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MASTER OF MEDICINE (MMED) IN MEDICAL GENETICS

The Characterization of Lowe Syndrome in a South African Cohort

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DECLARATION

I, Rizqa Sulaiman-Baradien, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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The Characterization of Lowe Syndrome in a South African Cohort

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CHAPTER 1: Introduction and Literature review

Introduction

Oculocerebrorenal or Lowe Syndrome (OMIM #309000) is an X-linked recessive condition characterized by a triad of ophthalmological, central nervous system and renal involvement (Lewis, Nussbaum, & Brewer, 1993; Loi, 2006). Lowe et al first described a small cohort of rachitic boys in 1952 with intellectual disability, hydrophthalmos, organic aciduria with decreased production of ammonia and a metabolic acidosis (Lowe, Terrey, & MacLachlan, 1952). This phenotype was not compatible with syndromes known to be associated with rickets or renal disease at the time. Lowe Syndrome is a pan ethnic, rare condition with an approximate frequency of 1:500 000 (Bokenkamp & Ludwig, 2016). Loss of function variants of the *OCRL* gene results in abnormal renal tubule and lens epithelium cell differentiation, migration, and function, resulting in the pathophysiological changes of Lowe Syndrome (Bokenkamp & Ludwig, 2016).

Lowe syndrome is diagnosed in males who have anomalies of the ocular, central nervous and renal systems with decreased OCRL-1 activity in skin fibroblasts and/or a hemizygous pathogenic variant in the *OCRL* gene (Lewis et al., 1993).

There is a dearth of knowledge regarding the clinical and molecular features of Lowe syndrome in Sub-Saharan Africa and the information gained may assist in improving identification and early management.

A literature search including “Lowe Syndrome”, “Oculocerebrorenal Syndrome”, “Renal Fanconi Syndrome”, “*OCRL* gene” and “congenital cataract” were entered into PubMed. A combined total of 11546 results were returned and only papers written in English describing the clinical characteristics of Lowe Syndrome and the *OCRL* variants implicated in Lowe syndrome were selected for review. A total of 43 of these papers have been cited. The search revealed only one published study conducted in 2009 in Durban, South Africa, describing the anaesthetic implications for patients who present with congenital cataract, referring to a boy with Lowe syndrome as a case in point (Ramanathan & Patil, 2009).

In the present study, we described a case series of three affected boys, which to the best of our knowledge, is the first attempt at clinically and molecularly characterizing Lowe Syndrome in South Africa.

Aims

This study aims to characterize the clinical phenotype and molecular genotype of Lowe Syndrome in South African males.

Objectives

1.) Describe the clinical phenotype of the affected male probands.
2.) Molecular analysis of the *OCRL* gene in the affected males in an attempt to identify the disease-causing mutation.
3.) If the disease-causing mutation is identified in a male proband, to analyse maternal DNA to confirm carrier status.

1.Clinical Manifestations of Lowe Syndrome

1.1 Ocular

The ocular findings in Lowe Syndrome are not only mandatory to make the diagnosis but may also be the earliest clinical manifestations in affected males. Congenital cataracts, with 100% prevalence in affected infants (Song et al., 2017), can be detected on antenatal ultrasound in some cases and has served as a marker for a foetus at an increased familial risk of Lowe Syndrome (Endres, Schaub, Stefani, Wirtz, & Zahn, 1977). Up to 50 % of patients will develop infantile glaucoma, some of which may be refractory to goniotomy following cataract surgery (Walton, Katsavounidou, & Lowe, 2005). Corneal keloids which may impair vision are also a well described post cataract surgery complication and it is recommended that contact lenses or any other risks of abrasion to the cornea be avoided if possible (Esquenazi, Eustis, Bazan, Leon, & He, 2005; Song et al., 2017). All affected males will have some degree of decreased visual acuity as a consequence of the ophthalmological manifestations (Loi, 2006).

1.2 Central Nervous System

Neonates with Lowe syndrome typically present with severe hypotonia and absent deep tendon reflexes (David et al., 2018; Lewis et al., 1993; Loi, 2006). There is delayed acquisition of milestones, notably motor milestones and ambulation may not be achieved in about 25% of patients (McSpadden, 2010). Intellectual disability (ID) and maladaptive behaviour is well described, and although the majority of affected boys will have developmental delay in the moderate range, there is a spectrum of cognitive function that ranges from normal to severe or profound impairment (Kenworthy, Park, & Charnas, 1993). A characteristic pattern of tantrums, aggression, stereotypy and restrictive behaviour with unusual preoccupation and obsessions is thought to be specific to the condition (Kenworthy & Charnas, 1995).
Seizures of various types and ages of onset are not infrequent, this includes an increased occurrence of febrile seizures (Lawrence Charnas, 1989; Erdogan, Ismailogullari, Soyuer, Ferahbas, & Poyrazoglu, 2007). Neuroradiological findings vary and mild ventriculomegaly, generalized brain atrophy, demyelination in both the cerebrum and cerebellum, pachygyria and polymicrogyria is seen. The clinical significance of this is yet to be determined as early demise is frequent, therefore brain imaging is not part of routine surveillance and management (Carvalho-Neto, Ono, Cardoso Gde, Santos, & Celidonio, 2009; L Charnas et al., 1988; Savolaine & Bielke, 1993).

1.3 Renal

Proximal renal tubular dysfunction is a hallmark of this condition and those affected are found to have renal Fanconi syndrome characterized by low molecular weight (LMW) proteinuria and clinical and biochemical manifestations of rickets (Bokenkamp & Ludwig, 2016). This is often refractory to vitamin D treatment (El Shafie, Samir, El Latif, El Sabagh, & Mahmoud, 2017). Renal disease, which is shown to be a more specific tubulopathy early in the course (Bockenhauer et al., 2008), only manifests between 6 and 12 months of age, but LMW proteinuria is found earlier and is a clue to diagnosis in boys born to known carriers. Progressive glomerulosclerosis occurs with chronic renal failure and end stage renal disease

between the second and fourth decade. Nephrocalcinosis and nephrolithiasis may also be visualized on kidney ultrasound (Bokenkamp & Ludwig, 2016; Loi, 2006).

Dent disease type 2 (Dent-2), allelic to Lowe Syndrome, is differentiated by the absence of extra renal manifestations. The pathophysiology of the exclusive renal phenotype is not well understood, and it is thought that modifier loci or epigenetic factors play a part (Bokenkamp et al., 2009).

