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Xq26.3 Duplication in a Boy With Motor Delay and Low Muscle Tone Refines the X-Linked Acrogigantism Genetic Locus

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We describe a 4-year-old boy with developmental delay who was found to carry by clinical grade (CG) molecular cytogenetics (MCs) a chromosome Xq26 microduplication. The report prompted a referral of the patient for possible X-linked acrogigantism (X-LAG), a well-defined condition (MIM300942) due to chromosomal microduplication of a nearby region. The patient was evaluated clinically and investigated for endocrine abnormalities related to X-LAG and not only did he not have acrogigantism, but his growth parameters and other hormones were all normal. We then performed high definition MCs and the duplication copy number variant (CNV) was confirmed to precisely map outside the X-LAG critical region and definitely did not harbor the X-LAG candidate gene, *GPR101*. The patient's phenotype resembled that of other patients with Xq26 CNVs. The case is instructive for the need for high definition MCs when CG MCs' results are inconsistent with the patient's phenotype. It is also useful for further supporting the contention that *GPR101* is the gene responsible for X-LAG.

Genomic disorders result from loss or gain of DNA material [1, 2]. Copy number variants (CNVs) can be pathogenic if they involve a dosage-sensitive gene(s) or if they influence genomic regions through regulatory elements [3]. This was recently found to occur in patients affected by a new genomic disorder, X-linked acrogigantism (X-LAG) [4]. These patients have germline (usually female) or somatic (usually male) Xq26.3 duplications and all present with GH and prolactin-secreting pituitary tumors or hyperplasia that are the cause of their gigantism. The size of these microduplications varies among patients and can thus include a different number of genes (reviewed in [5]). However, the smallest region of overlap was recently shown to encompass only one protein-coding gene, *GPR101* [6]. *GPR101* encodes for an orphan G protein-coupled receptor that is overexpressed in the pituitary lesions of affected individuals [4].

Testing for X-LAG syndrome can be performed by commercially available molecular cytogenetics (MCs) using clinical grade (CG) 60K array comparative genomic hybridization (aCGH).

Abbreviations: aCGH, array comparative genomic hybridization; CG, clinical grade; CNV, copy number variant; ddPCR, droplet digital PCR; HD-aCGH, high-density array comparative genomic hybridization; MC, molecular cytogenetic; X-LAG, X-linked acrogigantism.

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However, there are several limitations that may hinder a proper diagnosis: duplications can be smaller than the array resolution, and somatic mosaicism in male patients could lead to false negative results unless additional tissues are analyzed [6].

To minimize erroneous diagnoses due to the use of techniques with suboptimal resolution for this kind of analysis, a higher resolution aCGH platform is recommended. One such aCGH array specifically designed with high-density probes tiling the critical Xq26.3 region has been successfully used in different studies. Rearrangements identified by this method can then be confirmed using droplet digital PCR (ddPCR) [6, 7]. Ultimately, the use of these high-resolution techniques greatly benefits clinical counseling and assists researchers in defining the genetic maps harboring disease-responsible loci and elucidating potential gene(s) contributing to the phenotype.

1. Materials and Methods

A. Protocol

The patient was recruited under National Institute of Child Health and Human Development, National Institutes of Health protocol 97CH0076 for the study of patients with possible acrogigantism and related disorders. The parents were provided informed consent and signed the appropriate institutional review board approved consent forms.

B. DNA Studies

Proband and maternal DNA was extracted from peripheral blood using the QIAamp DNA Mini Kit (#51304, Qiagen, Valencia, CA) according to the manufacturer's protocol.

To precisely determine the size, genomic extent, gene content, and putative genomic boundaries of the rearrangement, we used an 8X60K format aCGH (Agilent Technologies, Santa Clara, CA) with high-density probes tiling the Xq26.3 region. The probe density averaged 5 oligonucleotides/kb for the critical X-LAG region and also interrogates the flanking genomic regions of up to 2 Mb in size with probe density of 1 to 2 per kb. The experimental procedures of aCGH, including DNA fragmentation, labeling, and hybridization, were performed as previously described [8].

