EDUCATION AND PRACTICE

CASE REPORT

Coexistence of genetic conditions: exploring a possible relationship

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ABSTRACT

We report on a 3-year-old boy who has congenital adrenal hyperplasia and a suspected Van Maldergem syndrome, another genetic condition, with the classic phenotype seen in our patient. The latter diagnosis was supported by a genetic test that showed a novel and likely pathogenic variant in a previously described gene of the syndrome. Paediatricians do encounter such a challenge of coexisting genetic conditions albeit infrequently, and advanced genetic analysis, example whole exome sequencing, increasingly report variants of unknown significance with a variable degree of potential pathogenicity. The treating physician needs to follow a systematic approach and entertain thorough literature search and brainstorming in order to prove or disprove any possible relationship between coexisting genetic conditions. The first step should be confirming the existence of the two conditions in the first place. In addition, when family segregation is unable to confidently make a sensible conclusion in such cases, a clinician should proceed to advanced functional studies to confirm pathogenicity. Then, one can explore further any hidden relationship between coexisting and possibly clinically-related genetic conditions.

KEYWORDS

Congenital adrenal hyperplasia; Hippo pathway; Van Maldergem syndrome.

INTRODUCTION

Van Maldergem syndrome (VMLDS) is a rare autosomal recessive genetic disease first described

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by Van Maldergem et al. [1]. Patients affected tend to have typical craniofacial dysmorphic features, limb anomalies, abnormal brain structures shown on magnetic resonance imaging (MRI), intellectual disability and auditory malformations leading to hearing loss (Table 1) [2]. Renal hypoplasia has also been reported in some patients [3].

There are two variations of the syndrome; VMLDS type 1 and VMLDS type 2. The cause of VMLDS-1 is a recessive mutation in the Dachsous Cadherin-Related 1 gene (DCHS1) located on chromosome 11p15.4, while VMLDS-2 is caused by recessive mutations in the Fat Tumour Suppressor Drosophila of 4 gene (*FAT4*), on chromosome 4q28.1 [4,5]. The *FAT4* gene can also cause Hennekam Lymphangiectasia Lymphedema syndrome, which overlaps in its features with VMLDS [6].

We report here on a 3-year-old boy with congenital adrenal hyperplasia (CAH) and a suspected VMLDS. To the best of our knowledge, there are no previous reports of CAH in patients with VMLDS. There are no known genetic or somatic associations between VMLDS and CAH apart from the anatomical proximity of the adrenal glands to the kidneys, and the latter could be affected in VMLDS. Few cases of VMLDS were reported with gonadal abnormalities, including hypogonadotropic hypogonadism and breast aplasia/hypoplasia in one female patient [7].

INITIAL PRESENTATION

We present a 3-year-old Saudi boy, who was born at 30 weeks of gestation at a district Hospital. Pregnancy events included a history of maternal gestational diabetes mellitus with insulin treatment and oligohydramnios. Baby was born via C-section due to foetal hypoxia, and he was 1,000 g of weight at birth requiring neonatal intensive care admission. Patient was noted to be over-masculinised and initially treated for a clinical diagnosis of non-salt wasting CAH due to 21-hydroxylase deficiency. Patient had other features that could not be fully explained by the CAH diagnosis, including microcephaly, long face and bi-frontal narrow open mouth. Parents are first-degree cousins and the patient has a sister with global developmental delay and similar facial dysmorphism. He has six other healthy siblings and they live in a good socioeconomic status. Our patient then developed generalised tonic colonic seizures at the age of 1.5 years when he was referred for further workup. The diagnosis of CAH was strongly supported by the patient's biochemistry [significantly high levels of: 17 OH progesterone 569 nmol/l (up to 3.3), ACTH = 178

Table 1. Characteristics of VMLDS type 2.

