Title

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Journal

American Journal of Human Genetics, 94(5)

ISSN

0002-9297

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Publication Date

2014-05-01

DOI

10.1016/j.ajhg.2014.03.015

Peer reviewed

REPORT

Mutations in *PIEZO2* Cause Gordon Syndrome, Marden-Walker Syndrome, and Distal Arthrogryposis Type 5

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Gordon syndrome (GS), or distal arthrogryposis type 3, is a rare, autosomal-dominant disorder characterized by cleft palate and congenital contractures of the hands and feet. Exome sequencing of five GS-affected families identified mutations in piezo-type mechanosensitive ion channel component 2 (*PIEZO2*) in each family. Sanger sequencing revealed *PIEZO2* mutations in five of seven additional families studied (for a total of 10/12 [83%] individuals), and nine families had an identical c.8057G>A (p.Arg2686His) mutation. The phenotype of GS overlaps with distal arthrogryposis type 5 (DA5) and Marden-Walker syndrome (MWS). Using molecular inversion probes for targeted sequencing to screen *PIEZO2,* we found mutations in 24/29 (82%) DA5-affected families and one of two MWS-affected families. The presence of cleft palate was significantly associated with c.8057G>A (Fisher's exact test, adjusted p value $<$ 0.0001). Collectively, although GS, DA5, and MWS have traditionally been considered separate disorders, our findings indicate that they are etiologically related and perhaps represent variable expressivity of the same condition.

Gordon syndrome (GS [MIM 114300]) is a rare autosomal-dominant disorder characterized by cleft palate and multiple congenital contractures of the hands and feet.[1–7] Gordon et al.[1] originally described a three-generation family with autosomal-dominant inheritance of camptodactyly, clubfoot, and cleft palate. Over the past few decades, several additional GS-affected families have been reported, although only a small percentage of affected individuals have had cleft palate.[3,6,8] The phenotypic characteristics of GS have also been noted to overlap with several other disorders, including Aase-Smith syndrome (MIM 147800),[9–11] Marden-Walker syndrome (MWS [MIM

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http://dx.doi.org/10.1016/j.ajhg.2014.03.015.

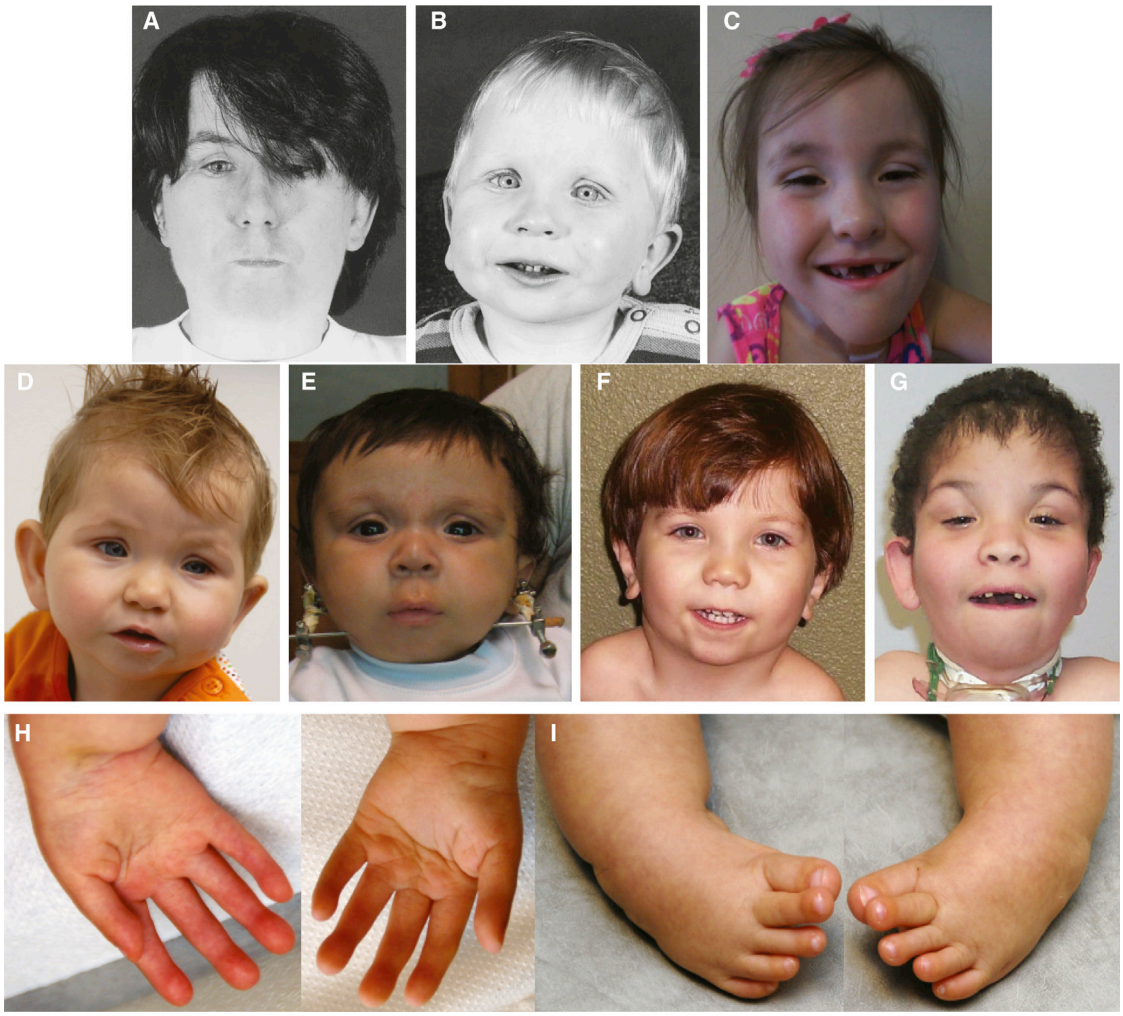

Figure 1. Phenotypic Characteristics of Each GS Individual Used for Exome Sequencing and the MWS Individual with a Mutation in *PIEZO2*

(A–F) Faces of individuals affected by GS. All individuals shown had *PIEZO2* mutations. Note the deep-set eyes and micrognathia. Each individual had either cleft palate or a bifid uvula.

(H and I) The hands (H) and feet (I) of an individual with GS demonstrate curved fingers with straight thumbs and clubfeet, respectively.

