Lathosterolosis: a relatively mild case with cataracts and learning difficulties

Anderson R[1], Rust S[2], Ashworth J[3], Clayton-Smith J[4,5], Taylor RL[5], Clayton PT[6], Morris AAM[1,5]

[1]Willink Metabolic Unit, [2]Paediatric Psychosocial Service, [3]Paediatric Ophthalmology Department & [4]Genomic Medicine, Manchester Academic Health Sciences Centre, Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom
[5]Division of Evolution and Genomic Sciences, Faculty of Biology, Medicine and Health, University of Manchester, United Kingdom
[6]Centre for Translational Omics, UCL Great Ormond Street Institute of Child Health, London, United Kingdom

Address of corresponding author:

Dr Andrew Morris PhD, FRCPCH
Consultant in Inherited Metabolic Medicine
Willink Metabolic Unit
Manchester Centre for Genomic Medicine
St Mary's Hospital
Manchester University Hospitals NHS Foundation Trust
Oxford Road,
Manchester, M13 9WL, UK
Ph: +44 (0)161-701-2137
Fax: +44 (0)161-701-2303
e-mail: andrew.morris@mft.nhs.uk

Word count
1 Figure
3 Tables

Abstract
Lathosterolosis is a rare defect of cholesterol synthesis. Only four previous cases have been reported, two of whom were siblings. We report a fifth patient, with a relatively mild phenotype. He presented at 5 years of age with bilateral posterior cataracts, which were managed with lensectomies and intraocular lens implants. He also had global developmental delay with a full scale IQ of 64 at 11 years of age. His head circumference is between the 0.4th and 2nd centiles and he has mild hypotonia and subtle dysmorphism (a high arched palate, anteverted nostrils, long philtrum and clinodactyly of toes). The diagnosis was established after sequencing a panel of genes associated with cataracts, which revealed compound heterozygous *SC5D* mutations: c.479C>G p.(Pro160Arg) and c.630C>A p.(Asp210Glu). The plasma lathosterol concentration was markedly raised at 219.8 μmol/l (control range 0.53-16.0), confirming the diagnosis. The c.630C>A p.(Asp210Glu) mutation has been reported in one previous patient[7], who also had a relatively mild phenotype. The mutation leads to a relatively conservative amino acid substitution, consistent with some residual enzyme activity. Our patient's family did not notice any benefit from treatment with simvastatin. In summary, milder patients with lathosterolosis may present with learning difficulties, cataracts and very subtle dysmorphism. The diagnosis will be missed unless plasma sterols are analysed or relevant genes sequenced.

Introduction

The importance of cholesterol in the human body has long been recognised[1]. Among other roles, cholesterol is needed for the structure of cell membranes and myelin; for the synthesis of multiple hormones and bile acids; and for signalling within the developing embryo, where cholesterol serves to modify Hedgehog protein maturation[1,2]. Furthermore, precursors within the cholesterol synthesis pathway may serve important roles in their own right, for instance 7-dehydrocholesterol is the immediate precursor for vitamin D synthesis[1]. More recently, the phenotypes of defects in several stages of cholesterol biosynthesis have been identified[1,3], with Smith-Lemli-Opitz Syndrome being the most common and well described[1,2] . It is not yet clear to what extent these diseases are caused by the reduction in cholesterol function, as opposed to the effects of accumulation of precursors and by-products[3].

Lathosterolosis is a defect of cholesterol synthesis caused by deficiency of 3-beta-hydrocholesterol-delta-5-desaturase (*SC5D*), thereby preventing conversion of lathosterol to 7-dehydrocholesterol[4]. Only four affected individuals have been reported[4,5,6,7], of whom one was a fetus aborted at 21 weeks gestation and one died at 18 weeks of age. The four patients all had microcephaly, post-axial polydactyly, syndactyly and variable hepatic involvement. The liveborn patients all had facial dysmorphism and cataracts and those surviving beyond 6 months also displayed central hypotonia and a degree of global developmental delay.

We describe a fifth case of lathosterolosis, confirmed by both genetic and biochemical analysis, with a relatively mild phenotype. The patient has been mentioned briefly in papers on the use of Next Generation Sequencing in children with cataracts[9,10].

Case report

The proband is the second child of healthy non-consanguineous Caucasian parents; his older sister is healthy with normal development. He was born at term following an uneventful antenatal course. There were no significant perinatal concerns but he did have neonatal jaundice and laryngomalacia.

The patient has always had good physical health but his developmental milestones were delayed, and he did not walk or talk until 3 years of age. He attended mainstream primary education with some additional support but from 11 years of age he has attended a secondary school for children with special needs. He has an autistic spectrum disorder: he becomes anxious very readily, finds it difficult to cope with changes to his routine and has poor inter-personal skills.

