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Asymptomatic retinal dysfunction in alpha-methylacyl-CoA racemase deficiency

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Purpose: Alpha-methylacyl-CoA racemase (AMACR) deficiency is a peroxisomal disorder due to biallelic mutations in *AMACR*. At least 13 genetically confirmed patients have been reported to date. Seven had obvious pigmentary retinopathy; however, for the other six, no retinal phenotype was mentioned. The purpose of this report is to document subtle retinal findings in an additional affected family.
Methods: Retrospective case series (three affected siblings and their unaffected parents).
Results: Three Arab siblings (16, 19, and 22 years old) with prior juvenile cholelithiasis had been diagnosed with AMACR deficiency based on biochemical analysis, whole exome sequencing, and confirmatory segregation analysis (AMACR NM_001167595.1: c.877T>C; p.C293R). For all three, there were no visual complaints, but retinal multimodal imaging and electroretinography suggested subtle retinal dysfunction.
Conclusions: Retinal dysfunction is a parameter that should be measured in patients with known or suspected AMACR deficiency even in the absence of visual symptoms. This may be helpful with clinical diagnosis and monitoring response to dietary interventions.

Peroxisomes are non-autonomous cellular organelles with important catabolic and metabolic functions, including lipid metabolism [1]. Peroxisomal alpha-oxidation breaks down phytanic acid, a dietary methyl-branched fatty acid mainly derived from dairy products, meat, and certain fish [1]. This yields pristanic acid, which, in turn, is degraded by peroxisomal beta-oxidation [1]. Peroxisomal beta-oxidation also enables the synthesis of docosahexaenoic acid, an omega-3 fatty acid enriched in neuronal tissue and photoreceptor outer segments, and of bile acids [2]. Peroxisomal disorders can be broadly classified as disorders of peroxisome biogenesis, such as Zellweger syndrome, and single peroxisomal enzyme deficiencies, such as alpha-methylacyl-CoA racemase (AMACR) deficiency [3]. AMACR catalyzes conversion of certain compounds with a 2R-methyl branch, such as pristanic acid and bile acid intermediates, to their S-stereoisomers, which is the form that is degradable by peroxisomal beta-oxidation. Thus, patients with AMACR deficiency have markedly elevated levels of (2R)-pristanic acid and of C27-bile acid intermediates. Phytanic acid can be mildly elevated as well. Thirteen patients with AMACR deficiency have been reported (11 genetically confirmed; four pathogenic variants), with hepatic and neurologic manifestations the most prominent features and a bimodal age of presentation (infancy or later adulthood) [4-14]. The clinical spectrum includes neonatal cholestatic liver disease or giant cell hepatitis or both, fat-soluble vitamin deficiency, bloody stool in early life secondary to vitamin K deficiency [5,7], learning difficulties [4,11], and neurologic disease, including encephalopathic episodes, sensory and motor neuropathy, seizures, and cerebellar signs [4,6,8-14]. Some patients develop recurrent rhabdomyolysis and stroke-like episodes, tremor, and hypogonadism [4,10,11].

Of the 13 previously reported patients [4-14], seven were noted to have clinically obvious pigmentary retinopathy [4,8,10,11,13,14]. However, for the other six [5-7,9,12], retinal appearance or function was not mentioned. Most of the previously reported patients with clinically obvious pigmentary retinopathy did not have electrophysiology studies, and for the few who did the details were limited. In this report, we document three additional siblings affected by this rare disorder and highlight that such patients should be evaluated for retinal dysfunction even if they do not have visual complaints.

METHODS

A retrospective chart review for an affected family was performed. Institutional review board approval was granted for this retrospective case series. The study adhered to the tenets of the Declaration of Helsinki and ARVO statement on human subjects.