Individual TaqMan CNV assays were performed to exclude duplication of the genes located in the X-LAG minimal region. CNV assays for *CD40LG*, *ARHGEF6*, *RBMX*, and *GPR101* consisted in a pair of unlabeled primers and a FAM-labeled MGB probe and were supplied from Thermo Fisher Scientific (assay ID: Hs02425845_cn, Hs01655699_cn, Hs01064297_cn, Hs01730605_cn, respectively). *RNaseP* (Thermo Fisher Scientific, #4403328, Waltham, MA) with a VIC-labeled TAMRA probe was used as reference gene. For more details on the protocol please refer to [4].

The DECIPHER v9.14 (https://decipher.sanger.ac.uk/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), dbVar (https://www.ncbi.nlm.nih.gov/dbvar), and Ecaruca (http://umcecaruca01.extern.umcn.nl:8080/ecaruca/ecaruca.jsp) databases were queried for CNV gains overlapping the duplicated Xq26.3 region that were comparable in size. We excluded patients harboring other concomitant genomic CNVs and those without available phenotypic information. All the cases retrieved are reported in Table 1 (no cases compatible with our search criteria were retrieved from the Ecaruca database).

2. Results

A. Case Report

The patient was first assessed at the age of 4 years when he presented with developmental delay and low muscle tone (Fig. 1). He was the second child, and first son, to Caucasian parents from a nonconsanguineous union. He was born at 39 weeks of gestation by

Table 1. Cases With Gains in the Xq26 Region Overlapping the Described Duplication (and Without Other CNVs) Listed in Databases of Genomic Variants (DECIPHER, dbVar, ClinVar)

Database	Code	ChrX Region Affected	Sex	Type	Size (bp)	Phenotype	Pathogenicity Contribution	No. of Genes Involved
	288958	134,824,559–134,910,208	NA	Gain	85,650	Abnormality of the cardiovascular system	Likely benign	3
	288811	134,766,956–134,910,208	NA	Gain	143,253	Abnormality of the nervous system; abnormality of the eye; growth abnormality	Likely benign	3
DECIPHER	283542	134,753,254–134,966,528	F	Duplication	213,275	Abnormality of head or neck; abnormality of metabolism/homeostasis; abnormality of the nervous system	NA	6
	280581	134,427,476–134,910,134	F	Duplication	482,659	Abnormality of the nervous system	NA	10
	341675	134,292,902–134,800,285	F	Triplication	507,384	Abnormality of the digestive system; abnormality of the nervous system	Uncertain	8
	262539	133,699,928–134,345,039	F	Duplication	645,112	Abnormality of the nervous system	NA	17
	251804	133,600,845–134,304,715	M	Duplication	703,871	Abnormality of the endocrine system; growth abnormality; abnormality of head or neck; abnormality of the nervous system; abnormality of the skeletal system	NA	25
ClinVar	221901	134,292,131–134,852,104	NA	Duplication	559,974	Premature ovarian failure	Benign	5
	254013	134,292,112–134,983,787	NA	Duplication	691,676	Autism[a]; global developmental delay	Uncertain	10
dbVar	nsv498263	134,293,036–134,330,110	M	Duplication	37,075	Developmental delay AND/OR other significant developmental or morphological phenotypes	Likely benign	1
	nsv931744	134,215,720–134,372,869	F	Duplication	157,150	Developmental delay AND/OR other significant developmental or morphological phenotypes	Benign	3
	nsv534415	134,725,220–134,910,134	M	Duplication	184,915	Developmental delay AND/OR other significant developmental or morphological phenotypes	Benign	4
	nsv995036	134,725,157–134,910,208	M	Duplication	185,052	Developmental delay AND/OR other significant developmental or morphological phenotypes	Likely benign	4
	nsv534162	134,427,695–134,613,879	F	Duplication	186,185	Developmental delay AND/OR other significant developmental or morphological phenotypes	Benign	4
	nsv917314	134,292,347–134,910,134	M	Duplication	617,788	Developmental delay AND/OR other significant developmental or morphological phenotypes	Uncertain	11
	nsv931406	134,292,347–134,800,326	F	Duplication	507,980	Congenital muscular torticollis; delayed gross motor development[a]; muscular hypotonia[a]; short stature	Likely benign	8

All coordinates correspond to the hg19 build of the human genome.
Abbreviation: NA, not available.
[a]Clinical features overlapping the phenotype of the patient described here.

spontaneous vaginal delivery. There were no complications during pregnancy other than nausea, and antenatal ultrasound scans were within normal limits. His birth weight was 3100 g (>10th < 25th centile). He had a history of mild flexural eczema and an abdominal capillary hemangioma that spontaneously resolved. He has remained well throughout his childhood, with no major illnesses or hospitalizations. He has a family history of mild gross motor delay, with both mother and maternal uncle walking at 18 months.