1.4 Other clinical features

Patients have normal birth lengths and exhibit postnatal growth slowing with short stature. Along with short stature, bone age may be delayed, but those with normal height tend to have a normal bone age (L. R. Charnas, Bernardini, Rader, Hoeg, & Gahl, 1991).

Variable expression of facial dysmorphic features described in the literature include frontal bossing, deep set eyes, full cheeks and fair complexion (Loi, 2006).

Joint hyper-mobility, subcutaneous nodules, arthropathy, tenosynovitis and non-tender joint swellings have also been described and pathological fractures with poor healing, in the absence of rickets or corrected Fanconi syndrome occurs in the presence of generalized poor bone mineralization (Athreya et al., 1983; L. R. Charnas & Gahl, 1991; Holtgrewe & Kalen, 1986).

2. Carrier phenotype in Lowe Syndrome

A 2/3 maternal carrier rate in sporadic cases has been described (Monnier, Satre, Lerouge, Berthoin, & Lunardi, 2000) and careful ophthalmological examination of suspected cases mothers is warranted as this may not only strengthen the evidence for a clinical diagnosis, but also guide recurrence risk counselling in the absence of genetic testing.

“Snowflake” lenticular opacities, visible on slit lamp exam, are a reliable indicator of carrier status. The sensitivity of slit lamp examination for detection of carrier status has been shown to be 97% and can be useful in detecting heterozygotes for cascade screening, particularly in the absence of genetic testing. Ten percent of female carriers will have cataracts that may be visually significant if large and these changes only become apparent in the post pubertal period. (Delleman, Bleeker-Wagemakers, & van Veelen, 1977; T. Lin, Lewis, & Nussbaum, 1999; Reilly, Lewis, Ledbetter, & Nussbaum, 1988; Roschinger, Muntau, Rudolph, Roscher, & Kammerer, 2000).

Affected females with the ocular, renal and CNS phenotype, have been described in the literature and one case showed completely skewed X-linked inactivation (100:0) (Cau et al., 2006).

3. Biochemical findings

Elevated serum creatinine kinase (CK), aspartate transaminase (AST) and lactate dehydrogenase (LDH) suggests abnormal muscle metabolism and highlight the association of Lowe syndrome with disfunction of the musculoskeletal system (L. R. Charnas et al., 1991).

OCRL-1 activity can be measured in cultured skin fibroblasts. This is decreased (<10%) in affected individuals and has a high negative predictive value in males (T. Lin et al., 1997). Some carriers may have decreased enzyme activity, this is however not reliable for carrier screening as false negative results may occur due to skewed X-linked inactivation (Cau et al., 2006; Lewis et al., 1993).

4. Molecular Genetics

Variants in the *OCRL* gene on chromosome Xq26.1 have been implicated in the pathogenesis of Lowe Syndrome. *OCRL*, has 24 exons and encodes for the enzyme inositol polyphosphate 5-phosphatase OCRL-1 which plays an important role in membrane cellular traffic control and regulation of actin-dependent processes including the overall formation and management of tight junctions in the kidney epithelium (M. Lowe, 2005). Decreased OCRL-1levels results in elevated intracellular levels of its substrate, phosphatidylinositol (4,5) bisphosphate (PtdIns (4,5) P_2) which further impairs intracellular protein trafficking and endocytic tubular transport. Furthermore, abnormalities of primary ciliary function as a result of increased substrate is thought to contribute to the ophthalmological and renal phenotype observed in Lowe Syndrome (Luo et al., 2012).

Over 200 variants in the *OCRL* gene have been described in Lowe Syndrome (Hichri et al., 2011). Variants lead to null effects and include largely frameshift, nonsense and splice site variants, but missense variants and micro-deletions have been previously reported on, albeit in much fewer numbers (Bokenkamp & Ludwig, 2016). Novel variants may seem to be the trend, but a hotspot region from exons 9-24 has been identified with 97% of variants detected in this area. (Zhang, Wang, Ding, Yan, & Yang, 2013). A pathogenic mutation in *OCRL* can be found in 95% of patients on sequence analysis and a further 5% on targeted deletion/duplication analysis. De novo mutations are identified in a third of patients and germ line mosaicism (4-5%) has been described (Loi, 2006).

Genotype-phenotype associations have not been shown and there is phenotypic variability in individuals with the same variant.

Complete loss of function of *OCRL* has been demonstrated in both Lowe Syndrome and the allelic Dent-2 Disease, however no family has yet exhibited both conditions. Frameshift and nonsense variants in Dent-2 disease occurring in the first 7 exons and missense variants overlap with Lowe Syndrome variants in exon 9-15 of the *OCRL* gene (Lewis, Nussbaum, & Brewer, 1993; Bokenkamp et al., 2009).

5. Prenatal Diagnosis

Given the potential poor prognosis with high morbidity and absence of a cure, prenatal diagnosis is important in a family with a known variant or at risk of recurrence of Lowe Syndrome. Ultrasound screening can augment risk if the foetus is determined to be a male as opposed to a female and increased nuchal translucent >95% has been shown to be associated with an increased risk to be affected (S. Y. Lin, Lee, Shih, Lin, & Su, 2011). Prenatal and preimplantation genetic diagnostic testing can be offered when the family variant is known (Sethi, Lunardi, Kabra, Deka, & Bagga, 2010) (Lewis, Nussbaum, & Brewer, 1993). With a germ line mosaicism rate of 4-5%, prenatal testing should be offered to mothers of an affected male even in the absence of family history and a normal slit lamp examination (McSpadden, 2000). Early foetal sexing by ultrasound can avoid the risk of miscarriage

associated with invasive testing in a female foetus. Unfortunately, foetal sex can only be reliably determined by ultrasound from 12 weeks' gestation (Lubusky, Studnickova, Skrivanek, Vomackova, & Prochazka, 2012) and thus delays a possible early diagnosis.

In the absence of a known genetic mutation Northern analysis of OCRL mRNA in amniocytes of a confirmed male foetus at risk is sufficient to determine if the foetus is affected (Tsuru, Yamagata, Momoi, & Okabe, 1999), but this is a laborious process. Alternatively, measurement of inositol polyphosphate 5-phosphatase activity in cultured amniocytes can be performed (Suchy, Lin, Horwitz, O'Brien, & Nussbaum, 1998

Neither prenatal enzyme testing nor gene sequencing with/without deletion/duplication analysis of the *OCRL* gene is currently available in South Africa. There is limited access to international next generation sequencing panels and single gene testing, but the logistics of transporting amniotic fluid or cultured amniocytes, high cost involved, and unpredictable turnaround time is not always feasible for prenatal testing in our setting.