(G) The face of an individual with MWS and a mutation in *PIEZO2*.

Case identifiers for the individuals shown in this figure are A:I-2 (A), A:II-1 (B), B:II-1 (C), C:II-1 (D, H, and I), D:II-1 (E), E:II-1 (F), and II:II-1 (G) and correspond to those in Table 1, which includes a detailed description of the phenotype of each affected individual. Figure S1 provides a pedigree of each GS-affected family, and Figure S3 provides a pedigree of the MWS-affected family (II).

248700]),[12–14] distal arthrogryposis type 5 (DA5 [MIM 108145]),[13,14] and Schwartz-Jampel (MIM 255800).[13] Furthermore, in the absence of cleft palate, GS can be virtually indistinguishable from distal arthrogryposis type 1 (DA1 [MIM 108120]) and distal arthrogryposis type 2B (DA2B [MIM 601680]). Accordingly, Bamshad et al. categorized GS as distal arthrogryposis type 3 (DA3) in the revised classification of distal arthrogryposis (DA) syndromes but questioned whether GS was an etiologically distinct syndrome.[7]

In an effort to ascertain GS cases for gene-mutation-discovery studies, we reviewed phenotypic data from 170 families affected by DA1, DA2B, DA3, or DA5 of unknown genetic etiology and identified 12 families affected by a phenotype consistent with GS (Figure 1; Table 1; Figure S1, available online). At least one affected individual in each of these GS-affected families had cleft palate and congenital contractures affecting both the hands and feet. All studies were approved by the institutional review boards of the University of Washington and Seattle Children's Hospital, and informed consent was obtained from participants or their parents. To identify causative mutations for GS, we first used Sanger sequencing to screen the proband of each GS-affected family for mutations in genes including *TPM2* (MIM 190990), *TNNT3* (MIM 600692), *TNNI2* (MIM 191043), and *MYH3* (MIM 160720), known to associate with DA1 or DA2B. We also screened *CHRNG* (MIM 100730), mutations in which cause congenital contractures in Escobar syndrome (MIM 265000).[21]

Table 1. Mutations and Clinical Findings of Individuals with GS, DA5, or MWS

		Mutation Information (*PIEZO2*)			Clinical Findings								
Family	**Subject**	**Exon**	**cDNA Change**	**Predicted Protein Alteration**	**CP or BU**	**Short Stature**	**Micrognathia**	**Ptosis**	**Ophthalmoplegia**	**Scoliosis**	**Pulmonary Disease**	**Cognitive Delay**	**Cerebellar Malformations**
GS													
A	I-2[11]	52	c.8057G>A	p.Arg2686His	BU	+	–	–	–	+	–	–	–
	II-1[11]	52	c.8057G>A	p.Arg2686His	CP	+	+	–	–	–	–	–	–
	II-2[11]	52	c.8057G>A	p.Arg2686His	CP	+	+	–	–	–	–	–	–
	II-3	52	c.8057G>A	p.Arg2686His	CP	+	+	–	–	–	–	–	–
B	II-1	52	c.8238_8245del8	p.Trp2746*	CP	+	+	+	–	+	–	–	–
C	II-1	52	c.8057G>A	p.Arg2686His	CP	ND	+	–	–	rigid	–	–	–
D	II-1	52	c.8057G>A	p.Arg2686His	CP	ND	+	–	–		–	–	–
E	II-1	52	c.8057G>A	p.Arg2686His	CP	–	+	–	+	–	–	–	ND
F	II-1	52	c.8057G>A	p.Arg2686His	BU	–	+	mild	–	–	–	–	–
G	I-2	52	c.8057G>A	p.Arg2686His	BU	ND	+	–	–	+	–	–	ND
	II-1	52	c.8057G>A	p.Arg2686His	CP	ND	+	+	ND	–	–	+/–	ND
H	II-1	52	c.8057G>A	p.Arg2686His	CP	+	ND	–	–	+	–	+/–	Chiari I malformation
I	II-1	52	c.8057G>A	p.Arg2686His	CP	+	+	+	–	+	–	–	Chiari I malformation
J	I-2	52	c.8057G>A	p.Arg2686His	CP	+	–	–	+	+	–	–	Chiari I malformation
	II-1	52	c.8057G>A	p.Arg2686His	CP	+	–	mild	–	–	–	–	–
DA5													
K	I-2	52	c.8153G>T	p.Arg2718Leu	–	–	–	–	+	–	+	–	ND
	II-2	52	c.8153G>T	p.Arg2718Leu	–	–	–	–	+	–	+	–	ND
	II-3	52	c.8153G>T	p.Arg2718Leu	–	–	–	–	+	–	–	–	ND
	II-5	52	c.8153G>T	p.Arg2718Leu	–	–	–	–	+	–	+	–	ND
	II-6	52	c.8153G>T	p.Arg2718Leu	–	–	–	–	–	–	–	–	ND
	III-1	52	c.8153G>T	p.Arg2718Leu	–	–	–	–	+	–	–	–	ND
	III-4	52	c.8153G>T	p.Arg2718Leu	–	–	–	–	+	–	–	–	ND
	III-6	52	c.8153G>T	p.Arg2718Leu	–	–	–	–	+	–	–	–	ND
	III-7	52	c.8153G>T	p.Arg2718Leu	–	–	–	mild	+	–	–	–	ND

(*Continued on next page*)

