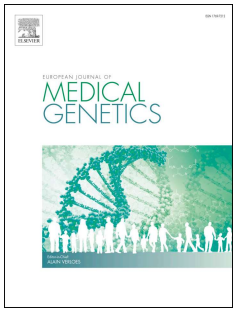

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PII: S1769-7212(20)30282-2

DOI: https://doi.org/10.1016/j.ejmg.2020.104009

Reference: EJMG 104009

To appear in: *European Journal of Medical Genetics*

Received Date: 31 March 2020

Revised Date: 5 June 2020

Accepted Date: 10 July 2020

Please cite this article as: M. Aerden, M. Bauters, K. Van Den Bogaert, J.R. Vermeesch, M. Holvoet, P. Frank, K. Devriendt, Genotype-phenotype correlations of *UBA2* mutations in patients with ectrodactyly, *European Journal of Medical Genetics* (2020), doi: https://doi.org/10.1016/j.ejmg.2020.104009.

Genotype-phenotype correlations of *UBA2* mutations in patients with ectrodactyly

Mio Aerden[1], Marijke Bauters[1], Kris Van Den Bogaert[1], Joris R. Vermeesch[1], Maureen Holvoet[1], Plasschaert Frank[2], Koenraad Devriendt[1]

1. *Center for Human Genetics, University Hospital Leuven and KU Leuven, Leuven, Belgium*
2. *Department of Physical Medicine and Orthopaedic Surgery, Ghent University, Ghent, Belgium*

Abstract

Interstitial 19q13.11 deletions are associated with ectrodactyly, which has recently been linked to loss-of-function of the *UBA2* gene. We report a boy with a de novo frameshift mutation in *UBA2* (c.612delA (p.(Glu205Lysfs*63)), presenting with ectrodactyly of the feet associated with learning difficulties and minor physical anomalies. We review genotype-phenotype correlations in patients with chromosomal 19q13.11 microdeletions compared to those with intragenic *UBA2* mutations.

Introduction

Ectrodactyly is a clinically and etiologically heterogeneous condition characterized by median clefts of the hands and/or feet due to the absence of the central digital rays. Other features include aplasia or hypoplasia of the phalanges, metacarpals and metatarsals and syndactyly. (Gurrieri and Everman, 2013; Jindal et al., 2009).

Recently, loss-of-function mutations of *UBA2* (ubiquitin-like modifier-activating enzyme 2, OMIM * 613295) have been identified as a rare cause of ectrodactyly. In a study of fifteen patients with 19q13.11 microdeletions encompassing the *UBA2* gene, clinical features were found to overlap the ectrodactyly ectodermal dysplasia-clefting syndrome (Abe et al., 2018). The hypothesized role of *UBA2* in ectrodactyly was subsequently confirmed by reports of intragenic pathogenic variants in

UBA2 in persons with ectrodactyly, often associated with other minor and major anomalies (Marble et al., 2017; Wang et al., 2019; Yamoto et al., 2019).

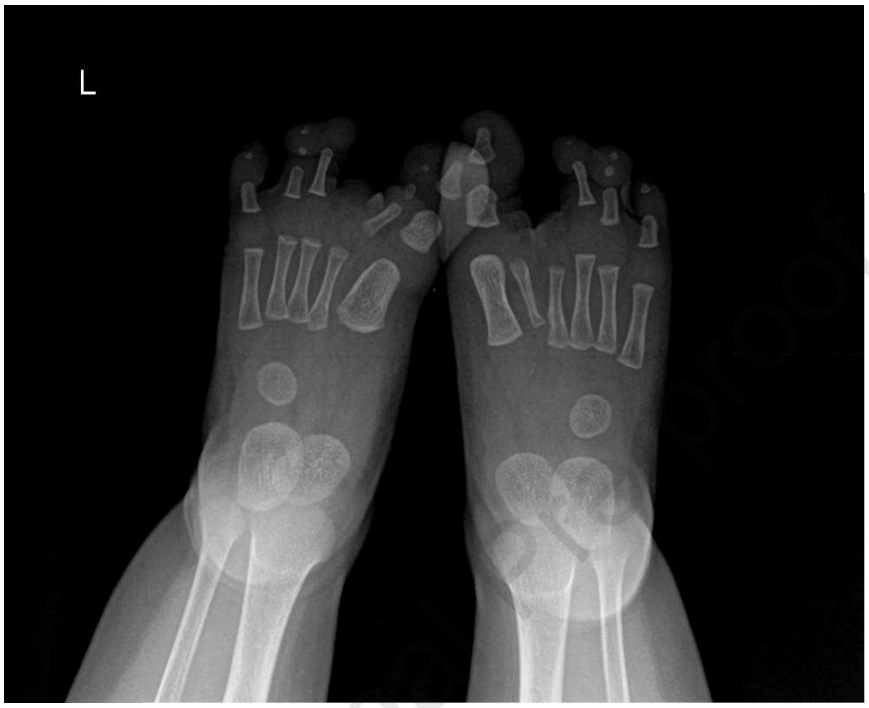
We here report a patient with ectrodactyly with additional manifestations carrying a *de novo* frameshift mutation in the *UBA2* gene. We review the literature of both *UBA2* microdeletions and intragenic pathogenic variants, in order to delineate the phenotype associated with *UBA2* haploinsufficiency.