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RESULTS

The first three of five siblings born to first-cousin Emirati parents (Figure 1) were diagnosed with AMACR deficiency based on biochemical analysis and whole exome sequencing in one sibling (Sibling 1) with confirmatory targeted segregation analysis. Their main symptoms were learning difficulties and juvenile cholelithiasis. Other features of AMACR deficiency, such as sensorimotor neuropathy, cerebellar dysfunction, and seizures, were not evident. The phenotype segregated with the novel homozygous *AMACR* (OMIM 604489; Gene ID 23600) variant NM_001167595.1: c.877T>C; p.C293R. All three siblings were confirmed as homozygous for the variant, and both parents, who did not have medical issues, were confirmed as carriers [15]. In addition to results of segregation analysis and biochemical analysis (described below), the following also supports pathogenicity of this novel missense variant: the highly conserved nucleotide and amino acid position, the large physiochemical differences between cysteine and arginine, and prediction of the variant as probably damaging by software analyses (PolyPhen-2 [Polymorphism Phenotyping-2], SIFT [Sorting Intolerant From Tolerant], Align-GVGD [Align-Grantham Variation Grantham Difference]). Neither parent and none of the three affected siblings had any visual complaints other than need for refractive correction. When asked specifically, none of the three siblings complained of nyctalopia, photophobia, or visual field constriction. Ophthalmic examination of both parents revealed the mother had fine rare fine lens opacities, which were not visually significant. Electrophysiology (full-field electroretinography [ffERG] and pattern electroretinography [pERG]) was performed with the Diagnosys E3 desktop system using DTL electrodes and the manufacturer's control database. Guidelines of the International Society for Clinical Electrophysiology of Vision were followed [16]. Mydriasis and compliance were as required during electrophysiology testing.

Sibling 1 was a 16-year-old male with learning difficulties and a history of infantile cholelithiasis (initial presentation at 2 years old). The biochemical workup revealed markedly elevated pristanic acid levels (9.29 µg/ml; normal <0.3 µg/ml), normal phytanic acid levels, elevated liver enzymes, the presence of 25R-trihydroxycholestanoic acid, and the absence of 25S-trihydroxycholestanoic acid. This biochemical profile strongly suggested AMACR deficiency, and whole exome sequencing confirmed the homozygous *AMACR* variant c.877T>C; p.C293R. Segregation analysis confirmed the parents to be heterozygous for the variant. He was placed on cholic acid, fat-soluble vitamin supplements, and a low phytanic acid and pristanic acid diet but was poorly compliant. He was referred for ophthalmic examination although he had no visual complaints. His best-corrected visual acuity was 20/20 in each eye (wearing his cycloplegic refraction of −1.50 + 1.00 × 096, −0.75 + 1.50 × 084). There were mild intermittent exotropia and red-green color deficiency with Ishihara color plate testing. Slit-lamp examination was notable for rare fine white lens opacities in both eyes that were not visually significant (Figure 2B). Retinal appearance and short-wave autofluorescence were unremarkable (Figure 2A,C,F,H). Macular spectral-domain optical coherence tomography (SD-OCT) revealed subtle abnormalities (Figure 2D,E,G). ffERG showed normal waveforms with mildly decreased photopic amplitudes (Table 1). pERG showed a decreased P50 amplitude (and thus, a decreased downstream N95 amplitude; Figure 3A). This was consistent with subnormal macular function [17].

Sibling 2 was a 19-year-old female with learning difficulties and a history of juvenile cholelithiasis (initial presentation at 12 years old). The biochemical workup revealed marked elevated pristanic acid levels and elevated liver enzymes. Targeted sequencing confirmed the homozygous *AMACR* variant c.877T>C; p.C293R identified in her affected brother (Sibling 1). She was placed on cholic acid, fat-soluble vitamin supplements, and a low phytanic acid and pristanic acid diet but was poorly compliant. Her best-corrected visual acuity was 20/20 in each eye (wearing her cycloplegic refraction of −2.50). Ishihara color plate testing was normal. The ophthalmic examination, retinal multimodal imaging, and ERG results were similar to those of her affected brother (Sibling 1). ffERG showed normal waveforms with mildly decreased photopic amplitudes (Table 1, Figure 4). pERG showed a decreased P50 amplitude (and thus, a decreased

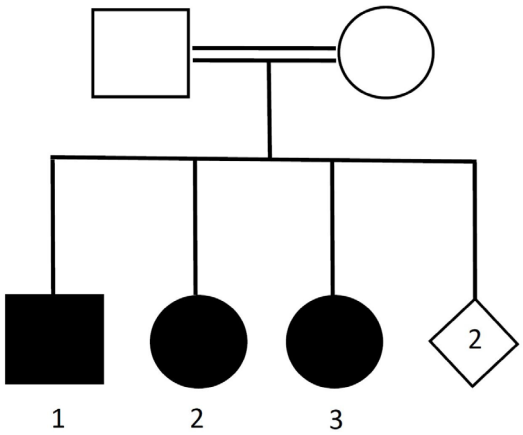

Figure 1. Pedigree of the three affected siblings.

downstream N95 amplitude; Figure 3B). This was consistent with subnormal macular function.