Table 1. *Continued*

		Mutation Information (*PIEZO2*)			Clinical Findings								
Family	**Subject**	**Exon**	**cDNA Change**	**Predicted Protein Alteration**	**CP or BU**	**Short Stature**	**Micrognathia**	**Ptosis**	**Ophthalmoplegia**	**Scoliosis**	**Pulmonary Disease**	**Cognitive Delay**	**Cerebellar Malformations**
L	II-5[15]	52	c.8181_8183delAGA	p.Glu2727del	–	+	–	mild BL	BL	+	+	–	ND
	II-7[15]	NT	NT	NT	–	+	–	–	BL	stiff	+	–	ND
	III-7[15]	52	c.8181_8183delAGA	p.Glu2727del	–	–	–	BL	BL	–	+	–	ND
M	II-4[16]	15	c.2134A>G	p.Met712Val	–	+	–	ND	–	–	–	–	ND
	III-1[16]	15	c.2134A>G	p.Met712Val	–	+	–	mild BL	+	+	–	–	ND
	IV-3[16]	15	c.2134A>G	p.Met712Val	–	+	–	mild	–	+	+	–	ND
	IV-4[16]	NA	no c.2134A>G	NA	–	+	–	ND	–	–	–	–	ND
N	III-3	45	c.7067C>T	p.Thr2356Met	–	–	+	+	+	–	–	–	–
	IV-3	45	c.7067C>T	p.Thr2356Met	–	–	–	+	+	stiff	–	–	ND
	IV-5	45	c.7067C>T	p.Thr2356Met	–	ND	–	mild	+	–	–	–	ND
	V-3	45	c.7067C>T	p.Thr2356Met	–	ND	–	+	+	–	–	–	ND
O	II-5	52	c.8181_8183delAGA	p.Glu2727del	–	+	–	mild	+	–	–	–	ND
	III-4	52	c.8181_8183delAGA	p.Glu2727del	–	–	–	–	+	–	–	–	ND
	III-5	52	c.8181_8183delAGA	p.Glu2727del	–	–	–	ND	+	–	–	–	ND
P	I-1[17,18]	52	c.8153G>T	p.Arg2718Leu	–	+	–	BL	BL	stiff	–	–	ND
	II-1[17]	52	c.8153G>T	p.Arg2718Leu	–	ND	–	BL	BL	–	–	–	ND
Q	II-1[19]	52	c.8215T>C	p.Ser2739Pro	–	+	–	R	BL	–	–	–	ND
	III-2[19]	52	c.8215T>C	p.Ser2739Pro	–	–	–	mild BL	+	–	–	–	ND
R	II-1	52	c.8181_8183delAGA	p.Glu2727del	–	+	ND	mild BL	BL	ND	ND	–	ND
	III-2	52	c.8181_8183delAGA	p.Glu2727del	–	+	ND	mild BL	BL	ND	ND	–	ND
S	II-2	43	c.6662C>T	p.Thr2221Ile	–	–	+	mild	+	+	–	–	ND
	III-1	43	c.6662C>T	p.Thr2221Ile	–	–	+	BL	+	+	–	–	ND
T	II-1	52	c.8181_8183delAGA	p.Glu2727del	–	–	–	mild	+	↓ ROM	–	–	ND

(*Continued on next page*)

Table 1. ***Continued***

		Mutation Information (*PIEZO2*)			Clinical Findings								
Family	**Subject**	**Exon**	**cDNA Change**	**Predicted Protein Alteration**	**CP or BU**	**Short Stature**	**Micrognathia**	**Ptosis**	**Ophthalmoplegia**	**Scoliosis**	**Pulmonary Disease**	**Cognitive Delay**	**Cerebellar Malformations**
U	II-2	52	c.8181_8183delAGA	p.Glu2727del	ND	ND	–	mild	ND	ND	ND	–	ND
	III-1	52	c.8181_8183delAGA	p.Glu2727del	ND	ND	–	mild	ND	ND	ND	–	ND
V	II-1	52	c.8181_8183delAGA	p.Glu2727del	–	ND	ND	ND	+	rigid	ND	–	ND
	III-1	52	c.8181_8183delAGA	p.Glu2727del	–	ND	ND	ND	+	rigid	ND	–	ND
W	II-2	15	c.2134A>G	p.Met712Val	–	–	–	+	BL	+	+	–	–
	III-1	15	c.2134A>G	p.Met712Val	–	+	–	BL	BL	–	–	–	ND
X	II-2[20]	52	c.8057G>A	p.Arg2686His	–	–	–	BL	BL	stiff	–	–	–
Y	II-1	52	c.8057G>A	p.Arg2686His	–	–	–	mild	+	+	+/–	–	ND
Z	III-1	52	c.8181_8183delAGA	p.Glu2727del	–	–	–	+	+	–	–	–	ND
AA	III-2	52	c.8181_8183delAGA	p.Glu2727del	–	+	–	mild	–	stiff	–	–	ND
BB	III-1	43	c.6662C>T	p.Thr2221Ile	–	–	–	mild	BL	–	–	–	ND
CC	II-1	43	c.6668C>T	p.Ser2223Leu	–	+	–	+	+	–	–	–	–
DD	II-1	52	c.8181_8183delAGA	p.Glu2727del	–	–	–	+	+	–	–	–	ND
EE	II-2	52	c.8208delA	p.Tyr2737Ilefs*7	–	+	–	+	+	–	–	–	–
FF	II-1	52	c.8153G>C	p.Arg2718Pro	–	–	–	+	+	+	+	–	ND
GG	II-2	20	c.2993T>C	p.Met998Thr	–	+	–	BL	BL	+	+	–	–
HH	II-2[10]	52	c.8181_8183delAGA	p.Glu2727del	–	+	–	ND	+/– §	+	–	–	–
	III-1[10]	52	c.8181_8183delAGA	p.Glu2727del	–	–	–	mild BL	+/– §	+	+	–	–
	III-2[10]	52	c.8181_8183delAGA	p.Glu2727del	–	+	–	mild BL	BL	+	+/–	–	Dandy-Walker malformation
MWS													
II	II-1	52	c.8056C>T	p.Arg2686Cys	CP	–	+	+	ND	+	–	+	Dandy-Walker malformation

This table provides a summary of clinical features of affected individuals from families in which *PIEZO2* mutations were identified. Clinical characteristics listed in the table are primarily features that distinguish the different diagnoses (GS, DA5, and MWS). In addition to showing the characteristics listed in the table, affected individuals had contractures of the hands and feet, which are characteristic of DA disorders. Abbreviations are as follows: +, presence of a finding; –, absence of a finding; +/–, possible or very mild features; BL, bilateral; BU, bifid uvula; CP, cleft palate; ND, no data available; NT, not tested; ROM, range of motion; and §, described as Brown syndrome.

Next, we selected five GS-affected families in which the affected individual had facial characteristics similar to those of affected individuals from GS-affected families in which vertical transmission of GS was evident. Each GS proband was screened for copy-number variations (CNVs) by array comparative genomic hybridization on the Illumina HumanCytoSNP-12. No pathogenic mutations or shared CNV regions were identified. Next, exome sequencing was performed on four GS parent-child trios (three simplex cases [from families B–D] and one family with an affected mother and son [family A]) and a single affected individual (from family E) (Figure 1; Figure S1).