6. Management

The management of patients with Lowe syndrome is largely symptomatic and involves a multi system approach addressing the three key affected areas: the eyes, central nervous system and kidneys. Early detection and removal of congenital cataracts is vital, but impaired visual acuity due to glaucoma is inevitable thus monitoring of intraocular pressure six monthly is recommended (Lewis et al., 1993).

Surveillance and treatment include detection and aggressive correction of the biochemical effects of renal tubular function and metabolic acidosis with the aim of ultimately preventing or treating the characteristic renal rickets (Lewis et al., 1993; Loi, 2006; Song et al., 2017). Oral medication includes sodium or potassium bicarbonate to correct the acidosis and phosphate and calcitriol to slow down the skeletal deterioration (Lewis et al., 1993). Phosphate supplementation does not have an effect on overall growth and there is no difference in final height between those who receive phosphate and those who do not (L. R. Charnas et al., 1991). Growth hormone may be considered in those with significant slowing of growth velocity (Lewis et al., 1993).

Perioperative correction and management of electrolyte and acid base imbalance along with managing hypotonia and frail bone is critical as these pose an anaesthetic risk (Chung et al., 2009). Primary haemostasis disorders as a result of platelet disfunction results in increased risk of intra operative bleeding and must be carefully assessed preoperatively (Lasne et al., 2010).

Early referral to neurodevelopmental services with speech therapy is recommended. Behavioural problems may require medical management and/or behaviour modification therapy and should ideally be formally assessed.

Conclusion

As with many rare genetic conditions, Lowe Syndrome has not been well characterised in African populations and particularly lacking is the sequence data for the *OCRL* variants. This missing knowledge may contribute to missed opportunities of diagnoses and therefore timeous and appropriate management, but also to genetic counselling of recurrence risk

which may be life altering with respect to reproductive choices in some families. The more clinical and genetic information we discover and contribute, the richer our understanding, diagnostic skills and ultimately, our surveillance and management of rare genetic diseases becomes.

Word count: 2297

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Chapter 2

Manuscript for submission to the American Journal of Medical Genetics

The Characterization of Lowe Syndrome in a South African Family of mixed ancestry

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Conflict of interest disclosures:

Dr Rizqa Sulaiman-Baradien has no conflict of interests to disclose.
Dr Careni Spencer has no conflict of interest to disclose.
Dr Gloudi Agenbag has no conflict of interest to disclose.
Professor Ambroise Wonkam has no conflict of interests to disclose.

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Editorial Policies and Ethical Considerations:

This study received ethics approval from the Human Research Ethics Committee (HREC) of the University of Cape Town in South Africa (HREC 773/2018). Informed consent was sought from the parent/s/guardian of the participants. This included minors not able to give assent and majors that are not of the cognitive ability to give consent. Mothers of participants included in the study were given their individual consent. Adult and parent/guardian consent forms were used. (Appendix 2 and 3)

This consent included permission to perform a medical examination, record medical information and collect blood samples for DNA extraction and analysis of the *OCRL* gene. Consent to take photographs of the affected male participants was also requested.

Abstract
Oculocerebrorenal or Lowe Syndrome (OMIM #309000) is an X-linked recessive condition characterized by a triad of congenital cataracts, proximal renal tubular dysfunction, and variable central nervous system involvement. Nearly all affected boys will be hemizygous for a pathogenic variant in the *OCRL* (NM_000276.4 c.2615delC) gene.
We present a clinical and molecular characterization of an extended multiplex family of three affected boys with Lowe Syndrome and describe a novel variant, predicted to be pathogenic, in the *OCRL* gene. This is to the best of our knowledge the first description of its kind in South African patients.

Introduction
Lowe Syndrome (OMIM 309000) was first described in 1952 (Lowe, Terrey, & MacLachlan, 1952). It is a pan ethnic congenital metabolic syndrome with an approximate frequency of 1:500 000 (Loi, 2006). Lowe Syndrome, an X-linked recessive condition, is characterized by the triad of congenital cataracts, variable central nervous system abnormalities and predominantly proximal renal tubular dysfunction (Bockenhauer et al., 2008; Bokenkamp & Ludwig, 2016).

The cataracts seen in Lowe Syndrome are largely diagnosed postnatally, but if detected on ultrasound antenatally it may serve as a marker for disease in at risk males (Endres, Schaub, Stefani, Wirtz, & Zahn, 1977; Song et al., 2017). All affected males will experience decreased visual acuity and up to 50% of patients will develop infantile glaucoma which may be refractory to goniotomy (Loi, 2006; Walton, Katsavounidou, & Lowe, 2005). Other variable ophthalmological findings are also described (Esquenazi, Eustis, Bazan, Leon, & He, 2005; Song et al., 2017).

Central nervous system features first manifest as severe hypotonia and absent deep tendon reflexes in the neonate (David et al., 2018; Lewis, Nussbaum, & Brewer, 1993; Loi, 2006). There is delayed milestones and motor delay may be particularly significant with up to 25% of patients not achieving ambulation. Intellect is usually in the moderate range but can vary from normal to more profound. (Kenworthy, Park, & Charnas, 1993). Maladaptive behavior is well described with characteristic patterns of temper tantrums, aggression, stereotypy and restrictive behavior. Moreover, seizures of all types are not infrequent (Kenworthy & Charnas, 1995).

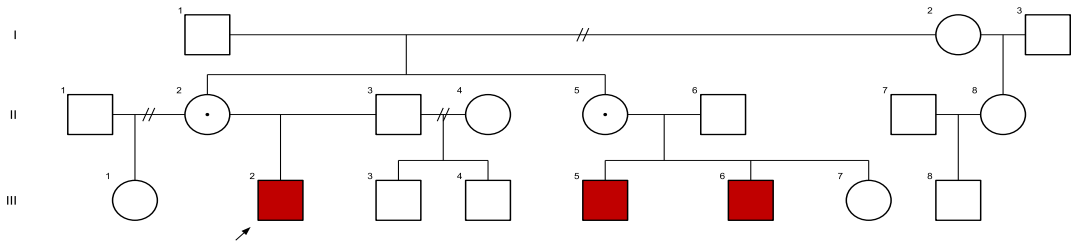
Proximal renal tubular dysfunction is a hallmark of this condition and those affected progress to renal Fanconi syndrome characterized by proteinuria, hypophosphatemia, acidosis and clinical and biochemical manifestations of rickets which is often refractory to treatment (Bokenkamp & Ludwig, 2016; El Shafie, Samir, El Latif, El Sabagh, & Mahmoud, 2017). Severe postnatal growth restriction is independent of renal dysfunction (Bokenkamp & Ludwig, 2016; Charnas, Bernardini, Rader, Hoeg, & Gahl, 1991). More specific tubulopathy only manifests between 3 and 12 months of age, but low molecular weight (LMW) proteinuria can present earlier. Progressive glomerulosclerosis occurs with chronic renal failure and end stage renal disease occurring between the second and fourth decade. Nephrocalcinosis and nephrolithiasis may also be visualized on kidney ultrasound (Bokenkamp & Ludwig, 2016; Loi, 2006).

