with thrombocytopenia [58]. *ALOX12* expression is also regulated epigenetically, as indicated by the increase in DNA methylation of *ALOX12* genes in myelodysplastic syndrome and acute myeloid leukemia patients with megakaryocytic dysplasia [61,62]. It has been suggested that *ALOX12* is associated with diminished bone mineral density as well [54–57]. Given that 12-LOX produces endogenous lipid ligands for nuclear receptors, such as PPAR-γ, which facilitate adipocyte differentiation from mesenchymal stem cells, the number of osteoblasts decreases, followed by impairment of bone mineral density [55].

An earlier study suggested that the 12-LOX-mediated pathway is associated with the risk of colorectal cancer [63]. The best-studied example includes mutation of E261R (835A > G), which causes an increase in 12-LOX activity, with a potential link to esophageal squamous cell carcinoma [64]. This mutation has also been associated with colorectal cancer [47,65] and breast cancer [48]. An in vitro and in vivo study has shown that 12-LOX plays a role in the proliferation and antiapoptosis of hepatocellular cells, suggesting that this carcinogenic function of *ALOX12* requires endogenously generated lipid mediators [66].

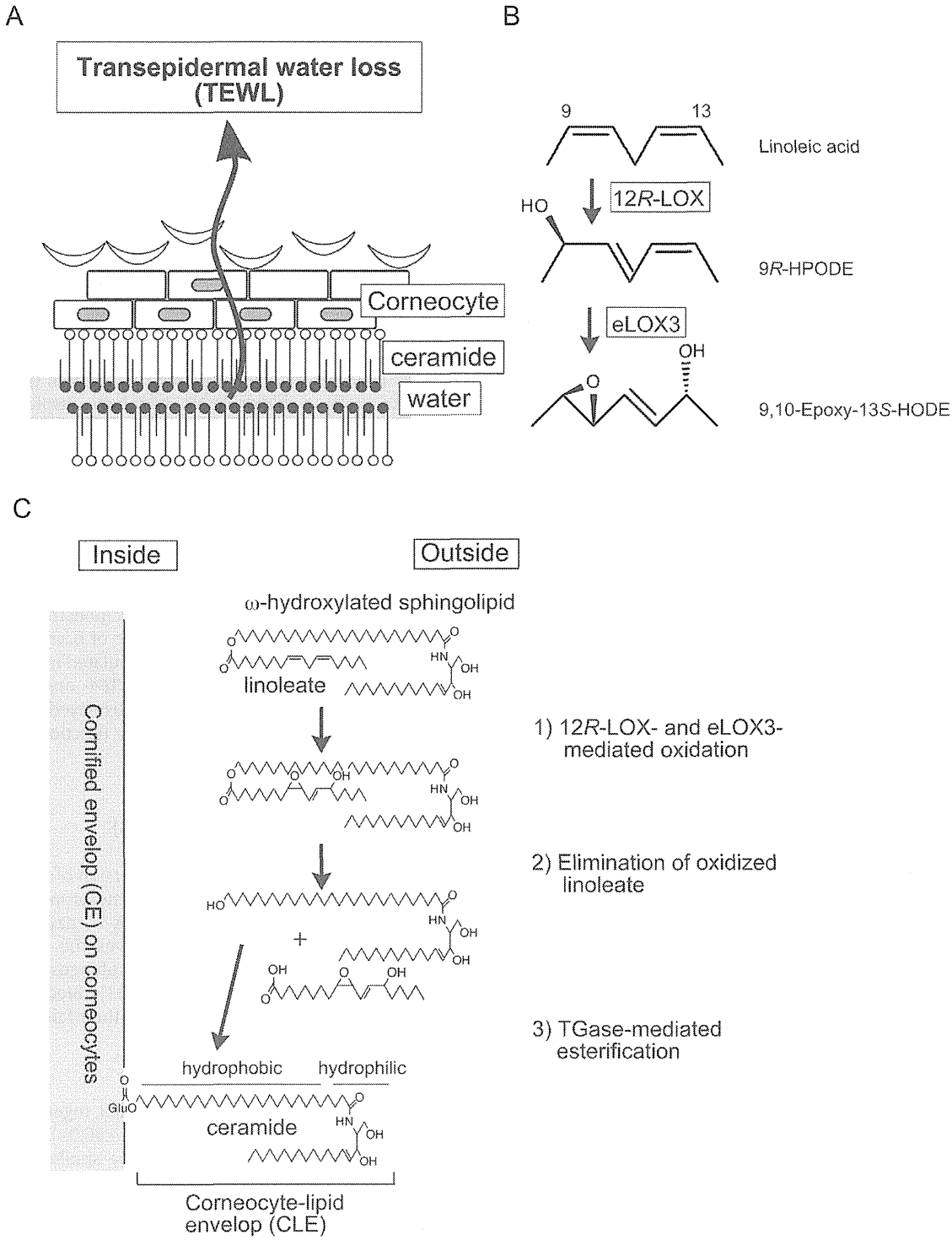

Fig. 1. A. Schematic representation of transepidermal water loss (TEWL) from the skin. B. Reaction of 12R-LOX and eLOX-3. C. Development of corneocyte-lipid envelop (CLE) over cornified envelop (CE) on corneocyetes.

4.2. Alox12

Consistent with the expression of *ALOX12* in the platelets of humans, mice lacking *Alox12* have shown increased platelet sensitivity and mortality due to thrombosis in response to the administration of adenosine diphosphate, whereas aggregation and secretion in response to most agonizts seemed normal [67]. *Alox12* deficiency has led to a reduced incidence of carcinoma in a C57BL6/129 genetic background and of papilloma in a tumor-sensitive SENCAR genetic background, showing that *Alox12* is involved in tumorigenesis in the skin in a context-dependent manner [68]. Most notably, *Alox12* deficiency has caused basal transepidermal water loss in the skin with unaltered inflammatory responses in *Alox12b*- and *Aloxe3*-deficient mice [69]. This finding suggests that *Alox12* has a critical role in the maintenance of the skin barrier in association with other isoforms, as explained in detail later.

5. 12-Lipoxygenase, 12R type (12R-LOX)

In humans, 12R-LOX has been detected in keratinocytes, tonsil squamous epithelial cells, bronchial epithelial cells, and psoriasis scales, as well as in B cells [8,70–74]. In mice, its expression has been induced at embryonic day (E) 15.5 in the epidermis, nasal epithelium, and surface of the tongue, suggesting that 12R-LOX is required for the proper development of neonates [71].

The physiological role of 12R-LOX is rather specific, in conjunction with its limited expression profile in epithelial cells. The best-characterized example is the skin. The important physiological role in the skin is to maintain appropriate moisture by preventing unnecessary water evaporation through epithelial cells, called transepithelial water loss (TEWL) (Fig. 1A). TEWL results in a loss of water from the skin, leading to excessively dry skin, as found in ichthyosis. In humans, this inherited disease is known as autosomal recessive congenital ichthyosis (ARCI) (MIM#s 190195, 242100, 242300). Biochemically, 12R-LOX reacts readily with linoleate rather than arachidonate to produce 9R-HPODE (Fig. 1B). This hydroperoxide is converted into its associated epoxide derivatives through the isomerase activity of eLOX-3 [75]. In the skin, the best substrate for these enzymes is linoleate, which is esterified with ω-hydroxylated sphingolipids, usually found outside corneocytes during the immature stage of skin development (Fig. 1C). As mentioned previously, this esterified linoleate is further converted into oxygenated linoleate, followed by elimination from sphingolipids by hydrolysis. The newly formed ceramide is ω-terminally hydroxylated; therefore, it is subsequently linked covalently to a carboxyl acid moiety of glutamine in cornified envelope (CE) proteins. The established lipid layer is called a corneocyte-lipid envelope (CLE), and it plays a crucial role in holding water in the hydrophobic group, in ceramides in the CLE. The completion of CLE formation requires transglutaminase-1, which catalyzes the cross-linking of the ceramides with the carboxylic acid in the side chain of glutamate in CE proteins, as many mutations of 12R-LOX enzymes cause ARCI in humans [76]. Mice lacking the transglutaminase-1 gene have consistently exhibited defective skin formation and high TEWL [77]. Both enzymes act critically prior to this transglutamination, as previous observations have suggested that a deficiency of 12R-LOX, as well as eLOX-3, causes failure of the skin barrier due to defective formation of enzymatic lipid oxidation products [78–80].

5.1. ALOX12B

Genetic failure of the above process leads to ARCI, a heterogeneous skin disease characterized by rough and scaly skin, with a prevalence of one in 200,000 newborns throughout the world [81–86]. The affected skin generally improves during either childhood or puberty, and these patients have a normal life span. Among various ARCI diseases, *ALOX12B* is mainly, but not solely, involved in nonbullous congenital ichthyosiform erythroderma. Mutations, mostly found as missense, termination, and frameshift in *ALOX12B*, are widely found in its entire molecule, involving both in a C-terminal catalytic domain and an N-terminal β-barrel structure [82]. Some mutants have lost enzymatic activity, as confirmed by biochemical assays.

5.2. Alox12b

Disruption of the *Alox12b* gene in the murine model provides an effective means of studying human ichthyosis. *Alox12b*-deficient mice suffer postnatal death characterized by a severely impaired barrier function of the skin [87]. This defective epidermal barrier appears around E17.5 in wild-type (WT) controls, prior to which the expression of *Alox12b* reaches its maximal level beginning at E15.5 and continues after birth. Thus, there is a strong correlation between *Alox12b* expression and the formation of functional epidermis.