In brief, 1 μg of genomic DNA was subjected to a series of shotgun-library-construction steps, including fragmentation through acoustic sonication (Covaris), end polishing (NEBNext End Repair Module), A-tailing (NEBNext dA-Tailing Module), and ligation of 8 bp barcoded sequencing adaptors (Enzymatics Ultrapure T4 Ligase). Prior to exome capture, the library was amplified via PCR (BioRad iProof). One microgram of barcoded shotgun library was hybridized for capture of probes targeting 64 Mb of coding exons (Roche Nimblegen SeqCap EZ Human Exome Library v.2.0) according to the manufacturer's protocol, and custom blockers complimentary to the full length of the flanking adaptor and barcodes were added. Enriched libraries were amplified via PCR before sequencing (BioRad iProof). Library quality was determined by examination of molecular-weight distribution and sample concentration (Agilent Bioanalyzer). Pooled, barcoded libraries were sequenced via paired-end 50 bp reads with an 8 bp barcode read on Illumina HiSeq sequencers.

Demultiplexed BAM files were aligned to the human reference genome (hg19, UCSC Genome Browser) with the Burrows-Wheeler Aligner (BWA).[22] Read data from a flow-cell lane were treated independently for alignment and quality-control purposes in instances where the merging of data from multiple lanes was required. All aligned read data were subjected to (1) removal of duplicate reads (Picard), (2) indel realignment by the Genome Analysis Toolkit (GATK) IndelRealigner, and (3) base-quality recalibration by the GATK TableRecalibration. Variant detection and genotyping were performed with the UnifiedGenotyper tool from GATK (v.1.529). Variant data for each sample were formatted (variant call format) as "raw" calls that contained individual genotype data for one or multiple samples and were flagged with the filtration walker (GATK) for marking sites that were of lower quality and potential false positives (e.g., quality scores $\leq$ 50, allelic imbalance $\geq$ 0.75, long homopolymer runs $>$ 3, and/or low quality by depth $<$ 5). Variants were annotated with SeattleSeq Annotation 134, and variants with an alternative allele frequency $>$ 0.01 in the NHLBI Exome Sequencing Project Exome Variant Server (ESP6500) or 1000 Genomes Project[23] or $>$ 0.2 in an internal database derived from other University of Washington Center for Mendelian Genomics exomes were excluded prior to analysis. Additionally, intergenic variants or variants that were flagged as low quality or potential false positives (quality scores $\leq$ 30, long homopolymer runs $>$ 5, low quality by depth $<$ 5, within a cluster of SNPs) were also excluded from analysis. Variants that were only flagged by a strand bias (strand bias $\geq$ 0.10) or allele-balance filter (allelic imbalance $\geq$ 0.75) were included in further analyses because both flags have previously been found to be applied to valid pathogenic variants. CNV calls were also generated from exome data with CoNIFER.[24]

Analysis of variants from exome sequencing under a model matching the predicted pattern of inheritance (autosomal dominant in family A and de novo in families B–E; Figure S1) identified mutations in a single gene, piezo-type mechanosensitive ion channel component 2 (*PIEZO2* [MIM 613629; RefSeq accession number NM_022068.2]), in all five GS-affected families. Specifically, in four families (A and C–E), a recurrent missense mutation (c.8057G>A) predicted to cause a nonconservative arginine-to-histidine substitution (p.Arg2686His) was found in each family. An 8 bp deletion (c.8238_8245del8) predicted to result in an immediate stop codon (p.Trp2746*) was found to be de novo in a single trio (family B). Neither mutation was found in ESP6500, the 1000 Genomes Project (phase 1 release),[23] or internal databases (>1,400 chromosomes). Sanger sequencing confirmed each variant identified. In families C–E, the c.8057G>A mutation was found to have arisen de novo. In family A, c.8057G>A was transmitted from an affected mother to her affected offspring, and Sanger sequencing confirmed that the two other affected individuals in family A also carried the c.8057G>A mutation.

To determine the extent to which mutations in *PIEZO2* cause GS, we used molecular inversion probes (MIP)[25,26] for targeted sequencing of *PIEZO2* in seven additional GS-affected families. MIPs can be used for multiplex targeted sequence capture, followed by massively parallel sequencing of capture products. This strategy is efficient and cost effective for sequencing large genes, such as the 52-exon gene *PIEZO2*, in modest to large sample sets. Pooled and phosphorylated MIP probes targeting 8,259 bp of *PIEZO2* coding sequence plus 20 bp of intron-exon flanking regions were designed[10] and used in capture reactions with 100 ng of genomic DNA from each individual. PCR was performed with universal primers, and unique 8-base sample indexes were introduced on the tagged reverse primer. The individual samples were then pooled, and the resulting library was purified with magnetic beads (Agencourt AMPure XP). After Picogreen quantification for determining the appropriate dilution, the library was sequenced on an Illumina MiSeq system. The alignment (BWA v.0.5.9-r16), analysis (GenomeAnalysisTK-2.3-4-g57ea19f.), and filtering (SeattleSeq Annotation 137) of the variants were similar to the filtering of exome variants in that candidate nonsynonymous, nonsense, and deletion and/or duplication coding variants or splicing variants that were not seen in

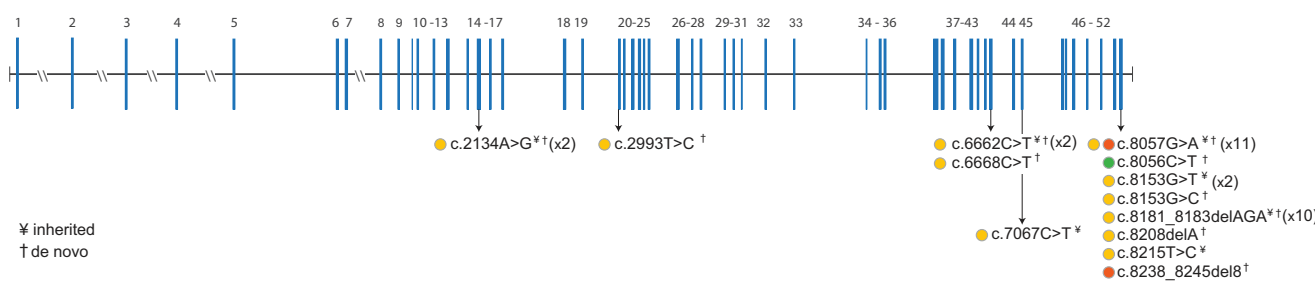

Figure 2. Genomic Structure and Spectrum of *PIEZO2* Mutations that Cause GS, DA5, or MWS
PIEZO2 is composed of 52 exons that encode protein-coding sequence (blue). Arrows indicate the locations of 13 different mutations found in 35 families affected by GS, DA5 or MWS. The ¥ symbol indicates mutations that were inherited in families with an autosomal-dominant pattern of inheritance, and the † symbol indicates mutations that were confirmed to be de novo in simplex cases. *PIEZO2* mutations identified in GS, DA5, and MWS individuals are indicated by red, yellow, and green circles, respectively.