Variants in the *OCRL* gene on chromosome Xq26.1 have been implicated in the pathogenesis

of Lowe Syndrome. *OCRL* codes for inositol polyphosphate 5-phosphatase (OCRL-1). OCRL-1 plays an important role in membrane cellular traffic control and regulation of actin-dependent processes including the overall formation and management of tight junctions in the kidney epithelium (M. Lowe, 2005). Furthermore, abnormalities of primary ciliary function are thought to contribute to the ophthalmological and renal phenotype observed in Lowe Syndrome (Luo et al., 2012). Over 200 variants in the *OCRL* gene have been described in Lowe Syndrome (Hichri et al., 2011). Variants lead to null effects and include largely frameshift, nonsense and splice site variants. Missense variants and micro-deletions have been previously reported on, albeit in much fewer numbers (Bokenkamp & Ludwig, 2016). Novel variants may seem to be the trend, but a hotspot region from exons 9-24 has been identified with 97% of variants detected in this area. (Zhang, Wang, Ding, Yan, & Yang, 2013). A pathogenic mutation in *OCRL* can be found in 95% of patients on sequence analysis and a further 5% on targeted deletion/duplication analysis. De novo variants are identified in a third of patients and thus 2/3 of mothers of affected boys will be carriers. Germ line mosaicism is not insignificant at 4-5% (Loi, 2006), it is thus important, in the absence of a family history to determine if the variant is indeed de novo as it will infer recurrence risk for the mother.

Clinical History

We present a case series of three South African boys of mixed ancestry with Lowe syndrome. Two brothers and a first maternal cousin presented with a history of bilateral congenital cataracts. One of the brothers was born during the time of recruitment to this study.



Family Pedigree: III-2, III-5, III-6 are affected with Lowe Syndrome and II-2 and II-5 are carriers.

Case1 (III-2):

Case 1 was referred for genetic review at 18 months. He was born at term after an uneventful pregnancy with a birth weight of 2810g (-1.23 SD), birth length of 46cm (-1.49 SD) and head circumference of 33cm (-1.32 SD). Bilateral congenital cataracts were diagnosed at age 3 months of age and he received surgery which resulted in him being aphakic. He was also noted to have bilateral microphthalmos. At initial presentation he was found to have global developmental delay with significant fall off in growth parameters (Table 1). Special investigations revealed a normal audiology screen, CT brain, thyroid function tests and urine organic acids analysis. His karyotype was that of a normal male profile, 46, XY.
At last evaluation, age 3 years, he had still not achieved walking, had only single word utterances and aggressive behavior. His mother reported scratching and biting and having more temper tantrums than usual. Growth faltering was persistent, and he had notable dysmorphic features including a tall prominent forehead, epicanthic folds with very long widely spaced eyelashes, thick flared eyebrows a long philtrum and thin upper vermillion with sharp pointed chin and low set ears (Figure 1). He was noted to have a bowed right lower limb and a marked kyphosis.
His urine and serum chemistry indicated renal tubular acidosis and suggested renal dysfunction of the Fanconi type (Table 1A and 1B – supplementary information). A clinical diagnosis of Lowe Syndrome was made on the basis of congenital cataract, developmental delay and Renal Fanconi Syndrome.

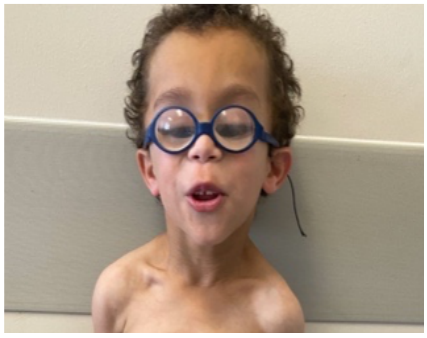


Figure 1
Case1: Cataract removal rendered him aphakic. Dysmorphic features: tall prominent forehead, thick flared eyebrows, long philtrum, thin upper vermillion, sharp chin and low set ears.

Case 2 (III-5):
Case 2 first presented at 19 months with a history of bilateral congenital cataracts, relative macrocephaly and failure to thrive. A largely uneventful pregnancy with a history of smoking during pregnancy was reported. He was delivered at 36 weeks' gestation with Apgar scores of 9 and 10 and intrauterine growth restriction (IUGR) was noted at birth with a weight of 1840g (-2.51SD), birth length of 43cm (-2.58SD) and head circumference of 30,5cm (-2.20SD), plotted on growth charts for premature babies. Congenital bilateral cataracts were diagnosed at 4 weeks old and he had successful surgery at 6months of age.
At initial examination he had significant fall of in growth parameters (Table 1), however he had achieved all developmental milestones timeously and displayed appropriate sociable behavior. He was noted to have sparse scalp hair, a prominent metopic suture, epicanthic folds, deep set eyes and a broad nasal bridge with retrognathia. He was also found to have a pectus carinatum. X-rays revealed diffuse osteopenia with irregular and flared femoral metaphyses.
On follow up exam at 3years old, additional dysmorphic features described included a tall prominent forehead and large prominent ears with thick flared eyebrows, not in keeping with the familial pattern and a smooth philtrum and thin upper vermillion (figure 3B). At this stage he had developed a marked kyphosis and had bowed lower limbs (figure 2). His urine and

serum chemistry suggested a renal tubular acidosis (table 2A and 2B – supplementary information) and a clinical diagnosis of Lowe Syndrome was made based on congenital cataracts, renal Fanconi Syndrome and an apparent X-linked pattern of inheritance as at this stage his maternal cousin was diagnosed with Lowe Syndrome.

