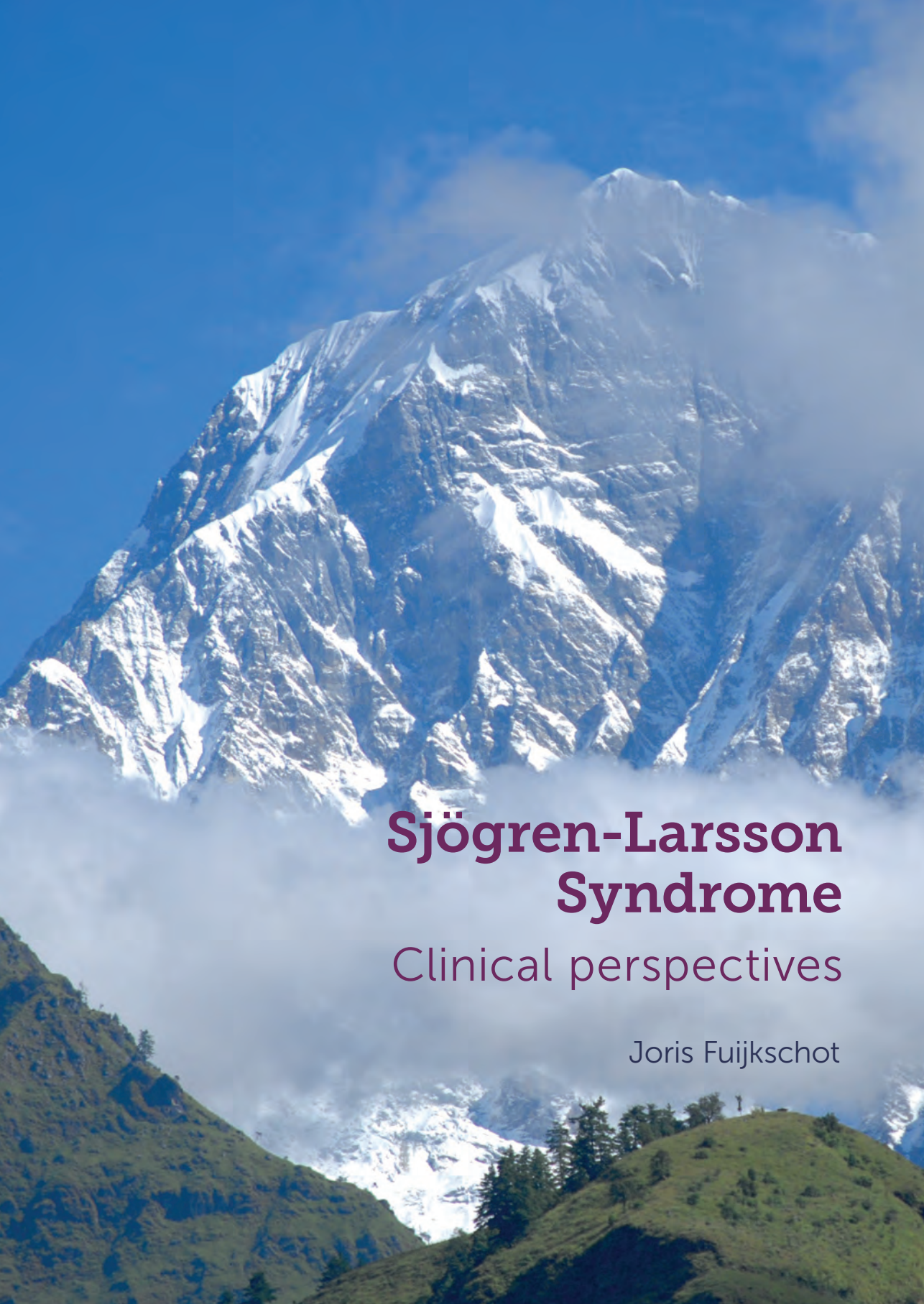**Radboud Repository**

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Sjögren-Larsson Syndrome

Clinical perspectives

Joris Fuijkschot

Colofon

stichting Spoedeisende Hulp
bij Kinderen

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Mt Annapurna I (8091m), Nepal (foto: Joris Fuijkschot)

Design and lay-out
Promotie In Zicht, Arnhem

Sjögren-Larsson Syndrome
Clinical perspectives

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. dr. Th.L.M. Engelen,
volgens besluit van het college van decanen
in het openbaar te verdedigen op vrijdag 17 april 2015
om 10.30 uur precies

door

Joris Fuijkschot
geboren op 10 november 1977
te Eindhoven

Voor mijn ouders

Contents

1

**General introduction
& outline of the thesis**

The Sjögren-Larsson syndrome (SLS) is hallmarked by a triad of clinical symptoms, namely ichthyosis, spasticity and mild-to-moderate intellectual disability. Though convincing clinical descriptions of Sjögren-Larsson patients can be found in literature dating back to 1935,[1] it was the Swedish psychiatrist and geneticist Torsten Sjögren (1896 - 1974) who in 1956 defined the combination of these symptoms as a new clinical entity.[2] Together with Tage Larsson (1905 - 1998) he made a report in 1957 upon a cohort of 28 patients with these cardinal symptoms and unraveled its autosomal recessive mode of inheritance.[3] The original Swedish study cohort was later expanded to a total of 58 patients originating from 41 families and again used to increase awareness and understanding of this rare neurocutaneous disorder.[4] Although SLS is still considered a rare disease, the prevalence reported here (8.3 per 100,000) was relatively high due to significant consanguinity within the study population.

Additional reports from partially different and genetically unrelated patients improved insights into the disease, creating a clinical picture with variable predominance, especially towards the degree of spasticity and ichthyosis.[5,6] Furthermore, additional clinical features were described such as premature birth, a peculiar crystalline maculopathy and impaired speech–language performance.[7,8]

In the majority of the patients, the ichthyosis is congenital or develops very early in life. It has a generalized distribution pattern and the central face is often spared. The striking pruritic character of the ichthyosis in SLS differentiates it from other cornification disorders.

Neurological symptoms gradually appear in the first 2 years of life and are often described as a spastic diplegia or tetraplegia with subsequent contractures and wheelchair dependency. Also, a delayed and disturbed acquisition of speech and language skills becomes apparent along with subsequent limitations in communication. Intellectual disability is described as mild to moderate.

In early childhood, a peculiar crystalline macular dystrophy develops in patients with SLS characterized by glistening macular dots. Clinically, this maculopathy is accompanied by decreased visual acuity and by photophobia.[7]

Biochemical studies

In 1988, Rizzo et al. discovered the primary biochemical defect in SLS.[9] Cultured skin fibroblasts from Swedish SLS patients showed a defective oxidation of the fatty alcohol *hexadecanol* into fatty acid indicating a defect in fatty alcohol metabolism. The deficiency of the microsomal fatty aldehyde dehydrogenase (FALDH) proved to be the underlying biochemical defect in SLS. The subsequent accumulation of both

fatty aldehydes and alcohols in tissues was considered the principal causative mechanism leading to the overall clinical phenotype of SLS.[10]

In the following years, Rizzo et al. unraveled much from what is now known as human fatty alcohol and aldehyde metabolism, by using some of the pathophysiological concepts derived from SLS.[11-13]

To gain an insight on the complexity of the biochemical pathogenesis in SLS and find new approaches for therapy, an understanding of FALDH and the role that in plays in SLS is needed.

Fatty alcohol and aldehyde metabolism

fatty (or long-chain) alcohols are formed in nature usually as waxes (e.g. beeswax or plant waxes) which are esters from fatty acids and fatty alcohols. Vegetables and fruits are the major dietary sources of fatty alcohols. However, the human intestine eliminates most fatty alcohols after ingestion by direct oxidation into fatty acids.[13]

In the human body cells synthesize fatty alcohols as well, mainly to act as substrate for the production of wax esters and ether glycerolipids. In the skin, for instance, wax esters are used for lubrification and protection. Ether glycerolipids, a subgroup of phospholipids, are mainly found in specific tissues such as tissues of the skin, heart, brain and myelin.[12] In total, fatty alcohols and aldehydes only comprise a very small portion of the total lipid composition of human tissues.

