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Chitayat Syndrome: hyperphalangism, characteristic facies, hallux valgus, and bronchomalacia results from a recurrent c.266A>G p.(Tyr89Cys) variant in the ERF gene

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Abstract:

Background: In 1993, Chitayat et al., reported a newborn with hyperphalangism, facial anomalies, and diffuse bronchomalacia. We have identified three additional families with similar findings. Characteristic features include bilateral accessory phalanx resulting in shortened index fingers; hallux valgus; facial features including prominent eyes, hypertelorism, depressed nasal bridge and upturned nose; respiratory compromise due to bronchomalacia necessitating ventilatory support and pectus excavatum.

Objective(s): To identify the genetic aetiology of Chitayat syndrome and identify a unifying cause for this specific form of hyperphalangism.

Methods: Through ongoing collaboration, we had collected patients with strikingly similar phenotype. Trio-based exome sequencing was first performed in Patient 2 through the Deciphering Developmental Disorders study. Putative de novo variants were identified from exome data using DeNovoGear software and validated using Sanger sequencing. Proband-only exome sequencing had previously been independently performed in Patient 4. Following identification of a candidate gene variant in Patient 2, the same variant was subsequently confirmed on analysis of exome data in Patient 4. Sanger sequencing was then used to validate the candidate gene variant in Patients 1 and 3 and to confirm paternal inheritance in Patient 5.

Results: A recurrent, novel variant NM_006494.2:c.266A>G p.(Tyr89Cys) in ERF was identified in five affected individuals: de novo (Patient 1, 2 and 3) and inherited from an affected father (Patient 4 and 5). p.Tyr89Cys is an aromatic polar neutral to polar neutral amino acid substitution, at a highly conserved position and lies within the functionally important ETS-domain of the protein. This variant has not previously been reported (1000 Genomes, dbSNP build 144, EVS or ExAC). The recurrent ERF c.266A>C p.(Tyr89Cys) missense variant causes Chitayat syndrome.

Discussion: ERF has been shown to suppress ets-induced transformation and be regulated by phosphorylation throughout the cell cycle via Ras/ MAPK signalling pathway. ERF variants

have previously been associated with complex craniosynostosis. In contrast, none of the patients with the c.266A>G p.(Tyr89Cys) variant have craniosynostosis.

Conclusions: We report the molecular aetiology of Chitayat syndrome characterised by the triad of hyperphalangism, characteristic facies and respiratory abnormalities. We discuss potential mechanisms for this distinctive phenotype associated with the p.Tyr89Cys substitution in ERF and explore why this variant does not present with craniosynostosis.

Keywords: ERF, hyperphalangism, bronchomalacia, craniosynostosis, Chitayat syndrome

Introduction

Hyperphalangism is a term used to describe the presence of an additional phalanx between the phalanges of the fingers (except the thumb where an extra phalanx is labelled as a 'triphalangeal thumb'). Leboucq of Belgium was first to describe hyperphalangism in 1869 based on anatomical observation [1]. Both males and females are equally affected and hyperphalangism is almost always bilateral. The main difficulties associated with this finding are the inability to use the index finger because of ulnar deviation and overlapping of other digits necessitating surgical correction. Hyperphalangism is often but not invariably associated with involvement of the feet including congenital talipes equinovarus (clubfoot), brachyphalangism, and clinodactyly amongst other deformities. There are 38 syndromes reported with accessory phalanges in the London Dysmorphology database (LMD v1.036) [2].

Chitayat syndrome is a rare condition associated with hyperphalangism, and respiratory distress presenting at birth [3]. Tanaka et al., 1994 published a patient with similar clinical features [4], although it is not clear if the patient reported has the exact same features as seen in Chitayat syndrome and no follow-up data is available for comparison. Low et al., 2013 published a mother-daughter pair that presented with similar hand and foot deformities, pectus excavatum, recurrent respiratory infections and tracheomalacia (in the daughter alone); however, consent for facial photos were declined and no molecular aetiology was identified [5].

