

# Prenatal diagnosis of a complex skeletal dysplasia case deciphered by exome sequencing and postmortem pathological examination

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The authors declare no conflict of interest

Control No. 2020-A-2103-ESHG

E-P01.33

## INTRODUCTION

We present a prenatal and postmortem diagnosis of diastrophic dysplasia (DD) in a fetus conceived by a mother with hypochondroplasia.

**Hypochondroplasia** is a skeletal dysplasia characterized by short stature, stocky build, disproportionately short arms and legs, broad short hands and feet, mild joint laxity and macrocephaly. The diagnosis is difficult in children under the age of three years, as skeletal disproportion tends to be mild and many of the radiographic features are subtle during infancy. It can occur randomly for unknown reasons with no apparent family history of the disorder. However there are also cases that the disorder is familial with autosomal dominant inheritance, correlated to pathogenic variants detected in ***FGFR3*** gene.

Overlapping prenatal phenotypes are observed between **hypochondroplasia** and **diastrophic dysplasia**, which is characterized by limb shortening, normal-sized skull, hitchhiker thumbs, spinal deformities, contractures of the large joints with deformities and early-onset osteoarthritis. Additionally typical findings are ulnar deviation of the fingers, gap between the first and second toes, and clubfoot. Most affected individuals survive the neonatal period and develop physical limitations with normal intelligence. Diastrophic dysplasia is considered a familial disorder with autosomal recessive inheritance, correlated to homozygous or compound heterozygous variants detected in ***SLC26A2*** gene

## MATERIALS AND METHODS

Prenatal testing at 13 weeks gestation was requested due to a maternal hypochondroplasia phenotype of unknown genetic background. Clinical exome sequencing was performed on DNA extracted from chorionic villi and maternal blood, using Sophia Genetics' Clinical Exome Solution v2. Following preparations according to the manufacturer's protocol, DNA libraries were sequenced on an Illumina NextSeq-500 genetic analyser. Data processing, variant calling and pre-classification were conducted by SOPHiA DDM® bioinformatics pipelines.

## GENES ANALYSED RELATED TO SKELETAL DYSPLASIA

*ACAN, ACP5, ADAMTS10, ADAMTSL2, AGPS, ALPL, ANKH, ARSE, B3GALT6, BMP1, BMPR1B, CA2, CANT1, CDC6, CDKN1C, CDT1, CHST3, CLCN7, COL10A1, COL11A1, COL11A2, COL1A1, COL1A2, COL2A1, COL9A1, COL9A2, COL9A3, COMP, CRTAP, CTSK, CUL7, CYP27B1, DHCR24, DLL3, DYM, DYNC2H1, EBP, EIF2AK3, ENPP1, ESCO2, EVC, EVC2, FAM20C, FGF23, FGFR1, FGFR2, FGFR3, FKBP10, FLNA, FLNB, GDF5, GNPAT, HSPG2, IFT140, IFT172, IFT80, IHH, KAT6B, LBR, LIFR, LMX1B, LRP5, LTBP2, MATN3, MMP9, NEK1, NPR2, OBSL1, ORC1, ORC4, ORC6, P3H1, PAPSS2, PCNT, PEX7, PHEX, PLOD2, PPIB, PTH1R, ROR2, RUNX2, SERPINF1, SERPINH1, SHOX, SLC26A2, SLC34A3, SLC39A13, SMAD4, SMARCAL1, SOX9, TCIRG1, TGFB1, TNFRSF11A, TNFRSF11B, TRIP11, TRPV4, TTC21B, VDR, WDR19, WDR35, WISP3, WNT5A, XYLT1*

# RESULTS

A *FGFR3* pathogenic variant was identified in the mother, confirming her phenotype; this variant was not detected in the fetus. However, one maternally inherited pathogenic and one of unknown significance *SLC26A2* variants were identified, related, according to the literature, to four distinct genetic skeletal dysplasias of various severity and prognosis. On 2nd trimester ultrasound the fetus presented short long bones and, following clinical genetic counselling, the pregnancy was terminated. Postmortem radiographical and histopathological findings were consistent with Diastrophic Dysplasia.