His parents describe poor visual attention from an early age and he failed a routine school vision test when aged five years. He was found to have bilateral posterior lens opacities, as well as astigmatism and myopia. The following year, the proband underwent bilateral lensectomies and intraocular lens implants. YAG capsulotomies were performed for posterior capsular opacification when the proband was 7 years old. The patient's current vision is stable at 0.5 right and 0.3 left LogMar.

In view of the cataracts and the developmental delay, the proband was referred to the Manchester Centre for Genomic Medicine when he was eight years old. Subtle dysmorphic features were noted (Figure 1), including a broad forehead, prominent ears, a high arched palate with crowding of teeth, anteversion of the nostrils, a long philtrum and clinodactyly of toes. The occipito-frontal head circumference was 51.1 cm at 11 years of age (between 0.4th and 2nd centiles). Mild hypotonia and joint hyper-extensibility were present.

Initial investigations were unremarkable, including array comparative genomic hybridization. When the patient was 10 years old, Next Generation Sequencing was undertaken on a panel of 115 genes associated with congenital or childhood cataracts[9]. This identified two heterozygous mutations in the *SC5D* gene: c.479C>G p.(Pro160Arg) and c.630C>A p.(Asp210Glu). The latter mutation has been reported in a previous patient[7]. The parents were each heterozygous for one mutation.

The diagnosis of lathosterolosis was confirmed by plasma sterol analysis by GC-MS, which revealed a markedly elevated plasma lathosterol concentration of 219.8 μmol/l (control range 0.5-16.0 μmol/l). There was also a raised concentration of 8(9)cholestenol, the immediate precursor of lathosterol, but other intermediates of cholesterol synthesis were normal (Table 1). The total plasma cholesterol concentration was 3.4 mmol/l (reference range <4.0 mmol/l).

Further assessment showed a mild iron deficiency anaemia (haemoglobin 105 g/l), normal plasma electrolytes, urea and creatinine, bilirubin, albumin, alanine transaminase and alkaline phosphatase with a minimally-deranged coagulation profile (PT 12.6 seconds, reference range 9.9-11.8 seconds; APTT 21.6 seconds, reference range 23.0-31.9 seconds). There was vitamin D level deficiency (plasma total 25-hydroxy vitamin D concentration 16.4 nmol/l, reference range >50 nmol/l) but other fat-soluble vitamins were normal.

Cognitive assessment was undertaken, using the Wechsler Intelligence Scale for Children, 4th UK Edition (WISC-IV) and the "Word Reading" and "Pseudoword Decoding" subtests of the Wechsler Individual Achievement Tests, 2nd UK Edition (WIAT-II). The patient scored within the *extremely low* range of the WISC-IV (standard score = 64 = 1st centile), indicating developmental delay in cognitive ability. He scored within the *borderline* ranges of Perceptual Reasoning and Processing Speed Indices of the WISC-IV, and the "Pseudoword Decoding" subtest of the WIAT-II, and the *extremely low* range of ability on the "Word Reading" subtest. The patient demonstrated greater aptitude for tasks involving concrete visual stimuli, with marked weakness in language comprehension (Table 2).

A trial of treatment was commenced with Simvastatin 40 mg daily and cholesterol 1.75 g daily. In a previous patient, treatment with simvastatin had normalised the plasma lathosterol concentration and coincided with an increase in developmental quotient from 55 to 64[7]. Our patient's family elected to discontinue the treatment after 4 months, having observed no benefit, particularly in terms of behaviour.

Discussion

The main clinical features reported in lathosterolosis have been multiple malformations, learning disability, cataracts and liver involvement (see Table 3). Many features resemble those of Smith-Lemli-Opitz syndrome, the commonest defect of cholesterol synthesis. Smith-Lemli-Opitz syndrome is known to have a wide range of severity, from cases that are lethal *in utero*

to others with minimal learning difficulties or dysmorphism. Lathosterolosis has generally been considered a very severe condition: of the four previous cases, one died aged 18 weeks with intractable myoclonus and respiratory failure and one was aborted at 21 weeks gestation due to multiple malformations. Milder forms of the condition may, however, have been missed. The patient reported here had learning difficulties and bilateral cataracts but only a few minor malformations and no liver involvement.

Microcephaly has been a universal finding in all reported cases of lathosterolosis. Our patient has learning difficulties with a full scale IQ of 64. The two previous patients who survived beyond infancy also had cognitive impairment. One of these had a developmental quotient of 64 at the age of 3 years 9 months (Griffiths Mental Developmental Scales)[7], suggesting that the degree of cognitive impairment was similar to our patient's. Developmental assessments were not reported for the other patient[6]. Our patient has autistic features but this is not specifically mentioned in previous reports.

