	Redaelli *et al.*, 2022	Yurchenko *et al.*, 2022	Present study 1	Present study 2
Cases	1F	3M/1F	1M/1F	1M	2M/2F	1M	1M/1F	1F	1M	1M	1F	6M/1F	1F	3M/6F	5M/3F	M	F
Deletion 8p	P12 p21.2	p23.1 p23.1	P12 p21.3	p23.1 p23.1	p22 p23.1	p21.2 p21.3	p23.1 p23.3	p11.23 p22	p23.2 p23.3	p23.1 p23.1	p23.1 p23.3	p23.2 p23.3	p11.22 p21.2	p23.1 p23.3	p23.1 p23.3	p21.2 p23	p12 p22
Neurodevelop-mental delay	+	2/4	2/2	+	4/4	+	1/2	+	+	+	+	4/7	+	ND	8/8	+	+
Seizures	ND	¼	-	-	-	-	-	ND	-	-	-	ND	ND	ND	1/8	+	-
Brain anomalies	+	¼	½	-	ND	+	-	+	-	-	+	ND	-	ND	6/8	+	+
Agenesis or hypogenesis of corpus callosum	+	¼	½	-	ND	+	-	ND	-	-	-	ND	-	1	5/8	+	+
Microcephaly	+	-	2/2	+	-	-	2/2	+	+	+	-	1/7	+	ND	-	+	+
Facial dysmorphism	+	2/4	2/2	+	1/4	+	2/2	+	+	-	+	3/7	+	ND	8/8	+	+
Malformed Ears	ND	-	½	+	-	-	1/2	-	+	+	+	2/7	+	ND	8/8	+	+
Genitourinary anomalies	+	-	½	ND	-	ND	ND	-	-	+	-	ND	ND	ND	ND	-	-
Congenital heart defect	-	4/4	½	+	-	ND	-	+	-	+	-	1/7	+	ND	-	+	-
Hands/feet abnormalities	ND	¾	½	+	-	ND	ND	+	ND	+	+	2/7	+	ND	ND	+	+
Anonychia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-

+: present; -: absent; ND: no dat.