Other conditions that present with hyperphalangism include Catel-Manzke syndrome, Desbuquois syndrome, Temtamy (1988) with brachydactyly, hyperphalangism, deafness, intellectual disability amongst others. Catel-Manzke syndrome is associated with brachydactyly, hyperphalangism, cleft palate and micrognathia (OMIM #616145) and is associated with homozygous or compound heterozygous mutations in the TGDS gene [6,7]. Desbuquois syndrome is associated with hyperphalangism and joint dislocations (OMIM #251450 and #615777) and is caused by mutations in the CANT1and XYLT1genes [8,9]. Temtamy hyperphalangism (OMIM #605282) was reported to be caused by mutations in the CHSYl gene [10,11]. These hyperphalangy genes encode enzymes that appear to be involved in proteoglycan synthesis or sulfation.

Hallux valgus can present in association with Fibrodysplasia ossificans progressiva (OMIM #135100) caused by heterozygous variants in the ACVR1 gene and Van Maldergem syndrome (OMIM #601390) caused by homozygous variants in DCHS1 gene amongst others but additional characteristic features would help define these conditions and distinguish them from Chitayat syndrome [12,13].

Here we report five individuals from four unrelated families who present with the characteristic triad of hyperphalangism, facial dysmorphism and respiratory involvement as reported by Chitayat et al., 1993 [3] and provide the first evidence of the molecular aetiology for this rare form of hyperphalangism.

Clinical Report:

Patient 1:

This is the first reported patient [3]. This patient was the second child of healthy, non-consanguineous, French-Canadian parents and his family history was non-contributory. He currently has two siblings who are well. He was born following an uncomplicated pregnancy at 39-weeks gestation with a birth weight of 3.55 kilograms (75th-90th centile) and presented with severe respiratory distress with no evidence of diaphragmatic eventration needing assisted

ventilation for 10 days. On examination, soon after birth, he was noted to have: hyperphalangism, facial anomalies (prominent eyes with hypertelorism, a depressed nasal bridge, short pointed nose with anteverted nostrils, a long philtrum and full lips), hallux valgus, and bronchomalacia identified on CT-chest scan. A skeletal survey confirmed the above skeletal findings and his early development was reported as normal. A small atrial septal defect identified shortly after birth did not necessitate any intervention. Chromosome analysis showed a 46, XY karyotype.

Subsequent follow-up showed normal growth but delayed developmental milestones with walking at 18 months and at 4 years, gross and fine motor skills were a year behind his chronological age. Expressive speech delay was also noted with first words at 2.5 years of age. His receptive language was within the normal range. Audiology assessment revealed mild conductive hearing loss and hearing aids were prescribed. He required speech therapy initially but this has resolved with no evidence of significant learning difficulties.

In terms of his pulmonary phenotype, for the first five years of life, he experienced respiratory difficulties secondary to his bronchomalacia. He required home oxygen supplementation during the first 18 months of his life. During this period, he had episodes of severe respiratory infections and severe bronchospasm which required repeated admissions to intensive care. His respiratory condition gradually improved and he currently has obstructive pulmonary disease [decreased FEV_1 of 2.3 liters and normal FVC of 4.4 liters with FEV_1/FVC of 52% (normal range >80% FEV1/FVC ratio)] with no response to bronchodilators and mild exertion dyspnea. His most recent chest CT-scan performed at 18-years of age was unremarkable.

The hyperphalangism involving the 2nd finger of his right hand required surgical correction of this finger and the soft tissue syndactyly between the 1st and 2nd fingers on both hands were repaired resulting in normal function.

On recent evaluation at 21-years of age, he had similar facial dysmorphism and surgically corrected limb deformities as described before (Figure 1a-d). His growth parameters were height~ 168 cms (10th centile), weight~ 75 kilograms (50-75th centile) and head circumference~

58 cms (50th-75th centile) with no clinical evidence of craniosynostosis. At the age of 21, he is currently studying at University and doing well academically.