He was first noted to have motor delay at 6 months of age, when he was unable to sit. He went on to sit unaided at 10 months. He never crawled, and mobilized by bottom shuffling from 18 months, and eventually walked at 32 months. He had delays in fine motor movements, no discernible speech, and delayed receptive language skills at 32 months. He had no behavioral or sleep disturbances. At age 4, he could follow simple commands and communicate with some single words and spontaneous signs. A formal diagnosis of autism was recently confirmed.

On examination he had dysmorphic features: deep set eyes, with a long and thin face, prominent forehead, and a prominent chin (Fig. 1). However, he did not display features of gigantism or acromegaly typical of patients with X-LAG. His height tracked along the 75th centile in keeping with his midparental height, whereas his head circumference measured at the 50th centile. He had low subcutaneous fat stores, and his weight tracked between the 5th and 10th centiles for age. He had low peripheral muscle tone and joint

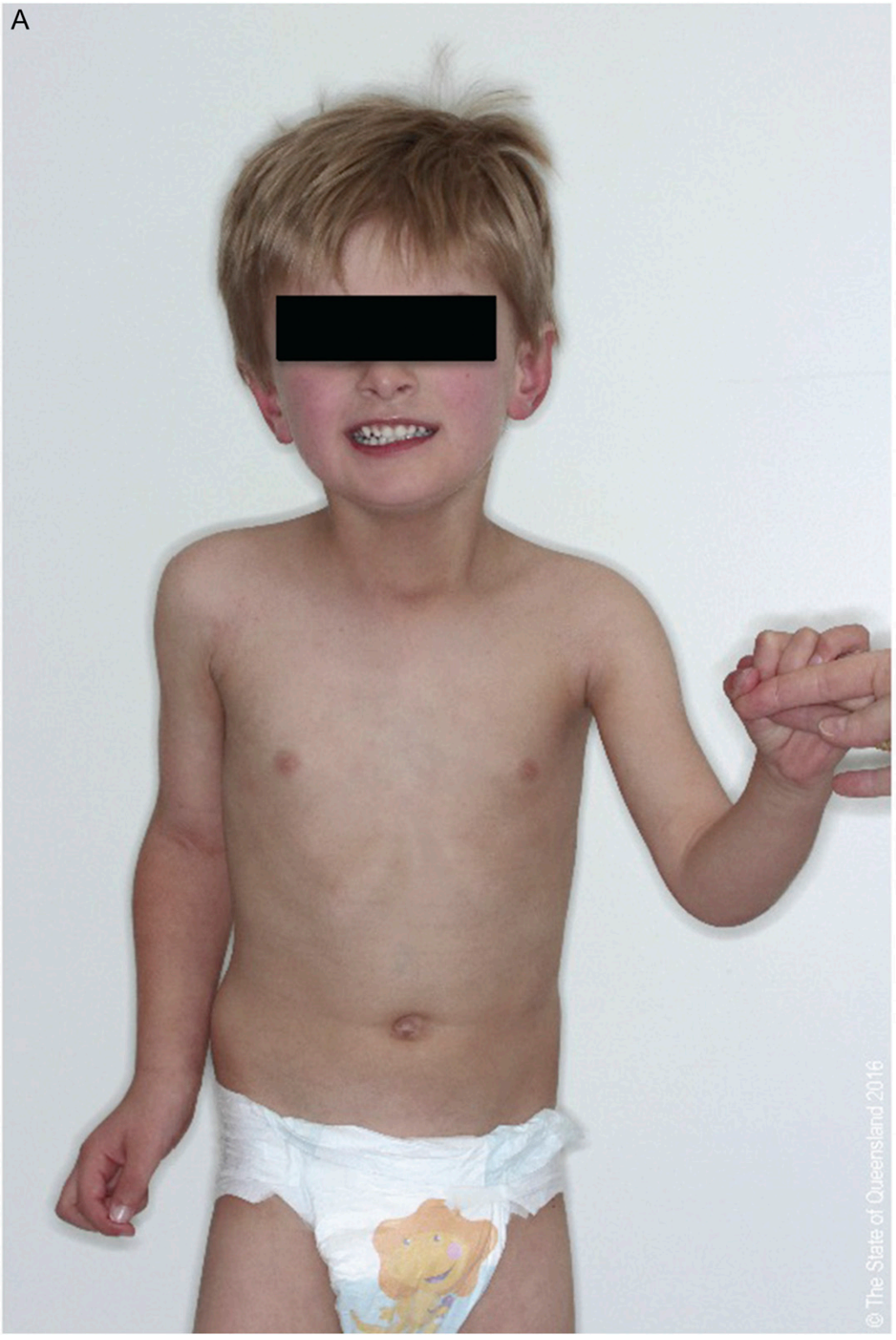

Figure 1. (A) Proband at 4 years of age. (B) Proband's height and weight growth charts showing he does not manifest gigantism.

hypermobility particularly at the wrists and ankles. His deep tendon reflexes and sensation were normal. His cardiovascular, respiratory, abdominal, and genital examinations were also normal.

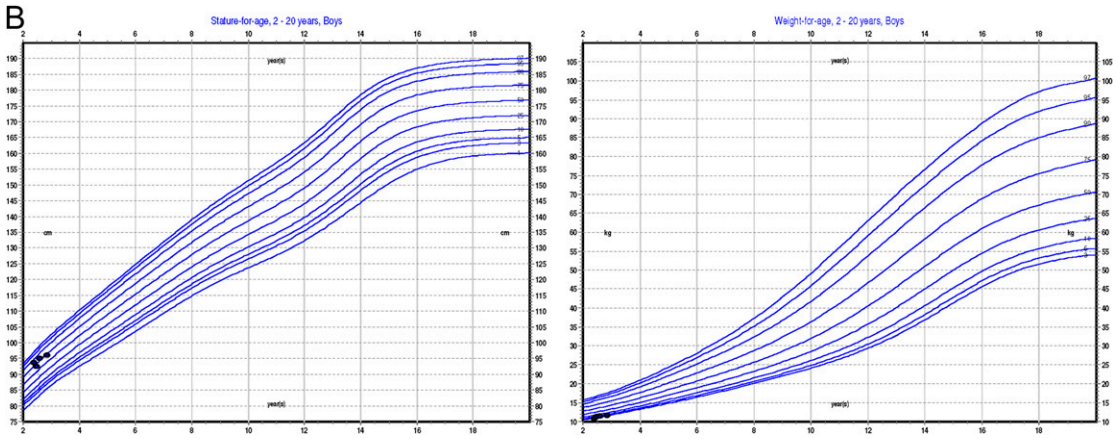

Figure 1. (Continued)