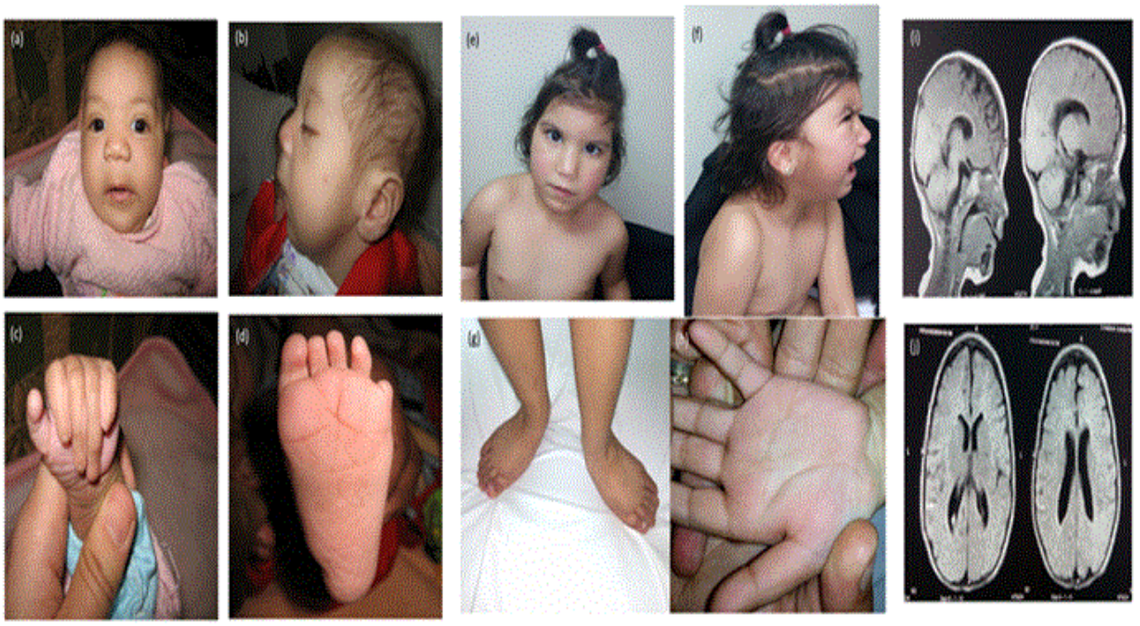

Figure 1: Patient 1: Squint, bilateral epicanthic folds, blue sclera, depressed nasal root, broad bulbous nose, long flat philtrum, thin lips, microretrognathia, bilateral malformed posteriorly rotated low set ears with absent tragus and prominent antitragus (a, b). Hands and feet showed complete absence of nails (total anonychia), hypoplastic 5th. toe with bilateral deep plantar creases (c, d). Patient 2: Narrowing forehead, sparse hair, bilateral epicanthic folds, hypertelorism, squint, full cheeks, depressed nasal bridge, bulbous broad nose, long flat philtrum, retromicrognathia, bilateral low set small malformed ears with abnormal thick folding, short neck, wide space nipples (e, f). incomplete simian crease on left hand, talipes valgus, bilateral syndactyly between 3rd and 4th toes, and bilateral flat foot were noticed (g, h). MRI brain showed atrophic brain changes and hypogenesis corpus callosum (i, j).

Also, the data of the associated genes with Anonychia (HP:0001798) was retrieved from OMIM database under [anonychia OR "absent nails"] terms.

Insertion of all anonychia relevant genes in STRING server to explore the potential protein, protein interaction (PPI) enrichment analysis.

We convert the coordinates of deletions reported by genome build hg38 into hg19, (https://genome.ucsc. edu/cgi-bin/hgLiftOver).

RESULTS

Karyotype of patient No. 1 was 46,XY,del(8)(p21.2p23), with normal parents karyotypes. Karyotype of patient No. 2 was 46,XX,del(8)(p12p22) and parental karyotypes were normal.

FISH Using mix 8 [ToTel Vysion probe mixtures, Abbot], 8p spectrum green, 8q spectrum orange and 17p spectrum orange and spectrum green) showed normal signals (no subtelomere deletion) (Figure 2) for the two patients. Probe for chromosome 8 Locus Specific Identifier LSI (Abbot Laboratories, Illinois, USA) (8p22) (spectrum Orange) and (8q24.12-q24.13) (spectrum Green) showed a deletion of 8p22 signal (Figure 2), in patient 1.

Array CGH:

Array CGH demonstrated an interstitial deletion of 18.5Mb in 8p23.2p21.3. arr[hg19]8p23.2p21.3(4476000_23115000)x1 elicited involvement of *GATA4* and *MCPH1* genes in patient 1 (Figure 3a). An interstitial deletion of 25.6 Mb in 8p22p12 in patient 2 arr[hg19]8p22p12(14471078_40093664)x1 (Figure 3b).

Figure (4) represents a diagram for the extent of the deleted 8p region in our two patients in comparison to the findings of other authors.

Table (1): showing the clinical manifestations related to 8p deletions in our two patients and that reported by other authors.

Sequencing Data Analysis:

The sequencing data were analyzed by DNA Baser Sequence Assembler v4.7 (2014), Heracle BioSoft SRL, [www.DnaBaser.com]. The analysis revealed two variants; NM_001308093.3:c.1000+56C>A (rs804280) and NM_001308093.3:c.1132A>G (p.Ser378Gly; rs3729856) as shown in Figure (5a,b).

Pathogenicity of rs804280 and rs3729856

Using Ensemble Variant Effect Predictor (VEP), Mutation Taster (MT) and SKIPPY (The National Human Genome Research Institute, NIH) to predict effect of the

detected two variants, we found that rs894280 is intronic variant probably led to marginally splice site change, and it is not a conserved residue, so it may be considered as benign variant.

While rs3729856 led to change amino acid serine to glycine (Ser378Gly). this residue is highly conserved and predicted to change splice site to produce non-functional transcript because it causes loss three exonic splicing enhancers (ESEs) motifs, additionally it may lead to loss of phosphorylation legend in aa 406 else. According to alternative splicing effect of this variant, we submitted it previously in ClinVar database under accession SCV000172172.1 (2014) as likely pathogenic variant.

We explored the shared genes between our two patients, the obtained gene list was subjected to Venn classification. The Venn diagram showed that there are 47 shared genes between the studied two patients, beside 93 differential genes as shown in Figure (6).

Phenomizer-Human Phenotype Ontology (HPO) server (**Köhler *et al.*, 2021**) was used to annotate the observed patients' clinical features, where absent nails (Anonychia; HP:0001798) was mainly observed in patient 1. Anonychia wasn't committed with the relevant clinical features to the deleted regions, therefore, outlier shell reactants proteins were hypothesized.