Patient 2: This patient was the first child of healthy, non-consanguineous Caucasian parents with no significant family history. She has a younger sibling who is well. In the pregnancy, polyhydramnios was identified at 28[+4] weeks gestation. The pregnancy was closely monitored until spontaneous labour at 32[+1] weeks gestation. No antenatal steroids were administered prior to delivery. She was born with a birth weight of 2.2 kilograms (91st-98th centile) and was in a poor condition at birth with an APGAR score of 2 and 8 at 1 and 5 minutes respectively. She required resuscitation and mechanical ventilation with no evidence of diaphragmatic eventration. She received two doses of surfactant and required ventilatory support for one month. Investigations in the neonatal period included: normal cranial and renal ultrasound, TORCH screen and sweat test. An echocardiogram identified a 3-4mm wide patent ductus arteriosus (PDA) which was treated with Ibuprofen. She was discharged home on nasal cannula, oxygen and diuretics at 58-days of age.

Growth parameters, at 2-months of age (corrected gestation of 39[+3]-weeks) were weight 2.49 kilograms (2nd-9th centile), length 48.5 cms (50th centile) and head circumference 33.5 cms (9th-25th centile). At 29-weeks of age (corrected 21-weeks), weight was 5.47 kilograms (2nd centile), length 62 cms (25th-50th centile) and head circumference 42 cms (25th-50th centile).

Genetics evaluation at 5-months of age noted greyish-blue sclerae, hypertelorism, and depressed nasal bridge with upturned nose, short index fingers bilaterally with ulnar deviation and bilateral hallux valgus. All growth parameters were on the 9th-25th centile and she was still on low-flow oxygen in view of chronic lung disease. Developmentally, she could roll over from back to front, had a palmar grasp and was babbling. Skeletal survey showed a bony spur arising from the lateral end of the proximal metaphysis of the proximal phalanx of the index finger, the proximal phalanges of her toes were dysplastic with bilateral hallux valgus (Figure 2d). The rest of her skeletal survey and her bone density were normal. Chest X-ray showed diffuse changes bilaterally consistent with pulmonary interstitial emphysema. Chromosome analysis showed a 46, XX karyotype.

At 14-months of age, she had been weaned off home oxygen. Developmentally, she was able to sit on her own, crawled and had a few words. She was recruited to the Deciphering Developmental Disorders (DDD) research study [DECIPHER ID: DDD-SGS266723].

On recent evaluation at 5.5 years of age, her weight was 15 kilograms (9th centile), height was 105 cms (9th centile) and head circumference was 50.5 cms (9th centile). She has noisy nocturnal breathing and suffered from recurrent chest infections needing prolonged treatment with antibiotics. She had surgical correction of her 2nd fingers and both big toes resulting in normal function. She attends a mainstream school with no learning difficulties and there were no concerns regarding her speech. She had similar facial dysmorphism and limb deformities as noted previously (Figure 2a-c). She was also noted to have a pectus excavatum but no clinical evidence of craniosynostosis. Following the identification of the ERF variant, she was assessed by the Craniosynostosis service and a CT-head scan showed no evidence of craniosynostosis.

Patient 3: This patient is a 6-year old boy, second child of healthy, non-consanguineous Caucasian parents. Family history was complicated by four miscarriages in the mother with no identifiable cause. He has two healthy siblings. In the pregnancy, which was conceived on Clomiphene, there was polyhydramnios detected in the third trimester of pregnancy. He was born at term with a birth weight of 3.74 kilograms (50th-75th centile). He was in a poor condition at birth with APGAR scores of 2, 6 and 8 at 1, 5 and 10 mins respectively. He was ventilated and placed on a high-frequency oscillation ventilator for significant respiratory distress, with no evidence of diaphragmatic eventration. As a newborn, he continued to remain ventilated until day 19 when he was first evaluated by Genetics.