Cataracts were a prominent feature in our patient and were the clue that led to the diagnosis. Cataracts were also noted in all previous patients except the aborted fetus. The cataracts were present at birth in one patient[5] (Parnes et al, 1990) & in the others were first noted at the age of 4 years[7], 5 years (our patient) and 6 years[4] (Table 3). The cataracts were bilateral in all cases but asymmetrical in one case, only requiring extraction in one eye. One patient only had small dot cataracts at 4 years with normal vision[7].

Abnormalities of the digits have been the commonest malformations in previous patients with lathosterolosis, possibly due to the role of hedgehog signalling in limb development. All 4 previous patients have had postaxial polydactyly (on feet ± hands) and syndactyly of the toes but our patient has neither feature, demonstrating that these are not always present in lathosterolosis. The only limb abnormality in our patient was clinodactyly of the toes.

The current patient has a high arched palate, mildly anteverted nostrils and a long philtrum, as have two previous patients[4,5]. He does not, however, have the other reported dysmorphic features, namely bitemporal narrowing, epicanthic folds, a broad nasal tip, micrognathia and a small chin. Malformations in previous patients have also included ambiguous genitalia[5], horseshoe kidneys[6], bilobed gall bladder[4], T8 butterfly vertebra[4], lumbosacral meningomyelocele and Arnold Chiari Type 2 malformation[6] (Table 3). None of these have been found in our patient but he has not had abdominal imaging or a skeletal survey.

Variable hepatic involvement has been reported in previous cases of lathosterolosis. For the fetus aborted at 21 weeks gestation, liver histology showed extramedullary hematopoiesis & atrophy of hepatocytic laminae[6]. Two other patients had persistently raised plasma levels of bilirubin, transaminases and alkaline phosphatase. One of these developed liver failure and portal hypertension at 7 years of age, with cholestasis and cholangiolitis on histology[6]; the other had hepatosplenomegaly and histology showed cirrhosis and vacuolation, especially of histiocytes, with storage of lipids and mucopolysaccharides (Parnes et al, 1990). The final patient had normal plasma bilirubin, transaminases and alkaline phosphatase levels and no hepatic symptoms but ultrasound showed mildly increased heterogenicity[7]. Our patient had neonatal jaundice but no subsequent symptoms or biochemical evidence of liver disease; he has not had imaging of his liver.

Like all previous cases, our patient has 2 missense mutations in *SC5D*. Both affect highly conserved amino acids, are predicted damaging *in silico*[9], and are extremely rare according to

the reference datasets, GnomAD and ExAC. The p.(Asp210Glu) variant has previously been associated with lathosterolosis in an unrelated patient who also had a relatively mild phenotype[7] and it is possible that this mutation leaves some residual enzyme activity, accounting for the milder phenotype. It has been suggested that the phenotypic severity may correlate with the lathosterol level in cultured fibroblasts[7] but our patient declined a skin biopsy so this analysis was not performed. His plasma lathosterol concentration was over 2.5 times that in the previous mild patient[7] but this may be influenced by the greater age of our patient. The plasma cholesterol concentration has been normal in all patients in whom it has been measured, in contrast to the mouse model[5].

In conclusion, this patient displays a milder phenotype than previously described in lathosterolosis, with bilateral cataracts, microcephaly and learning difficulties as the main features. Of particular note, the polydactyly and liver involvement seen in the other cases were absent. There may be a number of patients like this, with cataracts and learning difficulties, who will only be diagnosed if plasma sterol analysis or sequencing of relevant genes is undertaken.

References

1. Herman GE. *Disorder of cholesterol biosynthesis: prototypic metabolic malformation syndromes.* Hum Mol Genet. 2003 Apr 1;12 Spec No 1: R75-88
2. Merkens et al. *Smith-Lemli-Opitz Syndrome and inborn errors of cholesterol synthesis: summary of 2007 SLOS/RSH Foundation scientific conference sponsored by National Institutes of Health.* Genet Med. 2009 May; 11(5): 359-364
3. Waterham Hans R. *Defects of cholesterol biosynthesis*. FEBS Letters, 580, 2006
4. Brunetti-Pierri et al. *Lathosterolosis, a novel multiple-malformation/mental retardation syndrome due to deficiency of 3beta-hydroxysteroid-delta5-desaturase.* Am J Hum Genet. 2002 Oct;71(4):952-8. Epub 2002 Aug 20.
5. Krakowiak et al. *Lathosterolosis: an inborn error of human and murine cholesterol synthesis due to lathosterol 5-desaturase deficiency.* Hum Mol Genet 2003 Jul 1;12(13):1631-41.
6. Rossi et al. *Clinical phenotype of lathosterolosis.* Am J Med Genet Part A 143A:2371–2381.
7. Ho et al. *Lathosterolosis: A Disorder of Cholesterol Biosynthesis Resembling Smith-Lemli-Opitz Syndrome.* JMID Rep 2014; 12: 129–134.
8. Svodoba et al. *Treatment of Smith-Lemli-Opitz Syndrome and Other Sterol Disorders.* Am J Med Genet C Semin Med Genet. 2012 Nov 15; 0(4): 285-294
9. Gillespie RL, O'Sullivan J, Ashworth J, Bhaskar S, Williams S, Biswas S, Kehdi E, Ramsden SC, Clayton-Smith J, Black GC, Lloyd IC. Personalized diagnosis and management of congenital cataract by next-generation sequencing. Ophthalmology. 2014 Nov 30;121(11):2124-37.
10. Gillespie RL, Urquhart J, Anderson B, Williams S, Waller S, et al. Next-generation Sequencing in the Diagnosis of Metabolic Disease Marked by Pediatric Cataract. Ophthalmology. 2016 Jan;123(1):217-20.