A study using skin transplantation from *Alox12b*-deficient neonates into nude mice revealed ichthyosiform formation, typically characterized by a thickening of the epidermis and severe hyperkeratosis, with a phenotype similar to that of patients with *ALOX12B* mutations in the grafted mice [88]. Essentially, the skin grafted from the neonates became thicker than that from the WT controls, with a hyperplastic histology displaying epidermal acanthosis and severe hyperkeratosis. Further investigation of this hyperkeratosis by electron microscopy revealed that the stratum corneum of skin grafted from *Alox12b*-deficient mice was abnormally overlaid, indicative of aberrant proliferation. In addition, both the size and number of keratohyaline granules increased, indicating hypergranulosis in mutant skin grafts. Functional assays that measured TEWL identified a marked increase in the skin from the neonates, as well as a marginal but significant increase in mature skin grafted in these mice, demonstrating that *Alox12b* plays a critical role in the maintenance of barrier function in the skin (Fig. 1A). Among genetically manipulated mice with defects in barrier function in the skin, such as *KLF4*- and *Claudin*-deficient mice [89,90], *Alox12b*-deficient mice displayed an extremely defective phenotype in epidermal barrier function, but not in tight junctions.

6. Epidermal lipoxygenase 3 (eLOX-3)

As mentioned previously, the conversion of linoleoyl ceramide in CLE into CE plays a crucial role in the proper maintenance of epidermal barrier formation. The eLOX-3 enzyme plays a major role in the second step, which involves the conversion of 9R-HPODE esterified with ω-hydroxyacyl-sphingosine into its related epoxylated derivative (Fig. 1B) [91–93]. Biochemical reactions catalyzed by eLOX-3 are essential, and their failure leads to ARCI.

6.1. ALOXE3

Genetically, many mutations in *ALOXE3*, together with *ALOX12B*, have been found in ichthyosis [81–84,86,94]. The outcome of disease in *ALOXE3* variants seems to be similar to that found in *ALOX12B* variants, showing clearly that this sequential oxidation by *ALOXE3* and *ALOX12B* are equally important. As a substrate, it is known that eLOX-3 favors oxygenated lipids like 12R-HPETE rather than unoxidized compound such as arachidonic acid (Fig. 1B). Other than ARCI, other diseases associated with *ALOXE3* variants

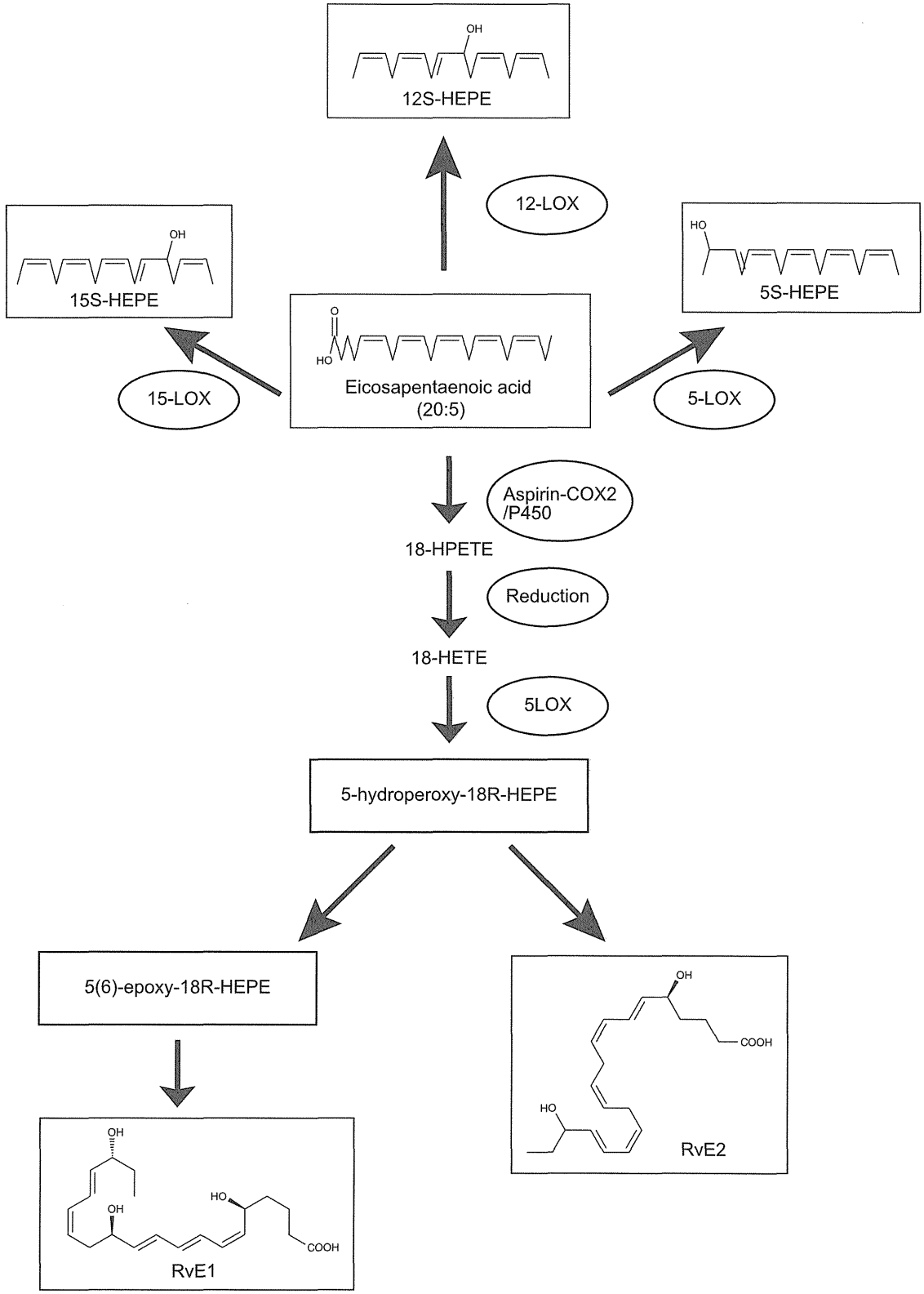

Fig. 2. Formation of resolvins from eicosapentaenoic acid (20:5).

have not been reported.

6.2. *Aloxe3*

A previous study showed that *Aloxe3*-deficient mice also exhibit a similarly severe ichthyosis phenotype with the loss of covalently bound ceramides and impaired CLE development [86]. As expected from its hepoxilin synthase activity of eLOX-3, in vivo results also showed a marked reduction of its metabolites in the skin. Similar to *Alox12b*-deficient mice, *Aloxe3*-deficient mice displayed a postnatal lethal phenotype [95].

7. 5-Lipoxygenase (5-LOX)

5-LOX plays an important role in the control of asthma. Under asthmatic conditions, activated immune cells first produce arachidonic acid by an enzymatic action of phospholipase A_2 from the plasma membrane, followed by 5-HPETE production through the 5-LOX enzyme. The produced 5-HPETE then converts into leukotrienes that have a potent biological effect on the constriction of bronchioles through cysteinyl leukotriene receptor 1, which is expressed exclusively onto bronchiolar smooth muscle cells, but not epithelial cells [96]. Conversely, cysteinyl leukotriene receptor 2 is expressed strongly in pulmonary interstitial macrophages and weakly in smooth muscle cells.

7.1. *ALOX5*

The expression of the *ALOX5* gene is transcriptionally regulated at a basal level and regulated by external stimuli such as Ca^{2+}. Numerous agents have been developed as antiasthmatics. A potent LOX-5 inhibitor zileuton introduced in the United States blocks a considerable amount of cysteinyl leukotriene production for a short period of time. Although the prevalence is low, a genetic E254K (760G > A) mutation in *ALOX5* has been reported in bronchiolar asthma patients [97]. This mutation causes an alteration in the electronic charge of the C-terminal catalytic domain from negative to positive, implicating defective changes in enzymatic activity and protein interaction. Mutations in the Sp1 binding site in the *ALOX5* promoter has been associated with airway hyperresponsiveness, but not with asthma [98,99]. Some evidence suggests that low 5-LOX expression in tumors found in humans might lead to greater 15-LOX expression followed by cancer formation through impaired apoptotic activity [47,100,101].

7.2. *Alox5*

7.2.1. *Inflammation*

As mentioned previously, immune cells express leukotriene receptor 1 and 2 as well as cysteinyl leukotriene receptor 2; as such, the role of *Alox5* could account for the migration of these immune cells. As expected, neutrophilic inflammation is one of the apparent phenotypes in *Alox5*-deficient mice. These mice have been shown to be resistant to anaphylaxis induced by platelet-activating factor (PAF), showing that *Alox5* seems to be closely involved in this process [102,103]. Similarly, *Alox5*-deficient mice exhibit a suppressed response to chemically induced local inflammation [104,105]. These mice are also susceptible to *Borrelia burgdorferi*-induced arthritis [106]. In this study, the authors showed that enzymatic activity of 5-LOX is not required for the initiation of infection, but it is required for earlier joint swelling and retarded arthritis recovery, suggesting a potential increase in the accumulation of neutrophils. Similarly, in a *Toxoplasma gondii* infection model, *Alox5*-deficient mice exhibited suppressed leukotriene A_4 production and increased interleukin-12 and interferon-γ production, followed by an increase in mortality rate due to marked encephalitis [107]. Such altered cytokine production seems to be explained, at least in part, by impaired neutrophilic inflammation.

In an ovalbumin-induced asthma model, *Alox5*-deficient mice exhibited a suppressed methacholine-induced response to airway hyperresponsiveness with impaired eosinophilic inflammation in the lung [108]. Thus, the production of lipid products from 5-LOX plays an important role under physiological conditions, and its level is tightly regulated by the balance between LOX and other enzymes, such as COX, as shown in an earlier study. Another study reported an increased mortality rate in *Alox5*-deficient male mice with an autoimmune-prone MRL-lpr/lpr genetic background, raising the possibility that an enhanced renal autoimmune inflammation could be involved in this process [109].

Recently, the roles of oxidation products derived from eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6) have been studied extensively (Fig. 2). As seen in their chemical structures, 20:5 and 22:6 have four and five bisallylic carbon atoms, respectively, in their molecules, providing a variety of oxidation products through free radical-mediated mechanisms. For example, 20:5 is a primary substrate for conventional LOX enzymes such as 15-LOX, 12-LOX, and 5-LOX. In addition, 18-hydroperoxy-5,8,11,14,16-eicosapentaenoic acid (18-HPETE), a free radical-mediated oxidation product of 20:5, can be further metabolized by 5-LOX to produce a novel class of oxidation product collectively called resolvins (Fig. 2) [110,111]. Emerging evidence has shown that resolvins assist in terminating inflammation through specific GPCR ChemR23 at nM concentrations in vitro [112]. The formation of resolvins seems to be critically regulated by local O_2 concentration, as well as the expression and activity of multiple LOX enzymes, both of which influence the final yield of resolvins from its initial substrate 20:5. Due to its anti-inflammatory function, whether these oxidized lipids might modulate the functions of microRNAs is under investigation [113].