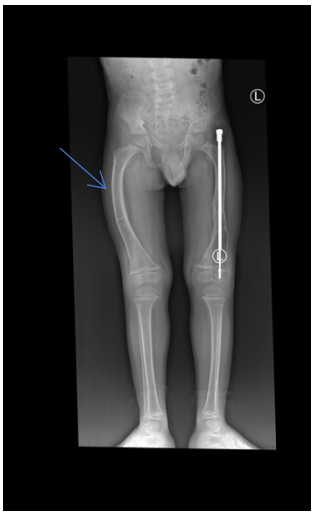


Figure 2
Case 2 at age 3: Bowed femurs (arrow) with left femur pin post orthopedic surgical correction of bowing. Diffuse osteopenia and flared metaphysis of the femur present.

Case3 (III-6):

Case 3 was born during the time of recruitment of this study. His mother received genetic counselling and ultrasounds at the Fetal Medicine Unit as due to the family history of Lowe Syndrome. Congenital cataracts were screened for at the 2nd trimester fetal anomaly scan and were not detected. His mother was offered prenatal testing but declined. He was born at 40 weeks' gestation with Apgar scores of 9 and 10 and a birth weight of 2210g (-2.07SD), birth length of 49cm (-0.37SD) and head circumference of 49cm (-2.04). Bilateral congenital cataracts were diagnosed on day 2 of life and had surgery at 8 weeks old. He was referred to the renal unit early and started on phosphate and sodium bicarbonate supplementation at 6months old.

On review at 8 months old, he displayed growth faltering (table 1) but had met his developmental milestones and was able to sit independently, was reaching to grab objects, cooing and babbling appropriately. He had subtle dysmorphology with deep set eyes, a flat nasal bridge, long philtrum and thin upper vermillion (figure 3C). Despite early treatment, a left hand Xray done at the time revealed early rickets like changes with a thin bony cortex and loss of distinction of the ulna metaphysis.

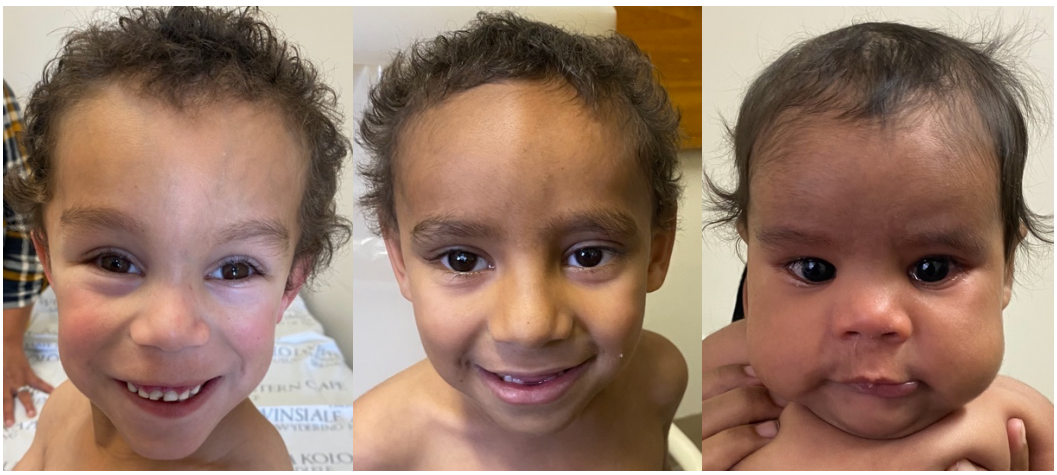


Figure 3 A B C

This figure shows the dysmorphic features of all 3 cases. Case 1 (A), Case 2 (B) and Case 3 (C) all display tall prominent forehead with variable degrees of frontal bossing, thick eyebrows, long smooth philtrum and thin upper vermillion.

	Birth Length	Length at 1st consult	Height at 2nd consult
Case 1	46cm (-1.49SD)	66cm (-5.43SD) 18months old	73cm (-6.78SD) 3years old
Case 2	43cm (-2.58SD)	62.4cm (-6.93SD) 19months old	66cm (-9.55SD) 3 years old
Case 3	49cm (-0.37SD)	58cm (-5.64SD) 8months old	

Table 1: This table summarises the significant growth faltering over time seen in all three cases

At diagnosis	Case 1	Case 2	Case 3
Urine protein dipstix	-	-	2+
Urine protein:creatine ratio (g/mmol)	1.307 H	1.44 H	-
Serum bicarbonate (mmol/l)	17 (23-29) L	19 (23-29) L	17 (23-25) L
Serum inorganic phosphate (mmol/l)	0.60 (1.05-1.08) L	0.99 (1.0-1.95) L	1.05 (1.15-2.15) L

Table 2: This table summarises some of the urine and serum chemistry findings of case 1 and 2 at diagnosis and case 3 between 6-8months old. Case 1 and 2 both had raised protein:creatinine ratios indicative of the loss of protein in the urine and case 3 had 2+ protein on urine-dipstix at 6months old. All of the cases had low serum bicarbonate and low inorganic phosphate at diagnosis. These findings are consistent with proximal renal tubule dysfunction.

Molecular Findings

DNA was extracted with Chemagic™ (PerkinElmer) instrument, quantified with a Nanodrop ND-1000 spectrophotometer (ThermoFisher Scientitic) and diluted accordingly. Twenty-three pre-designed sequencing primer sets were selected from ThermoFisher Scientific to flank the coding regions of the *OCRL* gene (NM_000276.4), which includes 24 exons. The proband (Case 1) was sequenced bidirectionally using these primers in conjunction with the BigDye™ Direct Cycle Sequencing Kit (ThermoFisher Scientific). A novel single base deletion (NM_000276.4 c.2615delC) in the *OCRL* gene was identified. This variant in exon 24 causes a frameshift and extends the protein by six amino acids. Segregation analysis performed on II-2, II-5,III-2, III-5 and III-6 revealed the same variant while II-7 and III-8 did not have the variant supporting it as the causative variant in this family. *OCRL* is the only gene which has

been associated with Lowe syndrome, and no other coding sequence changes have been found in this gene in this family. The variant (NM_000276.4 c.2615delC) was annotated and classified as pathogenic using the American College of Medical Genetics and Genomics guidelines(Richards et al., 2015).

Discussion

This research describes the phenotypic and molecular features of three maternal cousins with Lowe Syndrome. To the best of our knowledge this is the first description of its kind in South African patients.