Fatty alcohols are synthesized from fatty acids through the formation of acyl-CoA which in turn is used by the enzyme fatty acyl-CoA reductase (FAR) to produce the fatty alcohols. The excess of fatty alcohols produced is recycled back into fatty acids to prevent accumulation. This step completes the process referred to as the fatty alcohol cycle. This reaction is catalyzed by the fatty alcohol: nicotinamide adenine dinucleotide oxidoreductase (FAO). FAO is a multi-enzyme complex consisting of the two consecutive enzymes fatty alcohol dehydrogenase (FADH) and fatty aldehyde dehydrogenase (FALDH). FADH converts fatty alcohols into aldehydes which in turn are oxidized to acids by FALDH. Bodily cells have different capacities to synthesize fatty alcohols mainly depending upon tissue type and its need for wax esters or ether glycerolipids. This capacity is mainly regulated by the coordinating enzymes FAR and FAO. For instance, in keratinocytes, where the productive capacity of wax esters is high, high activities of FAR and low activities of FAO are seen. On the contrary, liver cells display low activities of FAR and high activities of FAO.
The complete fatty alcohol cycle is given in Figure 1.

In addition to its role in the breakdown of fatty aldehydes from wax ester and ether glycerolipid metabolism described previously, FALDH is also involved in the breakdown of fatty aldehydes from the following lipid pathways, as indicated by research and depicted in Figure 2.
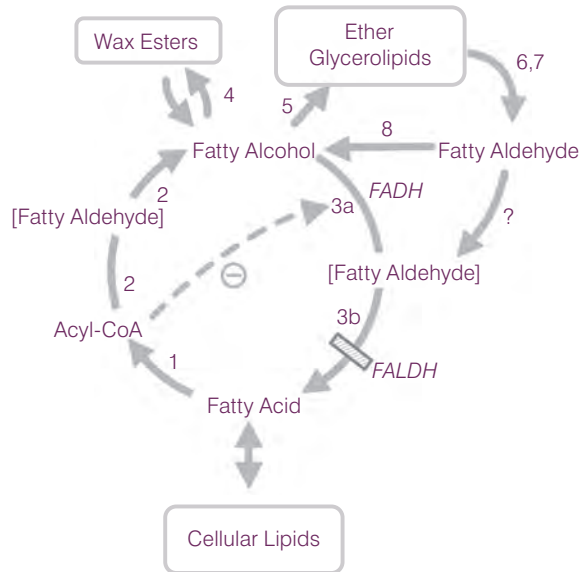
Figure 1 The fatty alcohol cycle. From Rizzo et al.[13]

The cycle consists of the following enzymes:
1: acyl-CoA synthetase
2: fatty acyl-CoA reductase (FAR)
3: FAO complex consisting of 3a: fatty alcohol dehydrogenase (FADH)
  and 3b: fatty aldehyde dehydrogenase (FALDH)
4: wax synthase
5: alkyl-DHAP-synthetase
6: alkylglycerol monooxygenase
7: lysoplasmalogenase
8: fatty aldehyde reductase

1. Branched chain alcohols

Phytol, a dietary derived fatty alcohol, is oxidized into the fatty aldehyde phytal which subsequently is oxidized by FALDH into phytanic acid. Phytanic acid is catabolized into the aldehyde pristanal which again is oxidated into pristanic acid by FALDH.[14]

Furthermore, farnesol and geranyl-geraniol are both fatty alcohols which function as intermediates in the synthesis of cholesterol and glycoproteins from mevalonic acid. Both alcohols are depended upon FALDH for its breakdown by oxidation.[14]

2. Sphingosine-1-P

Ceramides are lipid molecules made of sphingosine and fatty acids. The lipids found in the stratum corneum that lubricate the skin are comprised of these ceramides and play part in the formation of the water barrier that protects the skin from water leaking.

Intracellular catabolism of ceramides generates sphingosine-1-P. Its subsequent degeneration produces hexadecenal which needs FALDH for its breakdown into fatty acids.[15]

3. Fatty acid omega (ω) oxidation

Omega oxidation is an alternative pathway to mitochondrial beta oxidation in the process of fatty acid metabolism which takes place in the endoplasmatic reticulum. During the process the acid is oxidized into an alcohol which subsequently is catalyzed by an alcohol dehydrogenase and by an aldehyde dehydrogenase to generate the end product- a dicarboxylic acid.

Again, FALDH has shown to play a role in the ω-oxidation of specific fatty acids. This role is eminent in the breakdown of the pro-inflammatory mediator leukotriene B4 (LTB4). FALDH is needed for the inactivation of LTB4 by the oxidation of 20-CHO-LTB4 into 20-COOH-LTB4. Deficiency of FALDH in SLS results in the accumulation of LTB4 and its metabolites as was demonstrated earlier by our study group.[16,17]

Faldh deficiency in SLS

In SLS, the deficient activity of FALDH results in a defective functioning of FAO and consequently culminating in the accumulation of both fatty aldehydes and alcohols in tissues. As demonstrated here, FALDH is involved in several lipid pathways that are also more prevalent in the central nervous system and the skin which correspond with the main organ systems affected in SLS. In the last few decades many different studies have reported on the accumulation of specific metabolites in SLS and have deducted possible clinical consequences. However, the precise biochemical mechanisms responsible for all or some of the clinical features in SLS remain largely to be elucidated. In general, it is now believed that clinical symptoms evolve from one of the following two factors: (1) an accumulation of (toxic) lipid metabolites which cannot be metabolized by FALDH (or FAO), their diversion into other metabolic products or a combination of both, or (2) the deficiency of critical fatty acid products of FALDH.[18]

Genetic studies

In 1996, the genetic basis of SLS was unraveled by De Laurenzi et al.[19] The FALDH coding gene, recently renamed ALDH3A2, is located on chromosome 17p11.2, it consists of 11 exons and spans about 31 kb. The amount of ALDH3A2 mRNA produced corresponds to the FALDH activity. The mutations seen in SLS mostly result in a complete loss of mRNA production and consequently the loss of FALDH activity as well, although some mutations with residual FALDH activity have been reported.[20]

By now, more than 70 different mutations in the ALDH3A2 gene have been identified in SLS patients from around 120 different families. Most mutations in SLS
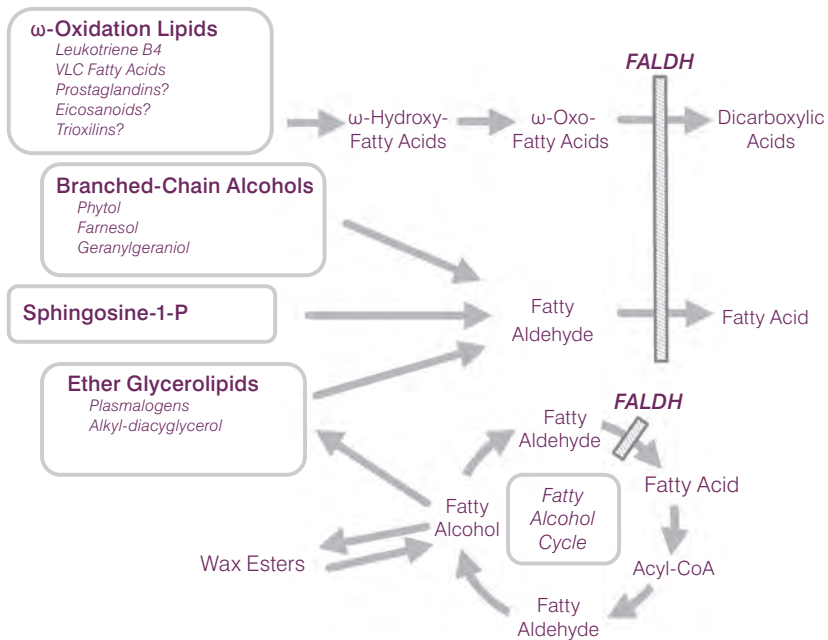
Figure 2 The central role of FALDH in fatty aldehyde/alcohol metabolism. From Rizzo et al.[13]

are scattered throughout the ALDH3A2 gene and they are generally private mutations, occurring in single cases. This makes it hard to study the correlation between genotype and phenotype. Original reports from the Swedish study cohort showed little variation in phenotype among patients with consanguinity. However, some reports have been made by multiple cases within the same family that relevant differences in corresponding phenotypes are spotted even though these cases share the same genotype and genetic background due to them being from the same family.[21,22] Other factors such as genetic background and environment probably play a part in the phenotypic appearance of SLS besides the factor of mutation.

Outline of the thesis

This thesis is the second Sjögren-Larsson doctoral thesis from the Radboudumc Nijmegen. For more than two decades, our research group has studied the clinical, biochemical, and genetic aspects of SLS. During the years, we have extend our study cohort to more than 30 genetically and biochemically proven SLS patients.

This thesis is divided into four parts.

Part 1 focuses on the daily functioning of patients with SLS. The purpose of this part is to provide patients, parents and clinicians a further insight into the clinical course of SLS and to provide prognostic information on intellectual disability. **Chapter 2** describes data from a cross-sectional study of motor performance and everyday functioning. **Chapter 3** provides a detailed insight into cognitive functioning and speech-language pathology in SLS. Also, it studies a possible correlation between motor performance results from chapter 2 and scores for developmental age, speech-language pathology as well as cerebral imaging.