Protein-Protein interaction (PPI) network construction (**Szklarczyk *et al.*, 2019**), to test a hypothesis that the outlier shell reactants proteins may be interpret the observed anonychia in patient no.1, further computational analysis was done using construct PPI network.

Data of the associated genes with anonychia was retrieved from OMIM database under [anonychia OR "absent nails"]. All the retrieved anonychia-relevant genes were input in STRING v11.5 server to explore the potential PPI enrichment analysis. We selected the interactions pertaining to Homo sapiens with highest interaction scores confidence= 0.9. Further, to obtain a better understanding of the PPI interaction network, we clustered the interactors using STRING k-Means clustering algorithm. Number of clusters was predetermined to be 2 clusters, based on the rule of thumb k= √(n/2) (**Kumar et al., 2020**), where n means number of nodes (protein interactors) in the network.

The STRING data showed that one of the differential genes at patient no.1, called *PINX1* (chr8:10,697,536-10,704,019/hg19), is enriched within anonychia relevant genes having PPI enrichment (*p*-value= 4.33e-15) with relevant significant enriched phenotypes that are; HP:0008398 [Hypoplastic fifth fingernail, *p*-value= 3.21e-13] and HP:0011937 [Hypoplastic fifth toenail, *p*-value= 6.38e-13].

PPI network showed that *PINX1* has highly experimental interaction score with *SMARCA4* and *SMARCB1* genes as 2nd shell and 3rd shell interactors respectively as shown in Figure (7). Both *SMARCA4* and *SMARCB1* genes are known to be associated genes with hypoplastic or absent nails features which included in Coffin-Siris syndrome type 3 and 4 diseases.

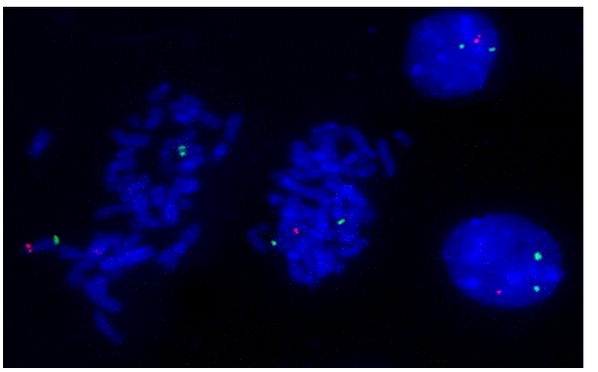

Figure 2: Patient 1: FISH using To Tel Vysion probe mixture 8 (8p spectrum green, 8q spectrum orange and 17p spectrum orange and spectrum green) showed normal signals, probe for chromosome 8 Locus Specific Identifier LSI (8p22) (spectrum Orange) and (8q24.12-q24.13) (spectrum Green) showed a deletion of 8p22 signal.

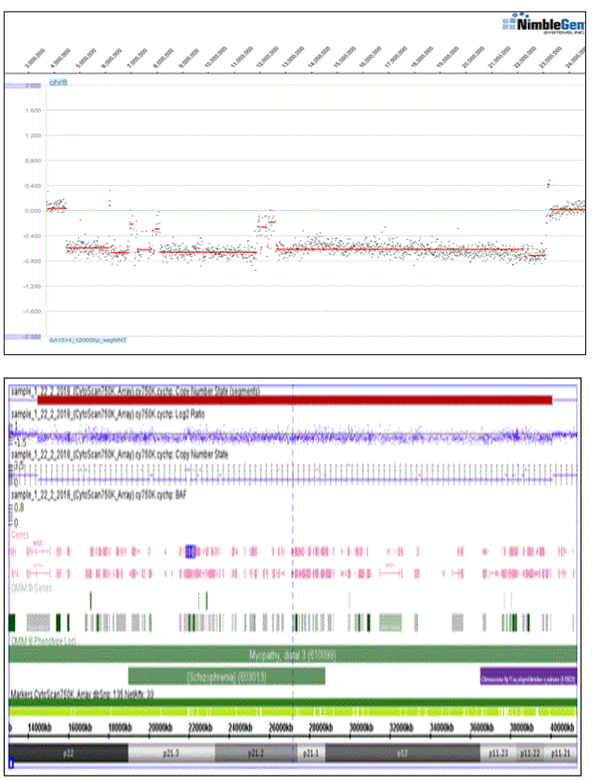

Figure 3: (a) Patient 1: Array CGH showing an interstitial deletion of 18.5Mb in 8p23.2p21.3. (3.b) Patient 2: Array CGH showing an interstitial deletion of 25.6 Mb in 8p22p12.