Clinical report

The proband is a male child of nonconsanguineous, parents. He was born at term after a normal pregnancy, with birth weight 3.350 kg (p 50) and length 50 cm (p 50). He presented bilateral malformations of the feet (**Fig. 1**). On the left foot, there were five metatarsals. There was a complete cutaneous syndactyly of the first two rays and of the third and fourth ray. The first metacarpal was broadened. The fifth toe lacked the middle phalanx and the third toe lacked the middle and terminal phalanges. There was a broad gap between the first toe and the middle toes. The right foot had six metatarsals with a supernumerary hypoplastic metatarsal between the first and second metatarsal. There were no phalanges on the second and third metatarsals. As on the left foot, there was a cutaneous syndactyly of the middle toes. There was a wide and deep gap between the first and middle toes. Similar to the left foot, the fifth toe lacked the middle phalanx, and the second complete toe lacked the middle phalanges. The hands were normal. He had mild hip instability, which resolved spontaneously. He underwent surgery at the right foot with removal of the second and third metatarsal bones, to reduce the wide gap between the first and middle toes. At the age of 3.6 years, weight was 14.3 kg (-1SD), height 99 cm (-0.4SD) and head circumference 48.7 cm (-1.1SD). He had a supernumerary nipple, increased hair growth on the back and dry, sparse scalp hair. There was retrognathia, low set and prominent ears (for which he had surgery later in life), fullness of the upper eyelids and strabismus with hypermetropia (+4 D and +4.5 D). During infancy, he had feeding difficulties and recurrent respiratory infections, associated with failure to thrive. Motor

milestones were normal, but speech development was delayed. He presented with learning difficulties. At the age of 8 years, the diagnosis of autism spectrum disorder was made and he had a total intelligence quotient of 76 (WISC-V).

(A)



(B)

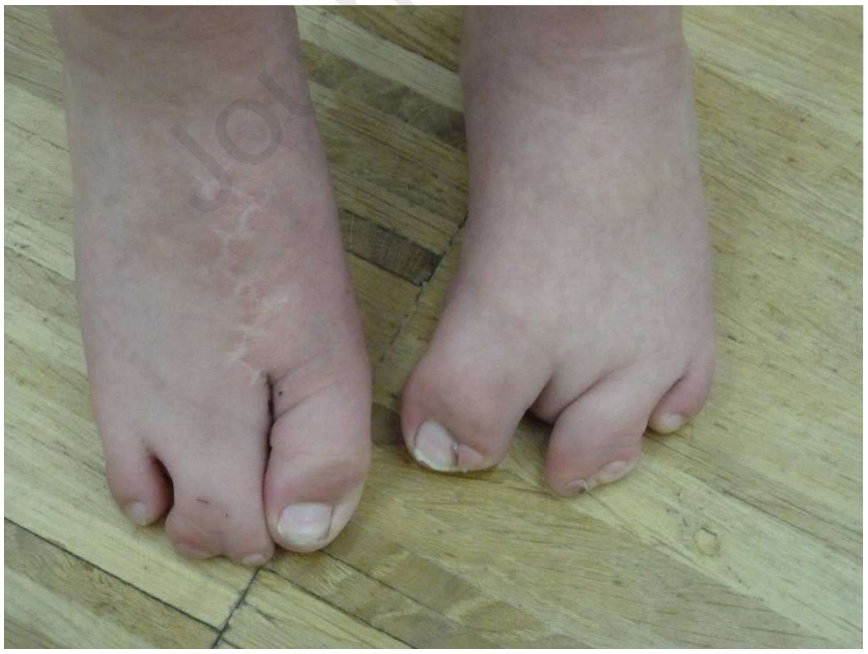


Fig. 1. (A) X-rays of the feet (age 16 months) and (B) clinical picture of the feet (post-surgery, at age 3.6 years).