7.2.2. *Atherosclerosis*

Apart from reactions in asthma and neutrophilic inflammation, there is some research showing that 5-LOX plays a key role in the initiation and/or development of atherosclerosis. Impaired expression of functional 5-LOX in LDL receptor-deficient mice has revealed suppressed atherogenesis, suggesting that 5-LOX plays a causative role in this disease [114]. Consistently, another atherosclerotic model induced by COX-2 disruption attenuated disease formation in *Alox5*-deficient mice [115]. Given that LOX-15 is involved in atherosclerosis, these studies provide examples that atherosclerosis can be induced by lipid peroxidation products formed from any isoforms. This finding is entirely consistent with observations indicating that antioxidants generally exhibit protective effects in experimental models.

7.2.3. *Neuronal disorder*

Alox5 is known to be highly expressed in neuronal tissue, particularly in Alzheimer's disease; thus, its role in neuronal disorders has been actively characterized [116]. A recent study reported that aged female *Alox5*-deficient mice exhibited protective effects against anxiety-like behavior on a C57BL/6 genetic background, raising the possibility that *Alox5* could modulate neuronal function [117]. A subsequent study using a transgenic mouse model of Alzheimer's disease demonstrated the efficacy of a 5-LOX inhibitor zileuton and hypothesized that *Alox5* could facilitate the initiation or progression of this disease [118]. Using such a disease model, *Alox5* deficiency consistently improved disease phenotypes [119].

7.2.4. *Tumor*

Colorectal cancer is often caused by mutation of the tumor suppressor Adenomatous polyposis coli (*APC*) gene. Among many mouse models generated by *Apc* mutations, $Apc^{\Delta 468}$ mice specifically bear a truncated *Apc* gene that develops severe polyposis by four months [120]. Interestingly, immunohistochemistry showed an increase in *Alox5* expression in $Apc^{\Delta 468}$ mice, suggesting that LOX-5 might contribute to tumorigenesis in colorectal cancer [121–123]. Mast cells play an important role in the development of colorectal cancer in this animal model, as they induce epithelial proliferation [122]. Consistently, the number of mast cells increased in $APC^{\Delta 468}$ mice compared to WT controls. In this model, a deficiency in *Alox5* led to impairment, implying that LOX5 acts as an important role in colorectal tumorigenesis [122].

8. 5-Lipoxygenase activating protein (FLAP)

FLAP is a small protein that activates 5-LOX through protein interaction. The formed complex of 5-LOX and FLAP in the nucleus efficiently generates 5-HEPE from arachidonic acid, which is subsequently converted into various leukotrienes. FLAP protein stays on the nuclear membrane and acts as a transporter for 5-LOX. FLAP expression is limited in myeloid cells.

8.1. *ALOX5AP*

Drugs targeting FLAP protein have been actively developed in humans. DG-031 (veliflapon, BAY x 1005), first licensed by DeCode Genetics and then developed by Bayer, is one example. This compound also reduces the incidence of ischemic myocardial infarction by reducing LTB_4 production [124]. A recently developed AM-103/GSK2190914 was designed based on the three-dimensional structure of FLAP protein [125]. In asthma, two intronic single-nucleotide polymorphisms have been associated with *ALOX5AP*, suggesting that these mutations can be used for diagnostic markers [126].

8.2. *Alox5ap*

Alox5ap-deficient mice exhibited unique phenotypes similarly observed in *Alox5*-deficient mice, such as an impaired response to PAF-induced anaphylaxis and zymosan-induced peritonitis [127]. In a collagen-induced arthritis model, *Alox5ap*-deficient mice displayed impaired arthritis, whereas the accumulation of antibody against collagen remained unchanged, suggesting that 5-LOX positively regulated inflammation without affecting the immune response. In a cerebral artery occlusion model, disruption of the *Alox5ap* gene caused impaired median infarct size and a better functional score, demonstrating that FLAP protein positively regulates cerebral inflammation [128]. The expression of *Alox5ap* is independent of the expression of 5-LOX. *Alox5ap* induces 12S-HETE production in the 12-LOX-induced signaling pathway, suggesting that 5-HPETE or downstream leukotriene metabolites might be involved in this process [129].

In atherosclerosis, FLAP inhibitor MK-886 and BAY x 1005 effectively attenuated disease formation in mice lacking *ApoE* and LDL receptors [130,131]. Similarly, another atherosclerotic model developed by transgenic mice expressing a dominant negative form of TGFβ receptor II in *ApoE*-deficient mice was suppressed by MK-886 [132]. These examples strongly suggested that FLAP protein is required for atherogenesis in these mouse models. Consistently, *Alox5ap*-deficient mice exhibited attenuated disease formation in an experimental model generated by *Cox-2*-deficient mice [115].

Alox5ap-deficient mice displayed an improved Alzheimer's disease-like phenotype [133] and an apparent increase in anxiety-like behavior in aged mice [134]. These results supported the phenotype of *Alox5*-deficient mice, showing that both 5-LOX and FLAP mutually and collaboratively play critical roles in the leukotriene pathway in neurological disorders.

9. Clinical Trials

There are many studies reporting synthesis and characterization of lipoxygenase inhibitors (reviewed in [135,136]). Generally, substances sharing similarity to either lipids or phenolic antioxidants have lower inhibitory activity for LOX enzyme. Furthermore, due to the presence of multiple isoforms, the development of selective inhibitor seems to be challenging. Therefore, there is a limited number of successful drugs that can be used for therapeutic purposes. One clinically applicable example includes zileuton that acts as anti-asthmatic agent by inhibiting 5-LOX. Apart from direct regulation of enzymes, there are some studies targeting FLAP for modulating 5-LOX under pathophysiological conditions in clinical trials.

9.1. *GSK2190915*

This is a FLAP inhibitor that inhibits the production of LTB_4 and other cysteinyl leukotrienes. The results of Phase I study showed that there was no clear difference in adverse events between placebo and drug-treated subjects in Western Europe (EUDRACT2007-00484872) and Japan (NCT00955383) [137]. Plasma concentration of GSK2190915 reaches maximal at two hours after oral administration. Consistently, LTB_4 production in drug-treated subjects was significantly impaired (EC_{50} in plasma is approximately 85 nM). Its beneficial effect is proven in adults and adolescents with persistent asthma (NCT01147744) [138]. Comparison between GSK2190915 and established asthma treatment such as montelukast and the inhaled corticosteroid fluticasone propionate revealed that GSK2190915 30 mg once daily has similar effect compared to montelukast 10 mg once daily as assessed by forced expiratory volume in 1 s (FEV_1), a widely used measure for asthma evaluation. A subsequent study reported that GSK2190915 50 mg daily showed clear attenuation in early asthmatic response induced by inhaled allergens in a placebo-controlled double-blind randomized study in UK (NCT00812773) [139].

10. Conclusions

The physiological roles of LOX enzymes have been studied extensively, due to the close link between them and various diseases. Apparently, the best characterized example includes the relation between 5-LOX and asthma, because cysteinyl leukotriene receptor 1, a GPCR activated by leukotrienes produced by 5-LOX, is widely expressed on bronchiolar smooth muscle cells. Genetically, both *ALOX12B* and *ALOXE3* play a critical role in the development of ichthyosis through TEWL. In humans, some mutations in *ALOX12* are found in tumor cells, suggesting this isoform might have anti-tumor effect. An increased expression of LOX enzymes in response to Th2 cytokines has been well established; how their expression is controlled and its consequences need to be investigated in the future.

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Identifying the need for a multidisciplinary approach for early recognition of mucopolysaccharidosis VI (MPS VI)

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ABSTRACT

Mucopolysaccharidosis VI (MPS VI, Maroteaux–Lamy syndrome) is caused by deficient activity of the enzyme, *N*-acetylgalactosamine-4-sulfatase, resulting in impaired degradation of the glycosaminoglycan dermatan sulfate. Patients experience a range of manifestations including joint contractures, short stature, dysostosis multiplex, coarse facial features, decreased pulmonary function, cardiac abnormalities, corneal clouding and shortened life span. Recently, clinicians from institutions in the Asia-Pacific region met to discuss the occurrence and implications of delayed diagnosis and misdiagnosis of MPS VI in the patients they have managed. Eighteen patients (44% female) were diagnosed. The most common sign presented by the patients was bone deformities in 11 patients (65%). Delays to diagnosis occurred due to the lack of or distance to diagnostic facilities for four patients (31%), alternative diagnoses for two patients (15%), and misleading symptoms experienced by two patients (15%). Several patients experienced manifestations that were subtler than would be expected and were subsequently overlooked. Several cases highlighted the unique challenges associated with diagnosing MPS VI from the perspective of different specialties and provide insights into how these patients initially present, which may help to elucidate strategies to improve the diagnosis of MPS VI.

1. Introduction

Mucopolysaccharidosis VI (MPS VI, Maroteaux–Lamy syndrome) is an autosomal recessive lysosomal storage disorder caused by deficient activity of the enzyme, *N*-acetylgalactosamine-4-sulfatase (arylsulfatase B, or ASB), resulting in impaired degradation of the glycosaminoglycan (GAG) dermatan sulfate (DS). The progressive accumulation of DS results in a multisystemic disorder including joint contractures, short stature, dysostosis multiplex, coarse facial features, decreased pulmonary function, cardiac abnormalities, corneal clouding and, ultimately, shortened life span [1]. MPS VI is a heterogeneous disease with a wide, continuous spectrum of manifestations, severity, and natural course. Within the first few years of life, patients at the more rapidly progressing end

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of the disease spectrum present with skeletal abnormalities, joint stiffness and deformities, cardiovascular symptoms, short stature with reduced growth velocity, coarse facies, and recurrent upper airway obstructions and infections [2–4]. Patients at the more slowly progressing end of the disease spectrum may present later in life, with variable symptoms and disease progression over several decades. This wide range of phenotypic presentation and lack of clinician awareness of the disease may contribute to the difficult diagnostic journey for some patients with MPS VI.