In SLS the central nervous system and the skin are the organ systems mostly affected by disturbed fatty alcohol and aldehyde metabolism. The retina is often considered the most peripheral part of the central nervous system. Therefore, **part 2** focuses on the pathognomonic crystalline maculopathy seen in the retina of SLS patients. **Chapter 4** studies morphologic changes in the macula by optical coherence tomography (OCT) in order to find out what specific retinal cell layers are involved in SLS. This chapter provides an insight into retinal lipid metabolism and forms the basis for a discussion towards which retinal cell types could be responsible for the crystalline maculopathy. The study in **chapter 5** describes the novel finding of a macular pigment deficiency in SLS. Macular pigment and its metabolism are used to give better understanding of retinal lipid metabolism in SLS.

Part 3 focuses on the skin. Epidermal dysfunction in SLS is rather complex since it contains various FALDH depended lipid pathways and hence it offers multiple potential approaches to reduce cutaneous symptoms. In **chapter 6,** a prospective, randomized, double-blinded and placebo-controlled trial was performed to assess the effect of blocking leukotrienes synthesis upon the general skin condition and especially pruritus in SLS.

Part 4 starts with Chapter 7 which gives a state-of-the-art synopsis of current patho-physiological concepts in SLS mainly based upon original research data from this study group. Furthermore, **chapter 8** presents a discussion on current insight and future perspectives. **Chapter 9** summarizes and discusses important findings from this thesis in English and Dutch respectively.

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Part one

**Daily functioning in
Sjögren-Larsson syndrome**

2

Sjögren-Larsson syndrome: motor performance and everyday functioning in 17 patients

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Jan Willem Gorter

Abstract

Sjögren-Larsson syndrome (SLS) is an autosomal recessive neurometabolic disorder characterized by spasticity, learning disability, and ichthyosis. To our knowledge, there is no detailed report in the literature concerning the functional consequences of SLS. Therefore, we performed a cross-sectional study of motor performance and everyday functioning in 17 patients with this rare disorder. Nine female and eight male patients with SLS (age range 1–35 years) were investigated. Data were obtained by structured interview with parents and patients with SLS, a telephone-conducted questionnaire, and physical examination. Motor performance was measured by the Gross Motor Function Measure; everyday functioning was assessed using the Pediatric Evaluation of Disability Inventory and the Vineland Adaptive Behavior Scale. In most patients, spasticity was bilaterally present in hamstrings, hip adductors, and gastrocnemic muscles. All participants above 7 years had contractures in the lower extremities. Limitations were present in all gross motor dimensions, except for lying and rolling.

Participants had developmental ages far below their chronological age.

This study revealed that patients with SLS have limitations in gross motor performance. Although some patients can reach a certain level of independence, most have activity limitations and restrictions in their participation in society.

Introduction

Sjögren-Larsson syndrome (SLS) is an autosomal recessive disorder originally described by Sjögren and Larsson in 1957 and characterized by the clinical triad of spastic diplegia or tetraplegia, learning disability and ichthyosis.[1–3] SLS is caused by a disturbance of lipid metabolism due to a deficiency of microsomal fatty aldehyde dehydrogenase (FALDH).[4] This enzyme catalyzes the oxidation of fatty aldehydes into fatty acids.[4,5] The clinical features of SLS are thought to be the result of the accumulation of lipid metabolites.[4] Congenital ichthyosis is usually the presenting symptom of SLS which brings the child to medical attention. Within the first 2 years of life, the neurological symptoms gradually appear. Despite the authors' longstanding interest in SLS, motor performance and everyday functioning was never systematically studied in detail in our patients. The motor problem described in our studies, and those published by others, is generally summarized as spastic di- or tetraplegia, with consequences in terms of contractures and wheelchair dependency.[1,3] A detailed description of daily functioning in SLS would help professionals to provide a basis for the prognostic information they give to patients and their parents.

The purpose of this study, therefore, was to describe the level of motor performance and everyday functioning at the International Classification of Functioning, Disability and Health (ICF)[6] levels of activities and participation, in patients with SLS. We aimed to give more insight into the natural history of SLS to allow for more precise counseling of parents. This information could also serve as reference for rehabilitation programs and future therapeutic trials.

Methods

Participants

Seventeen Dutch patients, from 14 different families, with biochemically and genetically proven SLS were investigated. Clinical characteristics of all participants are shown in Table 1. They all showed the classical clinical triad of congenital ichthyosis, spasticity and learning disability. Informed consent was given by all parents and the study was approved by the local Medical Ethical Committee of the Radboud University Nijmegen Medical Centre in Nijmegen and Rehabilitation Centre De Hoogstraat, Utrecht, the Netherlands.

Procedure

Data were obtained by structured interview with parents and children or young adults (if possible), a telephone questionnaire, and physical examination. The physical examination and structured interview took place at the Rehabilitation Centre De Hoogstraat,

Table 1 Clinical characteristics of participants with Sjögren-Larsson syndrome ($n=17$).

Patient	Sex/Age (years; months)	FAO/FALDH activity [a]	Ichthyosis [b]	Seizures [b]	Visual acuity [c]	Cognitive performance/ IQ level [d]	Retinal Crystals [b]	MR Imaging [e]	Proton (H) MR Spectroscopy [f]
1	M/1; 11	1.2	+	+	NR	LD	+	0	1
2	F/2; 1	0.8	+	–	NR	LD	–	0	1
3	F/3; 0	0.9	+	+	NR	LD	+	1	NP
4	F/5; 3	1.3	+	–	NR	LD	+	1	1
5	M/7; 11	0.0	+	–	NR	LD	+	1	1
6	M/9; 5	4.7	+	–	NR	LD	+	1	2
7	M/13; 6	3.2	+	–	0.16	LD	+	1	2
8	F/14; 3	3.3	+	–	0.4	61	+	1	1
9	M/14; 10	3.8	+	–	0.5	49	+	1	2
10	M/15; 0	3.7	+	–	0.4	LD	+	1	2
11	F/15; 2	6.3	+	–	0.6	55	+	1	1
12	F/19; 1	3.7	+	–	0.6	LD	+	1	–
13	F/20; 5	4.5	+	–	0.6	48	+	1	1
14	M/22; 1	5.1	+	+	0.25	48	+	2	2
15	F/23; 5	5.1	+	–	0.3	56	+	2	1
16	F/23; 8	5.2	+	–	0.25	54	+	1	2
17	M/35; 0	2.5	+	–	0.5	LD	+	1	1

[a] Fatty aldehyde oxidation/dehydrogenase (FAO/FALDH) activity, measured using C-18 fatty alcohol as a substrate; normal value ($n=13$), 19.7 to 54.3 pmol/ min.mg protein.[3]

[b] Ichthyosis, seizures, and retinal crystals noted as present (+) or absent (–).

[c] Visual acuity (both eyes have been averaged): acuity 0.3–0.8 = minor visual impairment, acuity <0.3 = major visual impairment. NR, not recorded.

[d] Cognitive performance given as total IQ score or described as learning disability (LD).

[e] Intensity of periventricular white matter lesions on T_2-weighted magnetic resonance (MR) images are depicted as absent (0), mild (1), or + (2).

[f] MR-spectroscopy score: height of characteristic lipid peak was compared with N-acetylaspartate (NAA) peak and scored as follows: 0 (no lipid peak visible), 1 (lipid peak < NAA peak), or 2 (lipid peak > NAA peak); NP: MR-spectroscopy not performed.[3,20]

Utrecht. Telephone questionnaires were conducted within a period of 4 months from the physical examination and structured interview. Data were collected between March 2005 and May 2006.

Measurements

For the present study, generic measures for children and adolescents were used as much as possible. For some variables for which a generic measure did not exist, measures primarily designed for patients with cerebral palsy (CP) were used. All measurements were performed by the same physician (JV).

Measures of body function and structure (ICF)

The level of spasticity, contractures, the presence of scoliosis, and ambulatory capacity were documented by physical examination. Also, to get an impression of functional muscle strength and selectivity of motor control, all participants were asked to squat 10 times in a standardized way.