Genetics evaluation noted severe hypotonia with pectus excavatum, large anterior fontanelle, facial dysmorphism with hypertelorism, depressed nasal bridge with upturned nose, brachydactyly with marked symmetric shortening of index and middle fingers on both hands and bilateral hallux valgus. Growth parameters tracked on the 50th centile for length, 25th centile for weight and 25th-50th centile for head circumference. Following discharge, he continued to have hypotonia, which gradually improved over time. He was unable to lift his arms at 4 months of

age and demonstrated gross motor delay as a young child. His medical history has been complicated by multiple respiratory infections, requiring oxygen supplementation. At 3 years of age, he had an RSV infection, which led to an intensive care unit admission with acute cardiomyopathy and ventricular tachycardia.

On recent evaluation at 6-years of age, his height and head circumference were on the 10th centile with weight on the 3rd centile. He was noted to have shortened second digits bilaterally, as well as shortened and laterally-deviated first toes bilaterally. He had a significant pectus excavatum and high-arched palate (Figure 3a-d). His hand and feet X-rays showed accessory phalanx with medial deviation of the index fingers bilaterally and bilateral hallux valgus (Figure 3e-f). He also had facial dysmorphism very similar to the other patients in the cohort. There are no particular concerns regarding his development.

Investigations including bone age, karyotype (46,XY), and a SNP microarray were normal. Metabolic testing was not informative. Sanger sequencing confirmed the same recurrent ERF variant in this patient with no evidence of craniosynostosis.

Patient 4 and 5: This patient (Patient 4) was the second child to parents of mixed European and Mexican ancestry. His sibling is well. The patient's mother has a history of two miscarriages. The patient's father (Patient 5) was hospitalised on several occasions in the first year of life for a total of three weeks, required ventilatory support and was diagnosed with "chronic lung disease". He was noted to have a pectus excavatum, hallux valgus and brachydactyly.

Pregnancy was complicated by maternal hypothyroidism needing replacement therapy and polyhydramnios was noted at 34 weeks gestation. The patient was born at 38^{+5} weeks gestation by caesarean section due to fetal distress with a birth weight of 2.99 kilograms (9th centile). Respiratory distress and hypotonia were noted at birth and the APGAR scores were 2, 6 and 8 at 1, 5 and 10 minutes, respectively. The patient was initially treated with CPAP and on day three of life required intubation and mechanical ventilation, with no evidence of diaphragmatic eventration.

At 10 days of age, his weight was 2.95 kilograms (10-50th centile), length 49 cms (10-50th centile) and head circumference 35 cms (50th centile). On examination after birth, he was noted to have normal tone and overfolded ear helices with hypertelorism, broad nasal root and a small nose. He had brachydactyly with fifth finger clinodactyly, mild pectus excavatum and bilateral hallux valgus with splayed second and third toes and broad distal phalanges.

Following failed extubation attempts, bronchoscopy at 2 weeks of life showed severe tracheo- and broncomalacia. Tracheostomy was performed at 2-3 months of age along with an insertion of gastrostomy tube and Nissen fundoplication.

Lung biopsy at 6-weeks of age showed irregularly enlarged and poorly septated alveoli. These findings were felt to be mild to moderate in severity. Skeletal survey at age 2 months showed small proximal phalanges of the first toes and brachydactyly on both hands with especially small middle phalanges of the second and fifth fingers (Figure 4a-b). Bilateral hydroceles were noted at five months of age that resolved spontaneously.

Despite some initial progress in the early years, he required repeated ventilatory support following severe decompensation with respiratory infections. On recent evaluation at five years of age, he was on ventilatory support only at night via his tracheostomy. His height and weight have tracked between the 5th and 10th centile with the head circumference between 10th to 15th centile. He has global developmental delay and first walked at 3-4 years of age with an unsteady gait. Although his receptive language was thought to be appropriate for age, he has very limited expressive language and speaks only a few words.