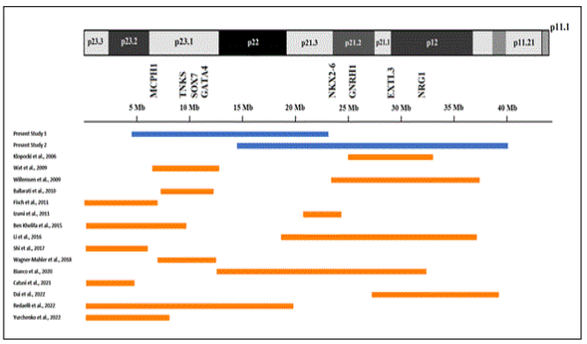

Figure 4: Illustration of the 8p regions showing relevant genes according to UCSC: CLDN23, Homo sapiens claudin 23. Blue bars describe deleted regions in our patients and orange bars in some published cases.

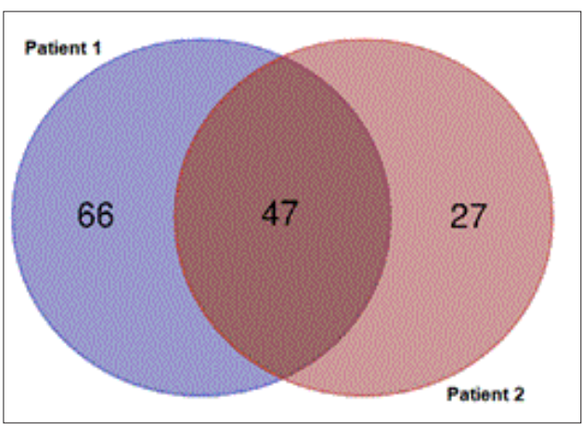

Figure 6: Showing the number of shared genes between patients 1 and 2.

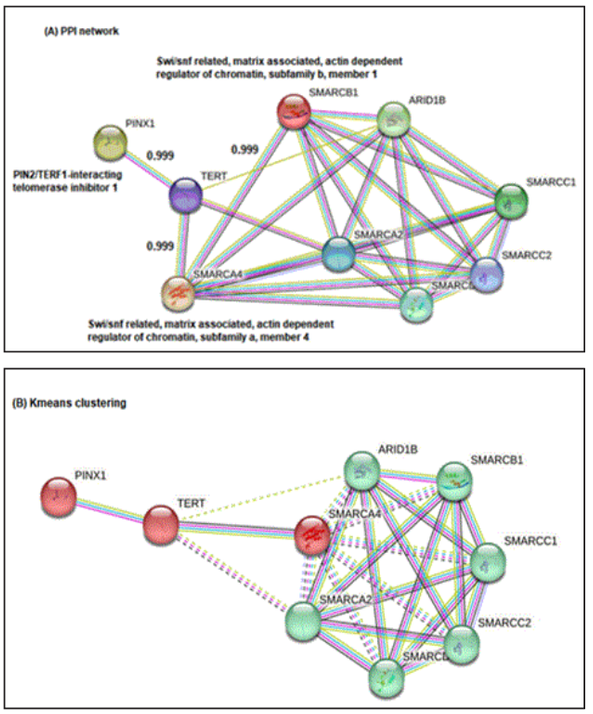

Figure 7: PPI network showing (A) the interaction scores between *PINX1* and its reactants, and (B) Kmeans clustering with two separate clusters, where each corresponding color represents a separate cluster. The two clusters showing that the interacted *PINX1*, *SMARCA4* and *SMARCB1* proteins were classified under separate clusters, but all were observed to be highly interconnected, reflecting a high degree of functional association and suggesting interplay between them.