Sibling 3 was a 22-year-old female with a history of learning difficulties and juvenile cholelithiasis (initial presentation at 13 years old). The biochemical testing revealed markedly elevated pristanic acid levels, elevated liver enzymes, and low fat-soluble vitamin levels. Targeted sequencing confirmed the homozygous *AMACR* variant c.877T>C; p.C293R identified in her affected brother (Sibling 1). During the ophthalmic evaluation, her best-corrected visual acuity was 20/20 in each eye (wearing her cycloplegic refraction of −1.25 + 0.25 × 090, −1.25 + 0.50 × 090). She had moderate-angle intermittent exotropia. Ishihara color plate testing was normal. Ophthalmic examination and multimodal imaging were similar to that of her two siblings (Siblings 1 and 2). ffERG showed normal waveforms with a decreased photopic flicker amplitude in the left eye (Table 1). pERG showed a decreased P50 amplitude (and thus, a decreased downstream N95 amplitude; Figure 3C). This was consistent with macular dysfunction.

DISCUSSION

For these three siblings with AMACR deficiency, there were no visual complaints, and the clinical appearance of the retina was normal. However, retinal multimodal imaging and electrophysiology revealed subtle abnormality of the central outer retinal layer and macular dysfunction, respectively. These findings highlight that even in the absence of visual symptoms or clinically obvious pigmentary retinopathy, AMACR deficiency may cause retinal dysfunction, a parameter that can be objectively measured. We suspect the six patients previously reported with AMACR deficiency for whom retinal findings were not mentioned [5-7,9,12] would have had signs of retinal dysfunction on multimodal imaging or retinal electrophysiology. Table 2 summarizes clinical features of the reported patients with AMACR deficiency.