Investigations including chromosomal microarray, plasma amino acids, urine organic acids and acylcarnitine profile, were normal.

Clinical evaluation of this patient's father (Patient 5) at age 40 showed pectus excavatum, bilateral hallux valgus and brachydactyly of the hands. He has not experienced significant respiratory disease since infancy. He has myopia since age 11. His skeletal survey at age 40 showed slightly shortened ulnae with mild radial bowing, brachydactyly with pronounced

shortening of the middle phalanges of the second and fifth digits and brachydactyly of the feet with pronounced shortening of the proximal phalanx of the first toe (Figure 4c-d).

Material and Methods:

Trio-based exome sequencing was performed for Patient 2 and her parents as part of the Deciphering Developmental Disorders study, as previously described [14]. Putative de novo variants were identified from exome data using DeNovoGear software [15] and were validated using targeted Sanger sequencing. Analysis revealed a candidate de novo variant in ERF. This was validated using targeted Sanger sequencing. Primers used for sequencing ERF exon 3 included: FWD: 5'-GTGTTAAGGTGTGGAGTCTAGACCTGGG-3'
REV: 5'-GAAGAGGGAAGATGAAGATGAAGAGC-3'

Patient 4 had previously undergone clinical whole exome sequencing and analysis of data identified the same ERF variant found in patient 2. Targeted Sanger sequencing of the ERF variant was performed on Patient 4 and his parents to evaluate inheritance; in the original patient described by Chitayat et al., 1993 (Patient 1) and an un-related patient identified through presentation with a similar phenotype (Patient 3).

Results:

Patient 1: Sanger sequencing identified a heterozygous variant NM_006494.2 ERF: c.266A>G p.(Tyr89Cys) (hg19.chr19:42,754,086 T>C). Sanger sequencing in the parents showed that the variant is de novo in this patient.

Patient 2: Trio whole exome sequencing identified a de novo heterozygous variant in ERF: c.266A>G p.(Tyr89Cys). Sanger sequencing of the patient and her parents confirmed the variant is de novo.

Patient 3: Sanger sequencing identified the same heterozygous variant in ERF: c.266A>G p.(Tyr89Cys). Sanger sequencing in the parents showed that the variant is de novo.

Patient 4 and 5: Analyses of clinical whole exome data showed a variant in ERF: c.266A>G p.(Tyr89Cys). Sanger sequencing was used to confirm this variant in the patient and his affected father, indicating autosomal dominant paternal inheritance.

Figure 6 shows the ERF sequence variant in patient 2, location of the variant within the ERF polypeptide and multiple sequence alignments demonstrating conservation of the Tyr89 in orthologues and other human ETS family members. Investigations into the pathogenicity of this variant showed that it had not been reported in the literature either as a pathogenic variant or a neutral polymorphism. This variant has not been reported previously in 1000 Genomes, dbSNP (build 144), the NLHBI GO Exome Sequencing project (ESP) or by the Exome Aggregation Consortium (ExAC) [16-19]. In silico prediction tools, including align GVGD, SIFT and Mutation taster, predict that this variant would be having a deleterious effect on the protein. (Alamut Visual version 2.7: Interactive Biosoftware, Rouen, France), providing further evidence regarding causality.

Discussion:

In 1993, Chitayat et al., [3] reported a newborn with hyperphalangism, facial anomalies, and diffuse bronchomalacia. We have identified three additional families with similar findings. Phenotypic features include respiratory compromise, bilateral accessory phalanx resulting in shortened index fingers, hallux valgus and characteristic facial features including prominent eyes, hypertelorism, depressed nasal bridge, full lips and upturned nose. A recurrent ERF: c.266A>G p.(Tyr89Cys) missense variant was identified in these four unrelated families supporting the conclusion that this particular ERF variant is the cause of Chitayat syndrome. Here we first report the molecular aetiology of Chitayat syndrome presenting with the characteristic triad of hyperphalangism, characteristic facial appearance and respiratory system abnormalities.