Spasticity was defined according to Lance: 'a motor disorder, characterized by a velocity-dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks, resulting from hyper-excitability of the stretch reflex as one component of the upper motor neuron syndrome'. [7] For the purpose of this study, we selected the modified Ashworth scale (MAS) and the Tardieu Scale to measure spasticity.[8,9] Although the validity of the MAS can be questioned, it is still one of the most commonly used methods for assessing spasticity in children and adults. It has been suggested that the Tardieu scale is more appropriate to measure spasticity.[9–11] The modified Tardieu Scale (MTS) quantifies muscle reaction to stretch and the joint angle at two specified velocities of stretch: fast passive velocity stretch (V3) and slow passive velocity stretch or passive range of motion (V1). The difference between the joint angles measured during the slow and fast velocity stretches is considered to be a clinical measure of spasticity. During our measurements, it was noticed by the examiner that spasticity in the participants was difficult to quantify due to the presence of contractures, especially in the older patients. Therefore, because we expected the MAS and MTS scores not to be reliable enough, we decided to use the measures only to report the presence or absence of spasticity. We, therefore, recoded the MAS score and the MTS score in a dichotomized way: '0' for the absence of spasticity and '1' for the presence of spasticity. If either the MAS or MTS indicated the presence of spasticity, it was recorded that spasticity was present.

To examine the presence of contractures, the passive range of motion (ROM) of the shoulder, elbow, wrist, hips, knees, and ankles was assessed using a goniometer with the participant in supine position. Full passive ROM was coded '0', and limited ROM, according to the reference values of the Spinal Alignment and Range of Motion Measure, was coded as '1'.[12]

Measures of activities and participation (ICF)

Ambulatory ability in our participant was classified using the Gross Motor Function Classification System (GMFCS).[13] Motor capacity was measured using the Gross Motor Function Measure (GMFM). The GMFM measures gross motor function in five domains: lying and rolling; sitting; crawling and kneeling; standing; and walking, running, and jumping. There is no age limit but all items should be accomplished by a 5-year-old with typical psychomotor development.[14]

To assess everyday functioning the Pediatric Evaluation of Disability Inventory (PEDI) and the Vineland Adaptive Behavior Scale (VABS) were used.[15,16] Both questionnaires were conducted by telephone interview. The PEDI is a parental questionnaire that measures both capability and performance of routine daily childhood activities in three domains: self-care, mobility, and social function. Capability is measured by the identification of functional skills for which the child has demonstrated mastery and competence. Performance of daily functional activities is measured by the level of caregiver assistance needed to accomplish them. In the present study, the Dutch version (PEDI-NI) was used, which has been shown to be reliable and valid.[17] The VABS is also a parental questionnaire. It measures the level of adaptive functioning in a child or adolescent with or without disability in four specific domains: communication, daily living skills, socialization skills, and motor skills. In the present study, the self-care and mobility domains of the PEDI and the communication, daily living skills, and socialization skills domains of the VABS were used.

Children's and adolescents' perspectives

In cases where the participants with SLS could be interviewed themselves, we asked for additional information on the type of school they attended, which sports they practiced, their leisure interests, and their own perception of competence. For the latter we made use of the Dutch version of the Perceived Competence Scale.[18]

Data analysis

Dichotomized scores (0 or 1) for spasticity of adductors, hamstrings, and gastrocnemius were counted, giving a possible score of 0 (no spasticity in one of the three muscle groups), 1, 2, or 3 (spasticity in three muscle groups) per leg. For the upper extremity, scores for biceps and flexors of the wrist were also summarized giving a possible score of 0, 1, or 2.

To indicate the number of contractures, the presence of contractures in each joint for each limb was summarized giving a possible score with a range from 0 to 3.

Gross motor function was measured by the GMFM with 88 items scored on a 4-point Likert scale. For each dimension a percentage score was calculated (child's score/maximum score $\times$ 100%). A total score was obtained by adding the percentage

scores for each dimension and dividing by the total number of dimensions (five). A higher percentage indicates better gross motor capacity.

According to the PEDI-manual, we calculated two scores for each domain scale: functional skill (FS) score and caregiver assistance (CA) score. These scaled scores (0–100) of the PEDI-NI are used in order to provide an estimate of the child's achievement of activities and amount of care assistance regardless of age. The Dutch translation of the VABS survey form was used.[16] Descriptive analyses were performed for all variables.

Results

Participant characteristics and test scores at the ICF body function and structure level are presented in Table 2a. Of 17 participants, eight were male and nine were female. Two sets of siblings participated in this study: patients 5 (age 7y) and 8 (age 14y), and patients 12 (age 19y) and 13 (age 20y) respectively. Patient ages at the time of investigation ranged from 1 to 35 years. In most cases, spasticity was bilaterally present in hamstrings, hip adductors, and gastrocnemic muscles. According to the measurement protocol in this study, in one adolescent (patient 15) spasticity could be confirmed in the gastrocnemii only and three out of 17 participants showed spasticity in the upper extremity. All participants above the age of 7 years had contractures in the lower extremities, with involvement of three levels (hips, knees, and ankles) from the age of 13 years. In one participant (patient 14) contractures were present in both elbows. In three participants (patients 10, 16, and 17) scoliosis was recorded. The two participants (patients 5 and 13) who were able to squat several times were classified at GMFCS Level II.

Motor functioning
Ambulatory ability according to the GMFCS is shown in Table 2b and ranged from Level II to IV. Percentage scores of all five dimensions and the Total score of the GMFM are also shown in Table 2b. All participants had high scores in Dimension A (lying and rolling). Sitting (Dimension B) and crawling and kneeling (Dimension C) were difficult for most children and adolescents. In children aged 5 years or older only two had a maximum score in sitting (patients 5 and 13), and only one had a maximum score in crawling and kneeling (patient 5). In these domains all children tried to compensate for the impairment of their legs by their arm strength. None of the participants achieved a maximum score in Dimension D (standing), or Dimension E, walking, running, and jumping. The capacity of walking in all participants was limited as the items walking, running, and jumping could only partly be carried out by seven out of 14 participants aged 5 years and older.

Table 2a Results at ICF body function and structure level.

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Sex/Age, years; months	M 1; 11	F 2; 1	F 3; 0	F 5; 3	M 7; 11	M 9; 5	M 13; 6	F 14; 3	M 14; 10	M 15; 0	F 15; 2	F 19; 1	F 20; 5	M 22; 1	F 23; 5	F 23; 8	M 35; 0
Spasticity																	
Up extr R/L	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2/2	0/0	0/0	1/1	0/0	2/2	0/0	0/0	0/0
Low extr R/L	3/3	3/2	3/3	3/3	3/3	2/2	3/3	3/3	3/3	1/1[a]	3/3	3/3	3/3	3/3	1/1	3/3	2/2
Contractures																	
Up extr R/L	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0
Low extr R/L	0/0	0/0	0/0	0/0	1/1	2/2	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Scoliosis	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+
Squats, *n*	0	0	0	0	3	0	0	0	0	0	0	0	3	0	0	0	0

[a] No data available on adductors and hamstrings.

Up extr.: upper extremity; Low extr.: lower extremity; R: right; L: left; –: not present; +: present.

ICF: International Classification of Functioning, Disability and Health.

Everyday functioning

PEDI and VABS data are missing from two patients (5 and 8) who could not be contacted by telephone. PEDI scores of the FS and CA scales of 15 participants are shown in Table 2b. Both scales range from 0 (minimum) to 100 (maximum) with higher scores indicating better performance and increased independence.

In the self-care domain, scores of the FS scale varied between 32.8 and 100.0. Only two out of six patients older than 18 years of age scored the highest possible level. In the CA scale, four out of the six patients older than 18 years of age and one child (9 years) scored the highest possible score. In the mobility domain, scores of the FS scale varied between 25.5 and 100.0 and in the CA scale between 32.7 and 100.0. Only one participant (patient 13) scored the highest possible score in the FS scale and four participants scored the highest possible score in the CA scale. For example, children with the lowest scores in the self-care domain have difficulties with items 'putting on socks and shoes' and 'putting on trousers'. In the mobility domain, lowest scores refer to difficulties with the items: 'walking up or down the stairs', 'self-mobility over different kinds of surface', 'transfers in and out of a car', and 'cycling'.

For the VABS, developmental ages for each domain are calculated and shown in Table 2b. In the communication domain, scores for 'expressive communication', and 'written communication' were lower than for 'receptive communication'. For the daily living skills domain, scores in 'domestic' and 'community daily living skills' were lower compared with 'personal daily living skills'. For the socialization domain, all three sub domains ('interpersonal relationships', 'play', and 'leisure time and coping skills') scores show a large variability.

Participation of children, adolescents, and young adults with SLS

All participants required the assistance of parents in the structured interview because speech production was affected in all patients. Of the participants aged 4 to 20 years, all went to special schools. Of the participants older than 20 years of age, only one (patient 13) had found a job in a social working program (special workplace/environment); the others went to day activity centers. All participants from the age of 7 years were active in sports. Eleven participants were able to answer questions about their perceived competence and they all were very positive about themselves. Eleven parents commented specifically on their son's or daughter's friendly and sociable character.

Table 2b Results at ICF activities and participation level.