In keeping with cases in the literature, they presented with congenital cataracts followed by renal dysfunction later in infancy and childhood.

The children display the facial dysmorphic features described in the literature including frontal bossing, deep set eyes and fair complexion compared to family members, but in addition they have a longer smooth philtrum with thin upper vermillion borders which has not been reported; these were not considered familial features.

All three cases had bilateral congenital cataracts and by the time of completion of this research project, had not developed glaucoma. Where all patients are expected to have some degree of visual impairment (McSpadden, 2000), interestingly Case 2 at age 6, still has normal vision and does not require any form of correction. Together with his brother (Case 3) and Case 1, he follows up with ophthalmology regularly for glaucoma surveillance.

All patients with Lowe Syndrome will have LMW proteinuria; it may be present at birth prior to any tubular dysfunction. (Charnas, Bernardini, Rader, Hoeg, & Gahl, 1991; Laube, Russell-Eggitt, & van't Hoff, 2004). Proteinuria with a raised ALP at age 6 months was the first sign of renal tubular dysfunction in Case 3 and despite treatment, he developed hypophosphatemia with a drop in his bicarbonate by 8 months. At the last consult, all three cases were being managed with phosphate and sodium bicarbonate supplementation and although Case 1 showed some improvement in his inorganic phosphate levels with treatment, it remained low, whereas Case 2 seemed to drop his levels further over time.

Growth faltering postnatally, independent of renal function, is in keeping and although our cases were all proportionally short statured at birth, they had significant fall off in height in excess of that described in the literature. Final average adult height of patients is expected to be around 155cm which is approximately at -2SD when plotted at 18 years old (McSpadden, 2000) (Bokenkamp & Ludwig, 2016). Our patients are already plotting below the -5SD. Significant interfamilial variability in development and behavioral disturbances is observed in this family, Case1 being more severely affected than Case 2 at the same age, however, it remains to be formally evaluated.

A novel pathogenic *OCRL* variant was found in exon 24. This is in keeping with previous literature which concluded that the majority of variants are private, and 97% of these will be in the hot spot region of exon 9-24 (Zhang, Wang, Ding, Yan, & Yang, 2013).

A molecular diagnosis of Lowe Syndrome allows for timeous family genetic counselling and carrier screening. With 2/3 of affected males' mothers being carriers, recurrence risk counselling is a critical part of management. Access to genetic testing in South Africa is limited in the public health system and careful clinical phenotyping is essential to offer

appropriate prenatal noninvasive testing and postnatal ophthalmological screening in at risk males. This case series may assist with making diagnoses of Lowe Syndrome in the South African population. A limitation of this study is that only one family was investigated, future research into more families with Lowe syndrome will be beneficial to knowledge about the condition in South Africa.
Word count (excluding abstract): 2392

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Supplemental Information:

Case1			
Urine Chemistry	At diagnosis (age 3)	**Urine dipstix**	6years old
creatinine	1.4mmol/l	blood	-
protein	1.83g/l	protein	3+
protein:creatine ratio	1.307g/mol H	glucose	trace
inorganic phosphate	3.71mmol/l		
glucose	Not done		

Table 1A

Case 1					
Serum Chemistry	At diagnosis (age 3)			At last consult (age 6)		
creatinine	32	(23-37)		48	(25-42)	
urea	3.6	(1.1-5.7)		3.4	(1.1-5.7)	
sodium	137	(136-145)		143	(136-145)	
potassium	3.7	(3.4-4.7)		4.4	(3.4-4.7)	
chloride	109	(98-107)	H	116	(98-107)	H

bicarbonate	19	(23-29)	L	17	(23-29)	L
anion gap	13	(9-16)		134	(9-16)	
calcium	2.28	(2.19-2.64)		2.24	(2.19-2.64)	
magnesium	1.03	(0.70-0.99)	H	1.03	(0.70-0.99)	H
inorganic phosphate	0.60	(0.13-0.28)	L	1.83	(1.05-1.80)	H
alkaline phosphatase	1128	(93-309)	H	493	(93-309)	H

Table 1B

Case 2		
Urine Chemistry	At diagnosis (age 3)	At last consult (age 6)
creatinine	2.0	2.4
protein	2.88	3.16
protein:creatine ratio	1.440g/mol H	1.317g/mmol H
inorganic phosphate	not done	not done
glucose	0.5	not done

Table 2A

Case 2					
Serum Chemistry	At diagnosis (age 3)			At last consult (age 6)	
creatinine	19	(23-37)	L	38	(25-42)
urea	5.2	(1.1-4.3)	H	5.9	(1.1-5.9)
sodium	134 (136-145)			136 (136-145)	
potassium	3.6 (3.4-4.7)			3.9 (3.4-4.7)	
chloride	106 (98-107)			105 (98-107)	
bicarbonate	19	(23-29)	L	21	(23-29) L
anion gap	13	(9-16)		14	(9-16)
calcium	2.35(2.19-2.64)			2.36(2.19-2.64)	
magnesium	1.07(0.70-0.99)		L	1.10(0.70-0.99)	L
inorganic phosphate	0.99(1.00-1.95)		L	0.72(1.05-1.80)	L
alkaline phosphatase	892 (104-345)		H	Not done	

Table 2B

Case 3		
Urine dipstix	6months old	8months old
blood	1+	-
protein	2+	2+
glucose	-	-

Table 3A

Case 3			
Serum Chemistry	4weeks old	6months old	8months old
creatinine	31 (10-56)	18 (14-34)	18 (14-34)
urea	2.3 (1.4-4.3)	2.1 (0.7-4.6)	2.4 (0.7-4.6)
sodium	136 (136-145)	136 (136-145)	136 (136-145)
potassium	4.8 (3.7-5.9)	4.7 (4.1-5.3)	4.3 (4.1-5.3)
chloride	102 (98-113)	107 (98-107)	107 (98-107)
bicarbonate	24 (23-29)	20 (23-29)	17 (23-25) L
anion gap	15 (9-16)	14 (9-16)	16 (9-16)
calcium	2.72(2.12-2.64)	2.57 (2.17-2.62)	2.53 (2.17-2.62)
magnesium	0.99(0.70-0.99)	1.06 (0.66-1.03)	0.98 (0.66-1.03)
inorganic phosphate	1.55	1.36 (1.15-2.15)	1.05 (1.15-2.15) L
alkaline phosphatase	Not done	581(82-383) H	614 (82-383) H

Table 3B

Appendices

Appendix 1

Information sheet and parent/guardian consent form for a research project

UNIVERSITY OF CAPE TOWN
DIVISION OF HUMAN GENETICS

Project title: **The characterization of Lowe Syndrome in South African families**

Principle Study Investigators:
Dr Careni Spencer, Division of Human Genetics, Department of Medicine, University of Cape Town (UCT)
Dr Rizqa Sulaiman-Baradien, Division of Human Genetics, Department of Medicine, UCT

You have been asked to be part of this study because your son/the child in your care has been diagnosed with Lowe Syndrome.