Conventional karyotyping (G-banding) and chromosomal microarray (200 kb mean resolution) showed a normal 46,XY karyotype. Whole genome sequencing and variant analysis were performed using a trio analysis. Sequencing libraries were generated after DNA shearing (Covaris LE220 plus) using the Kapa Hyper PCR free preparation kit (Roche). DNA fragments were then paired-end sequenced (150bp) on the Illumina NovaSeq 6000 system. Reads were aligned to the hg38 reference genome using BWA. Duplicate reads were marked using Picard MarkDuplicates and base quality score recalibration was performed using GATK BaseRecalibrator and ApplyBQSR. SNPs and small indels were called using GATK HaplotypeCaller. Trio members were jointly genotyped using GATK GenotypeGVCFs. Trio analysis revealed a *de novo* heterozygous deletion of a single nucleotide in exon 7 of the *UBA2* gene at 19q13.11, causing a frameshift mutation with a premature STOP codon (NM_005499.2:c.612del, Chr19(CRCh38):g.34443874del, p.(Glu205Lysfs*63)). This variant was submitted to the DECIPHER database (413410). No additional class 4 or 5 variants were identified.

Discussion

The present case confirms *UBA2* loss-of-function variants as a cause of ectrodactyly. Consistent with previous literature on genetic causes of ectrodactyly, we observe a reduced penetrance in patients with mutations affecting *UBA2*; 3/21 in chromosomal deletions and 3/5 in intragenic mutations (Sowińska-Seidler et al., 2014).

	19q13.11 microdeletions including UBA2																						Intragenic UBA2 mutations					
	Reported Pts												DECIPHER Pts															
	Kulharaya (1998)	Malan (2009)			Chowdhury (2014)		Venegas-Vega (2014)	Melo (2015)	Urquhart (2015)		Abe (2018)	Yamoto (2019)	127	3776	277936	283087	308407	331129	351864	397175	400842	**Total**	Marble (2017)	Yamoto (2019)	Wang (2019)		Present report	**Total**
		Pt 1	Pt 2	Pt 3	Pt 1	Pt 2			Pt 1	Pt 2															Pt 1	Pt 2		
Deletion size (Mb) or intragenic UBA2 mutation	11	6.16	4.27	3.14	8.16	2.30	2.49	4.91	3.22	3.22	3.72	1.35	3.16	5.69	11.62	2.45	0.18	3.31	4.30	10.98	2.64		c.71G>T	c.1324dupT	c.327delT	c.327delT	c.612delA	
Gender	F	M	M	M	F	M	M	F	M	M	M	M	M		M	F	F	F	M	F	M		F	M	M	F	M	
Preterm delivery (<37w)	+	+	+	+			+	-	-	-	+											6/9	-		-		-	0/3
Weight at birth (kg)	1.29	1.56	1.35	1.93	< 10%	<10%	< 3rd centile	1.95	1.98	1.79	1.79												2.87		2.2		3.35	
Age	3.0	6.0	9.2	5.0	5.6	1.6	6.7	0.7	44.0	44.0	10.2												2.6		4.0	35.0	8.3	
Developmental characteristics																												
Intrauterine growth retardation	+	+	+	+	-	+	+	+	+	+	+		+						+	+		13/14	-		+		-	1/3
Postnatal growth retardation	+	+	+	+	+	+	+	+	+	+	+			+							+	13/13	+		-		+	2/3
Recurrent infections	+			+			+	-	+	+										+	+	7/8	+				+	2/2
DD/intellectual disabilities	+	+	+	+	+	+	+	+	+	+	+		+	+	+			+		+		16/16	+				+	2/2
Language delay	+	+	+	+	+	+	+	+	+	+	+									+		12/12					+	1/1
Feeding difficulties	+	+	+	+	+	+	+	+	+	+	+		+	+						+		14/14					+	1/1
Microcephaly	+	+	+	+	+	+	+	+	+	+	+			+		+				+	+	15/15	-		-		-	0/3
Ectodermal dysplasia																												
Aplasia cutis	+	+	+	+	-	+	+	+	-	-	+		+	+								10/13	+		+	+	-	3/4
Thin/dry skin	-	+	+				+	+	+	+	-											6/8					-	0/1
Dysplastic nails	-	+	+		-	-	+	+	-	-	-										+	5/11					-	0/1
Teeth abnormalities		+			+	-	+	+	+	+	+	+	+								+	10/11					-	0/1
Hair/eyebrows/eyelashes anomalies	+	+	+	+	+	-	+	+	+	+	-	+										10/12					+	1/1
Supernumerary nipple				+																	+	2/2					+	1/1
Minor facial dysmorphic features																												
High forehead	+	+	+				+	+	+	+	+											8/8	+				-	1/2
Eye abnormalities	+		+				+	+	+	+	+									+	+	9/9	+		-		+	2/3
Micrognathia/retrognathia	+	+	+		-	+	+	+	+	+	+									+	+	11/12					+	1/1
Low set/poorly folded ears	+	-	+	+	+	+	+	+	+	+	+						+			+		12/13					+	1/1
Physical abnormalities																												
Fingers/toes syndactyly	-	-	+	+	+	+	+	-	-	-	+		+									7/12	-				+	1/2
Hand/foot ectrodactyly	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	3/21	-	+	+	-	+	3/5
Finger/foot clinodactyly	+	+	+		-		+	+	-	-										+		6/9	+				+	2/2
Hip dysplasia	+						+	+					+							+		5/5	+				+	2/2
Renal abnormalities	+										-						+			+		3/4	-		+		-	1/3
Genital abnormalities		+	+	+		+	+		+	+	+		+	+								10/10		+			-	1/2
Congenital heart defects	+		+		-	+	-	+	+	-	-		+				+			+		8/12	-					0/1