Patient	1	2	3	4	5	6	7
GMFCS level (I-V)	III	III	IV	III	II	III	IV
GMFM Scores (0-100)							
Lying&rolling	92.2	96.1	96.1	100	96.1	100	94.1
Sitting	38.3	45.0	78.9	88.3	100	90.7	91.2
Crawling& kneeling	11.9	38.1	66.7	78.6	100	73.8	85.7
Standing	0	0	5.1	15.4	82.1	7.7	46.2
Walk/run/jump	0	0	0	0	72.1	0	16.7
TOTAL (average)	28.5	35.8	49.4	56.5	90.0	54.4	66.8
PEDI (0-100)							
FS scale							
Self-care	34.1	51.7	32.8	57.7	-	70.1	62.0
Mobility	25.5	32.1	33.2	53.1	-	50.5	61.5
CA scale							
Self-care	26.3	42.6	26.3	26.3	-	100	68.4
Mobility	34.9	34.9	32.7	49.7	-	100	100
VABS							
Communication	1:0	1:1	0:1	2:2	-	3:3	5:7
Daily skills	0:10	0:10	0:4	1:9	-	3:3	2:11
Socialization	0:9	0:10	0:1	1:9	-	3:3	5:5

VABS scores are developmental age (y:mo). PEDI and VABS data missing from Patients 5 and 8 who could not be contacted by telephone. ICF, International Classification of Functioning, Disability and Health; GMFCS, Gross Motor Function Classification System; GMFM, Gross Motor Function Measure; PEDI, Pediatric Evaluation of Disability Inventory; FS scale, functional skills scale scaled scores; CA scale, caregiver assistance scaled scores; VABS, Vineland Adaptive Behavior Scales.

Discussion

This is the first study that has aimed to describe the level of motor performance and everyday functioning in a relatively large series of patients with SLS. Results show a large variability in functioning in this group. In line with the recent findings of Lossos et al., we found that two adult sisters (patients 12 and 13) differed in their level of self-mobility and daily functioning, indicating that phenotypic variability in SLS exists within families.[19] There seems to be a ceiling effect in motor performance and everyday functioning of most participants at or around a developmental age of 12 years.

As expected from our standardized neurological examination, we found that spasticity, as measured by the MAS and the MTS, was dominantly present in both legs in all participants, with only mild impairments in the upper extremities. These observations are in line with the original observations made by Sjögren and Larsson.[1] We made an attempt to quantify the level of spasticity but due to the presence of

	8	9	10	11	12	13	14	15	16	17
	III	III	IV	IV	IV	II	IV	IV	III	IV
	70.6	96.1	100	100	100	96.1	80.4	100	100	92.1
	98.3	68.3	26.7	73.3	93.3	100	16.7	87.7	88.3	13.3
	97.6	42.9	59.5	69.0	71.4	83.3	7.1	66.7	57.1	52.4
	64.1	10.3	15.4	0	7.7	59.0	2.6	56.4	38.5	2.6
	30.8	0	12.5	0	0	52.8	0	13.9	13.9	0
	72.3	43.5	42.7	48.5	54.5	83.3	21.4	64.9	59.6	32.0
	-	76.5	64.1	75.4	84.8	100	70.1	93.0	100	83.0
	-	59.6	53.1	54.0	54.0	100	45.3	55.8	54.9	54.9
	-	84.0	64.0	66.9	71.1	100	67.6	100	100	100
	-	64.5	62.2	69.3	60.9	100	53.1	90.7	100	100
	-	7:7	2:4	9:6	3:2	9:2	6:4	7:1	10:10	1:10
	-	5:9	3:3	8:9	5:9	15:9	5:2	6:10	12:3	5:0
	-	5:11	4:6	12:3	3:6	11:6	8:2	4:10	13:6	4:4

contractures in most participants the MAS and MTS scales could not be administered properly. For this reason, no valid conclusion could be drawn about the degree of spasticity in patients with SLS. In all participants, ambulatory ability was classified at GMFCS Level II, III, or IV, indicating that none of the participants was able to walk without restrictions and most of the patients with SLS use a wheelchair to move around outdoors and in the community. It is an important finding that none of the persons with SLS was severely limited in their self-mobility, even in the long term. In five patients, parents reported that their child had been able to walk with or without support but gait had deteriorated gradually over time. Sjögren and Larsson found that of 24 patients, 12 were unable to walk, five could walk only with support, and six others showed marked spasticity in their gait. In the remaining patient, gait was 'moderately spastic'. Reports were made about deterioration of gait in four patients but no cause for this deterioration was given. In SLS, spasticity might gradually worsen, with a subsequent increase in contractures, during the first decades of life.[3]

Scoliosis was recorded in three patients, but none of these had a severe scoliosis or had been operated on. Haddad also found mild spinal deformity was present in his group of nine patients with SLS.[20]

We found most patients had difficulties with sitting, crawling, kneeling, and standing. One explanation for this might be the presence of contractures. Another possible explanation might be a lack of muscle strength in combination with impaired selectivity of motor control in the lower extremities. Most children tried to compensate for the impairment of their legs with their arms.

Developmental ages of the VABS show a large variability, especially in the children aged above 5 years (Table 2b). However, all participants had developmental ages that were significantly lower than their chronological age and the majority of patients had developmental ages that were far below, or around, 12 years.

Although some patients reached a certain level of independence, self-care, and mobility in an adapted environment, most of them had limited social interaction with peers and their participation was restricted. This is in accordance with data from our structured interview that showed that only one of the all participants older than 20 years had a job (in a social working environment), while the rest went to day activity centers with special care facilities.

Limitations of the study

Several limitations should be considered when interpreting our results. First, due to the small sample size, no firm conclusion can be drawn from the findings. Although SLS is a very rare disorder we were able to include almost all known patients with SLS in the Netherlands. Second, due to lack of more suitable instruments we had to use generic instruments and instruments that have been validated only for patients with CP. Since the differences between both groups of patients are unknown, results must be interpreted carefully. We recommend further research that should focus on differences and similarities of patients with SLS and CP. Third, the present study design was cross-sectional. SLS is generally considered a non-progressive or only very mildly (over decades) progressive neurometabolic disorder. The results of our study confirm this hypothesis, but they do not, in the strictest sense, allow us to conclude that the consequences for daily functioning and participation are stationary during decades. Future prospective longitudinal studies are warranted to document and, if possible, predict the course in motor performance and everyday functioning in SLS.

Conclusion

In this study, most patients with SLS reached a level of everyday functioning that is around or below the level of a typically developing 12-year-old child. Limited communication, social interaction with peers, and restricted participation do have an impact on the outlook of patients with SLS. Spasticity and contractures, mainly in the legs, are major impairments but on the activity level these children and young adults are able to move around in the community (GMFCS Levels II–IV). In this cross-sectional study there was no sign that indicated a serious loss of motor performance or everyday functioning with increasing age. These findings can help professionals to provide parents of young children with SLS with general and specific information, to guide them to the services they need to minimize the constraints in their development and to enhance their quality of life.

Acknowledgement

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3

Speech-language performance in Sjögren-Larsson syndrome

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Abstract

Objective: To describe speech-language pathology in patients with Sjögren-Larsson syndrome (SLS) in relation to their cognitive and motor impairment.

Design: Observational case series.

Methods: Cognitive functioning was assessed in 16 patients with SLS (nine males; seven females) using different neuropsychological tests. Speech-language pathology was studied focusing on dysarthria, oral motor functioning, speech intelligibility and language development. Potential correlations between speech-language pathology and other neurological symptoms (e.g. spasticity) were studied.

Results: The median cognitive developmental age was 5 years; 8 months (n =13; range 3;5 – 8;0) years. A variable degree of mainly pseudo bulbar dysarthria was found. Speech intelligibility was influenced by dysarthria, but was also related to language pathology. No correlation between motor functioning and dysarthria or cognitive development was observed.

Conclusion: Dysarthria and language problems are important factors in daily life functioning of patients with SLS. Based upon the clinical profile found, early speech-language therapy is recommended in order to optimize their speech-language development.

Introduction

Sjögren-Larsson syndrome (SLS) is an autosomal recessively inherited disorder characterized by a clinical triad of congenital ichthyosis, spastic di-or tetraplegia and mental retardation. It is a rare neurocutaneous disease with a prevalence of < 0.4 per 100 000. A disturbance of lipid metabolism due to deficiency of the microsomal fatty aldehyde dehydrogenase (FALDH) underlies SLS.[1-3]

This enzyme catalyses the oxidation of many medium and long chain fatty aldehydes into fatty acids. A deficiency of FALDH results in the accumulation of fatty alcohols and aldehydes in body tissues and it is this accumulation that is thought to be the main cause of symptoms most clinical in SLS.