Early diagnosis of MPS VI is imperative due to the availability of galsulfase (recombinant human ASB; rhASB; Naglazyme®), which has been shown to slow the progression of the disease with a more significant impact on clinical outcomes the earlier the treatment is initiated [5, 6]. Early diagnosis also provides the family with vital genetic information, which may influence future reproductive decisions. Unfortunately, delays to or missed diagnoses are common when patients present with seemingly common childhood disorders and single organ involvement as patients with MPS VI do not have cognitive impairment and some with more slowly progressing disease may not have the characteristic coarse facies commonly associated with MPS VI [7]. Even clinicians familiar with the manifestations of MPS diseases may not identify slowly progressing patients immediately [8]. Therefore, it is not surprising that clinicians who are less experienced with MPS diseases may diagnose patients as having diseases with overlapping symptoms similar to MPS that are more commonly seen within their specialty. Busy specialists may assess and treat the patient for the manifestations related to their particular specialty without looking at the whole patient, and therefore do not identify the underlying MPS disease.

Recently, a group of healthcare professionals from several countries in the Asia-Pacific region met to discuss the occurrence and implications of delayed diagnosis of MPS VI in the patients they have managed. Several cases highlighted the unique challenges associated with diagnosing MPS VI from the perspective of different specialties. These cases provided insights into how these patients initially present, which may help to elucidate strategies to improve the diagnosis of MPS VI.

2. Methods

A group of healthcare professionals (HCPs) currently or previously involved in diagnosing and treating patients with MPS from the Asia-Pacific region were invited by BioMarin Pharmaceutical Inc. to gather for a two-day meeting in Hong Kong in September 2013 to discuss the diagnostic pathway for MPS VI at their institutions. All historical medical records available to the HCPs were reviewed prior to the meeting, with a focus on symptoms that led to specialist referrals and the subsequent diagnostic journey experienced by patients diagnosed with MPS VI. Cases of delayed diagnosis that involved unusual symptoms, referral patterns or misdiagnoses were explored and analyzed to understand possible underlying causes of misdiagnoses and discuss potential solutions to these issues.

3. Results

Eighteen patients (44% female) were diagnosed with MPS VI from 19 participating institutes in the Asia-Pacific region. Six patients (33%) were from Australia, five (27%) from Malaysia, two (11%) from Taiwan, two (11%) from Japan, and one (6%) from each of South Korea, Thailand, and India. Five patients (29%; three from Malaysia and two from Japan) were products of consanguineous marriages (Table 1).

The most common sign presented by the patients was bone deformities such as kyphoscoliosis found in 11 patients (65%). Nine patients (50%) presented with joint stiffness in early childhood but the two patients with a more slowly progressive type did not experience joint stiffness until adolescence or adulthood. Seven patients (41%) presented in infancy or early childhood with upper respiratory tract disorders, including recurrent sinusitis, otitis media, and hypertrophic tonsils. Two patients presented with obstructive sleep apnea (OSA) and one had a tonsillectomy long before diagnosis. Three infants (18%) were treated for inguinal hernia. Other common symptoms during infancy and toddler years in our cohort included progressive coarsening of the face (43%), dermal melanocytosis (36%), short stature (21%), cardiac murmurs due to mitral regurgitation (21%), and gross as well as fine motor delay (21%). Only two patients presented with the combination of inguinal hernia, skeletal abnormalities, hepatosplenomegaly, kyphoscoliosis, and macroglossia — manifestations that commonly lead to diagnosis. Overgrowth was a less common feature experienced by one patient in this cohort, who was in the 95th to 99th percentile from birth until six months of age for both height and weight, and the 75th percentile by her first year. Her growth rapidly decelerated, with the patient being <0.3rd percentile by the age of five years. Other presenting symptoms in this cohort included one patient each with left talipes equinovarus, hearing loss, and spinal cord compression.

Table 1
Demographics from the medical chart review of patients diagnosed with MPS VI in Asia-Pacific.

Demographic	
Diagnoses identified for study inclusion – n (%)	N = 18
Australia	6 (33%)
Malaysia	5 (27%)
Taiwan	2 (11%)
Japan	2 (11%)
South Korea	1 (6%)
Thailand	1 (6%)
India	1 (6%)
Gender – n (%)	
Male	10 (56%)
Female	8 (44%)
Ageduring diagnostic process – mean (median; min, max) months	
Symptom onset	33.8 (13.0; 0.0, 108.0)
Presentation	34.3 (24.0; 0.0, 108.0)
Diagnosis	67.1 (45.0; 12.0, 275.0)
Consanguinity – n (%)	
Consanguineous	5 (28%)

[a] Two outlying patients were not included in the analysis including one patient diagnosed at 23 years and one at 50 years of age.

Time from presentation to diagnosis of MPS VI varied significantly. Two outlying patients were diagnosed later in life and had more slowly progressing disease phenotypes. When removing these patients from the analysis, the mean age was 33.8 months at onset of symptoms and signs, 34.3 months at presentation, and 67.1 months at diagnosis, revealing a mean lapse of 33.3 months between onset and diagnosis. The minimum and maximum ages for both onset of symptoms and signs and presentation were from neonate to nine years of age, and for diagnosis 12 months and 23 years of age. One patient who presented in infancy with an inguinal hernia was not diagnosed until 23 years of age based on cardiac abnormalities (Case 3). Another patient presented at 45 years of age with cardiac abnormalities yet was not diagnosed until 50 years based on mild corneal clouding (Case 5) (Table 1).

Pre-diagnostic referral data was available for 10 of the 18 patients (56%). The most common pre-diagnostic referrals were to pediatricians (57%), geneticists (38%), and cardiologists (31%). The specialists most frequently diagnosing MPS VI were pediatricians (57%), geneticists (14%), ophthalmologists (7%), and cardiologists (7%).

A variety of reasons were reported as causes for the delay to diagnosis of MPS VI. These included the lack of or distance to diagnostic facilities for four patients (31%), alternative diagnoses that led to a delayed diagnosis of the underlying MPS disease for two patients (15%), and misleading symptoms experienced by two patients (15%). Several patients experienced symptoms and signs that are common for patients with MPS VI, but at presentation these were subtler than would be expected (Fig. 1). Some abnormalities of the hand (Fig. 1a) may not have been recognized as related to MPS VI had it not been for other symptoms such as joint stiffness and abnormalities in the hip (Fig. 1b). Subtle corneal clouding (Figs. 1c and d) was also overlooked.

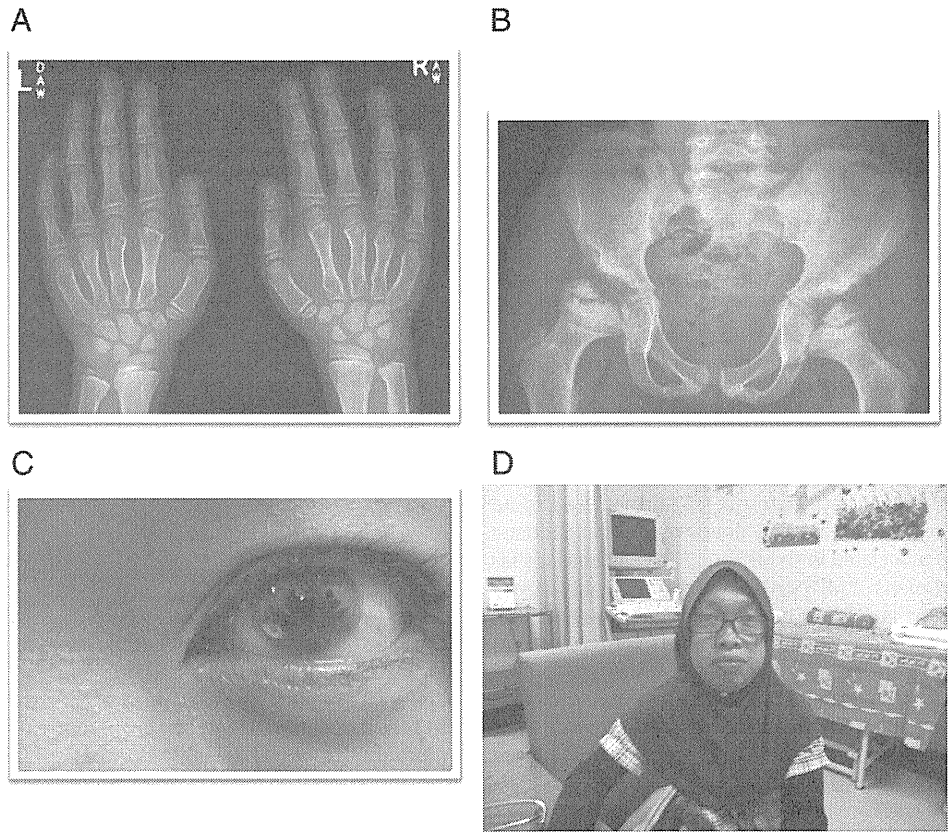

Fig. 1. Images of patients with confirmed MPS VI illustrating the range of abnormalities present in this cohort. Panel a shows a hand radiograph of a nine-year-old female patient (Case 8) with confirmed MPS VI, which revealed subtle skeletal abnormalities in the hand that may not have been recognized had it not been for the more obvious abnormalities of the pelvis with rounded iliac wings, tapered ilium distally, irregular and shallow acetabula and avascular necrosis of femoral heads (panel b). Panel c shows the mild corneal clouding with bluish hue from Case 3, which was not identified until the diagnosis of MPS VI. Panel d, also of Case 3, shows the milder coarsening of the facial features.