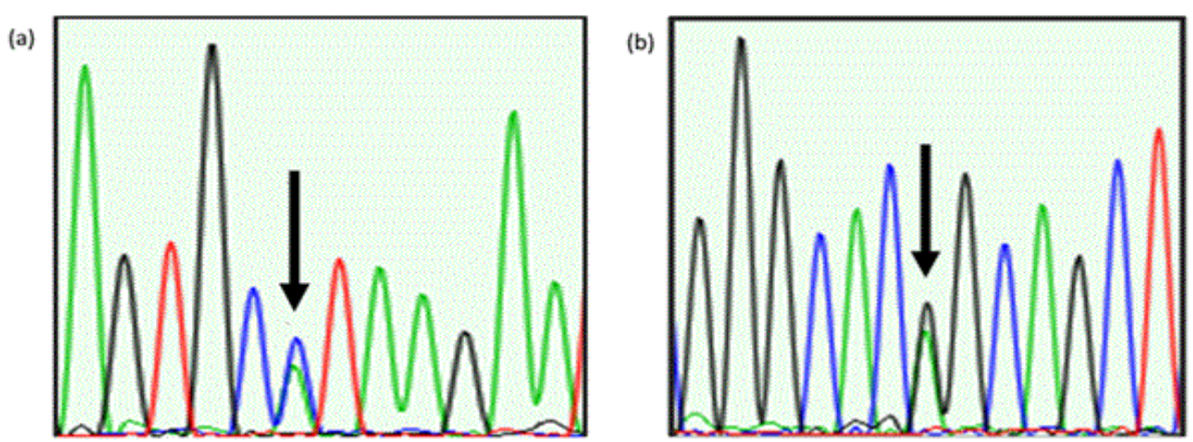

Figure 5: (a) Partial sequence chromatogram displaying the *GATA4* gene sequence in patient 1. The arrowed nucleotide indicates the position of the heterozygous intronic variant (NM_001308093.3:c.1000+56C>A); (b) Partial sequence chromatogram displaying the *GATA4* gene sequence of patient 1. The arrowed nucleotide indicates the position of the heterozygous variant (NM_001308093.3:c.1132A>G).

DISCUSSION

In this study we present two patients with interstitial 8p deletion; the first is a male patient presented with failure to thrive, recurrent chest infections and intractable seizures. He was dysmorphic with absent nails, congenital heart disease and hypogenesis of corpus callosum. Cytogenomic studies revealed 46,XY,del(8)(p21), array CGH revealed a 18.6Mb interstitial deletion. The second patient was a female presented with developmental delay, dysmorphic features, microcephaly and neurological abnormalities, MRI brain showed atrophic brain changes and hypogenesis of corpus callosum, Karyotype detected del(8) (p12), array CGH detected a 25.6Mb interstitial deletion.

For genotype phenotype evaluation and detection of the responsible gene for anonychia we divided the deleted regions in our patients into three parts. The 1st deleted region (OMIM: 65 entities) present in the patient one in 8p23.2p23.1 (4,476,000- 14,471,078), and not shared by patient two and includes four well known genes; *MCPH1* (chr8:6,264,148-6,506,029), *TNKS* (chr8:9,413,422-9,639,856), *SOX7* (chr8:10,581,278-10,588,021), and *GATA4* (chr8:11,534,444-11,617,511). The 2nd region shared between our two patients (OMIM: 63 intities), 8p22p21.2 (14,471,078-23,115,000). The 3rd region (OMIM: 102 entities), present in patient two and not shared by patient one in 8p21.2p12 (23,115,000-40, 093,664) and includes four important genes: *NKX2-6* (chr8:23,559,253-23,564,269), *GNRH1* (chr8:25,276,774-25,282,556), *EXTL3* (chr8:28,600,469-28,756,561), and *NRG1* (chr8:31,496,761-32,631,564), (*https://www.genecards.org/, GRCh37/hg19*).

Patients either have only 8p deletion or inv/dup del, the deleted regions always give its effect in the form of developmental delay, delayed speech, hypotonia, microcephaly, dysmorphic features, and when involve GATA4 gene it gives CHD.

We compared these three regions in our patients with the findings of other patients literatures and to patients reported in DECIPHER.

The 1st region (4,476,000-14,471,078), 8p21.3p23.2 which includes *GATA4* gene, the deleted regions include 380 patients in DECIPHER, the most important clinical manifestations reported in deleted 8p23.1p23.2 patients are congenital heart defect (CHD) in the form of atrial septal defect, atrio ventricular canal defect and tetralogy of Fallot. The other clinical manifestations are congenital diaphragmatic hernia, microcephaly, intellectual disability/developmental delay (ID/DD), delayed speech, pulmonary stenosis, abnormal pinna, low set ears, syndactyly, hypotonia, autism, hypoplasia of corpus callosum, cryptorchidism, hypospadias, attention-deficit/ hyperactivity, and aggressive disorders.

The 2nd region (8p21.2p22), (14,471,078-23,115,000) in DECIPHER the deleted regions include 100 patients, the most important clinical manifestations reported by DECIPHER in deleted 8p21.2p22 are atrial septal defect, ventricular septal defect, abnormal pinna, low set ears, ID, delayed speech, trigonocephaly, hypogenesis of corpus callosum, cryptorchidism, autism, shortening of distal phalanges, agenesis of permanent teeth, unilateral renal agenesis, behavioral abnormality and attention-deficit/ hyperactivity disorders.