Table 1 . Overview of clinical features of reported patients with a 19q13.11 microdeletion including *UBA2* or an intragenic *UBA2* mutation.

Whereas the overall appearance fits with ectrodactyly, the foot malformations in the proband are more complex, with additional polydactyly and syndactyly. In addition, he has several other manifestations such as developmental delay, borderline intelligence, autism spectrum disorder and minor physical anomalies. Previously, several patients with a chromosomal imbalance including *UBA2* have been reported, typically involving additional genes and thus additional features. It is now evident that the phenotype of intragenic *UBA2* loss-of-function mutations is also broader than an isolated ectrodactyly, with various other manifestations that occur in the five reported patients to date (**Table 1**). Although we note a significant overlap of clinical characteristics between patients with microdeletions and patients with intragenic mutations, some important differences between both groups can be observed. Microcephaly (15/15), genital abnormalities (10/10) and congenital heart defects (8/12) are more often present in patients harboring 19q13.11 microdeletions compared to patients with intragenic *UBA2* mutations (0/3, 1/2 and 0/1 respectively). Ectrodactyly however is more specific to patients with intragenic *UBA2* mutations (3/5 compared to 3/21).

Recurrent features in both groups include developmental delay, aplasia cutis and hip dysplasia, but more patients are needed to appreciate the full extent and variability of the phenotype associated with *UBA2* mutations.

The pathogenesis of ectrodactyly due to *UBA2* haploinsufficiency is unclear. *UBA2* plays a role in SUMOylation, a post-translational modification process in which a small ubiquitin-like modifier (SUMO) protein is ligated to a target protein, affecting its structure, intracellular localization, or activity. SUMOylation is involved in various cellular processes, including cell proliferation and migration (Yang et al., 2017). UBA2 (also called SAE2) contributes to this pathway by forming a heterodimer with SAE1 to activate SUMO proteins. Studies in mice highlight a potential role of *UBA2* in embryonal development and formation of the limbs. UBA2 expression is widespread in developing

mouse embryos (8.5 to 11.5 days post-coitum), although more abundant at sites of intensive morphogenetic activity, such as the branchial arches and limb buds (Costa et al., 2011).

In summary, we report a novel patient with ectrodactyly, learning difficulties and minor physical anomalies harboring an intragenic *UBA2* mutation. This finding contributes to establishing the genotype-phenotype correlations of *UBA2* loss of function mutations in patients with ectrodactyly and additional manifestations.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

"This study makes use of data generated by the DECIPHER community. A full list of centres who contributed to the generation of the data is available from http://decipher.sanger.ac.uk and via email from decipher@sanger.ac.uk. Funding for the project was provided by the Wellcome Trust (Firth et al., 2009)."