In our cohort, reported misdiagnoses include primary valvular heart disease in seven patients (39%). Of these, five had mitral valve involvement (alternative diagnoses were mitral valve prolapse in two patients, chronic rheumatic heart disease with mitral regurgitation in two patients, congenital dysplastic mitral valve in one patient) and two (11%) had aortic valve involvement (Cases 7 and 8). One patient had aortic valve replacement and mitral valve repair before the diagnosis (Case 8). Other delayed diagnoses involved orthopedic disorders in four patients (23.5%; alternative diagnoses were Perthes disease, congenital talipes equinovarus, and spondyloepiphyseal dysplasia [SED]), another subtype of MPS in one patient (6%), and an unknown inborn error of metabolism with hydrops fetalis in one patient (6%).

Eight cases provided unique referral scenarios (Cases 1 to 8), underscoring the importance of a multidisciplinary approach of diagnosis. Several included unique symptoms not currently associated with MPS VI, some of which were experienced by more than one patient. Prediagnostic referral patterns and misdiagnoses not previously associated with MPS VI were also identified and further explored within Cases 1 to 8.

3.1. Case 1: hydrops fetalis as the key presenting feature

One of fraternal twins born to non-consanguineous parents presented with generalized edema at 18 weeks of gestation, requiring an intrauterine drainage of a pleural effusion. Immediately after birth, the female newborn had a weak, uncoordinated suck and swallowing, which required nasogastric tube feeding. At one month of age, she was noted to have macrocephaly, hypotonia and peripheral edema. One month later, she had fluctuating hypoglycemia and hypothermia. Urine tests reported increased levels of glycine, lactate, and changes suggesting a deficiency in 3-methylcrotonyl-CoA carboxylase. However, urinary GAG (uGAG) levels were not evaluated due to an insufficient sample.

By three months of age, she was developing more slowly than her twin, had edema due to protein-losing enteropathy, and had a partial bowel malrotation. At five months of age, magnetic resonance imaging (MRI) of the brain revealed generalized reduction of cerebral white matter and upper cervical cord compression. The patient was also noted to have mild to moderate mitral valve regurgitation, and peripheral pulmonary artery branch narrowing. At seven months of age, a peripheral blood film showed vacuolated white blood cells. She was admitted to ICU for bronchiolitis at nine months of age and she was noted to have thoracolumbar kyphosis, hepatomegaly, and mild splenomegaly. She had progressive reduction in limb movement at 12 months of age, and repeated MRI showed a further reduction in the volume of cerebral white matter and severe upper cervical cord compression.

Despite all these complications and the numerous specialists who managed her since birth, a diagnosis of MPS VI was only made at 13 months of age, when her facial features began to become coarse. At this time, a repeat urine metabolic screen revealed increased urinary GAG of 32 (reference range [RR] 8–22) mg/mmol creatinine, increased DS while enzyme assay revealed no arylsulfatase B activity (RR 12–30 pmol/min/mg protein) in the white blood cells. The specialists who had been consulted included neonatologists, pediatricians, a cardiologist, a respiratory physician, an otolaryngologist, a geneticist, a pathologist, general surgeons, neurologists, neurosurgeons, radiologists, a gastroenterologist, emergency physicians, internists, and an orthopedic surgeon.

3.2. Case 2: cardiac abnormalities as the key presenting features

A male Malaysian patient was born at term with normal birth parameters. He had an unremarkable infancy other than a history of nasal congestion, macroglossia, and extensive dermal melanocytosis. At the age of

four years, he underwent a tonsillectomy for frequent upper respiratory tract infections (URTI) and enlarged tonsils associated with OSA. He attended school and participated in regular school activities such as games and athletics, despite the complaints of subtle clumsiness and joint stiffness. He needed assistance combing his hair due to limited joint range of motion and his writing was poor due to stiffness in his hands. At the age of five, a cardiac murmur was noted during a febrile URTI. An echocardiogram (ECHO) revealed thickened mitral valve with regurgitation, leading to the diagnosis and treatment for chronic rheumatic heart disease. It was not until the age of six years that the treating pediatric cardiologist noted coarse facies, hepatosplenomegaly, claw hands and joint stiffness, and then referred the patient to a geneticist for further testing. Radiographs revealed dysostosis multiplex. Urinary GAG analysis was elevated at 22.78 g/mol creatinine (unaffected <3 g/mol creatinine) with DS bands 1 and 2 in electrophoresis. Confirmatory enzyme analysis revealed absent arylsulfatase B activity.

By the time of the referral to geneticists for MPS VI assessment, pediatricians, a pediatric pulmonologist, a team of cardiologists, and an otolaryngologist had examined or treated the patient. Several radiologists had reviewed the patient's chest X-rays without realizing the presence of skeletal changes suggestive of MPS diseases.

3.3. Case 3: cardiac abnormalities as the key presenting features

A female Malaysian patient presented and was treated for bilateral inguinal hernia in infancy. At nine years of age, she was found to have a heart murmur and was diagnosed with mitral regurgitation, which led to the treatment for chronic rheumatic heart disease. She had short stature and during adolescence, she experienced joint stiffness and had poor vision attributed to myopia. She was of normal intelligence and did well at school.

A team of pediatricians and pediatric cardiologists provided care to the patient until she was 18 years old. The patient was assessed for Turner syndrome early in her management, revealing a normal karyotype. Growth hormone deficiency was also ruled out by blood testing. Despite the patient's height being well below the third percentile, short stature was dismissed as familial as midparental height was also below the third percentile. A MPS disease was not considered due to the patient having normal cognitive ability and only mild facial changes rather than the significant coarse facies typical of MPS [1] (Fig. 1d).

The patient was seen by the cardiologist managing Case 2 who was thus aware of the association between valvular cardiac lesion, joint stiffness, and MPS VI. The patient was subsequently diagnosed with MPS VI at 23 years of age with a mildly elevated uGAG level of 15.2 (RR < 9) g/mol creatinine band with elevation of DS in electrophoresis. Enzyme analysis confirmed reduced arylsulfatase B activity of 4.8 (RR 28–93) nmol/mg protein/h. At the time of diagnosis, she had developed multiple joint contractures, mild kyphoscoliosis, and corneal clouding (Fig. 1c). Cardiac assessments revealed thickened myxomatous mitral valve, moderate mitral regurgitation, and mild tricuspid regurgitation. Restrictive lung function and carpal tunnel syndrome were also noted at the time of diagnostic evaluation.

3.4. Case 4: fine motor delay as the key presenting feature

A female Malaysian patient of Chinese descent presented at approximately three and a half years of age, due to a reluctance to use her hands to perform age-appropriate daily activities. She was born full-term to non-consanguineous parents with no family history of metabolic disease. Physical examination revealed short stature (85 cm, below the third percentile), weight of 12.6 kg (10th to 25th percentile), coarse facies, short neck, extensive dermal melanocytosis, and grade one hypertrophic tonsils. Her liver was enlarged at 2 cm below the costal margin, without splenomegaly. Detailed developmental assessment revealed average speech development and a history of mild delay in walking until 20 months, which was similar to the development experienced by her siblings. She ran well and took stairs one step at a time with one foot per step. She had an admission at the age of one year and three months for bronchopneumonia but was otherwise well. She snored during sleep, but had no other signs of OSA. Radiological assessments revealed short and wide metacarpals and phalanges. No obvious spinal abnormalities were noted, except for mild scoliosis.

Urinary GAG assessment revealed elevated GAG levels with a marked increase in DS. Enzyme analysis confirmed a low level of arylsulfatase B at 0.01 (RR 92.3 ± 49.6) nmol/mg protein/h. Cardiac ECHO revealed moderate-to-severe mitral regurgitation. An otolaryngology evaluation identified right otitis media, and an ophthalmologist identified bilateral mild haziness of the corneas and a visual acuity of 6/18 on the right eye and 6/15 on the left, with a borderline intraocular pressure of 21 mm Hg. The patient was diagnosed with MPS VI at the age of three years and nine months, approximately three months after the first presentation.

3.5. Case 5: gross motor delay as the key presenting feature

A female Taiwanese patient presented at approximately 15 months of age due to delayed walking. At the time of presentation, her anthropometric measurements were within the lower limit of normal: 8.5 kg for weight (9th to 25th percentile), 73.5 cm for height (9th percentile), and she had a head circumference of 44.8 cm (25th to 50th percentile). She had recurrent febrile episodes and diarrhea up to five times per day from the age of five to six days. Poor appetite and rhinorrhea were noted from two days of age. Her developmental milestones were initially thought to be normal.

At 15 months old, the patient was referred to an orthopedic surgeon for kyphosis and delayed walking. Radiographs revealed dysostosis multiplex with anterior inferior beaking of the L1 and L2 vertebral bodies and kyphosis of the thoracolumbar spine, rounded iliac wings with lower iliac tapering, shallow acetabula, short and thick clavicles, oar shaped ribs and metacarpal tapering towards the wrists.

The patient was referred to a medical geneticist who confirmed the diagnosis of MPS VI at 16 months of age. Urinary GAG analysis revealed significant GAG elevation of 593.51 (RR 20.26–312.38) mg GAGs/g creatinine. Leukocyte and blood plasma enzyme activity levels were analyzed confirming low arylsulfatase B activity at 5.54 (RR > 121) nmol/mg protein/h. A MRI of the brain and upper cervical cord identified GAG deposits in the periodontoid region, which resulted in cervical spinal cord stenosis at the craniocervical junction, as well as bilateral arachnoid cysts in the middle cranial fossa, a small pineal cyst, and prominent CSF space in the bilateral optic nerve sheaths. Abnormal increased signal intensity of the left optic nerve on T2-weighted image was noted in the latest MRI (Figs. 2a and b), which had not been previously noted.

3.6. Case 6: congenital talipes equinovarus deformity as the key presenting feature

A male Malaysian child of Indian descent, the first born to consanguineous parents, presented with left talipes equinovarus at birth. By the age of one week, two orthopedic surgeons and physiotherapists had seen the patient and his left foot was fitted with a splint. He had frequent visits to a pediatrician before the age of one year for recurrent fevers and coughs. At 5 months, he was admitted to ICU for high fever with peripheral cyanosis. An unusual frog-like crawl was noted at nine months of age (Fig. 3a). At 15 months of age, his parents requested a pediatric cardiologist referral as a cousin in India, later diagnosed with MPS VI, had been diagnosed with a heart anomaly. Echocardiography showed an abnormal mitral valve with insignificant mitral regurgitation resulting in a diagnosis of congenital dysplastic mitral valve.

