Lipoid proteinosis is a rare autosomal recessive disorder, characterized histologically by infiltration of periodic acid Schiff-positive hyaline material into the skin, upper aerodigestive tract, and internal organs. Classical clinical features include skin scarring, beaded eyelid papules, and laryngeal infiltration leading to hoarseness. Moreover, the infiltrates in the tongue and its frenulum limit lingual movements and cause speech difficulties. Usually, the hoarse voice is present at birth or in early infancy, as the first manifestation. In more severe cases, diffuse infiltration of the pharynx and larynx might cause respiratory distress, at times requiring tracheostomy. The disorder has recently been shown to result from loss-of-function mutations in the extracellular matrix protein 1 gene on chromosome 1q21. The function of the protein extracellular matrix protein 1 gene is still unclear, although an important role in skin physiology and homeostasis has been hypothesized. In this report, the case is described of a 6-year-old girl with lipoid proteinosis. Histopathological examination of a laryngeal biopsy specimen showed massive deposits of eosinophilic, periodic acid Schiff-positive, and diastase resistant material in the lamina propria corroborating the clinical diagnosis of lipoid proteinosis. Molecular analyses in this patient also confirmed the clinical diagnosis. The proposita was a compound heterozygote for a new small rearrangement (543delTG/ins15) in exon 6, and a nonsense mutation (Arg243Stop) in exon 7. Together with previously documented mutations in the extracellular matrix protein 1 gene, this study supports the hypothesis that exons 6 and 7 are the most common sites for extracellular matrix protein 1 gene mutations in lipoid proteinosis.
Introduction

Lipoid proteinosis (LP) (Urbach-Wiethe disease, MIM 247100) is a rare autosomal recessive genodermatosis first described in 1929; fewer than 300 cases have been reported in the literature to date. Although LP occurs worldwide, ~25% of all reported cases have been observed in South Africa, where many of the patients are of Dutch or German ancestry.

The clinical manifestations of LP are protean and may vary considerably between affected individuals. The disease may display multiple systemic involvement, although the skin and mucosal membranes of the upper aerodigestive tract are those primarily affected. Onset is usually in early infancy, and signalled by a weak cry and hoarse voice due to laryngeal infiltration. In more severe cases, this infiltration may lead to respiratory obstruction. Diffuse skin infiltration and thickening gradually occurs, resulting in papules and chicken pox-like scars. Infiltrates in the tongue and its frenulum limit tongue movements and cause speech difficulties. Calcifications in the temporal lobes or hippocampus have been reported, sometimes in association with neurological, psychiatric and neuropsychological sequelae. Prognosis of LP is, nonetheless, relatively good, if not quoad vitam, if not quoad valetudinem.

LP is characterized, histologically, by intercellular deposits of periodic acid Schiff (PAS)-positive hyaline material in the skin, mucous membranes, and internal organs. Loss-of-function mutations in the gene encoding extracellular matrix protein 1 (ECM1) on chromosome 1q21 have been identified in LP; fewer than 20 variants have been reported (Fig. 1), most of which occur in single families. ECM1 encompasses 11 exons spanning ~5,498 bp of the genomic sequence and codes for a protein of 540 amino acids (ECM1a) (ECM1; 602201). Two distinct isoforms of different lengths have been described for ECM1 depending on the presence of two alternatively spliced exons (exons 5a and 7) and both are expressed in the skin and upper respiratory tract, although ECM1a has a wider pattern of expression. A third isoform, ECM1c, contains all 11 axons but has been detected in only two cancer cell lines. The function of the protein ECM1 is still unclear, although an important role in skin physiology and homeostasis has been hypothesized.

The present report focuses on clinical and genetic findings in an additional LP patient and summarizes pertinent data from the literature.