The ERF (Ets2 Repressor Factor) protein is an ETS-domain protein first described by Sgouras et al., 1995 and was isolated due to its interaction with the ets binding site (EBS) within the ETS2

promoter [20]. It is the first mammalian member of this family identified that showed strong transcriptional repressor activity [21]. The ETS family of genes share a common DNA binding domain that recognises a purine-rich sequence and have been implicated in a number of biological processes, most commonly the reproductive and immune systems as well as in tumorigenesis. ERF has been shown to suppress ets-induced transformation. It is regulated by phosphorylation throughout the cell cycle and during mitogenic stimulation via the Ras/ MAPK signaling pathway [22]. It is ubiquitously expressed and is analogous to the only other ETS-domain protein that has transcriptional repressor function, the Drosophila gene, Yan [23]. There is a high degree of conservation across the species not just in terms of the structure and sequence of ERF but also in their promoter region [24]. However, outside of the highly conserved ETS binding domain, it does not share a substantial homology to other known members of the ETS family, except ETV3.

The human ERF gene is mapped to 19q13.2 and comprises of 4 exons. It is a ubiquitously expressed ETS transcription factor and acts as a negative regulator either by competing with other ETS family members for DNA binding or by interacting with other unique targets [20,25]. ERF is a potent transcriptional regulator [26] and appears to regulate genes involved in cellular proliferation. ERF activity is regulated by phosphorylation. It has also been shown to be essential for chorion cell differentiation and has been implicated as the first gene to affect the ecto-placental cone cavity closure in mouse embryonic development [21].

Pathogenic variants in ERF have been associated with complex craniosynostosis. Twigg et al., 2013 identified a diverse range of heterozygous variants in the ERF gene as a novel cause of syndromal craniosynostosis (Craniosynostosis 4) [27]. All the variants were reported to result in heterozygous loss-of-function i.e. haploinsufficiency of the ERF protein. Since then, there have been other reports of ERF-related craniosynostosis showing inter- and intra-familial variability in the clinical manifestations with a high penetrance; craniosynostosis may not be present in all family members [28, 29]. ERF variants are commonly found in association with multi-suture craniosynostosis; however, generally without involvement of the coronal suture, which is unusual as the coronal suture is most commonly affected in most monogenic forms of craniosynostosis. In the series reported by Twigg et al., 2013, 3/14 patients in the paediatric age

group with ERF variants had multi-sutural synostosis with involvement of the coronal suture as did both patients with ERF variants reported by Chaudhry et al., 2015 [27, 29].

The p.Tyr89 is highly conserved across species (down to C.elegans) and across ETS subfamilies in humans [24]. Members of the ETS family play an important role in normal cell proliferation and differentiation; they regulate gene expression by binding to a consensus DNA sequence through the highly conserved DNA binding domain (the ETS-domain). The p.Tyr89 variant is located within this functionally important domain, just outside of the helix-3 region. X-ray and NMR studies of another member of the ETS family, ETS1, showed that helix 3 binds to the major groove of the consensus DNA sequence [28]. A different amino acid substitution at the equivalent residue to Tyr89 in the C.elegans ETS domain protein, LIN-1, (p.Tyr127Phe), that results in a multivulval phenotype, showed very weak DNA binding [30].