At 18 months, the child underwent surgery to correct an inguinal hernia. At 2 years and 7 months of age, another pediatrician managing the child's frequent episodes of bronchitis noted short stature,

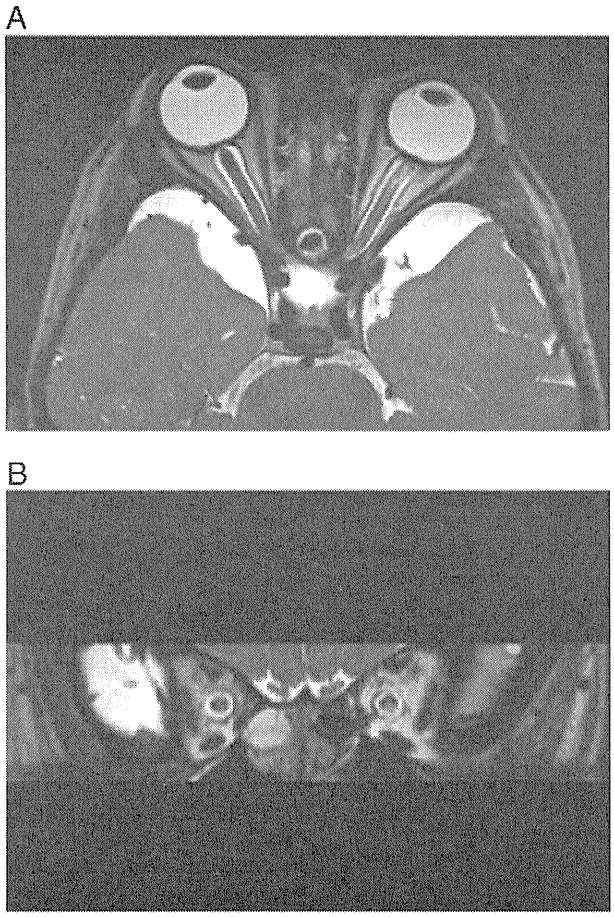

Fig. 2. a and b. (Case 5) MRI: T2-weighted axial and coronal MRI with single-slab 3D-TSE acquisition. Patchy increased signal intensity in the left optic nerve and dilated CSF spaces along the bilateral perineural sheaths. Note bilateral arachnoid cysts in the middle cranial fossa.

macrocephaly, thickened wrists, joint stiffness, and lumbar kyphosis, leading to clinical suspicion of a MPS disorder.

The patient was referred to an ophthalmologist who confirmed bilateral corneal opacity, and eventually to a geneticist in a tertiary care center 200 km away for diagnostic confirmation. There was radiographic evidence of lumbar kyphosis, gibbus, and anterior central beaking of the L1 and L2, a J-shaped sella turcica, and oar-shaped ribs, which were overlooked in a previous chest X-ray for pneumonia.

Urine GAG analysis revealed a high normal uGAG level of 19.9 (RR < 20) mg/mmol creatinine with significant bands of DS 1 and 2 at the age of two years and 10 months (Fig. 3b). Enzyme analysis confirmed the diagnosis of MPS VI with a very low level of 0.1 (RR 1.5–21.3, affected <0.3) pmol/min/mg protein of arylsulfatase B. At 5 years and 3 months of age (Fig. 3c), the patient was referred for evaluation for enzyme replacement therapy.

3.7. Case 7: ophthalmic manifestations as the key presenting feature

During the examination of a 50-year-old female patient referred for possible glaucoma, the ophthalmologist found corneal clouding, previously noted by the patient. Her visual acuity was 6/6 bilaterally and she had high hypermetropia. Intraocular pressure was normal. The patient had a history of aortic valve replacement and mitral valve surgery when she was 45 years old. She has normal intelligence and still participates in sports.

The ophthalmologist ordered uGAG analysis that revealed elevated GAG quantitation of 4 (RR < 3) mg/mmol creatinine. The MPS electrophoresis showed increased DS bands 1 and 2 and also mildly increased heparan sulfate, the latter of which can be normal at this age. Enzyme analysis confirmed the diagnosis of MPS VI with deficiency of arylsulfatase B of <0.1 (RR 0.8–2.4) U/g protein. Since diagnosis, the patient declined offers of appointments, primarily due to travel to the clinic involving approximately a two-and-half-hour flight.

3.8. Case 8: limited joint range of motion as key presenting feature

A nine-year-old Caucasian girl born to non-consanguineous parents presented with reduced range of shoulder movement that interfered with her swimming ability, and moderate lumbar scoliosis. She had a normal appearance and stature and was of normal intelligence. Her pediatrician, who was familiar with MPS diseases, ordered a uGAG electrophoresis which revealed GAG levels of 20 (RR 4–12) g/mol creatinine, with increased DS, and reduced arylsulfatase B activity of <0.1 (RR 0.8–2.4) u/g protein.

Following diagnosis, further investigation revealed sleep obstruction (snoring) without apnea and mild aortic valve thickening with grade 1-2/4 incompetence and mild mitral valve leaflet prolapse with no incompetence. Eye examination showed mild to moderate bilateral corneal clouding. Hand radiographs (Fig. 1a) revealed subtle skeletal abnormalities in the hand that may not have been recognized had it not been for the more obvious abnormalities of the pelvis, including rounded iliac wings, tapering ilium distally, irregular and shallow acetabula with avascular necrosis of femoral heads (Fig. 1b).

4. Discussion

Our cohort of patients with MPS VI presented with a wide variety of complaints at different ages and to different specialties. The diagnostic journey experienced by patients with MPS VI varied significantly. Although the average duration from the first presentation to final diagnosis in our cohort was less than three years, the range was from three months to 23 years from the initial presentation to diagnosis. The significant delays in diagnosis were often due to the variation in the phenotypic expression of the disease, severity, laboratory limitations, and lack of physician awareness of the complex spectrum of disease. While a clinician may be aware of the "classic" symptoms of MPS VI, a clinician may not recognize that patients with more slowly progressing disease may experience the common symptoms, but less severely, later in life, or not at all. Clinical diagnosis was still often suspected by the primary care doctor and confirmed with testing, with only 38% of patients referred to a geneticist, and only 14% of these referrals resulting in diagnosis. Furthermore, these patients often experience atypical symptoms not associated with MPS disease. All of these may contribute to the delayed diagnosis of MPS VI [4,9].

Radiographic evidence of MPS VI is classically a key component to the diagnostic process. While skeletal abnormalities associated with rapidly progressing MPS VI are easily identified in radiographs, images of more slowly progressing patients who do not display classic symptoms may actually detract from the clinical suspicion of MPS VI, directing the radiologist and orthopedist to more common but similar orthopedic diseases [8,10]. For several of our patients, features that are not currently recognized to be associated with MPS VI confounded clinicians, delaying the diagnosis. These included cases of developmental delay involving mainly the motor milestones, and the constellation of unrelated features.

Case 1, who presented with hydrops fetalis, provides significant insight into how unrelated symptoms can impede rapid and accurate diagnosis, even for a patient experiencing a more rapidly progressing form of MPS VI. Prior to diagnosis with MPS VI, the patient experienced symptoms commonly associated with MPS VI, yet due to the lack of clinical suspicion of MPS and unrelated results of the urine analysis, the clinicians did not pursue uGAG assessment when the initial urine sample was deemed insufficient. The diagnostic process was further complicated by several clinical features, such as narrowing of the peripheral pulmonary

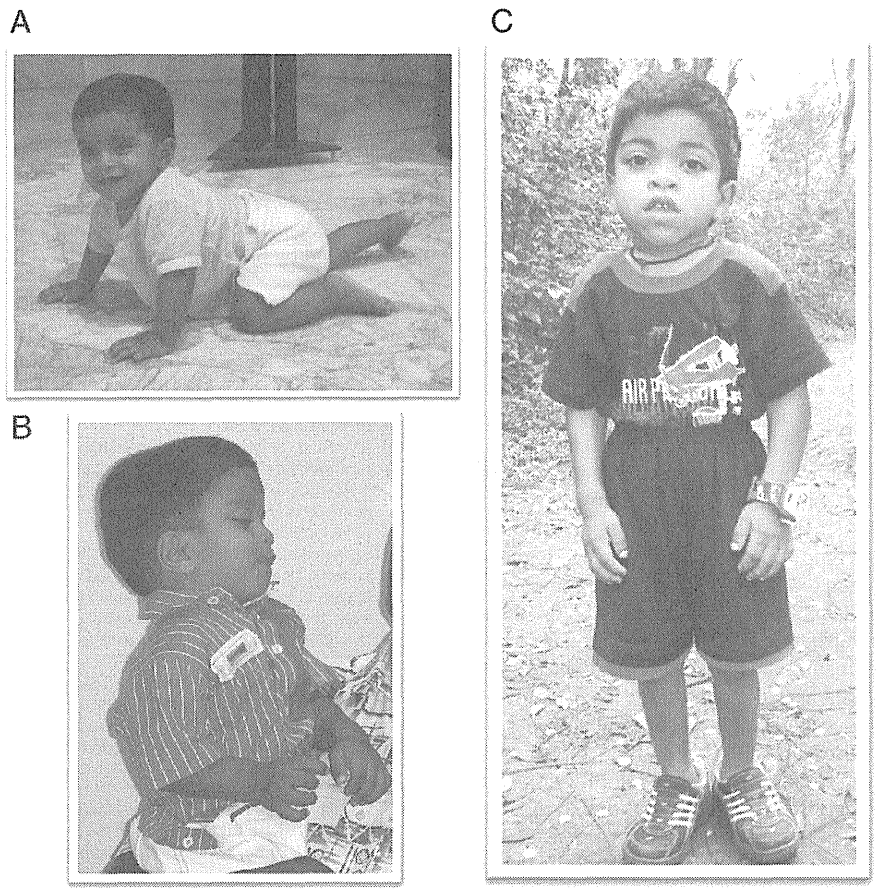

Fig. 3. A male Malaysian child at nine months (panel a), upon diagnosis at 2 years and 10 months of age and (panel b) at age 5 years and 3 months.

artery, protein-losing enteropathy and partial bowel malrotation which are not associated with MPS. Therefore, despite the patient presenting prenatally, experiencing several symptoms commonly associated with rapidly progressing MPS VI, and being assessed by specialists from fifteen different specialties, the patient was not diagnosed with MPS VI until she was 13 months of age.