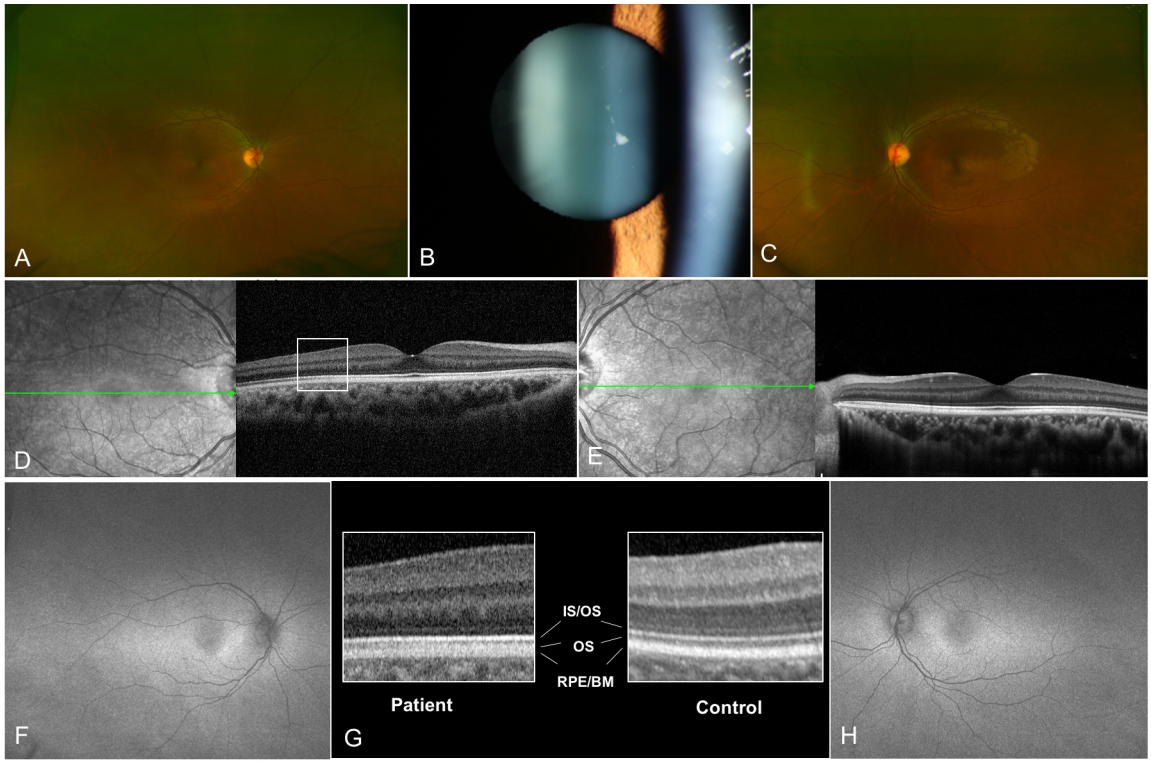

Figure 2. Sibling 1. **A**: The appearance of the right eye retina is normal. **B**: The slit-lamp examination of the right eye shows fine white opacities; these were in both eyes and not visually significant. **C**: The appearance of the left eye retina is normal. **D**, **E**: Macular spectral-domain optical coherence tomography (SD-OCT) of the right and left eyes reveals subtle findings, which are shown with magnification in **G**: The hyporeflective band (outer segments; OS) normally seen in the parafoveal area between the hyper-reflective layers associated with the inner/outer segment junction (IS/OS) and the RPE/Bruch's membrane (RPE/BM) complex is not seen; in addition, the RPE/BM layer has a blurred rather than a sharp appearance. **F**: Short-wave autofluorescence of the right eye is normal. **G**: Enlargement of right eye SD-OCT with control. **H**: Short-wave autofluorescence of the left eye is normal.

Table 1. ERG values in the siblings.

Sibling	Scotopic flash 0.01	Scotopic flash 3.0	Photopic flash	Photopic flicker	Interpretation
	b-wave, implicit time	a-wave, implicit time; b-wave, implicit time	a-wave, implicit time; b-wave, implicit time	trough time; peak, time	
Sibling 1	235uV, 96ms	−134uV, 15ms; 165mv, 50ms	−14uV, 12ms; 50uV, 29ms	12ms; 60uV, 25ms	decreased photopic amplitudes
	235uV, 92ms	−230uV, 15ms; 324uV, 54ms	−34uV, 15ms; −89uV, 29ms	12ms; 69uV, 25ms	
Sibling 2	259uV, 90ms	−183uV, 15ms; 146uV, 53ms	−17uV, 12ms; 67uV, 30ms	12ms; 54uV, 26ms	decreased photopic amplitudes
	149uV, 82ms	−137uV, 15ms; 203uV, 49ms	−18uV, 14ms; 72uV, 30ms	12ms; 64uV, 26ms	
Sibling 3	385uV, 85ms	−268uV, 16ms; 480uV, 56ms	−36uV, 15ms; 141uV, 30ms	14ms; 130uV, 27ms	decreased photopic flicker amplitude left eye
	206uV, 88ms	−154uV, 16ms; 257uV,54ms	−24uV, 14ms; 105uV, 29ms	11ms; 91uV, 27ms	
Normal range	235.4±151.4uV, 95.82±22.12ms	−175.1±146.7uV, 16.73±5.15ms; 230.1±273.2uV, 46.09±11.78ms	−64.17±38.03uV, 16.33±1.56ms; 183.5±116.9uV, 27.5±3.13 ms	9.08±2.62ms; 191.2±82uV, 24.17±4.33ms	

The three affected siblings had visually insignificant lens opacities. Although peroxisomal disorders can cause cataract [1], the lens opacities in these three siblings were likely unrelated to the AMACR deficiency. Visually insignificant lens opacities are often inherited as an autosomal dominant trait [18], and in this family, the mother had them as well. Similarly, the red-green color deficiency in the brother was likely the common form of color deficiency found in up to 8% of males and unrelated to AMACR deficiency.

There are different potential mechanisms by which AMACR deficiency could result in retinal dystrophy. Elevated pristanic acid induces reactive oxidative species and cell death in neural tissue such as the retina [19]. Cholestatic liver disease leads to malabsorption of fat-soluble vitamins such as vitamin A, and vitamin A deficiency can, in turn, result in retinal dysfunction [20]. Impaired synthesis of DHA is another potential mechanism for retinal dystrophy in AMACR deficiency, as this fatty acid is an essential component of photoreceptor outer segments [2]. Thus, it is not surprising that retinal dystrophy is part of AMACR deficiency.