The 3rd region (8p12p21.2), (23115000-40093664), DECIPHER reported 72 patients with deletions of this region, the main clinical manifestations are ID, delayed speech, obesity, microcephaly, blepharophemosis, scoliosis, seizure, dysmorphic features, behavioral abnormality, neurodevelopmental delay, syndactyly, tetralogy of Fallot, hypotonia, cryptorchidism, micropenis, abnormal pinna, abnormal ear, hypo genesis or agenesis of corpus callosum, hypertelorism, atrial septal defect, single transvers palmar crease, pulmonary stenosis, recurrent infection, abnormal erythrocyte morphology.

In DECIPHER they reported 26 patients with anonychia, only one patient (396051) with 8p23.1 deletion has anonychia.

Reviewing of the literatures for phenotypes related to each region revealed the following: in the first region. Patients either have only 8p deletion or inv/dup del, the deleted regions always give its effect in the form of developmental delay, delayed speech, hypotonia, microcephaly, dysmorphic features, and when involve *GATA4* gene it gives CHD.

Wat *et al.*, (2009) and **Ballarati *et al.*, (2011)** reported patients who had deleted segments shared with our first selected region that present in our first patient, they found nearly the same clinical manifestations.

Many authors reported deletion of 8p in patients who had deletion distal to *GATA4* gene, and they have no CHD, **Fisch *et al.*, (2011)** reported on 4 patients with inv/dup del 8p. Patients 1,2, and 4 had 6.9 Mb terminal deletion of 8p. the patients had neurodevelopmental manifestations with no CHD. **Khelifa *et al.*, (2015**) reported two female patients one with interstitial deletion of 8p23.1 and the other with del 8p23.1, both with no CHD as the deleted regions are distal to the *GATA4* gene.

Shi *et al.*, (2017) reported a boy patient and compared him with other 7 patients and all had isolated 8p23.2-pter deletions, they found the same clinical manifestations, but no CHD or congenital diaphragmatic hernia (CDH) and they identified the *DLG gene* as a candidate gene

for DD/ID, microcephaly and neurobehavioral disorders. **Wagner-Mahler *et al.,* (2019)** reported 46,XY interstitial del (8) (p23.1p23.1) presented with gonadal dysgenesis, microcephaly, with no characterized facial dysmorphology, its length was 5.6 Mb and contained the genes, *PINX1* (OMIM 606505), *SOX7* (OMIM 612202), and *GATA4*. **Lo Bianco *et al.,* (2020)** reported a female patient who had inv/dup del of 8p, the deletion was terminal 6,7Mb, she had developmental delay, delayed speech, hypotonia and dysmorphic features.

Catusi *et al.,* (2021) reported seven patients with 8p23.2-pter microdeletions, ranging from 71.79 kb to 4.55 Mb. They had variable clinical presentations including ID/DD, speech and language delay, autism, microcephaly, fingers/toes anomalies, epilepsy and dysmorphic features. All the patients had terminal deletion distal to our patient one deleted region except one patient with a very small overlap with our patient. **Yurchenko *et al.,* (2022)**, investigated 8 patients with inv/dup del of 8p. all had terminal deletion for approximately 7Mb, not involving *GATA4*. All patients were presented with a delay in psychomotor and language development, craniofacial dysmorphisms, and hypotonia. Five patients had agenesis/hypoplasia of the corpus callosum.

Redaelli *et al.,* (2022) reported 12 patients with rearrangement of chromosome 8p, ten patients had inv/dup del in chromosome 8p, with deleted segments in 8p23.1p23.3 ranged between 6-19 Mb, two patients had duplication of 8p23.3. They concluded that breakpoints seem more concentrated at three intervals in 8p, one at 8p23.3 and two in 8p23.1. The phenotype included congenital heart defect, developmental delay, facial dysmorphism, central nervous system abnormalities and hypotonia.

The second region (8p21.2p22) which is shared by our two patients and reported by **Klopocki *et al.,* (2006)**, who described a girl with interstitial 8 Mb deletion in 8p12p21.2, she has CHD and hypoplastic corpus callosum, also **Izumi *et al.,* (2011)** report a boy who has had a 3.6 Mb deletion of 8p21.2–21.3 between (20.7-24.2 Mb) from the 8p terminal. He had neurodevelopmental delay with delayed speech.