Of significance, none of the patients with the c.266A>G p.(Tyr89Cys) variant in ERF were found to have evidence of craniosynostosis despite investigations including CT-scans of the skull. Hence, this recurrent variant appears to only be associated with a hyperphalangism phenotype rather than a craniosynostosis phenotype. Interestingly, other nearby residues within the ETS-domain including p.(Arg65Gln) and p.(Arg86Cys) have been reported in association with craniosynostosis [27]. These also affect highly conserved residues within the DNA binding domain. Site-directed mutagenesis of equivalent or adjacent residues in other ETS-domain proteins abolishes DNA binding indicating that these mutations cause loss of protein function [31]. This is supported by functional studies that show that these mutations fail to repress promoters containing an ETS-binding site [27]. Deletions of the ERF gene have not so far been reported in craniosynostosis cases. However, patients with microdeletions that encompass ERF do show craniosynostosis indicating that the craniofacial phenotype may be caused by haploinsufficiency of ERF [32]. It is currently unclear why the p.Tyr89Cys variant produces a different phenotype, and further studies are required to determine its effect on DNA-binding.

Table 1 reports the common and distinguishing features in this cohort. The five patients reported (Patient 1-4 and father of Patient 4) all appear to have the same clinical phenotype comprising accessory phalanges at the base of the index fingers resulting in ulnar deviation and shortened

index fingers bilaterally, bilateral hallux valgus, similar facial features and abnormalities of the respiratory system including bronchomalacia/tracheomalacia, complicated by recurrent respiratory infections necessitating a period of ventilatory support. Other common features include, polyhydramnios (seen in 3 of 4 patients) and hyperphalangism not only of the index fingers but also the middle fingers (seen clinically in Patient 1 and in the X-rays of Patient 3). There was no evidence of diaphragmatic eventration in any of the patients reported here. However, as is evident from Patient 1 and Patient 5 (father of Patient 4) who are the oldest patients in this cohort, the condition does not necessarily result in long term respiratory dysfunction. All patients displayed a pectus excavatum.

Intelligence was reported to be normal in all patients except Patient 4 in whom the developmental delay could be secondary to his severe and prolonged respiratory complications. The characteristic facial features are striking especially when Patient 1, 2 and 3 were compared at the same age (Chitayat et al., 1993; Patient 2 and Patient-3 at 5-months of age- Figure 5). The common facial features consisted of prominent eyes with hypertelorism, depressed nasal bridge, short columella, high arched eyebrows, an upturned nose and full lips. As previously described, the phenotype of the mother-daughter pair reported by Low et al., 2013 are similar to the cohort here but the facial dysmorphism is dissimilar (Personal Communication). No DNA samples are currently available for genetic analysis [5].

As this ERF variant has not been previously reported in association with craniosynostosis, it appears that it has a different effect on the protein and thus results in a very specific phenotype. There are several examples of distinct phenotypes resulting from specific mutations in the same gene. One example includes variable phenotypic presentation of variants in the MSX2 gene, a homeodomain transcription factor, in which heterozygous, loss-of-function variants, result in skull defects, specifically, enlarged parietal foramina [33]. These variants seem to occur throughout the gene and include missense, nonsense, splicing and frameshift changes. There is however, specific missense changes occurring at a single amino acid in MSX2 (p.Pro148His and p.Pro148Leu), which have been reported in association with Boston-type craniosynostosis [34, 35]. This is a highly penetrant, autosomal dominant disorder leading to variable degrees of craniosynostosis. The p.Pro148His variant has subsequently been shown to have a dominant

negative effect on the MSX2 protein, which explains the different phenotypic presentation seen [36]. It is therefore probable that this ERF variant is having a very specific, possibly gain-of-function effect on the ERF protein, as compared to the ERF variants that have been reported in association with ERF-related craniosynostosis which lead to haploinsufficiency; however, further functional studies will be required to determine the precise mechanism of action.

Our report confirms that Chitayat syndrome as a specific clinical entity with an autosomal dominant pattern of inheritance and inter- and intra-familial variability in clinical manifestations. We have both confirmed and expanded the clinical manifestations of this condition and documented its association with a recurrent variant in the ERF gene. Further reports will enable additional delineation of this rare and important form of hyperphalangism.