Investigations to date revealed normal pituitary function and biochemistry including full blood count, electrolytes, renal and liver function, thyroid function (free T4 12.9 pmol/L, age-specific reference ranges: 9.0 to 19.0 pmol/L; TSH 3.2 mIU/L, age-specific reference ranges: 0.3 to 5.8 mIU/L), IGF-1, prolactin (197 mIU/L, age-specific reference: <500 mIU/L), ammonia, lactate, creatinine kinase, plasma amino acids, and urine organic acids. Studies done by MC CG using aCGH reported that the patient carried an Xq26.3 chromosomal defect (at max, chrX: 134,293,066 to 134,831,695; hg19) inherited from his mother. The mother, except for mild gross motor delay, did not manifest any other clinical features, however.

The subject underwent whole exome sequencing and revealed a variant in the *UPF3B* gene. There were no other relevant variants that could relate to the patient's phenotype. Currently, work is ongoing for proving the connection of this variant with the subject's phenotype and this is part of another investigation that may result in a separate manuscript as part of *UPF3B*-focused studies.

B. Precise MC Characterization of the Xq26.3 Structural Variation

We performed high-density aCGH (HD-aCGH) on leukocyte-derived DNA, which further refined the duplicated region by showing an Xq26.3 duplication spanning ~650 kb (at max, chrX: 134,248,528 to 134,903,125; hg19) (Fig. 2). The duplicated region, inherited from the mother, encompasses several genes including two long noncoding RNAs (*LINC00633* and *SMIM10L2A*), two transcription factors (*ZNF75D* and *ZNF449*), and cancer/testis antigens (*CT55* and the *CT45* gene family). The region is flanked by numerous low copy repeats and the duplication is potentially mediated by nonallelic homologous recombination. Individual TaqMan CNV assays confirmed that the four OMIM genes commonly duplicated in X-LAG patients were not affected by the rearrangement (Fig. 3).