Case report

A 6-year-old girl, born to unrelated parents, presented with progressive skin and mucous membrane changes since early childhood. The patient had developed hoarseness during the first few months of life and, at age 3 years, started to develop multiple warty papules on the dorsum of the hands, on the face and eyelid margins. Most papules were skin-coloured. Scarring was also evident. She had no history of frontal headache, seizures or visual distur-

![Fig. 1. Morbidity map of ECM1 gene. Illustration of position of new frameshift mutation (543delTG/ins15) (grey arrow) and previously identified mutations. Missense or nonsense mutations are depicted above and frameshift mutations below gene structure. Most mutations are nonsense or frameshift changes that occur within exon 6 or within differentially spliced exon 7.](image)
The patient underwent indirect laryngoscopy, on account of severe dysphonia, which showed thickening of the vocal cords and hyaline deposits in the larynx, oral cavity and oropharynx. As proposed elsewhere, the patient was submitted to a logopaedic programme; when this failed, the lesions were removed surgically, for temporary functional purposes. Light microscopy examination of laryngeal biopsy tissue showed massive deposits of homogeneous, eosinophilic, hyaline-like material in the lamina propria, without inflammation. The material was strongly PAS positive, diastase resistant, revealing the glycoproteic nature of the substance (Fig. 2).

In order to obtain definitive molecular confirmation of LP in this patient, we analyzed the ECM1 gene. Following parents’ informed consent, genomic DNA was extracted from peripheral blood and the coding sequence of ECM1 (mRNA Genbank accession number NM_004425.2) was amplified by polymerase chain reaction (PCR) using intronic oligonucleotide primers, as reported elsewhere. The PCR products were gel purified and directly sequenced using BigDye Terminator chemistry on an ABI 377 automatic sequencer (Applied Biosystems, Foster City, CA, USA). A small heterozygous deletion/insertion mutation in exon 6 was found. Subcloning into a PCR-compatible cloning vector (TOPO TA Cloning Kit, Invitrogen, Paisley, UK) and direct sequencing revealed a novel 543delTG/ins15, which results in frameshift and early translation termination at codon 187. By separation of PCR products, on acrylamide gel electrophoresis, the heterozygous mutation in the patient and in blood DNA from the healthy mother was confirmed (Fig. 3). A previously documented heterozygous 726C>T (Arg243Stop) mutation was detected on the paternal allele, and confirmed by primer extension using the ABI Prism SnaPshot Multiplex kit (Applied Biosystems, Foster City, CA, USA) (Fig. 4).

Discussion

Although several reports of LP patients have appeared, mostly in the dermatology literature, emphasizing the typical dermatologic features, the first clinical manifestation of LP is usually progressive hoarseness caused by diffuse deposition of hyaline material in the mucous membranes of the vocal cords. This hoarseness may be present at birth, as a weak cry, or develop later, within the first few years of life. The development of a hoarse voice was the first symptom noticed in our patient. Therefore, the importance of LP should not be underestimated by otolaryngologists and it should be included in the differential diagnosis of voice changes and hoarseness, in infancy and childhood.

The skin of children with LP is highly susceptible to damage, being reflected in the appearance of chicken pox-like scars and yellowish papules. These lesions involve primarily the face and extremities and rarely appear elsewhere. Subsequently, infiltration of the skin may present as groups of warty plaques on the axillae and elbows. Beaded papules on the eyelid margins (moniliform blepharosis) are a characteristic finding in about two-thirds of patients. Our patient presented typical skin lesions, which appeared at approximately 3 years of age.
In clinical practice, LP is rarely a life-threatening condition. Infiltration of the oral mucosa may lead to xerostomia and dysphagia. Other mucosal findings include thickening of the sublingual frenulum and tongue, limiting tongue movements and causing speech difficulties. Moreover, diffuse infiltration of the pharynx and larynx may cause respiratory distress, at times requiring tracheostomy. Our patient presented a speech impairment but no upper airway obstruction or dyspnoea. Hyaline deposits have been described in the conjunctiva, cornea, trabeculum, and retina. Corneal opacities or secondary glaucoma, due to infiltration in the trabeculum, may appear later. Deposition of hyaline material in the small bowel may lead to intestinal bleeding. Although autopsy studies have shown LP to be a generalized disorder with microscopic deposits of hyaline material in practically every organ, symptoms related to other viscera have not been described. Extracutaneous and extramucosal features may include neurological signs and symptoms, such as epilepsy, memory loss, and schizophrenic behaviour, sometimes in association with intracranial calcification in the temporal lobes or hippocampus, easily detected by brain computed tomography.