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	19q13.11 microdeletions including UBA2																						Intragenic UBA2 mutations					
	Reported Pts												DECIPHER Pts															
	Kulharaya (1998)	Malan (2009)			Chowdhury (2014)		Venegas-Vega (2014)	Melo (2015)	Urquhart (2015)		Abe (2018)	Yamoto (2019)	127	3776	277936	283087	308407	331129	351864	397175	400842	Total	Marble (2017)	Yamoto (2019)	Wang (2019)		Present report	Total
		Pt 1	Pt 2	Pt 3	Pt 1	Pt 2			Pt 1	Pt 2															Pt 1	Pt 2		
Deletion size (Mb) or intragenic UBA2 mutation	11	6.16	4.27	3.14	8.16	2.30	2.49	4.91	3.22	3.22	3.72	1.35	3.16	5.69	11.62	2.45	0.18	3.31	4.30	10.98	2.64		c.71G>T	c.1324dupT	c.327delT	c.327delT	c.612delA	
Gender	F	M	M	M	F	M	M	F	M	M	M	M	M		M	F	F	F	M	F	M		F	M	M	F	M	
Preterm delivery (<37w)	+	+	+	+			+	-	-	-	+											6/9	-		-		-	0/3
Weight at birth (kg)	1.29	1.56	1.35	1.93	< 10%	<10%	< 3rd centile	1.95	1.98	1.79	1.79												2.87		2.2		3.35	
Age	3.0	6.0	9.2	5.0	5.6	1.6	6.7	0.7	44.0	44.0	10.2												2.6		4.0	35.0	8.3	
Developmental characteristics																												
Intrauterine growth retardation	+	+	+	+	-	+	+	+	+	+	+		+						+	+		13/14	-		+		-	1/3
Postnatal growth retardation	+	+	+	+	+	+	+	+	+	+	+			+							+	13/13	+		-		+	2/3
Recurrent infections	+			+			+	-	+	+										+	+	7/8	+				+	2/2
DD/intellectual disabilities	+	+	+	+	+	+	+	+	+	+	+		+	+	+			+		+		16/16	+				+	2/2
Language delay	+	+	+	+	+	+	+	+	+	+	+									+		12/12					+	1/1
Feeding difficulties	+	+	+	+	+	+	+	+	+	+	+		+	+						+		14/14					+	1/1
Microcephaly	+	+	+	+	+	+	+	+	+	+	+			+		+				+	+	15/15	-		-		-	0/3
Ectodermal dysplasia																												
Aplasia cutis	+	+	+	+	-	+	+	+	-	-	+		+	+								10/13	+		+	+	-	3/4
Thin/dry skin	-	+	+				+	+	+	+	-											6/8					-	0/1
Dysplastic nails	-	+	+		-	-	+	+	-	-	-										+	5/11					-	0/1
Teeth abnormalities		+			+	-	+	+	+	+	+	+	+								+	10/11					-	0/1
Hair/eyebrows/eyelashes anomalies	+	+	+	+	+	-	+	+	+	+	-	+										10/12					+	1/1
Supernumerary nipple				+																	+	2/2					+	1/1
Minor facial dysmorphic features																												
High forehead	+	+	+				+	+	+	+	+											8/8	+				-	1/2
Eye abnormalities	+		+				+	+	+	+	+									+	+	9/9	+		-		+	2/3
Micrognathia/retrognathia	+	+	+		-	+	+	+	+	+	+									+	+	11/12					+	1/1
Low set/poorly folded ears	+	-	+	+	+	+	+	+	+	+	+						+			+		12/13					+	1/1
Physical abnormalities																												
Fingers/toes syndactyly	-	-	+	+	+	+	+	-	-	-	+		+									7/12	-				+	1/2
Hand/foot ectrodactyly	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	3/21	-	+	+	-	+	3/5
Finger/foot clinodactyly	+	+	+		-		+	+	-	-										+		6/9	+				+	2/2
Hip dysplasia	+						+	+					+							+		5/5	+				+	2/2
Renal abnormalities	+										-						+			+		3/4	-		+		-	1/3
Genital abnormalities		+	+	+		+	+		+	+	+		+	+								10/10		+			-	1/2
Congenital heart defects	+		+		-	+	-	+	+	-	-		+				+			+		8/12	-					0/1

Author statement.

Mio Aerden: Formal analysis , Investigation, Writing - Original Draft, Visualization

Marijke Bauters: Methodology, Validation, Formal analysis, Writing - Review & Editing

Kris Van Den Bogaert: Methodology, Validation, Formal analysis, Writing - Review & Editing

Joris R. Vermeesch: Conceptualization

Maureen Holvoet: clinical Resources

Plasschaert Frank: clinical Resources

Koenraad Devriendt, supervision, clinical resources, Writing - Review & Editing