A query of different databases of genomic variants for patients with overlapping rearrangements of similar size returned 13 entries (Table 1). Several of these patients show common phenotypes, including intellectual disability and developmental delay. Interestingly, one case was reported with delayed gross motor development. Furthermore, the mother also harbored an apparent gain at Xq22.2 (at max, chrX: 103,250,028 to 103,380,167), overlapping at least three genes. This Xq22.2 duplication, however, has not been inherited by her son (data not shown).

3. Discussion

We report a pediatric patient who was originally referred to us as harboring an Xq26.3 duplication that was detected by commercially available MC CG using aCGH. The patient

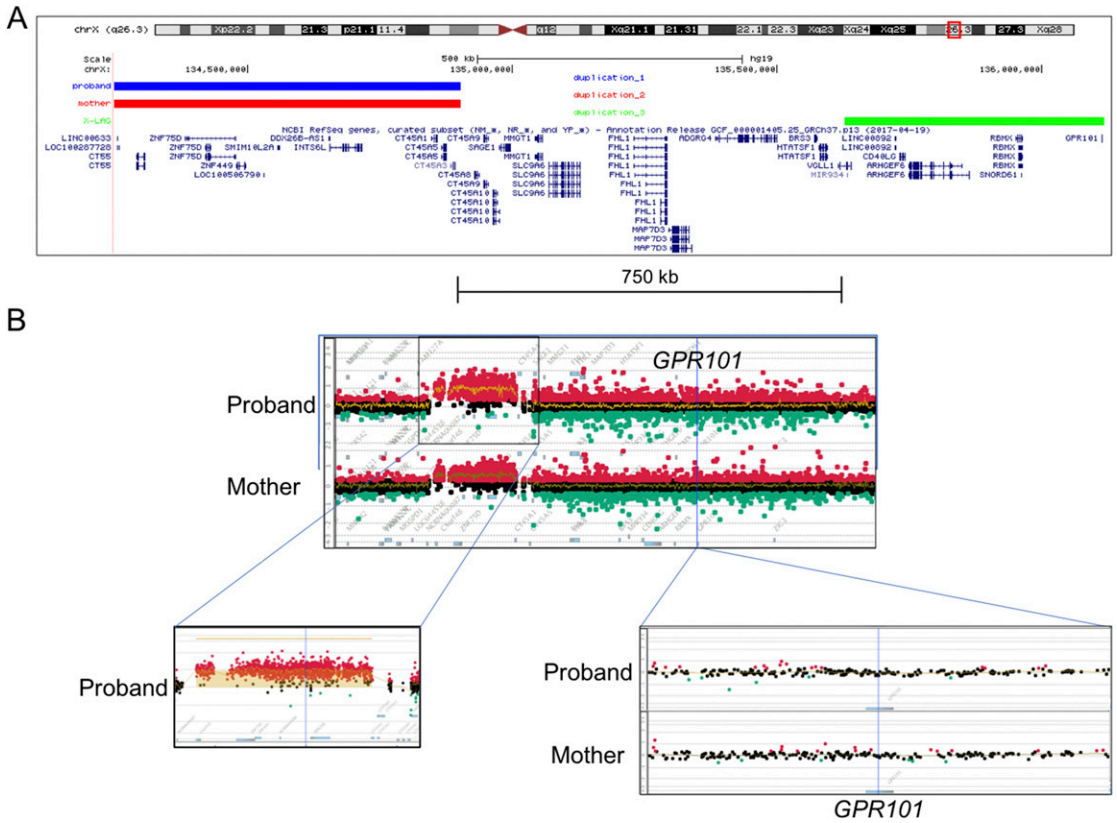

Figure 2. Diagrams depicting the chromosomal location and size of the Xq26.3 duplication detected in the proband. (A) The 650-kb duplication detected by HD-aCGH in the proband and his mother is shown in blue and red, respectively. The duplication is located ~750 kb upstream of the originally described X-LAG duplicated region (chrX: 135,627,637 to 136,118,269), depicted in green [4]. (B) aCGH $\log_2$ ratio plots showing the duplication identified in the proband and his mother. The cluster of red dots represents the copy number gain relative to the control. The left zoom in panel depicts in detail the duplicated area, whereas the panel on the right shows that the X-LAG minimal region is normal. All coordinates correspond to the hg19 build of the Human genome.

phenotype did not match the gigantism phenotype observed in all X-LAG cases reported so far (reviewed in [5]). We then performed HD-aCGH to precisely determine the boundaries of the duplication.