Li *et al.,* (2016) reported a female child with 18.5-Mb deletion at 8p11.23–p22 who had dysmorphic features and atrial septal defect, this region includes the cardiac-associated genes *NKX2-6* and *NRG1*. The deleted region reported by **Li *et al.,* (2016)** covered large segments in our regions 2 and 3. The patient had a clinical feature similar to our two patients. **Li *et al.,*** suggest that 8p21-8p12 may be another critical region for 8p-associated CHD, and some cardiac malformations might be due to *NKX2-6* haploinsufficiency. This deletion covers a large part of our second and third regions. Also, **Dai *et al.,* (2022)** reported a newborn female who had a deletion of 8p11.22-p21.2. She had developmental delays, microcephaly, ear anomalies, polydactyly, and gonadal hypoplasia. This large deletion covers our second and third regions and extended more proximal till 8p11.2.

Regarding the third region (8p12p21.2), (23115000-40093664), **Willemsen *et al.,* (2009)** reported the deletion of this region in two patients, the first was a boy with CHD, developmental delay, hypospadias and hypogenesis of corpus clausum, the second patient had developmental delay, dysmorphic features, without CHD or brain anomalies.

Mental impairment: *TNKS* gene is highly expressed in the brain, it is suspected to be related to mental impairment together with *PRAGMIN* and *CLDN23* genes that may contribute to neurodevelopment. Neurodevelopmental delay is well documented in all reported patients including ours. Meanwhile MRI brain anomalies documented in our two patients and in those of **Willemsen *et al.,* (2009); Li *et al.,* (2016); Lo Bianco *et al.,* (2020); Yurchenko *et al.,* (2022)**.

MCPH1 encodes Microcephalin 1, which have many functions in controlling telomere integrity regulation. Errors in spindle organization, premature chromosome condensation and misalignment were reported in the lack of Microcephalin 1 (**Liu *et al.,* 2016; Siskos *et al.,* 2021**).

Neurodevelopmental disorder and primary microcephaly: Liu *et al.,* (2021) confirmed *MCPH1* to be a candidate gene for neurodevelopmental *disorder* and primary microcephaly; having an important role in determining the size of the brain. Various *MCPH1* mutations were previously identified in microcephaly phenotype (**Trimborn *et al.,* 2004; Liu *et al.,* 2016**). *MCPH1* is within the deleted region in our presented patient 1.

CHD: The spectrum of CHD is more severe in interstitial and/or terminal deletions involving 8p23.1 region when compared to defects seen in patients with heterozygous *GATA4* gene mutations. That could be attributed to the deleterious effect of mutations of the other *GATA4* allele or may be due to the existence of other genes as *SOX7* in the deleted region which lies upstream of *GATA4*, as haploinsufficiency of *SOX7* gene impact heart development in individuals with *GATA4* deletion **(Digilio *et al.,* 1998; Tsukahara *et al.,* 1995; Wat *et al.,* 2009**).

Heart malformations are frequently described in patients with proximal 8p deletions that encompassed 8p21-p12 region (**Digilio *et al.,* 1998; Tsukahara *et al.,* 1995**) which could be considered another critical region accounting for CHD (**Li *et al.,* 2016**), specially affecting *NKX 2-6* genes (8p21.1) which were deleted in our two patients, however in patient No.2 no evidence of congenital heart defects was elicited, which agrees with other studies

as the rate of CHD in the deleted region encompassing 8p23.1 is much higher than in deletions involving 8p21.1 (**Willemsen *et al.,* 2009). Willemsen *et al.,* (2009)** referred to the non-penetrance of congenital heart defects or due to a multifactorial element. Additionally, *NRG1* was found to be involved in neural development as well as having notable role in many functions of nervous system (**Li *et al.,* 2016; Willemsen *et al.,* 2009**). Hence, **Li *et al.,* (2016)** postulated that *NRG1* haploinsufficiency may interpret the mental retardation in patients with 8p deletion.

We detected two genetic variants in *GATA4* gene. Using bioinformatics analysis by Ensemble Variant Effect Predictor (VEP), Mutation Taster (MT) and SKIPPY appeared that rs804280 considered as benign variant, but rs3729856 (p.Ser378Gly) is more likely affect splice sites. **Reamon-Buettner *et al.,* (2007)** found that rs3729856 affected septal defect cases in highly conserved amino acid (S378G), so it has been recommended to perform further study to measure effect S378G variant on protein expression. Furthermore, in 2013, revealed that there are a significant causative association for the rs3729856 A>G(p.S378G) (Odds ratio (95% confidence interval)= 1.76(1.19–2.61); p= 0.005) and the rs3729856_GG(5.09 (2.14–12.06); p <0.000001) with congenital heart disease.

rs3729856 variant was submitted in ClinVar database by eight submitters from 2014 to 2022, out of them seven submitters classified it as benign using clinical testing and one submitter classified it as likely pathogenic based on its splicing affect. However, further expression analysis is needed to classify it based on obvious experimental analysis evidence.