variant databases were chosen for further confirmation by Sanger sequencing. MIP sequencing of *PIEZO2* and variant confirmation in probands and family members by Sanger sequencing identified the same missense mutation (c.8057G>A [p.Arg2686His]) in *PIEZO2* in five additional families affected by GS. Altogether, mutations in *PIEZO2* were found in 10 of 12 GS-affected families, and nine of ten families shared the same missense mutation. All ten mutations occurred in exon 52 (Figure 2).

Mutations in *PIEZO2* have recently been reported to cause congenital contractures and ophthalmoplegia in two families affected by DA5.[27] Accordingly, the observation that the phenotypic characteristics of GS and DA5 overlap is consistent with the discovery that mutations in *PIEZO2* also cause GS. Indeed, among the 15 GS-affected individuals (from ten families) in whom we found *PIEZO2* mutations, five also had ptosis and two had ophthalmoplegia.

To further explore the genetic and phenotypic overlap with DA5, we used a combination of MIP and Sanger sequencing to screen *PIEZO2* in 29 families affected by DA5. Mutations in *PIEZO2* were found in 24/29 (83%) DA5-affected families (Tables 1 and 2; Figure 2; Figures S2 and S5), including 14/16 (88%) familial cases and 10/13 (77%) simplex cases. Forty-two percent (10/24) of DA5-affected families had the same recurrent c.8181_8183delAGA (p.Glu2727del) mutation (Table 2). In one DA5-affected family (family Z, Figure S2), the c.8181_8183delAGA mutation found in the proband was also detected at a low level (11%) in a DNA sample obtained from peripheral blood in his unaffected father (Figure S4). The presence of this mutation was confirmed in DNA sampled from both buccal cells (10%) and saliva (12%). This observation suggests that either the penetrance of c.8181_8183delAGA was incomplete and/or the level of mosaicism in the father was too low to cause the phenotypic characteristics of DA5. Two simplex cases of DA5 (in families X and Y, Figure S2) had a recurrent c.8057G>A (p.Arg2686His) mutation, the same one found to cause GS (Table 2). Neither individual had a cleft palate.

GS and DA5 are phenotypically similar to MWS, a rare disorder for which the genetic basis has not yet been discovered. MWS has been hypothesized to be etiologically related to both DA[12,28] and GS.[14] Therefore, we hypothesized that mutations in *PIEZO2* might also cause MWS. MWS is characterized by joint contractures including camptodactyly, cleft palate, blepharophimosis, "immobile facies," diminished muscular bulk, developmental delay, and hindbrain malformations, most commonly Dandy-Walker malformation. The latter is notable because hindbrain malformations were found in several individuals with *PIEZO2* mutations and either GS or DA5. Specifically, three of the families affected by GS had a Chiari I malformation, and one individual with DA5 had a Dandy-Walker malformation. Sequencing of *PIEZO2* in two cases of MWS revealed a de novo c.8056C>T (p.Arg2686Cys) mutation in one family. Whereas this mutation is predicted to cause a cysteine substitution in MWS, mutation of the adjacent nucleotide (c.8057G>A) is predicted to cause a histidine substitution for the same arginine in amino acid position 2686 (p.Arg2686His) in GS and DA5 (Table 2).

Collectively, we identified 13 different mutations in *PIEZO2* (Figure 2; Table 2) in 35 families affected by GS (n = 10), DA5 (n = 24), or MWS (n = 1). Ten of these mutations are missense, two are predicted to cause a frameshift, and one is a 3 bp in-frame deletion (Table 2). Recurrent mutations occurred at five sites, and two of these (c.8057G>A [p.Arg2686His] and c.8181_8183delAGA [p.Glu2727del]) were observed in ten or more families and arose de novo (six missense and three deletions) in nine of these families. Although the sample size is small, these sites could represent mutation hotspots in *PIEZO2*.

The phenotypes of GS, DA5, and MWS are distinguished from one another by just a few major characteristics. However, some individuals with mutations in *PIEZO2* had characteristics that spanned more than one of these diagnoses (Figure 3). Furthermore, the c.8057G>A

Table 2. Summary of *PIEZO2* Mutations Found in Individuals with GS, DA5, or MWS

Mutation Information					Number of Families				Inheritance	
Exon	cDNA Change	GERP	Protein Alteration	Type	Total	GS	DA5	MWS	De Novo	AD
15	c.2134A>G	4.80	p.Met712Val	missense	2	0	2	0	+	+
20	c.2993T>C	4.99	p.Met998Thr	missense	1	0	1	0	+	–
43	c.6662C>T	4.68	p.Thr2221Ile	missense	2	0	2	0	+	+
43	c.6668C>T	5.55	p.Ser2223Leu	missense	1	0	1	0	+	–
45	c.7067C>T	4.64	p.Thr2356Met	missense	1	0	1	0	–	+
52	c.8057G>A	4.62	p.Arg2686His	missense	11	9	2	0	+	+
52	c.8056C>T	4.62	p.Arg2686Cys	missense	1	0	0	1	+	–
52	c.8153G>T	5.24	p.Arg2718Leu	missense	2	0	2	0	–	+
52	c.8153G>C	5.24	p.Arg2718Pro	missense	1	0	1	0	+	–
52	c.8181_8183delAGA	NA	p.Glu2727del	in-frame deletion	10	0	10	0	+	+
52	c.8208delA	NA	p.Tyr2737Ilefs*7	single bp deletion	1	0	1	0	+	–
52	c.8215T>C	4.71	p.Ser2739Pro	missense	1	0	1	0	–	+
52	c.8238_8245 del8	NA	p.Trp2746*	8 bp deletion	1	1	0	0	+	–
Total					**35**	**10**	**24**	**1**		

This table summarizes the mutations identified in 35 families affected by GS (n = 10), DA5 (n = 24), or MWS (n = 1). GERP scores provide an estimate of conservation across species at a nucleotide site; a more positive score is associated with deleteriousness. Abbreviations are as follows: AD, autosomal dominant, GERP, Genomic Evolutionary Rate Profiling; and NA, not applicable.

