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Genetics Corner: An infant with a CHARGE-like syndrome and dual diagnoses: Xq28 duplication and Exon 38/39 KMT2D Missense Variant syndrome

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Case Summary

An eight-day-old term South Asian male with a postnatally diagnosed pathogenic duplication on chromosome Xq28 and bilateral hypoplastic ears was transferred from a community hospital intensive care nursery after two failed attempts at extubation. A genetics consultation was requested for multiple congenital anomalies.

He was born by scheduled C-section at 38 weeks gestation to a 30-year-old primigravida mother, whose pregnancy was complicated by IUGR and transverse lie. Apgar scores were 1^{1}, 1^{5}, 5^{10}, and 7^{15}. BW was 2510 grams (3rd %ile), BL 47 cm (6th %ile), HC 34.5 cm (51st %ile). Newborn screening was abnormal for severe combined immunodeficiency. A chromosome microarray confirmed a maternally derived pathogenic 439 Kb interstitial duplication on Xq28: [GRCHh37] Xq28(154,124,362_154,563,724) x2 mat.

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He had many congenital anomalies (Figure 1) in addition to small dysplastic ears: micrognathia, cleft palate, bilateral choanal atresia, dysplastic bilateral semicircular canals, vestibule and cochlea, absent nipples, postaxial polydactyly of both hands, micropenis, and left central calyceal dilation that progressed to bilateral hydronephrosis and proximal hydrometers. There were no colobomas on the ophthalmology exam. A brain MRI scan demonstrated the absence of the adenohypophysis. Panhypopituitarism, including hypothyroidism, was treated with replacement hormone therapy. He had an absent or hypoplastic thymus and diminished T cell subsets: CD4 (T helper) 10% low (normal 50-57%), count 522 low (normal 2,800-3,900), CD8 (T suppressor) 7% low (normal 8-31%), CD3 18% low (normal 55-82%), total count 940 low (3,500-5,000). The echocardiogram showed a PFO and a large PDA.

Figure 1. The left ear is small, dysplastic, posteriorly rotated, and low set. The lobule and superior elements of the helix and antihelix are absent. Note micrognathia and postaxial polydactyly of the right hand.

Following the repair of bilateral choanal atresia, he tolerated oxygen supplementation via nasal cannula but required high flow for desaturation events and increased work of breathing. Chest X-ray showed increased aeration. He had significant swallowing dysfunction with reflux and nasal regurgitation. He had persistent emesis with feeds and required prolonged total parenteral nutrition. A gastrostomy tube was placed, but he could not tolerate gastric feeds. NJ tube was placed and was tolerating full feeds. He required Calcium gluconate therapy for intermittently low Ca++.

Follow-up chest radiographs revealed bilateral interstitial prominence with worsening perihilar predominant airspace disease. He developed chronic respiratory failure and MSSA bacteremia. After almost four months in the NICU, he was transferred to the PICU. He developed severe pulmonary edema and was escalated to high-frequency oscillatory ventilation on the 10th day of transfer and died after a cardiopulmonary arrest.

Discussion:

As his clinical course worsened, it became more likely that our patient had another diagnosis in addition to his pathogenic Xq28 duplication, which primarily causes intellectual disability with occasional congenital anomalies (1). At first, we thought this child's anomalies were within the spectrum of Xq28 duplication syndrome, but in fact, congenital anomalies are infrequent in this condition. The Xq28 duplication (OMIM 300815) causes X-linked intellectual disability, developmental delay, mild dysmorphic features such as s high forehead, upper eyelid fullness, deep-set eyes, large ears,

and occasional congenital anomalies. Neonatal seizures have been reported. It is a recurrent copy number variant mediated by a mismatch during chromosome crossover between neighboring low copy repeat regions on the X chromosome called intron 22 homologous regions 1 and 2, in or near the F8 gene. Hence, this condition is also called int22h1/int22h2-mediated Xq28 duplication syndrome. It is diagnosed in hemizygous males and heterozygous females by detecting a 0.5-Mb duplication extending from 154.1 Mb to 154.6 Mb within the q28 region of the X chromosome using the reference build GRCh37/hg19. The mother is a carrier, but the family history did not suggest an X-linked pattern of intellectual disability.