Of all clinical symptoms the ichthyosis is usually the first symptom of SLS. Later in childhood the complete clinical picture gradually develops, consisting of spasticity in the extremities (diplegia more common than tetraplegia), mental retardation and often delayed and disturbed acquisition of speech and language skills with subsequent limitations in communication. Many of these patients receive multidisciplinary treatments in rehabilitation centers and schools for special education.

Cerebral magnetic resonance (MR) imaging shows the presence of a leukoencephalopathy, while MR spectroscopy studies reveal a characteristic peak reflecting the accumulation of lipids in cerebral white matter.[2] Furthermore, a peculiar crystalline retinopathy causes photophobia and subnormal visual acuity and is another early symptom of SLS. This retinopathy can be visualized by ophthalmoscopy and is mainly characterized by glistening macular dots.[4]

Many of the typical clinical symptoms occurring in SLS have been studied and described in the literature during the past decades. However, few data have been published regarding the speech language pathology and limitations in communication of patients with SLS.

In the preparation of this study, an extensive literature search was performed by PubMed using Medical Subject Heading (MeSH) database and the search term 'Sjögren-Larsson syndrome'. Besides the general descriptions of impaired speech-language performance and pseudo bulbar dysarthria in these patients, this search was unable to find any relevant previously published studies.[2,3]

The aim of this study is to provide a detailed description of the speech and language performance in SLS patients and to study the potential correlation between speech-language pathology and other neurological signs and symptoms such as spasticity scores and abnormalities on cerebral MR imaging and spectroscopy studies. This way, more insight is given into the everyday functioning of these patients, which serves professionals involved in their treatment.

Methods

Participants

This study is part of a larger research project on daily functioning in patients with SLS of which results recently have been published.[5] The same patients were included in the present cross-sectional observational case study, except for one patient who had to be excluded because of incomplete data. In all patients the diagnosis SLS had been proven at both the genetic and biochemical level. Local medical ethics committee approval and parental informed consent were both obtained for all study participants. The clinical and biochemical characteristics of the participants are summarized in Table 1.

Procedure

All participants were tested by the same members of this research group. Data were obtained by an experienced paediatric neuro-psychologist (BM) and an experienced paediatric speech-language pathologist (MG), at the Department of Paediatric Neurology of the Radboud University Nijmegen Medical Centre in Nijmegen, The Netherlands. For this study, standardized motor function testing of these patients took place at Rehabilitation Centre De Hoogstraat in Utrecht, The Netherlands.

Measurements
Mental performance

In a previous study the developmental age of the patients was estimated using the structured parental interviewing tests Pediatric Evaluation of Disability Inventory (PEDI) and Vineland Adaptive Behavior Scales (VABS).[5] PEDI and VABS provide an estimate of performance in daily life situations.[6,7] However, in order to gain further insights in neuropsychological development and intelligence of these individuals, the cognitive capacity was assessed in this present study using different tests. Cognitive tests were selected for the age-range 4–12 years, being the a priori estimated developmental age of the participants. Tests used in this study were the Kauffman-ABC sub-tests 'Number Recall' (auditory memory), 'Word Order' (auditory memory and motor output) and 'Hand Movements' (sequential motor memory) and two sub-tests of the verbal intelligence scale of the 'Leiden Diagnostic Test' (LDT).[8,9] To provide a valid assessment of the cognitive capacity, developmental age was assessed for each patient such that all five sub-test results scored within ±2 SD from the tests norm score at that particular developmental age.

Cognitive testing was not performed in patients 1 and 2 because their very young ages made testing unreliable.

Table 1 Clinical characteristics of participants with Sjögren-Larsson syndrome.

Patient	Verhoog et al. [a]	Sex [b]	FAO/FALDH Activity [c]	Ichthyosis [d]	Seizures [d]	MR Imaging [e]	Proton (H) MR Spectro-scopy [f]
1	1	M	1.2	+	+	0	1
2	3	M	0.9	+	+	1	NR
3	4	F	1.3	+	-	1	1
4	5	M	0.0	+	-	1	1
5	6	M	4.7	+	-	1	0
6	7	M	3.2	+	-	1	0
7	8	F	3.3	+	-	1	1
8	9	M	3.8	+	-	1	0
9	10	M	3.7	+	-	1	0
10	11	F	6.3	+	-	1	1
11	12	F	3.7	+	-	1	NR
12	13	F	4.5	+	-	1	1
13	14	M	5.1	+	+	0	0
14	15	F	5.1	+	-	0	1
15	16	F	5.2	+	-	1	0
16	17	M	2.5	+	-	1	1

[a] Corresponding patient number in *Verhoog et al.* [5]

[b] Sex marked as M (male) or F (female).

[c] Fatty aldehyde oxidation/dehydrogenase (FAO/FALDH) activity, measured using C-18 fatty alcohol as a substrate; normal value (n=13), 19.7 to 54.3 pmol/min.mg.

[d] Ichthyosis and seizures noted as present (+) or absent (-).

[e] Intensity of periventricular white matter lesions on T2 weighted magnetic resonance (MR) images depicted as 0 (moderate), 1 (mild), 2 (absent).

[f] MR-spectroscopy score: height of characteristic lipid peak was compared with N-acetylaspartate (NAA peak) and scored as follows: 0 (lipid peak >NAA peak), 1 (lipid peak < NAA peak), 2 (no lipid peak visible), NR (= not recorded).

Data from [e] & [f] were adapted from Willemsen et al.[2]

Speech-language performance

Dysarthria was scored using the 'Dysarthria Score' which is defined in Table 2. The Dysarthria Score is an English translation and adaptation of the nationally used Dutch 'Nijmegen Dysarthria Scale' (NDS).[10] The NDS is an outcome measurement scale which was recently developed by experienced speech language pathologists. It is used to systematically score the type and severity of dysarthria in different disease

modalities. The NDS evaluates the main physiological speech systems (respiration, phonation, resonance, prosody, articulation and lips/ tongue/jaw movements) and assesses the effectiveness of speech during communication in daily life. In fact, the NDS scoring system is a Dutch revision and translation of the internationally developed Therapy Outcome Measures (TOMs). TOMs are a series of 0–5 rating severity scales for different aspects of speech-language pathology (including dysarthria) developed in the UK by Enderby and John.[11] Several reliability research trials have been conducted in the UK and confirmed TOMs reliability and reproducibility.[12,13] Nationally performed research has indicated that the NDS has comparable reliability and reproducibility, though further research is pending.[14]

Because most patients with SLS suffer from mild-to-moderate spasticity it was expected that dysfunctional movements of lips, tongue and jaw (due to this spasticity) would be a major determinant in the dysarthria found in these patients. To test this hypothesis it was decided to further specify the dysarthria encountered in these patients by scoring the aspects of lips, tongue and jaw movements separately. The results of this separate score are indicated in the Oral Motor Score (OMS). Furthermore, to provide insight in everyday communicative functioning of these patients, the intelligibility of speech was also specified into a separate score (Speech Intelligibility Score; SIS). Both OMS and SIS are based upon clinical assessment and elements from the previously described Dysarthria Score.

Language production and perception tests consisted of the standardized Schlichting and Reynell tests used for the younger children and a specific validated Dutch language production/perception test ('Taal test voor Kinderen') used for the older children.[15-17] The calculated developmental age, determined in the first phase, was used to select the proper test. Results of language production and perception tests were converted to age equivalents.

Motor performance and neuro-imaging scores

Detailed results regarding motor performance in these SLS patients have recently been published.[5] For the purpose of the present study the results of motor testing were modified into a separate 0–12 rating 'Spasticity score'. This simplified score was calculated by summing the original upper and lower extremity spasticity scores in these patients and provided a valid impression of motor functioning (Table 3).

Furthermore, in Table 1 the scores were added for cerebral MR imaging and proton MR spectroscopy of these patients, which were obtained from another previous study in this patient cohort.[2]

Table 2 Dysarthria score.[10] Definition describing both functional and communicative characteristics of speech.