(p.Arg2686His) mutation, which explains most of the GS cases, was found in two DA5-affected families (i.e., families X and Y). This observation raises the question as to whether GS, DA5, and MWS are distinct syndromes or different manifestations of the same condition.

One explanation for the overlap of clinical phenotypes among these syndromes is that there is a strong genotype-phenotype relationship between *PIEZO2* mutations and the major characteristics that vary among GS, DA5, and MWS. Accordingly, we tested whether c.8181_8183delAGA and c.8057G>A were associated with the presence of cleft palate, pulmonary disease, ophthalmoplegia, scoliosis, or cerebellar malformation regardless of diagnosis. Cleft palate was significantly associated (Fisher's exact test, adjusted p value < 0.0001) with c.8057G>A, but no other associations were significant. All of the individuals with GS and a c.8057G>A mutation had either cleft palate (n = 12) or a bifid uvula (n = 2), but only four were reported to have ptosis and only one had ophthalmoplegia. These findings suggest that the phenotype caused by c.8057G>A is a distinct entity, namely GS. Yet, neither of the individuals with the c.8057G>A mutation in the two DA5-affected families had cleft palate or a bifid uvula. Given the reduced penetrance of cleft palate in GS, we suspect that the correct diagnosis in these two families is GS rather than DA5.

Eighty-five percent (11/13) of the mutations we identified in *PIEZO2* are predicted to alter amino acids in the intracellular C-terminal domain of PIEZO2. The C-terminal domain is highly conserved across metazoans, although its function is unknown. Nevertheless, its integrity appears to be vital to the function of PIEZO2 in normal physiology and development of skeletal muscle. Moreover, at least two *PIEZO2* regions that encode the C-terminal domain (c.8057G and c.8181_8183delAGA) appear to be hotspots for mutations, more intolerant of variation, or some combination thereof.

PIEZO2 and PIEZO1 are large, transmembrane protein components of mechanically or stretch-activated ion channels found in many tissues.[29] Recent electrophysiological studies of two individuals with DA5 have revealed that c.8181_8183delAGA (p.Glu2727 del) is a gain-of-function mutation that causes *PIEZO2*-dependent, mechanically activated currents to inactivate slower but recover faster from this inactivation. As a result, altered channels transduce repetitive mechanical signals more efficiently.[27] One of the more unusual clinical characteristics of individuals with DA5 is that their muscles, particularly the muscles of the anterior chest wall, feel firm or "woody" on palpation, suggesting that resting muscle tone might be higher. Additionally, some individuals with DA5 develop progressive pulmonary insufficiency partly because of restrictive chest disease. Furthermore, in contrast to other DA disorders, the contractures of some individuals with DA5 appear to worsen with age despite intervention (e.g., physical therapy). Collectively, these observations suggest that in addition to affecting the development of skeletal muscle, dysregulated mechanotransduction due to *PIEZO2* mutations might also affect the function of skeletal muscles after birth.

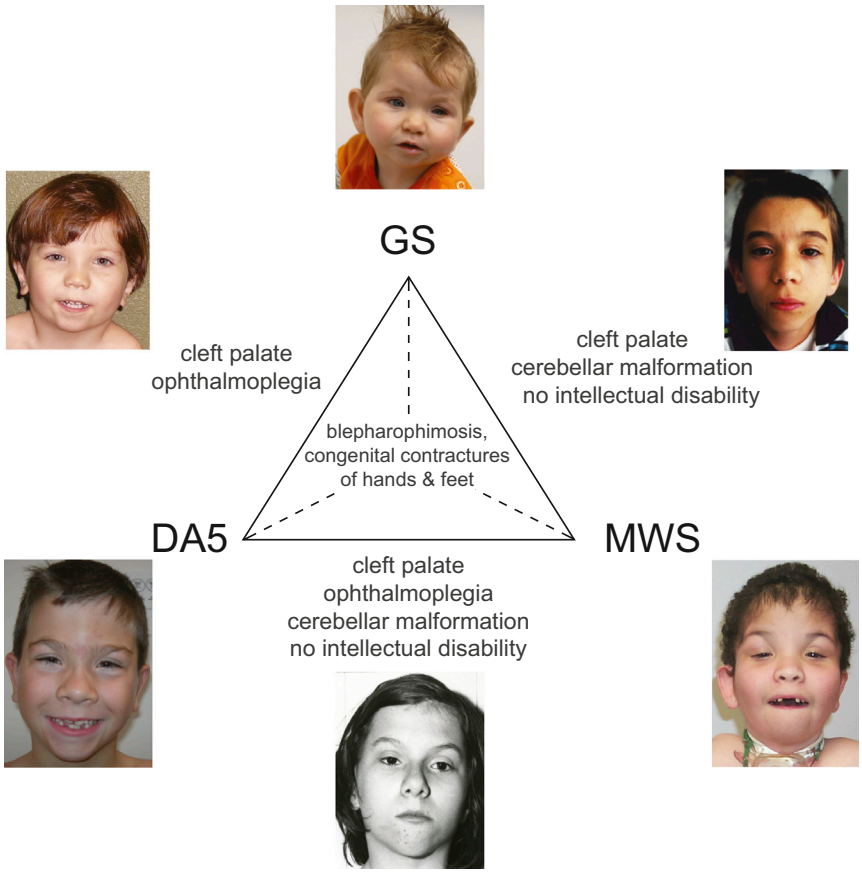

Figure 3. Phenotype Overlap among Families Affected by *PIEZO2* Mutations and GS, DA5, or MWS

Individuals with GS, DA5, or MWS share common clinical findings, including short stature, curved fingers with straight thumbs, and foot contractures and/or an increased space between the first and second toes (the so-called "sandal gap"). Specific additional clinical features (e.g., cleft palate in GS, ophthalmoplegia in DA5, and cerebellar malformations in MWS) are typically used for distinguishing these conditions from one another. Representative facial photos of individuals with GS (C:II-1), DA5 (Y:II-1), and MWS (II:II-1) are shown. In families affected by *PIEZO2* mutations, some individuals had "intermediate phenotypes," that is, they shared so-called "distinguishing features" among GS, DA5, and MWS. Individual E:II-1 had both cleft palate and ophthalmoplegia, individual I:II-1 had both cleft palate and a cerebellar malformation, and individual J:I-2 had cleft palate, ophthalmoplegia, and a cerebellar malformation.