Theoretically, treatment for AMACR deficiency is dietary modification. Restriction of phytanic and pristanic acids with supplementation of cholic acid could potentially limit retinal dystrophy as well as neuronal degeneration [1-4]. However, given the rarity of the disease, to date this has not been conclusively demonstrated. We suggest that multimodal and electrophysiological monitoring of retinal function, even in individuals without overt pigmentary retinopathy, is a potential method by which response to dietary modification could be assessed and monitored.

REFERENCES

1. Van Veldhoven PP. Biochemistry and genetics of inherited disorders of peroxisomal fatty acid metabolism. J Lipid Res 2010; 51:2863-95. [PMID: 20558530].

2. Das Y, Baes M. Peroxisomal Disorders and Retinal Degeneration. In: Bowes Rickman C, Grimm C, Anderson RE, Ash JD, LaVail MM, Hollyfield JG, editors. Retinal Degenerative Diseases. Cham: Springer International Publishing; 2019. p. 317–21. (Advances in Experimental Medicine and Biology).

3. Wanders RJA, Waterham HR. Peroxisomal disorders: the single peroxisomal enzyme deficiencies. Biochim Biophys Acta 2006; 1763:1707-20. [PMID: 17055078].

4. Ferdinandusse S, Denis S, Clayton PT, Graham A, Rees JE, Allen JT, McLean BN, Brown AY, Vreken P, Waterham HR, Wanders RJ. Mutations in the gene encoding peroxisomal

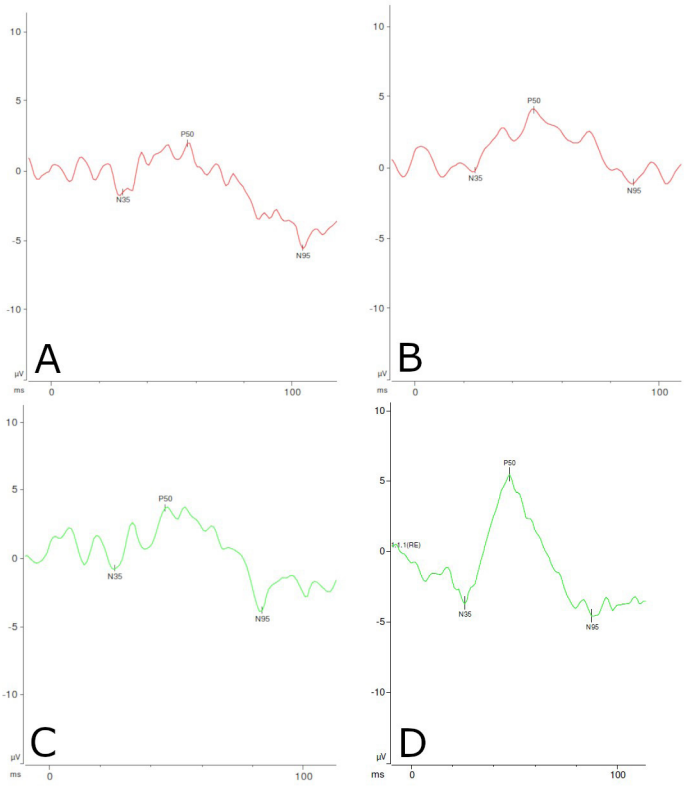

Figure 3. Siblings 1, 2, 3, and control. **A**, **B**, **C**: Pattern electroretinograms of the right eyes of siblings 1, 2, and 3 show a decreased P50 amplitude (and a decreased downstream N95 amplitude), indicative of macular photoreceptor dysfunction. The left eyes were similar. **D**: The right eye of a control subject.

alpha-methylacyl-CoA racemase cause adult-onset sensory motor neuropathy. Nat Genet 2000; 24:188-91. [PMID: 10655068].

5. Van Veldhoven PP, Meyhi E, Squires RH, Fransen M, Fournier B, Brys V, Bennett MJ, Mannaerts GP. Fibroblast studies documenting a case of peroxisomal 2-methylacyl-CoA racemase deficiency: possible link between racemase deficiency and malabsorption and vitamin K deficiency. Eur J Clin Invest 2001; 31:714-22. [PMID: 11473573].