Body site	Characteristic features
Head	Large anterior fontanels, thick skull base and frontal bones
Facial dysmorphism	Flattened face, Bitemporal constriction, Mandibular hypoplasia, Hypoplastic Maxilla. Short palpebral fissures, epicanthal folds, hypertelorism, ptosis. Broad nasal bridge, microtia. Downturned mouth, thick gums, tented upper lip.
Auditory	External auditory canal atresia, sensorineural and conductive hearing loss.
Skeletal	Narrow thorax, short clavicles, tracheomalacia. Osteopenia, lax joints, hypotonia, skeletal dysplasia, scoliosis. Hand deformity: finger webbing, short fourth metacarpals, syndactyly.
Neurological	Intellectual disability, structural abnormalities (thin corpus callosum, periventricular nodular heterotopia, subcortical band heterotopia)
Abdomen and pelvis	Anterior placement of the anus. Micropenis, cryptorchidism, hypospadias, bifid scrotum. Kidney hypoplasia.

pg/ml (4.7–48.8), Testosterone 18.7 pmol/l (0.6–2.6), Androstenedione 39.9 nmol/l (up to 10.5)]. The diagnosis was then confirmed by a genetic test that showed CYP21A2 mutation, a heterozygous variant in the c.518T > A (p.lle173Asn) and c.955C > T (p.Gin319*) genes. On examination, he had microcephaly and global developmental delay with a weight and a height within the 25th percentile for his age and ethnicity. His cardiovascular and gastrointestinal examinations were unremarkable. Genitourinary examination showed over-masculinisation with enlarged male genital structures for age, and pubic hair tanner stage 2 but no axillary hair.

A full neurological examination showed hypotonia with positive reflexes, power grade 3 and clubfeet, which were corrected by a bilateral tendon Achilles tenotomy. Sensory examination revealed grossly normal to fine touch and pain. Cranial nerve examination showed pupils to be equal, round and bilaterally reactive to light. No facial asymmetry was noted. Hearing was grossly intact. Palate was normal and symmetrically elevated with phonation. The tongue was midline at rest and moved with no deviation. Gum hyperpigmentation was noted. Ophthalmic examination showed right cataract, positive optokinetic nystagmus and decreased vision.

He had a normal brain MRI for his age. Abdominal ultrasound showed grade 1 left-sided hydronephrosis but did not show other signs of organomegaly although there was no report about the size of adrenal glands. Skeletal survey showed normal bone density and no evidence of deformity nor delayed or accelerated bone age. EEG was later done and showed artefacts but the parts that were readable were normal. The patient was diagnosed with epilepsy and treated with Keppra 300 mg that reasonably controlled his seizures. He continued on physiological replacements of steroids for his CAH and continued to be clinically stable.

First genetic result

Chromosomal analysis was done and revealed an apparently normal male karyotype (46,XY). Whole exome sequencing (WES) was performed for index patient and parents and a homozygous

Table 2. The result of WES for the present patient.

Gene (Transcript)	Nucleotide (protein)	Zygosity			*In silico* parameters *	MAF**	Variant classification	Disorder (OMIM#, inheritance)
		Index	Mother	Father				
FAT4 (NM_001291303.1)	c.8021A > T p.(Asp2674Val)	Homo-zygos	Hetero-zygos	Hetero-zygos	3\3 pathogenic; highly conserved aa	0.0022	Uncertain significance (class 3)	VMLDS 2 (61556, AR)

*Number of *in silico* prediction programs that perfect pathogenicity all applicable programs (SIFT, PolyPhen2, AlignGVD, MutationTastor).
**Highest minor allele frequency (MAF) of representative population [Exome Aggregation Constorium database, Exome Sequencing Project, or 1000Genome project (1000G)].
***Based on ACMG.

variant in the *FAT4* gene was detected. C.8021A > T p.(Asp2674Val); Chr4(GRCh37): g.126370186A > T. This variant was also detected in both parents in a heterozygous state. Table 2 and it is classified as class 3; a variant of uncertain significance according to the recommendations of ACMG.

Based on the in silico predictions for the detected variant and partial overlap of the phenotype described for the associated disorder with the patient's presentation, a diagnosis of VMLDS type 2 is possible. However, since the diagnosis of VMLDS-2 would not explain all the patient's symptoms, we further evaluated the data in search for a research variant (Table 2). Variants are identified in genes with no or only partial experimental evidence for their involvement in human disease.

Sequence Analysis of the *CYP21A2* gene (OMIM: 613815) was performed. We detected a heterozygous variant of the *CYP21A2* gene, c.518T > A (p.lle173Asn). Since we detected the variant, c.955C > T (p.Gin319*), we preformed also deletion/duplication analysis for the *CYP21A2* gene to exclude variation in gene dosage. No large deletions or duplications within or including the *CYP21A2* gene were detected by MLPA analysis.

Previous reports on this rare syndrome showed similarity in molecular and clinical features though not all of these features should be present in every patient (Table 3). However, no CAH association has been previously described in these patients.