Other patients in our cohort also experienced several symptoms not commonly associated with MPS VI, such as the 36% of patients who experienced extensive dermal melanocytosis. The increased signal intensity experienced by Case 5 may be due to GAG deposition, although other possibilities such as optic neuritis or other infiltrating lesions cannot be ruled out. Other symptoms were in direct contrast to symptoms reported in the literature, such as the overgrowth experienced by one patient (5%), which is unlike the short stature commonly experienced by many patients with MPS VI [4]. While these unusual or more subtle symptoms may not need to be included as part of a differential diagnosis for MPS VI, individual symptoms and that may be in contrast with the differential diagnosis of MPS VI should not necessarily exclude it as a potential diagnosis.

While it may be expected that unusual symptoms or those symptoms that conflict with what is reported in the literature would cause a delayed diagnosis, several patients in our cohort who have slowly progressing disease were treated for manifestations that are commonly associated with MPS VI — and yet their diagnoses were still delayed. This was especially notable by the significant number of patients in our cohort who were seen by cardiologists for valvular heart disease without identifying the underlying MPS disease, similar to previous reports [11–14]. Recently, a cardiac variant of MPS VI was described in 10 patients with homozygous p.R152W mutation in the ARSB gene where progressive valvular heart disease occurred with subtle manifestation of other typical features of MPS VI, delaying the diagnosis up to 23 years from symptom onset [15]. Case 7 had a similar experience, having undergone aortic valve replacement and mitral valve surgery, but not diagnosed until 50 years of age when she was evaluated for visual problems. Case 3 was diagnosed at 23 years of age. Her mildly coarse facies and short stature were not significant enough to immediately identify her as having MPS VI, despite being treated for manifestations commonly associated with MPS VI, yet the diagnosis of MPS VI did not occur until the cardiologist who was treating another patient with MPS VI recognized the cardiac manifestations commonly associated with the disease.

As is commonly reported with MPS VI, the patients in our cohort saw a wide variety of specialists [4] who successfully treated the manifestations of MPS VI related to their specialty, without identifying the underlying MPS disease. In our cohort, 21% of patients experienced ophthalmic manifestations of the disease, yet only one ophthalmologist suspected MPS VI. The lack of awareness of the association of MPS disease with ophthalmic manifestations, the more subtle changes experienced by our patients (Fig. 1d), and not having a complete medical history may all contribute to this delay in diagnosis. Had the ophthalmologist from Case 7 not taken a thorough medical history thereby discovering the cardiac manifestations, the late diagnosis of MPS VI in the 50-year-old patient may have continued to go unrecognized. The advantage of the initial specialist having knowledge of MPS is evident with Case 8, whose pediatrician recognized the symptoms of limited range of motion and lumbar scoliosis as potentially indicating a MPS disease. It also shows that additional features, which support the diagnosis, such as the cardiac and eye changes, may not be evident clinically until the appropriate investigations are performed.

Delays in diagnosis, even when clinical suspicion is raised, can also be due to logistical and cultural barriers. For some patients, visiting the necessary clinics can be hampered by the lack of transportation or economic

barriers. In our cohort, the doctors of five patients (36%) cited the lack of or distance to appropriate diagnostic facilities or genetics clinics as the reason for the delay. There is a cultural tendency in some Asia-Pacific countries to deny the potential for disease. In several countries in the Asia-Pacific region, laboratories and genetics clinics are available only in the large city centers, or sometimes not at all, and this presents a significant obstacle. In Malaysia, for example, the laboratory service for uGAG analysis is only available in the capital city of Kuala Lumpur and the service may not reach out to cases from East Malaysia, due to the distance requiring air transportation. Enzyme analysis was not available until recently in Malaysia. Therefore, in the past, laboratory samples were sent to Australia for analysis. Preparing these samples for transport can pose challenges, especially when international shipping is required.

Given the potential for false-negative uGAG assessments, which are stated to affect approximately 15% of MPS patients [16,17], analysis of enzyme activity is the gold standard for diagnosis when clinical suspicion exists. Although significant levels of DS are excreted in the rapidly progressing forms of MPS VI, limited amounts of DS can be present in the urine from patients experiencing a more slowly progressing form of the disease [9], potentially compromising quantitative uGAG analysis due to limited, if any, increase in total uGAGs. Given these complexities, relying on less-experienced laboratories for diagnosis holds its own risks and may result in even further delays in diagnosis. Equally important is the knowledge of the treating clinician to recognize the potential for false-negative uGAG analysis, as with Case 6. If the lab had only performed quantitative uGAG, as is the practice of some laboratories, this patient could have been missed and the diagnosis further delayed. When there is a high degree of clinical suspicion of MPS, it is important that a urine MPS electrophoresis is performed in addition to the uGAG quantitation and, even if both of these screening tests are normal, the patient should have enzyme analysis, which in Case 6, confirmed the diagnosis of MPS VI by revealing a very low level of enzyme activity despite a near normal uGAG level.

Overcoming these barriers is critical for the efficient diagnosis of MPS VI. Targeted population screening programs may make the diagnosis more efficient for patients. In spite of increasing interest in this group of disorders due to therapies becoming available, many doctors remain unaware of these rare diseases, and may not become interested unless directly involved in the management of a patient, as was the case for several authors of this paper. Educating general practitioners, pediatricians, cardiologists, rheumatologists, radiologists, and other specialists encountering patients with MPS, increasing awareness of the symptoms of slowly progressing phenotypes, and developing better biochemical screening tests may all contribute to earlier diagnosis for all patients with MPS VI, and not just those patients with the "classic" symptoms of the disease.

5. Conclusions

Our cohort reveals that patients with MPS VI in the Asia-Pacific region present with a variety of symptoms at variable ages, which may not conform to the more classical form of the disease. Educational and outreach programs for clinicians and specialists who commonly encounter these patients may enhance the diagnostic workup of these patients. Clinicians should potentially consider MPS VI in the differential diagnosis of the subtle or atypical features listed above, which may be the only symptoms that manifest in patients with more slowly progressing MPS VI disease.

Conflicts of interest

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Letter to the Editor

Effects of enzyme replacement therapy on immune function in ADA deficiency patient

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An accumulation of toxic metabolites of purine nucleotide due to inherited deficiency of adenosine deaminase (ADA) can cause metabolic disorders and severe combined immunodeficiency. Stem cell transplantation from human leukocyte antigen (HLA)-identical sibling donors is usually the first-line treatment option. For patients without suitable donors, polyethylene glycol-conjugated bovine ADA (PEG-ADA) was developed as a form of enzyme replacement therapy (ERT) for improvement of clinical symptoms [1,2]. In addition, stem cell gene therapy (SCGT) is recognized as a curative therapeutic option [3,4]. However, the success of SCGT depends on various factors such as the use of cytotoxic drugs or the level of residual thymic activity at the initiation of gene therapy [5].

The patient was a 14-year-old Japanese female with ADA deficiency. Due to unavailability of suitable bone marrow donors, she received SCGT at four years old at the Hokkaido University School of Medicine in Sapporo, Japan [6]. Normal life resumed without severe infection while receiving periodical intravenous immunoglobulin (IVIG) and prophylactic drugs. Four years later, however, she began to show gastrointestinal distress and failure to thrive due to severe appetite loss, likely caused by incomplete recovery of cellular and humoral immunity in SCGT without any conditioning chemotherapy. At 11 years of age, she developed pneumonia. ERT using PEG-ADA (375 U, 20 U/kg, once a week) was administered. Here, we described the improvement of her clinical condition and recovery process after ERT. All studies have been performed with the parents' informed consent and the patient's assent according to an IRB approved protocol.

Levels of total adenosine (AXP) and deoxyadenosine nucleotides (dAXP) in erythrocytes were measured as described previously [7]. Levels of T-cell receptor excision circle (TREC) were measured by quantitative PCR as previously described [8]. Peripheral blood lymphocyte subsets were determined with FACSAria III (Becton Dickinson, CA) using anti-human CD3, CD4, CD5, CD8, CD19, CD27, CD38, CD45RA, IgM, IgD and BAFF-R monoclonal antibodies (BioLegend, CA). Peripheral blood mononuclear cells (PBMCs) were stained with a set of 23 antibodies for TCRvβ (Beckman Coulter, CA) in TCRvβ spectratyping analyses. FlowJo software was used for all data analyses.

Serum ADA enzyme activity increased and reached a plateau of 400 U/L from 300 U/L after the fourth injection of PEG-ADA (reference range 5.0–20.0 U/L); dAXP in erythrocytes declined rapidly and was undetected after the second injection. Peripheral lymphocyte count increased and remained stable at around 250 /mm^3 after two months of ERT. $CD3^+CD4^+$ T cells exceeded $CD3^+CD8^+$ T cells in number after four months of ERT. ERT relieved her symptoms of diarrhea, appetite loss, failure to thrive and pneumonia; weight gain of 3.5 kg occurred over three months of ERT.

Notably, after 16 months of ERT, there was a delay in the increase of $CD4^+CD45RA^+$ naïve T cells, reaching a plateau of around 8/mm^3 (Fig. 1a). TCRvβ spectratyping analyses were performed at 24 and 36 months of ERT to assess the time course of T cell clonality in the patient's peripheral blood (Fig. 1b). While a restricted usage of patient's TCRvβ repertoire with a dominant oligoclonal expansion of the TCRvβ2 $CD8^+$ T cell population was recorded at the 24th month, it appeared to be relieved at the 36th month (demonstrated by an increase of other T cell populations). The value of TREC, which was undetected before ERT, increased to 4.3×10 copies/μg DNA at 36 months of ERT (reference range $8.2 \pm 6.3 \times 10^3$copies/μg DNA in 13–18 years old [8]).