6. McLean BN, Allen J, Ferdinandusse S, Wanders RJA. A new defect of peroxisomal function involving pristanic acid: a case report. J Neurol Neurosurg Psychiatry 2002; 72:396-9. [PMID: 11861706].

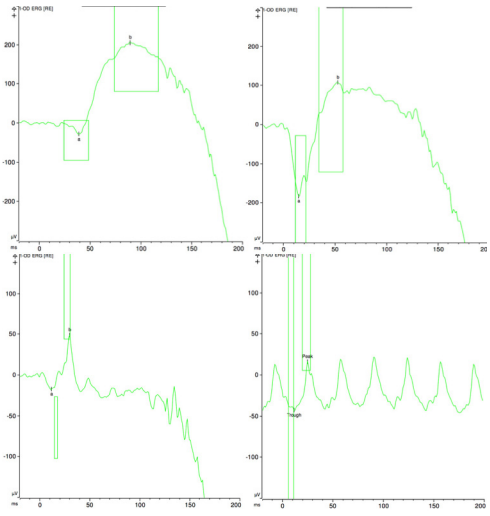

Figure 4. Sibling 2. Full-field electroretinography tracings (upper scotopic, bottom photopic) show normal scotopic (rod) function and mildly decreased photopic amplitudes (cone function). Rectangular blocks outline the upper and lower limits of normal values. Stimuli (DA, dark-adopted; LA, light-adapted) were as follows: upper left DA flash 0.01 cd·s·m^2, upper right DA flash 3.0 cd·s·m^2, lower left LA flash 3.0 cd·s·m^2, and lower right LA 0.0 cd·s·m^2, flicker at 30 Hz.

Table 2. Alpha-methylacyl-CoA racemace (AMACR) deficiency patients.

Author/year (reference)	No. of patients	Age of onset (years) /sex	Findings (no. of patients)	AMACR pathogenic variant (homozygous)
Ferdinandusse/2000 (4) McLean/2002† (6)	3	18/M†, 48/F, 1/M ‡	Seizures (1), encephalopathy (1), **retinitis pigmentosa (1)**, sensory-motor neuropathy (3), migraine (1), hypogonadism (1), learning difficulties (1)	c.154 T>C [p.S52P] in two, c.320 T>C [p.L107P] in the child
Van Veldhoven/2001 (5)	1	1/F	Hematochezia (secondary to a coagulopathy from vitamin K deficiency), giant-cell neonatal hepatitis	NA
Setchell /2003 (7)	1	1/F	Cholestatic liver disease, hematochezia (secondary to a coagulopathy from vitamin K deficiency), fat-soluble vitamin deficiency	c.154 T>C [p.S52P]
Clarke/2004 (8)	1	36/F	Seizures, encephalopathy, **retinitis pigmentosa**, cataract, tremor, cerebellar signs, neuropathy, depression, migraine	c.154 T>C [p.S52P]
Thompson/2008 (9)	1	13/F	Seizures, encephalopathy, sensory-motor neuropathy, cognitive decline, depression, homonymous hemianopia	c.154 T>C [p.S52P]
Smith/2010 (11)	1	Early adulthood/M	Seizures, encephalopathy, **retinitis pigmentosa**, sensory neuropathy, hypogonadism, learning difficulties	c.154 T>C [p.S52P]
Kapina/2010 (10)	1	23/M	Rhabdomyolysis, stroke-like episodes, seizures, encephalopathy, sensory neuropathy, **degenerative retinopathy**, schizophrenia	c.559 G>A [p.G187R]
Stewart/2011 (13)	1	25/M	Seizures, encephalopathy, **pigmentary retinopathy**, low testosterone level	NA
Dick/2011 (12)	1	50/M	Seizures, cerebellar signs, sensory-motor neuropathy, decline in short-term memory	c.154 T>C [p.S52P]
Haugarvoll /2013 (14)	2	30/M, 33/F	Seizures (2), encephalopathy (2), tremor (1), sensory-motor neuropathy (2), **pigmentary retinopathy (2)**, cataract (2), type 2 diabetes (2)	c.367 G>A [p.Asp123Asn]
Alsalamah/2020§	3	2/M, 12/F, 13/F	Cholelithiasis (2), cholestatic liver disease (3), **subtle retinopathy (3)**, fat-soluble vitamin deficiency (3), learning difficulties (3)	c.877T>C [p.C293R]

7. Setchell KDR, Heubi JE, Bove KE, O'Connell NC, Brewsaugh T, Steinberg SJ, Moser A, Squires RH. Liver disease caused by failure to racemize trihydroxycholestanoic acid: gene mutation and effect of bile acid therapy. Gastroenterology 2003; 124:217-32. [PMID: 12512044].