Results of a trio whole exome sequencing study, which were available only after his demise, detected a likely pathogenic de novo heterozygous missense variant in exon 39 of KMT2D: c.10763A>G, p.His3588Arg. Pathogenic variants in this gene can cause Kabuki syndrome (OMIM 147920), but this child did not have the characteristic features of Kabuki syndrome, such as long palpebral fissures or large prominent ears. Instead, missense variants are also responsible for another phenotype in a gene region spanning parts of exons 38 and 39, in which missense variants have been associated with features of both Kabuki and CHARGE syndromes (2, 3). This syndrome has been referred to (awkwardly) as either Exon 38/39 KMT2D Missense Variant Syndrome (Ex38/39 KMT2D MV) or as Branchial Arch Anomalies, Choanal Atresia, Athelia, Hearing Loss and Hypothyroidism Syndrome (BCAHH, OMIM 620186). Although our patient's variant has not been previously described in this disorder, it is a missense variant located within the targeted protein domain. His phenotype is a strong match with this condition, down to absent nipples and hypothyroidism, which were, in retrospect, overlooked as important clues to his diagnosis. Searching the OMIM database using the search terms "athelia" and "hypothyroidism" reveals only one gene match and one syndrome match: KMT2D and BCAHH. Many of our patient's features are consistent with CHARGE syndrome (OMIM 214800). See Table 1 for a comparison of clinical features between our patient and others with these conditions.

This infant had two distinct genetic disorders, both of which were important: a familial Xq28 duplication and a de novo missense variant in KMT2D. His microarray, which was performed first, identified a pathogenic familial X chromosome duplication that might have gone unrecognized had the exome sequence test been performed first. The Xq28 duplication poses a significant recurrence risk to future children in this family as the mother is a carrier. However, the features eventually attributed to his KMT2D missense variant dominated our patient's phenotype. This condition was most relevant to his multiple anomalies, chronic problems, and eventual demise.

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The early diagnosis of a pathogenic Xq28 duplication, which would generally be advantageous in this case, probably slowed the diagnosis of this infant's second and more consequential genetic disorder because once a genetic diagnosis was established, clinical attention naturally turned to treatment and management. It took some time to realize that another diagnosis was likely.

It may not be as rare as it may seem at first glance to have two genetic disorders. More than one genetic diagnosis has been documented in 4-6% of individuals with a genetic condition. In their study of 1000 exomes, Trujilliano et al.(4) reported that 3/307, or about 1% of their patients with a genetic disorder, had a second "dual" genetic diagnosis. Posey et al.(5) identified two or more disease loci in 101/2076 patients with genetic diagnoses for a yield of 4.9%. Even after establishing a genetic diagnosis, the astute clinician should consider further genetic studies in some patients.

Feature	Our patient	Ex38/39KMT2D MVs cohort (Cuvertino *et al*.(2))	Kabuki syndrome Type 1	CHARGE syndrome
Branchial sinus/neck pits	No	7/9	No	No
Hearing Loss		8/9	Common	Common
External ear anomalies	Yes	6/9	Common prominent and simple	Common simple and dysplastic
Eye anomalies	No	2/9	Rare	Common
Choanal atresia	Yes	7/9	Rare	Common
Cleft lip/palate	Yes, Cleft palate	0	Common	Common
Athelia	Yes	6/9	Not reported	Rare
Congenital heart defect	No	3/9	Common	Common
Renal anomalies	Yes	0	Common	Common
Feeding problems	Yes	5/9	Common	Common
Thyroid abnormality	Yes	6/9	Rare	Rare
Abnormal immune system	Yes	4/9	Common	Common

Table 1. A comparison of the phenotypes of our patient, Ex38/39 KMT2D MV syndrome cohort from Cuvertino et al.(2), Kabuki and CHARGE syndromes (Adapted from Cuvertino et al.(2) Table 2)

Beers et al.(6) found that chromosome microarray supplemented the yield of exome testing in their recent report on diagnostic yield in patients with errors of immunity. In their cohort: "three (2.2%) participants had diagnostic molecular findings from both ES [exome sequencing] and CMA [chromosome microarray], including …. one participant with two distinct diagnoses." They found that "overall, CMA contributed to 18/134 diagnoses (13.4%), increasing the overall diagnostic yield by 15.5% beyond ES alone." This may justify ordering whole genome sequencing (WGS) as a single test instead of exome and microarray, because WGS detects copy number variants and sequence variants. As costs come down, WGS may become the preferred first test for infants with severe multiple anomalies or complex clinical courses in the neonatal intensive care unit.

3. Utilize trio whole-exome gene sequencing testing earlier than later in the clinic for infants with complex problems and severe congenital anomalies.