Information about Lowe Syndrome:

Lowe Syndrome is an inherited genetic condition that affects boys. A genetic condition occurs when a genes function in our body is changed. Our genes are our body's building plans and are packaged in our chromosomes.

Boys with Lowe Syndrome are born with cataracts, develop more slowly when compared to other children their age and have a kidney condition called Fanconi Syndrome. Fanconi Syndrome affects how the boys absorb vitamin D and other important substances from their kidneys that help with bone growth. This is why boys with Lowe Syndrome are usually shorter than other boys their age and they may have soft bones (rickets).

Lowe Syndrome is caused by a change in the OCRL gene. The OCRL gene is located on the X chromosome, so we call this condition an X-linked inherited condition. Females have two X-chromosomes, one that they inherit from their mother and one from their father. Males only have one X-chromosome that they inherit from their mothers. This means that if a female child inherits the X-chromosome with the changed OCRL gene, she will be a carrier of the condition, but will not be affected or have very mild symptoms as the other X-chromosome with the normal OCRL gene is the back up. Males however only have one X-chromosome and therefore no back up in the event that they inherit the X-chromosome with the changed OCRL gene. These males will then be affected with Lowe Syndrome.
Family members at risk of being carriers or affected may want to have genetic counselling and can be referred to the Genetics Clinics at RCWMCH and GSH. It is particularly important that boys of presumed carrier mothers are seen by a doctor early, as the kidney

function can be monitored and managed appropriately. There is no cure for Lowe Syndrome, but treatment can slow down the progression of the kidney dysfunction and therefore the bone abnormalities that occur as a result.
Please contact the Division of Human Genetics on 021 4046235 to make an appointment.

Why are we doing this study?

Lowe syndrome is very rare and not a lot is known about the condition in South African children. We would like to improve this by describing the features of South African children with Lowe Syndrome. We would also like to determine if there are changes in the OCRL gene in these boys and their families.

What we will be doing:

If you agree to participation:

- We will be performing a medical examination on your son/ the child in your care.
- We would like a photograph of your son/child in your care.
- We will be performing an ophthalmological (eye) exam on all available mothers of affected boys.
- We will draw a blood sample from your son/the child in your care and their mothers to test for a change in the *OCRL* gene.

It is important to remember that even though your son/ the child in your care has been diagnosed with Lowe Syndrome, we may not get a positive genetic result, but this will not affect his current management.

Important information about this study:

1.) Participation is voluntary – **You don't have to agree to take part in this study. Your child's medical care will not in any way be affected by your decision or participation.**

2.) You can withdraw from participation at any time – **You can change your mind at any time,** and we will not use your medical information or blood sample in this study.

3.) We will only be analysing the *OCRL* gene – we will not be able to give you more information about your other health concerns or other genes.

4.) Research results cannot be used to test pregnancies or family members suspected to be affected or carriers of the change in the *OCRL* gene and need to be verified independently in an accredited genetic diagnostic lab.

Testing for the change in the OCRL gene via an accredited diagnostic lab can be facilitated through our Genetic Clinics at Red Cross War Memorial Children's Hospital and Groote Schuur Hospital. In certain cases, this costing might be covered by the hospital but in others the patients may be expected to pay for testing themselves.

5.) We will be collecting blood samples, and this will require a venepuncture (a needle prick and blood withdrawal from a vein on the body). This may feel uncomfortable but is not dangerous and wherever possible will be done at the same time blood is taken for medical reasons.

Are there any benefits to me?

There are not likely to be any direct benefits immediately, but this study may help us to gather more information about Lowe Syndrome that we can share with other health care professionals to facilitate easier diagnosis and management in the future.

We will make every effort to contact you to discuss the results. Please inform us if any of your contact information changes during the period of this research

Please feel free to contact the following people if you have and further questions or concerns about this study:

Dr Rizqa Sulaiman-Baradien
Tel: 021 4046235
Email: rizqa.sulaiman-baradien@uct.ac.za

Dr Careni Spencer
Tel: 021 4046235
Email: careni.spencer@uct.ac.za

Appendix 2

Affected male consent form

UNIVERSITY OF CAPE TOWN
DIVISION OF HUMAN GENETICS

Project title: **The characterization of Lowe Syndrome in South African families**

What we aim to do:

1) To perform a detailed clinical examination on males with Lowe syndrome
2) To have an eye examination performed on mothers of participating males with Lowe syndrome
3) To draw a blood sample from the affected males, their mothers and other potentially at-risk family members for genetic testing of *OCRL* gene
4) To describe the clinical and genetic findings of families with Lowe Syndrome in South Africa

Please complete the form in block letters and cross out the statement that does not apply

to you.

1.) I, ….....the parent/guardian of ….....voluntarily consent to his participation in this study, the purposes of which and limitations is outlined in the information sheet and has been explained to me by
….....

2.) I consent/do not consent to the taking of and publication of his photograph.
2.1) Please cover his eyes
2.2) His eyes do not have to be covered

3.) I understand and accept that DNA will be extracted from the blood sample for analysis of the *OCRL* gene

4.) I understand and accept that any results, even if shared with me were obtained in a research study and cannot be used to test future pregnancies or other family members as described in the information above unless confirmed independently by an accredited diagnostic genetic laboratory.

5.) I consent/do not consent to the possible reanalysis of my son/child in my care extracted DNA for other research purposes in the future

Signature of parent/guardian …..... Date.....

Witness Signature.....Date.....

Investigators signature.....Date.....

Appendix 3

Adult Consent Form/Potential Carriers

UNIVERSITY OF CAPE TOWN
DIVISION OF HUMAN GENETICS

Project title: **The characterization of Lowe Syndrome in South African families**

What we aim to do:

1) To perform a detailed clinical examination on males with Lowe syndrome
2) To have eye examinations performed on mothers of participating males with Lowe syndrome
3) To draw a blood sample from the affected males, their mothers and other potentially at-risk family members for genetic testing of *OCRL* gene
4) To describe the clinical and genetic findings of families with Lowe Syndrome in South Africa

Please complete the form in block letters and cross out the statement that does not apply to you.