Interpretation of the initial genetic result

Since the present patient is diagnosed with CAH and suspected with VMLDS-2, causality in this association has to be examined and further molecular studying would be required. To establish any functional relationship between the involved genes, we analysed the WES data focusing on variants affecting protein function (nonsense, frameshift, conserved splice site and missense with high pathogenicity predictions) in genes with supporting evidence from zygosity or segregation and additional evidence for a functional importance of the gene in the described phenotype based on available expression, experimental data or animal models. We also searched for regions of homozygosity and evaluated the genes and variants with respect to a possible so far undescribed involvement in human disease corresponding to our patient. However, no such variants could be identified.

Table 3. Variations in clinical findings in previously reported patients with VMLDS compared to the present patient.

Reported feature	Reference	Present patient
Neurological - No seizures Brain MRI findings - Corpus callosum dysmorphism or agenesis - Nodular periventricular hypotopia	[4] [3,4]	Normal MRI, seizures
Auditory Atresia of external auditory meatus (hearing loss)	[4]	Hearing is grossly intact
Urogenital - Genital abnormalities (micropenis, cryptorchidism) micro-phallus, bifid scrotum. - Small kidney	[5]	Enlarged penis, testis and pubic hair stage 2 Mild right-sided hydronephrosis
Musculoskeletal Inability to walk	[5]	Hypotonia, Clubfeet.

In the first type of the syndrome VMLDS-1, the mutated gene is DCHS1. This gene codes for a calcium-dependent transmembrane cell–cell adhesion molecule that is a member of the protocadherin family. DCHS1 molecule is a ligand for FAT4 receptor. Cell–cell adhesion is primal for cellular structure. To achieve this, many classes of adhesion molecules are involved including a major group of cadherins molecules [7]. In VMLDS-2, however, the mutation happens in the *FAT4* gene. This gene encodes a protein that belongs to an enormous group of protocadherins, it may play a role in planar cell polarity (PCP) regulation (OMIM #612411). The DCHS1-FAT4 signalling pathway is essential in the development of several organs including heart, lungs, kidney, intestines, brain, ear, cochlea and the skeleton. This may affect the development of adrenal glands as well that are anatomically closely related to the kidneys. Nevertheless, the two organs arise from different origins.

A POSSIBLE RELATIONSHIP BETWEEN VMLDS AND CAH

The above genes through Hippo pathway could probably influence the size of organs. This pathway (also known as Salvador–Warts–Hippo pathway) has been identified as one of the pathways that control cell growth and proliferation thereby affecting organ size [8]. The role of FAT4 and other genes of the FAT family in the Hippo signalling pathway are thought to be due to atypical cadherin that results from FAT4 mutation acting as a receptor for the Hippo pathway, thus affecting the organ size [9]. We hypothesise that CAH in our patient is a resultant of enlarged adrenals due to the mutation of FAT4 [10]. A study showed strong evidence demonstrating a regulatory role of ACTH on adrenal endothelial junctions via the effect of vascular endothelial-cadherin. In our review of the literature, we found that the *FAT4* gene (also known as FAT atypical cadherin 4) has a role in determining cell polarity [11].

The association between the two syndromes is then postulated to be due to the effect of FAT4-DCHS1 complex on the Hippo-pathway that is responsible for organ size development. We suggest a bidirectional relationship between the two syndromes. To elaborate this suggestion, we think CAH could be associated with Van Maldergem through the effect on the Hippo-pathway that affects organ size development leading to enlarged adrenal glands. CAH leads to high levels of ACTH, which in turn could stimulate the cadherin gene resulting in features of VMLDS. Thus, we postulated that the two