Hypogenesis of corpus callosum: Was reported in our 2 patients. **Vibert *et al.,* (2022)** reported 36 patients who had inv/dup del of chromosome 8p, Sixty-three percent of their patients presented with abnormal corpus callosum (AnCC), (n= 17/27). They tried to narrow down the region responsible for AnCC.The deleted segments in their patients are terminal and ranged from 5 to 7Mb, and they considered it not related to AnCC although they comment on other authors who found thinning of CC in patients with terminal deletions up to 7Mb. **Vibert *et al.,* (2022)** concluded that the duplicated regions are the responsible cause. They reviewed other patients with duplicated 8p and narrowed down the segment responsible for AnCC to be within 5Mb on 8p from 22,553,621 to 27,672,961 bp. Also, thy suggested that the *RHOBTB2* gene (chr8:22,844,764-22,877,712) is a candidate gene for AnCC. The formation of CC may be affected by gene dosage, deletion or duplication of a certain locus may affect the genesis of CC. Our two patients have hypogenesis of corpus callosum and share part with the suggested region as well as the suspected causative gene suggested by **Vibert *et al.,* (2022)**.

Absent nails (anonychia): It was reported related to deletion in 8p23.1 regions once before in a patient in DECIPHER (patient number 396051).

In the current study, PIN2/TERF1 interacting telomerase inhibitor 1 (*PINX1*) protein is one of differential proteins in patient 1 who has anonychia. Our PPI enrichment analysis showed that *PINX1* has highly confidential interaction score with *SMARCA4* and *SMARCB1* proteins as 2nd shell and 3rd shell interactors across Telomerase reverse transcriptase (TERT) at p-value= 4.33e-15 (**Maida *et al.,* 2014**).

The current computational analysis showed that TERT interacts directly with Both *SMARCA4* and *SMARCB1* at highly confidential scores. Interestingly, both *SMARCA4* and *SMARCB1* are involved in Coffin-Siris Syndrome 4; CSS4 (MIM#614609) and Coffin-Siris Syndrome 3; CSS3 (MIM#601607) respectively. Both *SMARCA4* and *SMARCB1* genes are known associated genes with hypoplastic or absent nails features which are included in Coffin-Siris syndrome.

In our patient anonychia may be due to the recessive inheritance due to haploinsufficiency deletion of *GATA4* and other allele mutation (rs3729856); serine to glycine [Ser378Gly]). This needs more data reporting. Also, we suggest that anonychia may be due to deletion of the *PINX1* gene which is involved through a network with other responsible genes that cause anonychia like *SMARCA4* and *SMARCB1* genes that are known associated genes with hypoplastic or absent nails features which are included in Coffin-Siris syndrome.

CONCLUSION

We predict that anonychia in our first patient may be due to deletion of *PINX1* gene, resulting in disturbance of interaction between *PINX1* and *SMARCA4* and *SMARCB1* across *PINX1*-TERT-SMARC crosstalk. This needs more reports. Also, anonychia may be due to deletion of one copy of *GATA4* gene and mutation in the other allele.

Comprehensive cytogenomic analysis, FISH, array CGH, gene sequencing and in silico analysis are crucial in diagnosis and to improve management in patients with complex phenotype of dysmorphic features, congenital anomalies and neurodevelopmental delay.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Signed informed consent for research and for publication from legal guardians of patients (the father) obtained. All methods were performed in accordance with the relevant guidelines.

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Medical Ethical Committee of the National Research Centre.

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CONFLICT OF INTEREST

There are no conflicts of interest.

AUTHORS CONTRIBUTIONS

AMM: Design and planning, interpretation of data, manuscript writing; **WMM:** Performed the FISH study, collection of data, share in writing the manuscript; **AF:** Performing the sequencing analysis and in silico analysis; **AE:** Collection of data and share in writing the manuscript; **EA:** Clinical examination and evaluation of patients; **MZ:** Clinical evaluation of patients and revision of the manuscript; **NH:** Cytogenetic and FISH evaluation; **OE:** Array CGH evaluation; **AKK:** Revision of the manuscript.

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