The absence of a *PIEZO2* mutation in two GS individuals and one MWS individual suggests that both could be genetically heterogeneous. However, it is likely that that our sequencing strategy using MIPs could not reliably detect some indels, including single-exon deletions. To assess whether deletions or duplications that include *PIEZO2* might indeed be responsible for some cases of GS or MWS, we queried the DECIPHER database and identified 44 individuals with either deletions (n = 22) or duplications (n = 22) over the region containing all or part of *PIEZO2*. For the 30 of these individuals for whom phenotypic information was available, ten had a feature that could be considered to be within the GS and DA5 phenotype spectrum. Specifically, seven had some type of congenital contracture, three had cleft palate, and two had ptosis. Only two of these ten individuals had more than one of these characteristics. None of the individuals with the smallest deletions that included *PIEZO2* had any of these features. This observation is consistent with the findings of Coste et al., who reported that DA5 is caused by gain-of-function, rather than loss-of-function, mutations in *PIEZO2*.[27]

The hypothesis that the DA5 phenotype in individuals with *PIEZO2* mutations might reflect ion channel hyperresponsiveness to forces engendered during development and later during postnatal life[27] could extend to GS and MWS as well. Mechanotransduction plays an important role during normal development of multiple-organ systems, including the CNS. In the brain, for example, mechanical interaction between cells and their environment affects the speed and directionality of neuronal migration.[30] How mechanical forces are sensed and transduced by such cells is unclear. *PIEZO2* is expressed in the brain, including the hindbrain, but there is virtually no information available about its role, if any, in the development of the CNS. The observation that some individuals with GS, DA5, or MWS and *PIEZO2* mutations have hindbrain malformations offers perhaps a window for further investigation.

In summary, we used exome sequencing to discover that mutations in *PIEZO2* cause GS and MWS. Furthermore, we found that mutations in *PIEZO2* also explain the overwhelming majority of cases of DA5. The genetic and phenotypic overlap among these three conditions supports a shared developmental mechanism, although the presence of a genotype-phenotype relationship in GS suggests that certain functions of *PIEZO2* might be perturbed by the disruption of specific residues or domains. Along with our recent report that mutations in *ECEL1* (MIM 605896) cause distal arthrogryposis type 5D[31] (DA5D [MIM 615065]), the present study suggests that DA5 and its subtypes are caused by a disturbance of the neuromuscular pathways that influence the development of skeletal muscle, whereas other DA types are caused by mutations in genes that encode proteins of the contractile apparatus. Although DA5 and its subtypes appear to be phenotypically distinct from other DAs, particularly the much more common DA1 and DA2B,[32] distinguishing DA5 from other DAs in a clinical setting is often challenging. Accordingly, the existing heuristic classification of DAs

appears to be a helpful tool for prioritizing the differential diagnosis. Yet, because natural history varies widely among different DA disorders, including between DA5D and DA5, identification of the underlying causal variant is essential.

Supplemental Data

Supplemental Data includes five figures and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2014.03.015.

Acknowledgments

We thank the families for their participation and support, Paul Frisby and Rosanna Pallotta for referral of affected individuals, and Christa Poel for technical assistance. Our work was supported in part by grants from the NIH National Human Genome Research Institute (1U54HG006493 to M.J.B., D.A.N., and J.S.; 1RC2HG005608 to M.J.B., D.A.N., and J.S.; 5R000HG004316 to H.K.T.), the NIH National Institute of Child Health and Human Development (1R01HD048895 to M.J.B.; 5K23HD057331 to A.E.B.), the Life Sciences Discovery Fund (2065508 and 0905001), and the Washington Research Foundation.

Received: January 6, 2014
Accepted: March 20, 2014
Published: April 10, 2014

Web Resources

The URLs for data presented herein are as follows:

DECIPHER, http://decipher.sanger.ac.uk/
FASTX-Toolkit, http://hannonlab.cshl.edu/fastx_toolkit/
Genome Analysis Toolkit (GATK), http://www.broadinstitute.org/gsa/wiki/
GeneCards, http://www.genecards.org
HumanCytoSNP-12 DNA Analysis BeadChip, http://www.illumina.com/products/humancytosnp_12_dna_analysis_beadchip_kits.ilmn
Human Genome Variation Society, http://www.hgvs.org/mutnomen/
NHLBI (Exome Sequencing Project) Exome Variant Server, http://evs.gs.washington.edu/EVS/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/
Picard, http://picard.sourceforge.net/
SAMtools, http://samtools.sourceforge.net/
SeattleSeq Annotation 137, http://snp.gs.washington.edu/SeattleSeqAnnotation137/