The microduplication in our patient did not overlap with the described X-LAG CNV region, being ~750 kb upstream and was, rather, associated with a different phenotype that matched some of those reported in other patients with Xq26 duplication CNVs (Table 1). Some of the duplicated genes showed testis-specific expression (*CT55*, *CT45A1*, *LINC00633*) (http://www.proteinatlas.org/), were reported to possibly regulate chondrogenesis (*ZNF449*) [9], and to promote (*CT55*, *CT45A1*) [10, 11] or inhibit (*SMIM10L2A*) [12] cell growth. Therefore, because there were no obvious candidates that could explain this patient's phenotype, further investigation to determine their potential contribution to the patient's final phenotype was pursued. Based on these considerations and following published guidelines for the assessment of the clinical relevance of a CNV [13, 14], we suggest that this duplication should be classified a variant of uncertain significance.

This case is instructive on how low-resolution CG aCGH in cases with doubt should be followed by HD-aCGH before giving advice to patients and their families. Such investigation should always be sought in cases where there are discrepancies between the clinical phenotype and the known manifestations for a given chromosomal defect. The fact that the patient did not have a chromosomal defect including the *GPR101* gene and he did not have

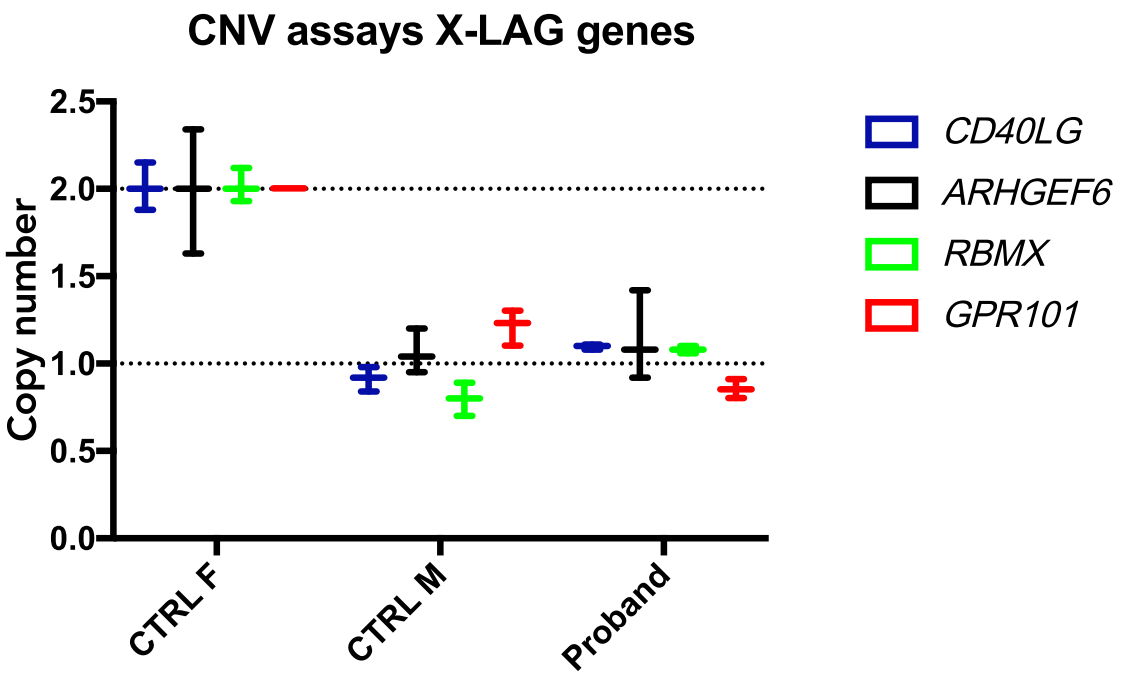

Figure 3. Individual CNV assays for *CD40LG*, *ARHGEF6*, *RBMX*, and *GPR101*. The proband did not show any duplication for the four OMIM genes commonly duplicated in X-LAG patients. The calculated copy number along with the minimum and maximum copy number values (copy number range bars) are shown for each gene. For details on the methods, please refer to Trivellin *et al.* [4]. CTRL F, normal female control; CTRL M, normal male control.