Dysarthria score	Definition
0	**very severe dysarthria/anarthria** • (Almost) complete loss of capability to move lips, tongue and jaw causing inability to produce separate speech sounds. Only some undifferentiated vocalization. Aphonia or severely disturbed quality of phonation. Not sufficient respiratory support for speech. • No effective communication possible.
1	**severe dysarthria** • Extremely disturbed muscle tone and impaired movements of lips, tongue and jaw causing production of some open vowels and some clearly aberrant consonants. Clearly disturbed quality of phonation. Very slow speech; short breathes of speech. • Occasionally able to communicate basic needs with familiar persons or trained listeners in familiar contexts.
2	**moderate/severe dysarthria** • Clearly disturbed muscle tone and movement abilities of lips, tongue and jaw causing aberrant sounding vowels and consonants. Aberrant rate of speech and quality of phonation. Aberrant control of breathing during speech. • Able to communicate basic needs. Frequent repetition required. Communication improves using trained listeners and family members or in familiar settings.
3	**moderate dysarthria** • Minimal disturbance of muscle tone and movement abilities of lips, tongue and jaw causing minimal aberrant sounding vowels and consonants. When focusing the patient is able to utter syllables and words correctly. Minimal aberrant rate of speech and quality of phonation. Minimally aberrant control of breathing during speech. • Communication with familiar persons is effective. With some help, communication with unfamiliar persons may be effective. Capable of communicating beyond here and now. Occasional repetition required.
4	**mild dysarthria** • Some minor problems with articulation, quality of phonation and/or control of breathing during speech. • Can be understood most of the time by any listener despite communicative irregularities.
5	**no dysarthria** • Speech according to expectations based upon corresponding chronological and developmental age. • Taking into account both chronological and developmental age as well as cultural aspects communication is effective in all situations.

Results

Mental performance

Table 3 depicts both chronological age at time of testing and the assessed developmental age. The mean chronological age was 17;10 years. The mean developmental age was much lower with a mean of 5;8 (range 3;5 – 8;0) years, describing the degree of mental retardation in these patients. In agreement with a relatively valid assessed developmental age, the majority of the intelligence subtest scores were within ±1 SD of the test's mean developmental age score, while nearly all results were within ±2 SD.

Study subject 3 had an estimated developmental age of <2 years. This however was not based upon test results and therefore this subject was not included in the calculations of mean age and mean developmental age.

Speeech-language performance

Table 3 summarizes the main test results regarding speech-language performance in these SLS patients.

Speech

In the greater majority of the study cohort significant dysarthria was found (Dysarthria Score ≤4; n =13), predominantly of the pseudo bulbar sub-type (n =10). Most study participants were at least moderately affected (Dysarthria Score ≤3; n =10). In two participants no dysarthria was found and in three participants the dysarthria was severe (Dysarthria Score 1). Two of the latter study participants used alternative forms of communication (e.g. speech computer) in daily life.

Oral facial motor functioning was impaired in most participants (OMS ≤4; n =15). It was noted that most SLS patients in this study cohort have poor functioning of the upper facial musculature. Remarkably severe dysphagia was not encountered in these patients and accordingly none depended on gastric tube feeding in daily life.

Speech intelligibility was likewise impaired in most of the tested participants (SIS ≤4; n =14).

Language

In all participants in whom language could be tested (n =14) at least one language production and one language perception test was administered, except for patient 9 (who could not produce intelligible speech and thus not be tested on production). Most of the study participants (n =9) were tested by two different tests for both language production and language perception. In these subjects the results of the different tests were averaged.

Table 3 Cognitive development and speech-language test results.

Patient	Chron. age (years; months)	Develop. age (years; months)	Dysarthria score [a]	Oral motor score [b]	Speech intell. score [c]	Language prod. (years; months) [d]	Language perc. (years; months) [d]	Spasticity score [e]
1	1; 9	NR	1/PB	1	0	NR	NR	6
2	2; 6	NR	UTR	4	0	NR	NR	6
3	5; 2	≤2	5	3	3	2; 9	2; 7	6
4	7; 8	5; 0	4/PB	3	3	3; 4	3; 7	6
5	9; 7	5; 6	4/PB	5	2	3; 3	4; 5	4
6	13; 4	7; 0	3/HK+PB	4	4	7; 2	6; 1	6
7	14; 2	5; 0	3/HK	2	4	3; 6	4; 2	6
8	14; 9	6; 6	3/PB	3	4	7; 5	5; 6	10
9	14; 10	4; 6	1/PB	2	0-1	NR	4; 7	UTR
10	15; 2	7; 0	4/PB	4	4	7; 0	8; 3	6
11	19; 0	3; 5	2/PB	3	3	3; 11	4; 6	8
12	20; 4	5; 7	5	4	5	6; 10	6; 10	6
13	21; 11	5; 0	2/PB	2	3	7; 9	6; 0	10
14	23; 4	8; 0	3/HK	3	4	8; 10	7; 1	2
15	23; 5	6; 11	3/PB	3	5	8; 0	8; 9	6
16	34; 11	4; 6	1/PB	3	1	4; 5	5; 3	4

[a] Dysarthria score ranging from 0 (very severe dysarthria)–5 (no dysarthria); pseudo bulbar (PB) or hypokinetic (HK) sub-types are indicated.

[b] Oral motor score ranging from 0 (very severe oral motor dysfunction)–5 (normal oral motor function).

[c] Speech intelligibility score ranging from 0 (very poor speech intelligibility)–5 (clear speech).

[d] Language production and perception. Test scores are depicted as age equivalent (years; months).

[e] Spasticity score ranging from 0 (no spasticity)–12 (severe spasticity in all extremities).

NR =not recorded. UTR =unreliable test result.

The mean age equivalent for language production was 5;8 (range 2;9 – 8;1) years and for language perception 5;6 (range 2;7 – 8;3) years. Thus, the mean ages and ranges for the language scores were rather similar to the developmental ages depicted earlier. Therefore, in the majority of the study cohort the language problems encountered in daily life were most likely strictly related to the global developmental delay. This was confirmed by the observation that the specific profile with poor language development is most pronounced in those patients with the lowest developmental ages. Significant correlations (spearman's rho) were found between developmental age on the one hand and language production (r =0.70; p < 0.01)

and language perception (r =0.77; p < 0.001) on the other. The correlation between language production and language perception age was even higher (r =0.85; p < 0.001)

It was noted that in the older patients the scores on the vocabulary tests were relatively high (> +2 SD; patients 9, 11–15). Apparently these patients had continued their vocabulary development beyond their language age equivalent.

Motor performance and neuro-imaging

Moderate-to-severe spasticity, most commonly in the lower extremities, was encountered in the majority of the study group. A possible correlation between spasticity and scores for developmental age, dysarthria (Dysarthria Score, OMS and SIS) and MR imaging was studied.

The individuals with the lowest motor functioning scores (patients 8, 11 and 13) were suffering only from a moderate (but not severe) dysarthria and also had only moderate oral motor impairment (OMS 2–3). Thus, in contrast to the hypothesis, no clear relationship between spasticity score and oral motor score or dysarthria was found (correlation coefficients of r = -0.12 and r = -0.06, respectively).

The developmental ages of participants 8 and 13 were within ±1 SD from average in this study group. Neuro-imaging data in participants 8 and 13 showed distinct abnormalities, but other participants with similar neuro-imaging scores did not seem to have the same extent of motor function impairment. Also, the participants with the best motor functioning (spasticity score 2; patients 9 and 14) differed with respect to cognitive functioning (developmental age 4;6 and 8;0 years, respectively) and dysarthria and neuro-imaging scores. Furthermore, the participants with the lowest developmental ages (patients 9, 11 and 16) had relatively mild neuro imaging scores.

In line with these observations, no correlations between extent of motor function impairment (spasticity score) and dysarthria (r = -0.06), cognitive impairment (r = -0.08) or results of neuroimaging were found when calculated.

Discussion

To the best of the authors' knowledge, this is the first study describing in detail the speech-language pathology encountered in SLS patients in relation to their cognitive development and spasticity. Detailed knowledge on speech-language development and acquisition of communication skills in SLS enables adequate parental counseling and optimizes potential benefits of early started speech-language therapy.

Mental performance

In contrast to most other neurometabolic disorders, SLS is considered a non-progressive disorder without any signs of progressive brain disease during childhood or early adulthood.[3] This is illustrated in this study by the observation that, although

all participants suffered from moderate mental retardation, none of the participants were severely affected. Also, the mean developmental age of all adult participants (chronological age > 18 years; n =6) was strikingly close to the mean of the total study group (mean of adult sub-group: 5;7 years; mean total study cohort: 5;8 years). Apparently, some kind of ceiling effect in cognitive development (developmental age 5–6 years) was reached at a certain chronological age without loss of acquired cognitive abilities later on in life. Of course, this is a cross-sectional observation and thus further longitudinal research is required to confirm this observation.

Speech-language performance

Although the patients with the poorest speech intelligibility (patients 1, 9 and 16) also had the most severe dysarthria, it was found that speech intelligibility was not always related to the severity of the dysarthria. For example, patients 3 and 5 suffered only from mild-to-absent dysarthria (Dysarthria score 5 and 4) but had relatively poor speech intelligibility (SIS 2 and 3) suggesting co-morbidity. In the latter patients, other factors such as low developmental age, developmental apraxia of speech and phonological problems may have directly influenced the speech intelligibility. Note that in patients 4 and 5 language production was severely affected. Phonological problems may be part of their co-existing language production impairment and directly affect speech intelligibility.