8. Clarke CE, Alger S, Preece MA, Burdon MA, Chavda S, Denis S, Ferdinandusse S, Wanders RJA. Tremor and deep white matter changes in alpha-methylacyl-CoA racemase deficiency. Neurology 2004; 63:188-9. [PMID: 15249642].

9. Thompson SA, Calvin J, Hogg S, Ferdinandusse S, Wanders RJA, Barker RA. Relapsing encephalopathy in a patient with alpha-methylacyl-CoA racemase deficiency. J Neurol Neurosurg Psychiatry 2008; 79:448-50. [PMID: 18032455].

10. Kapina V, Sedel F, Truffert A, Horvath J, Wanders RJA, Waterham HR, Picard F. Relapsing rhabdomyolysis due to peroxisomal alpha-methylacyl-coa racemase deficiency. Neurology 2010; 75:1300-2. [PMID: 20921516].

11. Smith EH, Gavrilov DK, Oglesbee D, Freeman WD, Vavra MW, Matern D, Tortorelli S. An adult onset case of alpha-methyl-acyl-CoA racemase deficiency. J Inherit Metab Dis 2010; 33:Suppl 3S349-53. [PMID: 20821052].

12. Dick D, Horvath R, Chinnery PF. AMACR mutations cause late-onset autosomal recessive cerebellar ataxia. Neurology 2011; 76:1768-70. [PMID: 21576695].

13. Stewart MW, Vavra MW, Whaley NR. FUNDUS FINDINGS IN A PATIENT WITH α-METHLYACYL-COA RACEMASE DEFICIENCY. Retin Cases Brief Rep 2011; 5:262-6. [PMID: 25390181].

14. Haugarvoll K, Johansson S, Tzoulis C, Haukanes BI, Bredrup C, Neckelmann G, Boman H, Knappskog PM, Bindoff LA. MRI characterisation of adult onset alpha-methylacyl-coA racemase deficiency diagnosed by exome sequencing. Orphanet J Rare Dis 2013; 8:1-[PMID: 23286897].

15. Al-Shamsi A, Hertecant JL, Al-Hamad S, Souid A-K, Al-Jasmi F. Mutation Spectrum and Birth Prevalence of Inborn Errors of Metabolism among Emiratis: A study from Tawam Hospital Metabolic Center, United Arab Emirates. Sultan Qaboos Univ Med J 2014; 14:e42-9. [PMID: 24516753].

16. McCulloch DL, Marmor MF, Brigell MG, Hamilton R, Holder GE, Tzekov R, Bach M. ISCEV Standard for full-field clinical electroretinography (2015 update). Doc Ophthalmol 2015; 130:1-12. [PMID: 25502644].

17. Holder GE. Pattern electroretinography (PERG) and an integrated approach to visual pathway diagnosis. Prog Retin Eye Res 2001; 20:531-61. [PMID: 11390258].

18. Khan AO, Aldahmesh MA, Alkuraya FS. Phenotypes of Recessive Pediatric Cataract in a Cohort of Children with Identified Homozygous Gene Mutations (An American Ophthalmological Society Thesis). Trans Am Ophthalmol Soc. 2015;113:T7.

19. Rönicke S, Kruska N, Kahlert S, Reiser G. The influence of the branched-chain fatty acids pristanic acid and Refsum disease-associated phytanic acid on mitochondrial functions and calcium regulation of hippocampal neurons, astrocytes, and oligodendrocytes. Neurobiol Dis 2009; 36:401-10. [PMID: 19703563].

20. Smith J, Steinemann TL. Vitamin A deficiency and the eye. Int Ophthalmol Clin 2000; 40:83-91. [PMID: 11064859].

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