clinically or biochemically X-LAG supports the notion that *GPR101* is the gene that confers most of the phenotypic findings associated with this rare and relatively unique form of gigantism. *GPR101* was suggested as the causative gene for X-LAG by the initial report of the syndrome [4] and this suggestion was further supported by the recent description of a patient with X-LAG who had an Xq26 microduplication that involved only *GPR101* [6]. The latter patient, however, had yet another X-chromosome variant, which appears to be also unrelated to X-LAG.

We recently published an algorithm for the genetic evaluation of GH excess [15]. This report and other recent studies showing the importance of performing HD-aCGH and/or ddPCR [6, 7] prompted us to highlight here some important considerations that clinicians should keep in mind for a correct molecular genetic diagnosis of X-LAG: (1) patients with a phenotype suggestive of X-LAG but that tested negative for Xq26.3 duplications by CG aCGH (first-tier analysis commonly performed in leukocyte- or saliva-derived DNA) should be genetically re-evaluated by HD-aCGH, possibly using DNA extracted from different sources (*e.g.*, buccal cells, skin forearm, or the resected pituitary lesion, if available). This second-tier test is especially important in sporadic male patients, because so far they presented as somatic mosaics. (2) A positive finding could be confirmed by employing *GPR101*-specific TaqMan hydrolysis probes used in conjunction with Real-Time PCR or ddPCR instruments. Borderline and negative findings should prompt a third-tier screening by ddPCR because HD-aCGH can still miss low levels of mosaicism (<10% to 20%), whereas ddPCR has the ability to detect mosaicism levels as low as 5%. (3) We are nonetheless aware that HD-aCGH assays are expensive and commonly limited to specialized laboratory; therefore, if performing HD-aCGH is not feasible for the referring laboratory, as an alternative to the approach outlined above we recommend ddPCR as second-tier test. Advantages of ddPCR include its ease of use and its suitability to screen large numbers of patients in a short time; however, investigators should keep in mind that this analysis does not return information on the extent of a possible duplication.

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Disclosure Summary: J.R.L. has stock ownership in 23andMe, is a paid consultant for Regeneron Pharmaceuticals, has stock options in Lasergen, Inc., and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, and bacterial genomic fingerprinting. The Department of Molecular and Human Genetics at Baylor College of Medicine derives revenue from molecular genetic testing offered in the Baylor-Genetics Laboratories. The remaining authors have nothing to disclose.