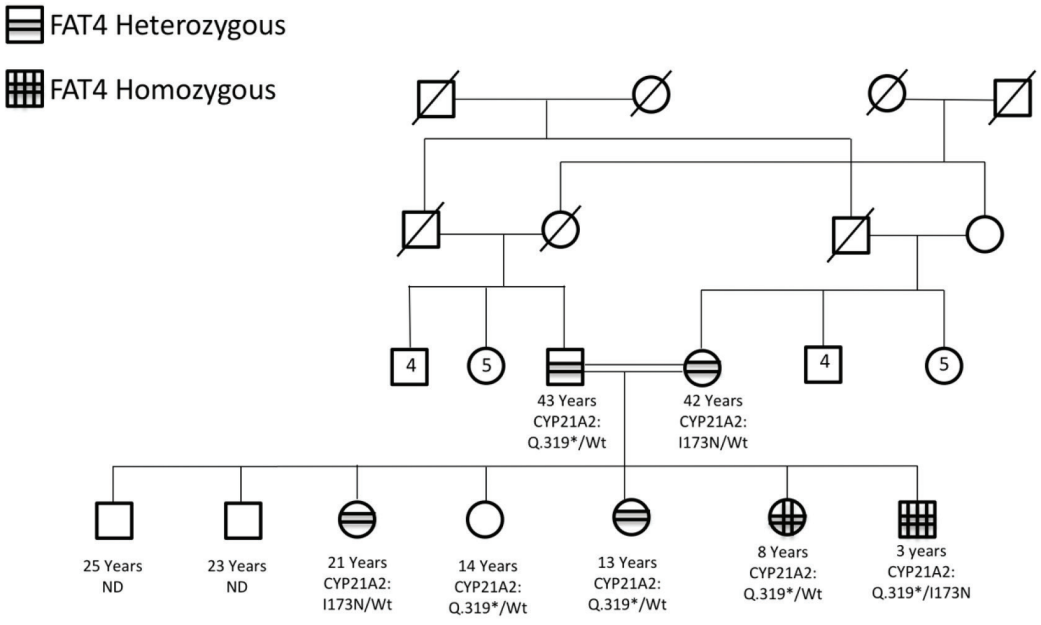

Figure 1. Family pedigree. ND, genetic testing not done; Wt, Wild type.

diseases might affect the appearance of each other in a loop picture.

FURTHER GENETIC STUDIES

However, the segregation analysis (Figure 1) in other family members did not support the postulation of FAT4 being a pathogenic variant resulting in VMLDS. This is because of the patient's sister who had similar homozygous mutation but did not show a classic phenotype of the disease. In a counter argument to this, a classic phenotype of a rarely described condition may not be fully relied on, i.e., there could be milder phenotypes that are not yet described. The only way to confidently rule in or rule out pathogenicity of the likely pathogenic variance, and hence further relationships with coexisting genetic conditions, is functional studies. These studies, namely; real-time polymerase chain reaction (PCR) and protein expression analysis are better in establishing causality rather than merely describing association. They may unveil a hidden relationship, in our case, between the suspected VMLDS and the evident CAH given the genetic existence of gene mutations for the two diseases in the entire family members.

If this mutation has been proven to be pathological by functional studies, an explanation should be given to the asymptomatic form in the homozygous sister. Intra-familial phenotypic variability has been previously described in many genetic conditions [12]. In general, the Mendelian mode of inheritance has proved to be an oversimplified way of looking at mode of inheritance when it comes to rare autosomal recessive disease. The rarer is the mutation, the most likely that it gets revealed with consanguinity. Consanguinity can result in the expression of relatively recent mutations, as recent as three generations in the case of first cousin marriage [13].

The relationship between homozygosity and autozygocity is interesting although homozygosity concludes autozygocity, but the relationship is more complex than that. In autozygocity, every block represents haplotypes that are indistinguishable since they originated from a common source (identical by decent), thus, homozygous. However, the opposite is not true, you can have similar haplotypes even if they originate from different origins (identical by state). Consequently, to assess autozygocity, one has to determine homozygosity first then differentiate whether it was identical by state or identical by decent [13]. Our patient's sister had a homozygous mutation of the FAT4 even though she did not express any obvious qualities of VMLDS, aside from the mild phenotype, which might suggest that the mutation is identical by decent but not identical by state.

CONCLUSIONS

Segregation analysis of a family may identify a phenotypically normal sibling of an index case with similar homozygous mutation of unknown significance. In that case, the final destination would be further functional studies such as real-time PCR, protein expression analysis and, if feasible, animal studies. When two genetic conditions strongly coexist in a family but one of them is a variance of unknown significance, a physician may proceed in a step-wise approach to functional genetic studies to confirm the significance of class 3 mutations. These may confirm the existence of autosomal recessive conditions with intra-familial variations in the phenotype.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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None.

ETHICS

The Ethics Committee of our institute; King Abdullah International Medical Research Center

(KAIMRC) granted ethics clearance and approval of the study. Informed consent for participation and publication of medical details was also obtained from the parents of the patient.

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