Histologically, LP is characterized by disruption/duplication of the basement membrane and deposition of hyaline material at the level of the basement membrane (resulting in its thickening at the dermo-epidermal junction), papillary dermis, surrounding capillaries, and around adnexal epithelia, especially sweat coils. The hyaline material is eosinophilic, PAS-positive, and diastase resistant, indicating the presence of glycoproteins. Accumulations of type IV and V collagen occur around the blood vessels and appendages; type I and III collagen is reduced.

Our patient was not subjected to skin biopsy. However, histopathological examination of a laryngeal biopsy specimen showed massive deposits of eosinophilic, PAS-positive, and diastase resistant material in the lamina propria corroborating the clinical diagnosis of LP. Molecular studies ultimately confirmed the clinical diagnosis. The proposita was a compound heterozygote for a small rearrangement (543delTG/ins15) in exon 6, and a nonsense mutation (Arg243Stop) in exon 7. Both mutations lead to early protein truncation and likely loss of function. This finding is in keeping with data reported in the literature, which suggest that LP patients usually harbour mutations in exon 6 and, especially, 7 of ECM1. Recent cell biology studies have provided new data that further our understanding of the function of ECM1 and that partially explain the disease phenotype in LP. ECM1 has been reported to regulate enchondral bone formation, to stimulate proliferation of blood vessel endothelial cells, to promote angiogenesis, and to be involved in the control of epidermal differentiation. However, clues to its physiological function in the dermis are now emerging. It has been demonstrated that ECM1 is a secreted glycoprotein that binds to perlecan, the major heparan sulphate proteoglycan of the basement membrane, as well as to growth factors and fibrillar proteins. Thus, ECM1 may act as a “biological glue” in the dermis, helping to regulate basement membrane and interstitial collagen fibril macro-assembly and growth factor binding. Therefore, loss of ECM1,
within the dermis may have profound effects on dermal homeostasis, leading to the clinical features of skin infiltration and scarring. Meanwhile, lack of ECM1 within the epidermis may alter the normal pattern of keratinocyte maturation and differentiation and give rise to the clinical features of warty hyperkeratosis.

Some Authors have attempted to correlate genotype with clinical manifestations. Nonsense or frameshift mutations in exon 7 lead to ablation of the ECM1a transcript, but not the shorter ECM1b transcript that normally lacks this exon and that is expressed in skin and upper respiratory tract. From a clinical point of view, it is suggested that the patients harboring mutations in exon 7 could have a slightly less severe skin and respiratory tract phenotype compared to cases bearing mutations not affecting exon 7. Our patient, despite the presence of nonsense mutation in exon 7, developed hoarseness during the first months of life and skin manifestations in early childhood. This finding favors the hypothesis that the isoform containing exon 7 (ECM1a) is of fundamental biological importance. Instead, no specific correlation between genotype and neurological manifestations has ever been shown. We are unable to support the hypothesis suggesting the lack of such a correlation since we cannot, at present, predict the evolution of the clinical syndrome in our patient or the possibility of her developing neurological complications. Furthermore, a detailed analysis of a large, inbred South African family demonstrated that individuals sharing the same genetic background and with an identical mutation can present vastly different phenotypes during disease progression. Other genetic and epigenetic factors might be invoked.

The prognosis of LP patients is generally good despite the progressive nature of the disease until early adulthood. However, there is currently no effective therapy. Laser microlaryngoscopy, dissection of the vocal cords and excision of deposits may be performed to preserve or improve the voice. Respiratory obstruction is infrequent and rarely requires tracheostomy. Dermabrasion and chemical skin peeling can be performed in some cases. Approaches reported in the literature include oral steroids, dimethyl sulphoxide, intralesional heparin, and etretinate. Irrespective of evolution of the disease and use of symptomatic treatment, it is important that parents of affected children be counselled concerning the risks of having other affected offspring.

References