Parnes S et al, (1990) Am J Med Genet 35:397-405

Acknowledgements

Next generation sequencing was supported by Fight for Sight UK (grant no. 1831). RLT is supported by a Medical Research Council/UK Research and Innovation Skills Development Fellowship (Ref: MR/R024952/1).

Table 1: Plasma sterol analysis by GC-MS

	Patient Sample (μmol/l)	Control Range (μmol/l)
8-Dehydrocholesterol	0.53	<2.0
7-Dehdrocholesterol	0.93	0.70-1.96
Desmosterol	0.23	2.65-9.22
8(9) Cholestenol	21.61	<4.0
Lathosterol	219.79	0.53-15.98
Cholestanol	6.95	3.87-18.04
Lanosterol	0.26	0.00-1.53

Courtesy of PT Clayton, Institute of Child Health, London

Table 2: Psychometric assessment

		Standard and Scaled Scores (Percentile)
Wechsler Intelligence Scale for Children, 4th UK Edition		
Full Scale IQ	-	64 (1st)
Verbal Comprehension Index	-	69 (2nd)
Perceptual Reasoning Index	-	73rd (4th)
Working Memory Index	-	68 (2nd)
Processing Speed Index		73 (4th)
Wechsler Individual Achievement Test, 2nd UK Edition		
Word Reading		63(1st)
Pseudo word Decoding		72 (3rd)

Table 3: Summary of the current and previously reported patients

	Brunetti-Pierri *et al* 2002[4], Rossi *et al* 2007[6]	Rossi *et al* 2007[6]	Krakowiack *et al*, 2003[5], Parnes et al 1990	Ho *et al* 2014[7]	Current patient
SC5D mutations	p.R29Q & p.G211D	p.R29Q & p.G211D	homozygous p.Y46S	p.K148E & p.D210E	p.P160R & p.D210E
Lathosterol plasma level	338 μmol/l	N/A	N/A	81.6 μmol/l	219.8 μmol/l
Cholesterol level	normal	N/A	N/A	normal	Normal
Age at diagnosis	2 years	post mortem (21 weeks gestation)	post mortem (aged 18 weeks)	22 months	10 years
Cognition	Impaired	N/A	N/A	DQ 64 at 3.8 years	IQ 64 at 11 years
Microcephaly	Yes	Yes	Yes	Yes	Yes
Cataracts	bilateral from 6 years	-	bilateral from birth	bilateral dot cataracts from 4 years	bilateral from 5 years
Liver	liver failure & portal hypertension with abnormal biochemistry; cholestasis & cholangiolitis on histology	extramedullary hematopoiesis & atrophy of hepatocytic laminae on histology	hepatospleno-megaly with abnormal biochemistry; cirrhosis and vacuolated cells on histology	normal biochemistry; mildly increased heterogenicity on ultrasound	normal biochemistry; imaging not performed
Dysmorphism	epicanthus, broad nasal bridge, anteverted nares, long philtrum, micrognathia, high palate	N/A	ptosis, short nose, micrognathia	micrognathia, bitemporal narrowing, broad nasal tip	high palate, very mild ptosis, anteverted nares & long philtrum,
Limbs	hexadactyly & syndactyly of left foot	hexadactyly of both hands and feet, bilateral talipes	hexadactyly & syndactyly of both feet	hexadactyly & syndactyly of both feet	clinodactyly of both feet
Other malformations	bilobed gallbladder, T8 butterfly vertebra	lumbosacral myelomeningo-cele, Arnold Chiari Type 2 malformation	ambiguous genitalia	single umbilical artery	

N/A not available

Figure Legend: Facial appearance of patient aged 11 years