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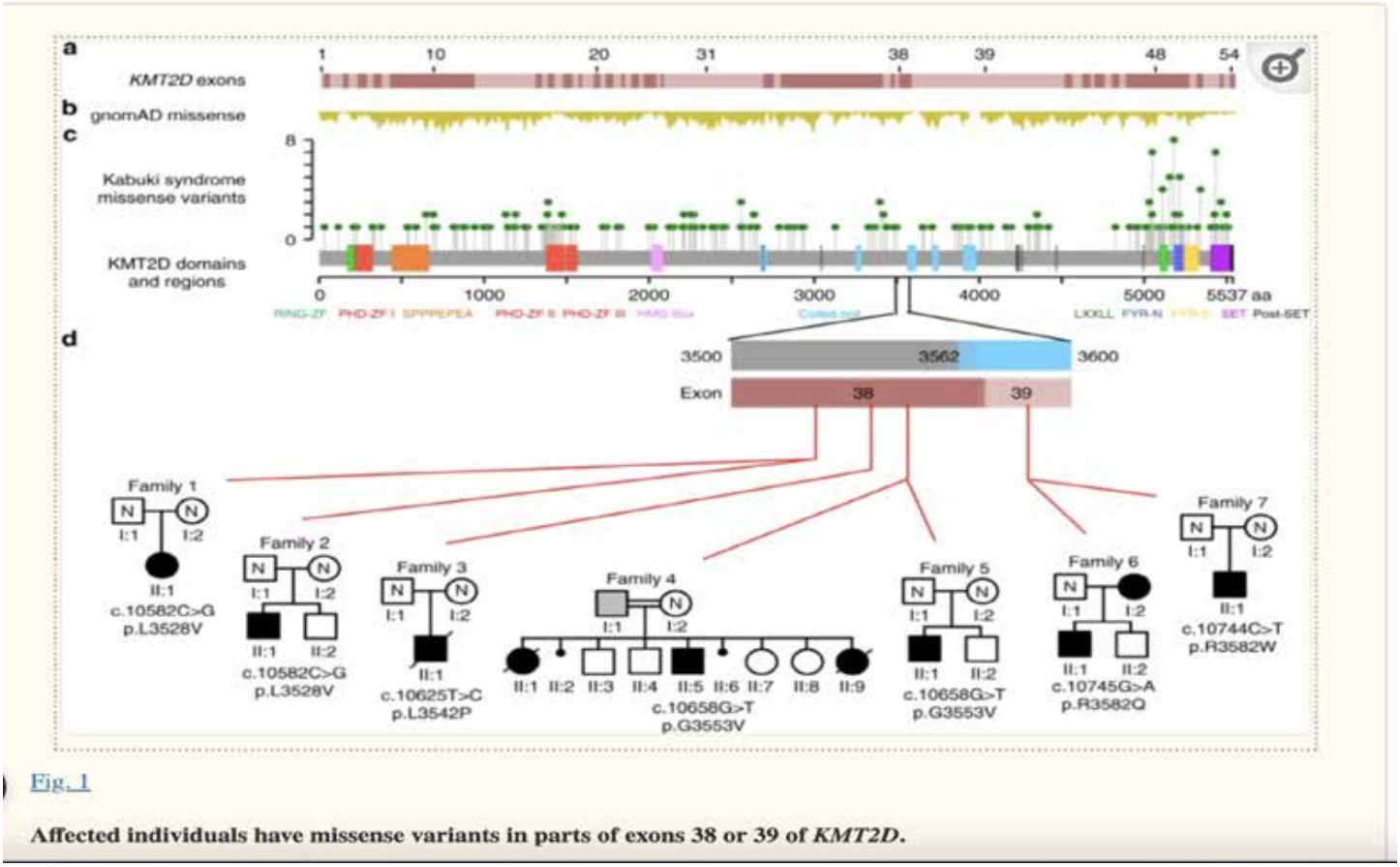

Figure 2. Adapted from Cuvertino et al.(2). The region of interest of the KMT2D gene is shown in more detail in the middle of this figure: the red horizontal bar shows parts of exons 38 (amino acid 3503-3580) and 39 (amino acid 3581-4510). The blue vertical bars denote the coiled-coil regions disrupted by missense variants in this area. The pedigrees are mapped to the locations of previously reported missense variants within exons 38 and 39. Our case would be mapped to the right of these families but still within the target region of exon 39.

Practical applications:

1. Consider a second genetic diagnosis if the first diagnosis does not explain the phenotype or clinical course. Recall that 4-6% of individuals with one genetic disorder have a second genetic diagnosis.
2. Pay attention to rare minor anomalies or rare combinations of anomalies, in this case, absent nipples and hypothyroidism, a rare combination that could have helped establish the diagnosis.

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