1.) I, ….....voluntarily consent to my participation in this study for purposes outlined in the information sheet and explained to me by:
….....

2.) I consent to an Ophthalmological (Eye) exam.

4.) I understand and accept that DNA will be extracted from my blood sample for analysis of the *OCRL* gene.

5.) I understand and accept that any results, although delivered to me are for research purposes only and cannot be used to test future pregnancies or other family members as described in the information above unless confirmed independently by an accredited diagnostic genetic laboratory.

6.) I consent/do not consent to the possible reanalysis of my extracted DNA for other research purposes in the future.

Signature of participant …..... Date.....

Witness Signature.....Date.....

Investigators signature.....Date.....

Appendix 4

Clinical Data Capturing Sheets:

Facility/Hospital:

Proband/Mother

Study number/Family number:

Contact details (phone numbers and email address):

- **Please complete all sections**

Background Information

Self- identified ethnolinguistic background:

Birth History:
Gestation at birth:
Birth weight:
Birth length:
Head circumference:

Pedigree:

Clinical examination and special investigation findings specific to Lowe Syndrome

Case number		
Age		
Anthropometry	Height (cm/percentile)	
	Weight (cm/percentile)	
	Head Circumference (cm/percentile)	
Nervous System Exam		
Cardiovascular System Exam		
Respiratory System Exam		
Abdominal Exam		

Tick features present and describe additional features not on this form

Dysmorphic features	• Frontal Bossing • Deep set eyes • Full cheeks • Fair complexion • Other (please describe)	
Ophthalmology examination	• Bilateral congenital cataracts • Glaucoma • Corneal Keloids • Amblyopia	
Skeletal exam/survey	• Features of rickets Describe findings	
Urine dipstix/chemistry	• Record findings	

Serum chemistry	• Record Findings	
Ultrasound KUB/Abdomen	• Nephrocalcinosis • Nephrolithiasis	
Brain Imaging	• CT Brain • MRI Brain • Describe findings	
Neurodevelopmental Assessment	• Developmental Delay (DD) • Intellectual disability Describe severity • No DD or ID	
Behaviour	• No concerns • Difficult to manage at times • Always difficult/Aggressive/Anger outbursts	

Western Cape Government
Health

DR N BEYERS
Manager: Medical Services
Red Cross War Memorial Children's Hospital
Email: Nellis.Beyers@westerncape.gov.za
Tel: +27 21 658 5788 Fax: +27 21 658 5006/5166

02 July 2019

Dr R Sulaiman-Baradien
Division of Human Genetics

Dear Dr Sulaiman-Baradien,

RESEARCH: RXH: RCC 194

PROJECT TITLE: The Characterization of Lowe Syndrome in a South African Cohort

It is a pleasure to inform you that the hospital Research Review Committee has approved your application to conduct above-mentioned study at Red Cross War Memorial Children's Hospital.

Kindly complete the attached trial registration form and forward to the Fees Department, for attention Natasha Jaftha (Natasha.Jaftha@westerncape.gov.za). Her telephone number is 021 658-5286 for any queries. Please forward a list with the details of the participating patients. Reception will be notified after the form has been processed.

Yours sincerely,

DR N BEYERS
MANAGER: MEDICAL SERVICES
(OBO Dr Parbhoo)

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

28 March 2019

HREC REF: 773/2018

Dr C Spencer
Division of Human Genetics
IDM -FHS

Dear Dr Spencer

PROJECT TITLE: THE CHARACTERISATION OF LOWE SYNDROME IN A SOUTH AFRICAN COHORT (Master's candidate-Dr R Sulaiman-Baradien)

Thank you for your response letter dated 28 February 2019, addressing the issues raised by the Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 March 2020.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student: Dr R Sulaiman-Baradien will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

pp UBurgess)
PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

American Journal of Medical Genetics Guidelines:

Case Reports in Diverse Populations. This section is aimed to photographically document the phenotypes of molecularly confirmed genetic syndromes from diverse populations. Brief reports of one or more patients with a genetic syndrome will be considered. **Examples of these articles are available here**. Preference will be given to reports of patients with rare syndromes, or syndromes not previously published in patients from diverse backgrounds. Emphasis will be on detailed photographic documentation, therefore authors are requested to provide as many figures as possible, with a few figures published in the print version and rest published as online supplementary figures. All manuscripts must have consent of the patient/family for publication of photographs. Authors are encouraged to follow the standard guidelines for describing the phenotype and genotype and use the prescribed nomenclature for sequence variants, copy number variations and chromosome results. Though novelty is not absolutely essential, papers describing a new mutation, novel features, a recently described syndrome or a rarely reported condition in the population are favorably considered. Case reports of patients with syndromes would be excluded if they have been published previously in large series, like Down syndrome, 22q11.2 deletion syndrome, Noonan syndrome, Williams syndrome, Cornelia de Lange syndrome, Beckwith-Wiedemann syndrome, or Turner syndrome in diverse populations. **See this editorial for more information.** ***FORMAT***: Articles should not exceed 2-3 printed pages in the journal (6-9 manuscript pages), with the following elements: Title Page (include funding information), Abstract, Introduction, Case Report, Discussion, and References (up to 5). Non- photographic clinical data (chromatograms, etc.) should be submitted as supplemental information.

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The Journal uses the terms “intellectual disability” or “cognitive disability” instead of "mental retardation".

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Please include a statement confirming that your study was approved by an ethics committee as the first sentence of your Methods section, under the subheading, “Editorial Policies and Ethical Considerations”.

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The Journal requires that all appropriate steps be taken in obtaining informed consent of any and all human and/or experimental animal subjects participating in the research comprising the manuscript submitted for review and possible publication, and a statement to this effect must be included in the Methods section of the manuscript, under the subheading, “Editorial Policies and Ethical Considerations”. Identifying information should not be included in the manuscript unless the information is essential for scientific purposes and the study participants or patients (or parents or guardians) give written informed consent for publication.

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While the manuscript will be processed upon submission, anything considered protected health information will be restricted from access prior to the receipt of documented permission. We caution you that the absence of material or cited figures may adversely impact the manuscript in the review process. The submission of masked photos without

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