## Variants detected in the fetus

Gene	Coding consequence	c.DNA	Depth	V% ref	alt	Protein	Exon	EAC	Freq.	ClnVar rating	ClinVar rating	
SLC26A2	missense	c.532C>G	120	46.3	T	p.(Arg77P)	2	1.0E-4	2	7	Pathogenic	
SLC26A2	missense	c.192G>T	37	56.8	A	p.(Arg64Cys)	23	2	0	0	0.0	
SLC26A2	missense	c.1152C>T	146	42.5	A	p.(Ser54K)	2	0.039	91	909	Likely benign	
SLC26A2	missense	c.3543A>G	113	46.9	T	p.(Ser84Ser)	17	0.0	2	0	0.0	
SLC26A2	missense	c.3142G>A	79	35.4	A	p.(Ser71A)	15	96	708	Uncertain significance...	0.0	
SLC26A2	missense	c.232C>G	93	57.0	G	p.(Ser77A)	16	96	713	Uncertain significance...	0.0	
SLC26A2	missense	c.2313A>G	86	100.0	G	p.(Lys77V)	16	680	5116	Benign	0.049	
SLC26A2	missense	c.3728G>A	215	100.0	C	p.(Gln85G)	11	0.053	109	700	Benign	2.0E-4
SLC26A2	missense	c.215G>C	90	37.8	C	p.(Thr72Ser)	2	2.0E-4	1	4	Conflicting interpret...	0.0
SLC26A2	missense	c.2025>G	64	39.4	T	p.(Leu72Val)	17	1	0	0	0.0	
SLC26A2	missense	c.5127A>A	101	38.6	T	p.(Leu171Leu)	2	1	0	0	0.0	
SLC26A2	missense	c.797T>C	36	55.6	C	p.(Leu25I)	8	0.443	538	6426	Benign	0.456
SLC26A2	missense	c.1538A>T	175	53.7	A	p.(Glu131Ala)	13	0.877	789	9221	Benign	0.801
SLC26A2	missense	c.836G>A	120	100.0	A	p.(Glu28Gly)	2	0.984	798	9221	Benign	0.984
SLC26A2	missense	c.573G>A	132	98.5	A	p.(Ala19I)	5	0.31	494	5417	Benign	0.303
SLC26A2	missense	c.1249G>C	104	100.0	G	p.(Leu15I)	11	0.993	798	10007	Benign	0.993
SLC26A2	missense	c.282T>T	64	45.3	A	p.(Pro18Asn)	1	0.488	611	7109	Benign/Risk factor	0.561
SLC26A2	missense	c.891A>G	106	38.6	A	p.(Thr25T)	9	0.956	798	10130	Benign/Likely benign	0.956
SLC26A2	missense	c.597G>C	123	53.7	G	p.(Ala31Val)	1	0.497	423	7314	Benign	0.5307
SLC26A2	intronic	c.401>ST-C	52	100.0	A		3	0.934	793	8560	Benign	0.9316
SLC26A2	missense	c.1893C>T	127	52.8	G	p.(Ile431I)	11	0.167	221	2387	Benign	0.1696
SLC26A2	missense	c.670A>C	104	55.8	T	p.(Arg224I)	3	0.633	422	7683	Benign	0.6299
SLC26A2	missense	c.601G>A	94	100.0	C	p.(Val20Ileu)	6	0.657	492	9624	Benign/Likely benign	0.6594
SLC26A2	missense	c.826A>G	79	100.0	T	p.(Thr276Asn)	8	0.985	796	10989	Benign/Likely benign	0.9847
SLC26A2	missense	c.1094T>C	124	51.6	G	p.(Ile392I)	10	0.334	511	6056	Likely benign	0.3284
SLC26A2	missense	c.271C>T	91	100.0	A	p.(Ile17I)	3	0.637	715	8622	Benign	0.6376
SLC26A2	missense	c.531A>C	105	52.4	T	p.(Gln18Arg)	2	0.107	201	2014	Likely benign	0.1039
SLC26A2	missense	c.2328A>G	112	35.7	T	p.(Glu43C)	22	0.473	548	4373	Benign	0.4732
SLC26A2	missense	c.184G>T	129	45.7	T	p.(Gln54His)	3	0.261	344	4253	Benign	0.2618
SLC26A2	missense	c.1598C>T	91	52.8	A	p.(Ala663I)	9	0.556	690	7416	Benign	0.5573
SLC26A2	missense	c.2631C>T	123	51.2	A	p.(Pro87I)	12	0.166	296	3128	Benign	0.1672

## Variants detected in the mother

Gene	Coding consequence	c.DNA	Depth	V% ref	alt	Protein	Exon	EAC	Freq.	ClnVar rating	ClinVar rating	
SLC26A2	missense	c.1618A>G	170	53.5	A	p.(Ser55Ser)	12	0.0	1	0	Pathogenic	
SLC26A2	missense	c.532C>T	120	46.3	T	p.(Arg77P)	2	1.0E-4	2	7	Pathogenic	
SLC26A2	missense	c.342C>T	60	50.0	T	p.(Ala154Val)	3	0.024	27	298	Benign	
SLC26A2	missense	c.192G>T	37	56.8	A	p.(Arg64Cys)	23	2	0	0	0.0	
SLC26A2	missense	c.1152C>T	146	42.5	A	p.(Ser54K)	2	0.039	91	909	Likely benign	
SLC26A2	missense	c.3543A>G	113	46.9	T	p.(Ser84Ser)	17	0.0	2	0	0.0	
SLC26A2	missense	c.3142G>A	79	35.4	A	p.(Ser71A)	15	96	708	Uncertain significance...	0.0	
SLC26A2	missense	c.232C>G	93	57.0	G	p.(Ser77A)	16	96	713	Uncertain significance...	0.0	
SLC26A2	missense	c.2313A>G	86	100.0	G	p.(Lys77V)	16	680	5116	Benign	0.049	
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SLC26A2	missense	c.2025>G	64	39.4	T	p.(Leu72Val)	17	1	0	0	0.0	
SLC26A2	missense	c.5127A>A	101	38.6	T	p.(Leu171Leu)	2	1	0	0	0.0	
SLC26A2	missense	c.797T>C	36	55.6	C	p.(Leu25I)	8	0.443	538	6426	Benign	0.456
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# DISCUSSION

Targeted *FGFR3* mutation testing, based on the mother's phenotype, would have missed the Diastrophic Dysplasia diagnosis in the fetus, preventing informed decision for this pregnancy and proper genetic counselling for the family. Therefore, this case highlights the utility of exome sequencing for complex overlapping prenatal phenotypes such as skeletal dysplasia, and underscores the contribution of postmortem pathological examination to the phenotype - genotype correlation, which is essential for the correct interpretation of exome sequencing results.