References and Notes

1. Harel T, Lupski JR. Genomic disorders 20 years on-mechanisms for clinical manifestations. *Clin Genet*. 2018;**93**(3):439–449.
2. Lupski JR. Structural variation mutagenesis of the human genome: impact on disease and evolution. *Environ Mol Mutagen*. 2015;**56**(5):419–436.
3. Carvalho CM, Lupski JR. Mechanisms underlying structural variant formation in genomic disorders. *Nat Rev Genet*. 2016;**17**(4):224–238.
4. Trivellin G, Daly AF, Faucz FR, Yuan B, Rostomyan L, Larco DO, Schernthaner-Reiter MH, Szarek E, Leal LF, Caberg JH, Castermans E, Villa C, Dimopoulos A, Chittiboina P, Xekouki P, Shah N, Metzger D, Lysy PA, Ferrante E, Strebkova N, Mazerkina N, Zatelli MC, Lodish M, Horvath A, de Alexandre RB, Manning AD, Levy I, Keil MF, Sierra ML, Palmeira L, Coppieters W, Georges M, Naves LA, Jamar M, Bours V, Wu TJ, Choong CS, Bertherat J, Chanson P, Kamenický P, Farrell WE, Barlier A, Quezado M, Bjelobaba I, Stojilkovic SS, Wess J, Costanzi S, Liu P, Lupski JR, Beckers A, Stratakis CA. Gigantism and acromegaly due to Xq26 microduplications and GPR101 mutation. *N Engl J Med*. 2014;**371**(25):2363–2374.
5. Trivellin G, Hernández-Ramírez LC, Swan J, Stratakis CA. An orphan G-protein-coupled receptor causes human gigantism and/or acromegaly: Molecular biology and clinical correlations. *Best Pract Res Clin Endocrinol Metab*. 2018;**32**(2):125–140.
6. Iacovazzo D, Caswell R, Bunce B, et al. Germline or somatic GPR101 duplication leads to X-linked acrogigantism: a clinico-pathological and genetic study. *Acta Neuropathol Commun*. 2016;**4**(1):56.
7. Daly AF, Yuan B, Fina F, Caberg JH, Trivellin G, Rostomyan L, de Herder WW, Naves LA, Metzger D, Cuny T, Rabl W, Shah N, Jaffrain-Rea ML, Zatelli MC, Faucz FR, Castermans E, Nanni-Metellus I, Lodish M, Muhammad A, Palmeira L, Potorac I, Mantovani G, Neggers SJ, Klein M, Barlier A, Liu P, Ouafik L, Bours V, Lupski JR, Stratakis CA, Beckers A. Somatic mosaicism underlies X-linked acrogigantism syndrome in sporadic male subjects. *Endocr Relat Cancer*. 2016;**23**(4):221–233.
8. Shinawi M, Liu P, Kang SH, Shen J, Belmont JW, Scott DA, Probst FJ, Craigen WJ, Graham BH, Pursley A, Clark G, Lee J, Proud M, Stocco A, Rodriguez DL, Kozel BA, Sparagana S, Roeder ER, McGrew SG, Kurczynski TW, Allison LJ, Amato S, Savage S, Patel A, Stankiewicz P, Beaudet AL, Cheung SW, Lupski JR. Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioural problems, dysmorphism, epilepsy, and abnormal head size. *J Med Genet*. 2010;**47**(5):332–341.
9. Okada K, Fukai A, Mori D, Hosaka Y, Yano F, Chung UI, Kawaguchi H, Tanaka S, Ikeda T, Saito T. Identification of SCAN domain zinc-finger gene ZNF449 as a novel factor of chondrogenesis. *PLoS One*. 2014;**9**(12):e115169.
10. Shang B, Gao A, Pan Y, Zhang G, Tu J, Zhou Y, Yang P, Cao Z, Wei Q, Ding Y, Zhang J, Zhao Y, Zhou Q. CT45A1 acts as a new proto-oncogene to trigger tumorigenesis and cancer metastasis. *Cell Death Dis*. 2014;**5**(6):e1285.
11. Tomiyoshi G, Nakanishi A, Takenaka K, Yoshida K, Miki Y. Novel BRCA2-interacting protein BJ-HCC-20A inhibits the induction of apoptosis in response to DNA damage. *Cancer Sci*. 2008;**99**(4):747–754.

12. Léveillé N, Melo CA, Rooijers K, Díaz-Lagares A, Melo SA, Korkmaz G, Lopes R, Akbari Moqadam F, Maia AR, Wijchers PJ, Geeven G, den Boer ML, Kalluri R, de Laat W, Esteller M, Agami R. Genome-wide profiling of p53-regulated enhancer RNAs uncovers a subset of enhancers controlled by a lncRNA. *Nat Commun*. 2015;**6**(1):6520.
13. Vallespín E, Palomares Bralo M, Mori MA, Martín R, García-Miñaúr S, Fernández L, de Torres ML, García-Santiago F, Mansilla E, Santos F, M-Montaño VE, Crespo MC, Martín S, Martínez-Glez V, Delicado A, Lapunzina P, Nevado J. Customized high resolution CGH-array for clinical diagnosis reveals additional genomic imbalances in previous well-defined pathological samples. *Am J Med Genet A*. 2013;**161A**(8):1950–1960.
14. Hehir-Kwa JY, Pfundt R, Veltman JA, de Leeuw N. Pathogenic or not? Assessing the clinical relevance of copy number variants. *Clin Genet*. 2013;**84**(5):415–421.
15. Lodish MB, Trivellin G, Stratakis CA. Pituitary gigantism: update on molecular biology and management. *Curr Opin Endocrinol Diabetes Obes*. 2016;**23**(1):72–80.