$CD19^+$ B cells increased approximately 10- to 20-fold in response to PEG-ADA until they reached a plateau at 8–12 months of ERT (Fig. 1c). Although $CD19^+CD27^+$ memory B cells also increased in response to PEG-ADA, they were stabilized at low values eventually (Fig. 1c) and the increased B cells were mostly naïve $CD19^+CD20^+$ B cells with expressions of IgM, IgD, CD10 and/or CD5, and $CD38^{high}$ (Fig. 1d). Interestingly, they did not express a receptor for a B cell-activating factor, BAFF-R (Fig. 1e), that should be expressed on peripheral B cells. No adverse effects of ERT were recorded during the observation period.

As ADA deficiency is classified as an inherited metabolic disorder, accumulated toxic nucleic-acid metabolites can damage various organs including the liver and neurons. When the population of the genetically-corrected cells circulating in peripheral blood is very small, as it was in our case, detoxification is particularly difficult. Hence, we propose ERT as a therapeutic option for patients who received SCGT but still experienced recurrent infection, gastrointestinal distress and failure to thrive.

Although ERT resulted in a positive outcome in our case, it has its limitation in terms of immunological recovery. $CD3^+CD4^+CD45RA^+$ naïve T cells were not accumulated until 16 months of ERT. A considerable period of time is likely to be required for re-cultivation of the thymus gland for thymopoiesis and generation of naïve T cells [9,10], although it remains undetermined if naïve T cells are derived from gene-corrected bone marrow cells. Consistent with previous reports [11,12], $CD19^+CD27^+$ memory B cells barely increased during ERT, but there was an increase in naïve B cells, particularly $CD19^+CD38^{hi}IgM^{hi}$ transitional B cells. The most possible explanation for this is a deficient in the number or function of mature $CD4^+$ T cells that would otherwise have been able to transduce signals for B cell maturation and induce class-switch recombination of immunoglobulin genes. Peripheral lymphoid tissues where maturation of B cells takes place by interacting with supporting cells such as follicular dendritic cells, may also be severely damaged in ADA-deficient patients; while the bone marrow is less damaged by the scavenger activity of

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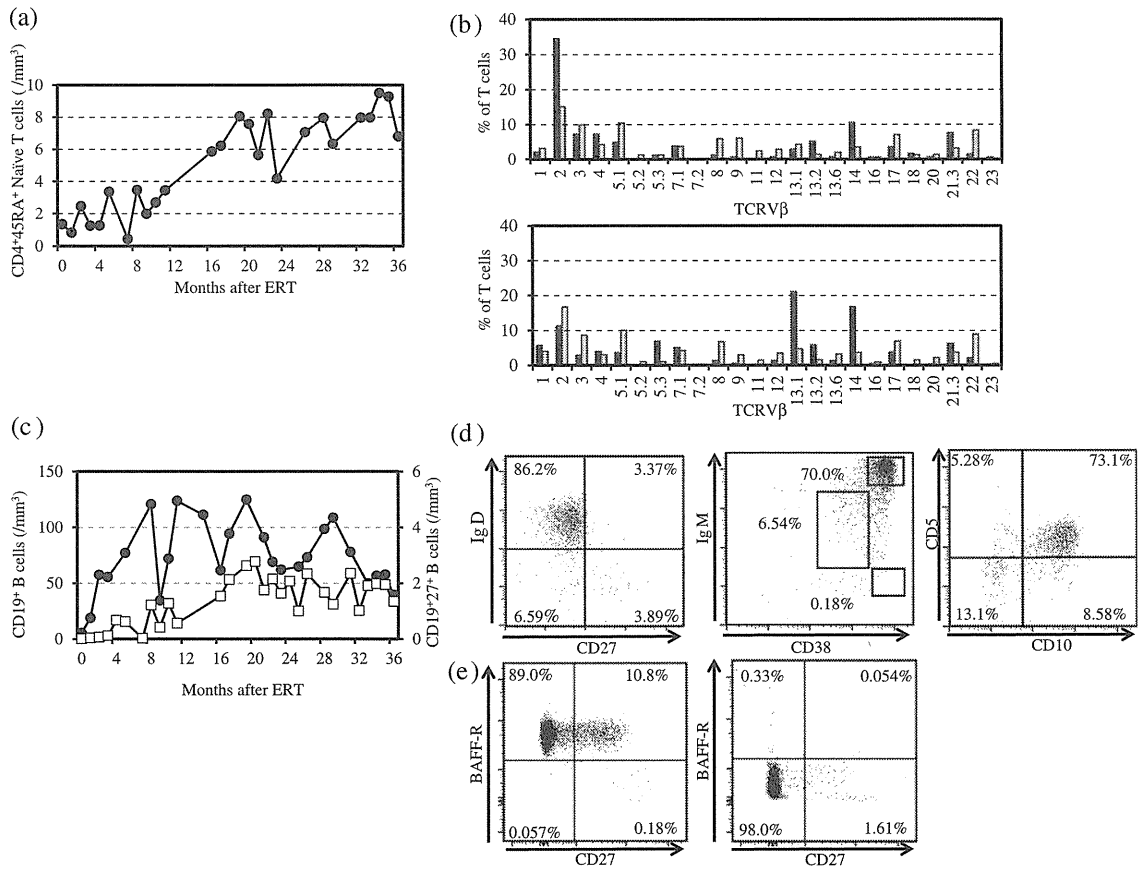

Fig. 1. Time course analyses of the patient's T and B cells after ERT. Peripheral lymphocytes of the patient were stained with antibodies against CD4 and CD45RA and the number of $CD4^+CD45RA^+$ naïve T cells was plotted on charts at the indicated time (a). They were also stained with antibodies against TCRvβ and analyzed at approximately 24 months (upper panel), and 36 months (lower panel) after initiation of ERT (b). Gray and black bars indicate the percentages of $CD4^+$ and $CD8^+$ T cells, respectively. For characterization of the patient's B cells, they were stained with antibodies against B cell-related antigens. The number of each population such as $CD19^+$ and $CD19^+CD27^+$ B cells (closed circles and open squares, respectively) were plotted on charts at the indicated time (c). They were analyzed by FACS at 36 months of ERT in terms of IgD^+CD27^-, IgD^+CD27^+, and IgD^-CD27^+ cells present naïve, marginal, and immunoglobulin class-switched B cells, respectively (left panel), $IgM^{hi}CD38^{hi}$, $CD38^+IgM^+$, and $CD38^{hi}IgM^-$B cells present transitional, mature, and plasmablastic B cells, respectively (middle panel), and $CD10^+CD5^+$ cells present immature B cells (right panel) (d). No expression of BAFF-R was observed on the patient's B cell surface in comparison to those of an age-matched healthy control (e).

macrophages [13]. Furthermore, maturation of B cells *per se* is hampered by the accumulated dAXP as demonstrated by the absence of BAFF-R that is essential for peripheral B cell homeostasis and peripheral maturation [14].

Our results suggest that ERT with PEG-ADA improves clinical symptoms of ADA-deficient patients whose immune system is not fully recovered by SCGT.

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CONCISE COMMUNICATION

Pathological characterization of pachydermia in pachydermoperiostosis

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ABSTRACT

Pachydermoperiostosis is a rare hereditary disease, which presents with the cutaneous manifestations of pachydermia and cutis verticis gyrata. Histological findings in pachydermia frequently include dermal edema, mucin deposition, elastic fiber degeneration, dermal fibrosis and adnexal hyperplasia. However, the severity of these findings varies between clinical reports, and a systematic multiple-case clinicopathological correlative analysis has not been performed to date. In the present study, we reviewed the skin biopsy specimens obtained from the pachydermia of six pachydermoperiostosis patients. The severity of the characteristic histological features was semiquantitatively evaluated and correlated with the grade of pachydermia. Dermal edema, mucin deposition and elastic fiber degeneration were observed in all cases. Patients with severe pachydermia had sebaceous gland hyperplasia and fibrosis. These results suggest that the triad of mucin deposition, dermal edema and elastic fiber degeneration are found from very early stage pachydermia, and could be considered diagnostic findings. To ensure an earlier diagnosis of pachydermoperiostosis, a biopsy should be taken when a patient has grade 1 pachydermia to determine the presence of this histological triad.

Key words: dermal edema, elastic fiber degeneration, fibrosis, mucin deposition, pachydermoperiostosis, sebaceous hyperplasia.

INTRODUCTION

Pachydermoperiostosis (PDP, Online Mendelian Inheritance in Man no. 614441) is a rare hereditary disease diagnosed by the presence of digital clubbing, periostosis and pachydermia, including cutis verticis gyrata (CVG).[1] Recent genetic analysis revealed that homozygous or compound heterozygous mutations in the solute carrier organic anion transporter family member 2A1 (*SLCO2A1*) gene, which is associated with prostaglandin (PG) metabolism, are significantly associated with PDP.[2,3] Clinically, three distinct forms of this syndrome have been proposed in accordance with the intensity of symptoms: the complete form, characterized by prominent furrowing of the face, CVG, digital clubbing, and primary hypertrophic osteoarthropathy; the incomplete form, in which CVG is absent; and the fruste form, characterized by one or more main skin changes and minimal skeletal involvement.[1]

Several studies have reported the following histopathological findings of pachydermia: sebaceous gland hyperplasia, dermal edema, mucin deposition in the dermis, elastic fiber loss and dermal fibrosis.[4–6] However, the severity of these findings varies among clinical reports, and a multiple-case clinicopathological correlative analysis of pachydermia has not been performed to date. To gain insight into the pathogenesis of pachydermia and CVG development, we histologically examined skin biopsy specimens of six PDP patients with known clinical information. We evaluated the degree of each of the histological findings semiquantitatively, and correlated these data with the severity of pachydermia.

PATIENTS AND TECHNIQUES

This study was approved by the ethics committee of the National Center for Child Health and Development and Keio University School of Medicine, and conformed to the provisions of

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