References

1. Gordon, H., Davies, D., and Berman, M. (1969). Camptodactyly, cleft palate, and club foot. A syndrome showing the autosomal-dominant pattern of inheritance. J. Med. Genet. *6*, 266–274.
2. Higgins, J.V., Hackel, E., and Kapur, S. (1972). A Second Family with Cleft Palate, Club feet and Camptodactyly. Am. J. Hum. Genet. *24*, 58a.
3. Halal, F., and Fraser, F.C. (1979). Camptodactyly, cleft palate, and club foot (the Gordon syndrome). A report of a large pedigree. J. Med. Genet. *16*, 149–150.
4. Say, B., Barber, D.H., Thompson, R.C., and Leichtman, L.G. (1980). The Gordon syndrome. J. Med. Genet. *17*, 405.
5. Robinow, M., and Johnson, G.F. (1981). The Gordon syndrome: autosomal dominant cleft palate, camptodactyly, and club feet. Am. J. Med. Genet. *9*, 139–146.
6. Hall, J.G., Reed, S.D., and Greene, G. (1982). The distal arthrogryposes: delineation of new entities—review and nosologic discussion. Am. J. Med. Genet. *11*, 185–239.
7. Bamshad, M., Jorde, L.B., and Carey, J.C. (1996). A revised and extended classification of the distal arthrogryposes. Am. J. Med. Genet. *65*, 277–281.
8. Ioan, D.M., Belengeanu, V., Maximilian, C., and Fryns, J.P. (1993). Distal arthrogryposis with autosomal dominant inheritance and reduced penetrance in females: the Gordon syndrome. Clin. Genet. *43*, 300–302.
9. Patton, M.A., Sharma, A., and Winter, R.M. (1985). The Aase-Smith syndrome. Clin. Genet. *28*, 521–525.
10. Pallotta, R., Ehresmann, T., and Fusilli, P. (2000). Occurrence of Dandy-Walker anomaly in a familial case of distal arthogryposis type IIB. Am. J. Med. Genet. *95*, 477–481.
11. Becker, K., and Splitt, M. (2001). A family with distal arthrogryposis and cleft palate: possible overlap between Gordon syndrome and Aase-Smith syndrome. Clin. Dysmorphol. *10*, 41–45.
12. Williams, M.S., Josephson, K.D., and Wargowski, D.S. (1993). Marden-Walker syndrome: a case report and a critical review of the literature. Clin. Dysmorphol. *2*, 211–219.
13. Gripp, K.W., Scott, C.I., Jr., Brockett, B.C., Nicholson, L., and Mackenzie, W.G. (1996). Extending the spectrum of distal arthrogryposis. Am. J. Med. Genet. *65*, 286–290.
14. al-Ghamdi, M.A., Polomeno, R.C., Chitayat, D., Azouz, E.M., and Teebi, A.S. (1997). Arthrogryposis multiplex congenita, craniofacial, and ophthalmological abnormalities and normal intelligence: a new syndrome? Am. J. Med. Genet. *71*, 401–405.
15. Williams, M.S., Elliott, C.G., and Bamshad, M.J. (2007). Pulmonary disease is a component of distal arthrogryposis type 5. Am. J. Med. Genet. A. *143*, 752–756.
16. Sahni, J., Kaye, S.B., Fryer, A., Hiscott, P., and Bucknall, R.C. (2004). Distal arthrogryposis type IIB: unreported ophthalmic findings. Am. J. Med. Genet. A. *127A*, 35–39.
17. Friedman, B.D., and Heidenreich, R.A. (1995). Distal arthrogryposis type IIB: further clinical delineation and 54-year follow-up of an index case. Am. J. Med. Genet. *58*, 125–127.
18. Altman, H.S., and Davidson, L.T. (1939). Amyoplasia congenita (arthrogryposis multiplex congenita). J. Pediatr. *15*, 551–557.
19. Lai, M.M., Tettenborn, M.A., Hall, J.G., Smith, L.J., and Berry, A.C. (1991). A new form of autosomal dominant arthrogryposis. J. Med. Genet. *28*, 701–703.
20. Schrander-Stumpel, C.T., Höweler, C.J., Reekers, A.D., De Smet, N.M., Hall, J.G., and Fryns, J.P. (1993). Arthrogryposis, ophthalmoplegia, and retinopathy: confirmation of a new type of arthrogryposis. J. Med. Genet. *30*, 78–80.
21. Morgan, N.V., Brueton, L.A., Cox, P., Greally, M.T., Tolmie, J., Pasha, S., Aligianis, I.A., van Bokhoven, H., Marton, T., Al-Gazali, L., et al. (2006). Mutations in the embryonal subunit of the acetylcholine receptor (CHRNG) cause lethal and Escobar

variants of multiple pterygium syndrome. Am. J. Hum. Genet. *79*, 390–395.
22. Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics *25*, 1754–1760.
23. Abecasis, G.R., Auton, A., Brooks, L.D., DePristo, M.A., Durbin, R.M., Handsaker, R.E., Kang, H.M., Marth, G.T., and McVean, G.A.; 1000 Genomes Project Consortium (2012). An integrated map of genetic variation from 1,092 human genomes. Nature *491*, 56–65.
24. Krumm, N., Sudmant, P.H., Ko, A., O'Roak, B.J., Malig, M., Coe, B.P., Quinlan, A.R., Nickerson, D.A., and Eichler, E.E.; NHLBI Exome Sequencing Project (2012). Copy number variation detection and genotyping from exome sequence data. Genome Res. *22*, 1525–1532.
25. O'Roak, B.J., Vives, L., Fu, W., Egertson, J.D., Stanaway, I.B., Phelps, I.G., Carvill, G., Kumar, A., Lee, C., Ankenman, K., et al. (2012). Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science *338*, 1619–1622.
26. Carvill, G.L., Heavin, S.B., Yendle, S.C., McMahon, J.M., O'Roak, B.J., Cook, J., Khan, A., Dorschner, M.O., Weaver, M., Calvert, S., et al. (2013). Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. Nat. Genet. *45*, 825–830.
27. Coste, B., Houge, G., Murray, M.F., Stitziel, N., Bandell, M., Giovanni, M.A., Philippakis, A., Hoischen, A., Riemer, G., Steen, U., et al. (2013). Gain-of-function mutations in the mechanically activated ion channel PIEZO2 cause a subtype of Distal Arthrogryposis. Proc. Natl. Acad. Sci. USA *110*, 4667–4672.
28. Fryns, J.P., Willekens, D., Van Schoubroeck, D., and Moerman, P. (1998). Marden-Walker syndrome versus isolated distal arthrogryposis: evidence that both conditions may be variable manifestations of the same mutated gene. Clin. Genet. *54*, 86–89.
29. Coste, B. (2011). [Feeling the pressure? Identification of two proteins activated by mechanical forces]. Med. Sci. (Paris) *27*, 17–19.
30. Franze, K. (2013). The mechanical control of nervous system development. Development *140*, 3069–3077.
31. McMillin, M.J., Below, J.E., Shively, K.M., Beck, A.E., Gildersleeve, H.I., Pinner, J., Gogola, G.R., Hecht, J.T., Grange, D.K., Harris, D.J., et al.; University of Washington Center for Mendelian Genomics (2013). Mutations in ECEL1 cause distal arthrogryposis type 5D. Am. J. Hum. Genet. *92*, 150–156.
32. Beck, A.E., McMillin, M.J., Gildersleeve, H.I., Kezele, P.R., Shively, K.M., Carey, J.C., Regnier, M., and Bamshad, M.J. (2013). Spectrum of mutations that cause distal arthrogryposis types 1 and 2B. Am. J. Med. Genet. A. *161A*, 550–555.