Statements:

We would like to thank all the local clinicians and other healthcare professionals involved with these patients. Finally, we would of course especially like to thank all these families for collaborating with this project and consenting to publication here.

C. Contributorship Statement:

MB planned the study; recruited Patient 2 to DDD; wrote manuscript. HL, HG, AMW, TL undertook sequencing and analyses. SL, FT, GS recruited Patient 1, JAB and DLS recruited Patient 4 and 5. HG, AMW and GB conducted analysis of exome sequencing data for patient 4. DSJ, JB clinical care of Patient 2. ARC, DHV recruited Patient 3. DDD Study undertook exome sequencing of Patient 2. JAB and DC senior authors conceived the study, assembled the patient cohort and prepared the manuscript; all authors reviewed and contributed to the manuscript.

D. Competing Interest: M. Balasubramanian: None. J.A. Bernstein: None. H. Lord: None. S. Levesque: None. F. Thuriot: None. A. Wenger: None. G. Sillon: None. H. Guturu: None. D.L. Sureka: None. G. Bejerano: None. T. Lester: None. A. Calhoun: None. D. Viskochil: None. D. Chitayat: None.

Figure Legends:

Figure 1a-d: Patient 1 showing facial features as an adult and hand deformities.

Figure 2 a-d: Patient 2 showing similar facial features to Patient 1 with hand and feet deformities; Figure 2d shows a bony spur arising from the lateral end of the proximal metaphysis of the proximal phalanx of the index finger.

Figure 3a-d: Patient 3 showing similar features to Patient 1 and 2 with shortened second digits bilaterally and shortened, laterally-deviated first toes bilaterally. He has a significant pectus excavatum.

Figure 3e-f: X-rays of hands and feet of Patient 3 showed similar findings to other patients in the cohort i.e. accessory phalanx with medial deviation of the index fingers bilaterally and bilateral hallux valgus.

Figure 4: X-rays of hands and feet of Patient 4 and 5. Figure 4a does not show hyperphalangism in Patient 4. His father's hand x-ray (Figure 4c) shows an unusual shape of the proximal end of the proximal phalanx of the index finger, rather than true hyperphalangism. Both patients show bilateral hallux valgus.

Figure 5: Comparison of facial dysmorphism of Patient -2 with Patient-1 and Patient -3 at the same age as an infant demonstrating prominent eyes with hypertelorism, depressed nasal bridge, short columella, high arched eyebrows, an upturned nose and full lips.

Figure 6: A: Schematic showing the ERF polypeptide including the position of the p.Tyr89Cys variant; B: Conservation of p.Tyr89 (marked with an arrow) and the surrounding amino acids; C: Sequencing electropherogram for Patient 2 compared with a normal control. The heterozygous c.266A>G p.(Tyr89Cys) variant in the patient is marked with an arrow.

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Table 1

Patient Number	Patient 1(Chitayat et al., 1993)	Patient 2	Patient 3	Patient 4	(Patient 5)Patient 4' father
Age in years	18	5.5	6	5	40
Polyhydramnios in pregnancy	-	+	+	+	U
Facial dysmorphism	+	+	+	+	+
Respiratory distress	+	+	+	+	+
Bronchomalacia	+	+	+	+	+
Interstitial lung disease	+	+	+	+	+
Pectus excavatum	+	+	++	+	+
Brachydactyly	+	+	+	+	+
Hallux valgus	+	+	+	+	+
Hyperphalangism index finger ± middle finger	+	+	+	-	-
Other digital anomalies	+	-	-	-	-
Other anomalies	Umbilical hernia, hypospadias	None	High-arched palate	Cleft palate	Webbed neck
Development	Early language and motor delay, now resolved	Normal	Normal	Moderate expressive language delay, motor delay	Normal
Craniosynostosis	-	-	-	-	-

+: Present; -: absent; NR: Not reported ; U: Unknown