In general, it is stated that both dysarthria and language developmental problems (related to the cognitive impairment) are important factors determining the speech intelligibility in daily life. As stated earlier, no clear relation between motor functioning (spasticity) and dysarthria or cognitive development was observed in this study. Thus, given the variability in cognitive and speech-language development within different levels of motor impairment, it was concluded that it is not likely that spasticity is the limiting factor in these children's cognitive and speech-language development.

Speech and language therapy (SLT)

SLT aims to optimize the capabilities of patients with broad ranging disorders to communicate and consists of different treatment modalities. Most patients with SLS receive therapy from speech-language pathologists. However, the effect of this treatment regarding outcome in communicative skills of SLS patients is not clear. Because the neurological features of SLS are usually stable, the literature regarding the management of speech-language pathology in children with cerebral palsy (CP) was studied. Speech-language pathology is common in CP (estimated incidence approximately 20%).[18] A recent Cochrane Database review showed some positive effects on communication skills assigned to SLT in a very diverse patient population with CP; conclusive evidence for the beneficial effect of (early) SLT on the improvement of communication skills, however, was lacking.[19]

In The Netherlands a national guideline on CP was recently implemented which also covers this topic.[20] This guideline proposes early started SLT in children with CP to improve speech language performance and thereby daily functioning in these patients. Especially children with a disharmonic profile (e.g. low speech intelligibility combined with relatively spared cognitive functioning) should receive SLT as early as possible. SLT should focus on stimulating the child to actively communicate with its communication partners (e.g. siblings and peers) and may involve the use of alternative forms of communication (e.g. speech computer).

Limitations in communication and restrictions in social participation are important factors influencing daily functioning of patients with SLS, as was demonstrated by Verhoog et al.[5] Realizing the limitations of comparing SLS with a highly diverse patient population such as CP, it is believed that starting SLT early could as well be advantageous to SLS patients. Based upon the authors' own experience and the above-mentioned beneficial effect of SLT in CP patients, this study therefore proposes early started SLT in all patients with SLS. The duration and focus of SLT depends on individual progress, speech-language and developmental profile as well as existing co-morbidity (e.g. visual impairment). It should involve both patient and parents. Progress of speech-language and communication performance should be monitored frequently.

Counseling parents

This cross-sectional study clearly shows the complex clinical character of SLS. Yet, it provides detailed information when counseling the caregivers of a newly diagnosed SLS patient. Based upon these findings, the expected clinical profile relating to cognitive functioning, speech-language and communication performance as well as motor functioning can be drawn. Clinical variability urges one to use prognosis with caution.

Conclusion

This study provides an overview of the impairment in cognitive development and speech-language performance in a relatively large cohort of SLS patients. The tests performed in this study show that most of the patients suffer from moderate cognitive impairment which typically seems to reach a ceiling at 5–6 years of developmental age. There are no signs of progressive neurological disease contrasting to the common experience in most other neurometabolic disorders. Cognitive impairment is associated with language impairment that is more severe in those patients who reached the lowest developmental ages. Almost all patients suffer from a mild-to-moderate (pseudo bulbar) dysarthria which influences speech intelligibility and aggravates their already poor communicative daily life functioning. Taken together

the test results show that impairments in speech-language performance and communication are common and important clinical features of SLS. Early started SLT may play an important role to optimize speech-language performance and (augmented) communication and thereby improve the daily functioning of patients with SLS.

Acknowledgement

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Part two

Ophthalmological findings

4

Subclinical changes in the juvenile crystalline macular dystrophy in Sjögren-Larsson syndrome detected by optical coherence tomography

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Thomas Theelen

Abstract

Purpose: To study morphologic changes in the macula by optical coherence tomography (OCT) in patients with a crystalline macular dystrophy due to the autosomal recessive neurocutaneous Sjögren-Larsson syndrome (SLS).

Design: Retrospective observational case series.

Participants: Twenty-seven eyes of 14 patients, mean age 14.6 (range 3–24) years, with biochemically and genetically proven SLS underwent clinical and OCT investigation between September 2004 and September 2006.

Methods: All patients underwent full ophthalmologic examination including slit-lamp biomicroscopy and binocular ophthalmoscopy. Optical coherence tomography of all eyes was performed using the macular thickness map protocol of Stratus OCT.

Main Outcome Measures: Macular morphology in clinical examination and OCT.

Results: Beside clinically visible perimacular crystalline deposits in all eyes of all study participants, macular morphology and reflectivity were significantly changed on OCT compared with healthy eyes. We found focal hyperreflectivities in all study eyes within the perifoveal ganglion cell layer and the inner plexiform layer, corresponding to the clinical localization of retinal crystals. More interestingly, a cystoid foveal degeneration on OCT was present in the majority of patients with SLS (18/27 eyes, or 67% of all eyes studied), varying from multiple micro-cystoid spaces to cystoid foveal atrophy. In general, patients who were severely affected on OCT showed intense changes on previously performed cerebral magnetic resonance spectroscopy.

Conclusions: Patients with SLS show a childhood-onset crystalline macular dystrophy with cystoid foveal atrophy on OCT in most cases. The intra-retinal deposition of lipid metabolites may lead to Müller cell degeneration with subsequent formation of cystoid spaces or atrophic changes within the fovea. Because this macular dystrophy is present in all examined patients with SLS, familiarity with this maculopathy seems important for the diagnosis of this rare systemic disease.

Introduction

Sjögren-Larsson syndrome (SLS) is an autosomal-recessive, neurocutaneous disorder with a prevalence of <0.4 per 100 000 population.[1] The disease, which was originally described by Sjögren and Larsson in 1957, consists of a clinical triad of spastic diplegia or tetraplegia, mental retardation, and ichthyosis.[2] Sjögren-Larsson syndrome is caused by a disturbed lipid metabolism due to a deficiency of the microsomal fatty aldehyde dehydrogenase (FALDH) enzyme, which catalyzes the oxidation of many different medium- and longchain fatty aldehydes into fatty acids. Therefore, FALDH deficiency results in the accumulation of fatty alcohols and fatty aldehydes in body tissues. The accumulation of these lipid metabolites is considered the principal cause of the clinical symptoms of SLS.[3] In addition to the neurologic symptoms, most SLS patients suffer from photophobia and subnormal visual acuity. A crystalline maculopathy, which develops gradually within early childhood in patients with SLS, can be observed by ophthalmoscopy (Figure 1).[4] The crystalline dots are clinically scattered throughout the perifovea within the inner retinal layers. Although the central fovea seems to be uninvolved by crystal deposition, virtually all SLS patients have subnormal visual acuity. We hypothesized the presence of faint changes of the subclinical foveal anatomy, which could explain the disturbed visual function. The present paper describes the anatomic changes within the fovea of patients suffering from SLS, which were perceived by optical coherence tomography (OCT).

Figure 1 Color fundus photograph representative of the clinical appearance of juvenile macular dystrophy in Sjögren-Larsson syndrome. Note the white-yellow perifoveal crystals oriented alongside Henle's layer. The fovea of the patient seems clinically unremarkable, which is in contrast with the central cystoid space found on optical coherence tomography of the same eye (inset).

Materials and Methods

Patients

We included patients with SLS treated at the Department of Pediatric Neurology, Radboud University Nijmegen Medical Center, in our retrospective, observational case series. Our study was performed at the Department of Ophthalmology, Radboud University Nijmegen Medical Center, in accordance with the tenets of the Declaration of Helsinki 1975 (1983 revision). Local ethics committee approval was obtained, and informed consent to participate in this study was acquired for all subjects. In preparation for this study and when finishing our manuscript, we performed an extensive literature search by PubMed using the Medical Subject Heading (MeSH) database and the search terms "Sjögren-Larsson syndrome," "tomography, optical coherence," and "macula lutea." In doing so, we were unable to find any previously published literature covering our research topic. All study participants underwent a complete ophthalmologic examination, including slit-lamp biomicroscopy, Goldmann applanation tonometry, and binocular ophthalmoscopy. If possible, we determined the patients' best-corrected visual acuity. In addition, digital color fundus photographs (Imagenet, Topcon Corporation, Tokyo, Japan) of the posterior pole were acquired.

We included patients with no other accompanying ocular disorders that could affect retinal morphology, such as macular diseases or retinal dystrophies, diabetic retinopathy, inflammatory retinal disorders, or increased intraocular pressure. Diagnosis of juvenile macular dystrophy in SLS was established by characteristic, intraretinal, glistening yellow-white dots in the perimacula of both eyes accompanied by deficient FALDH activity in cultured skin fibroblasts and proof of the diagnosis at the genetic level by mutation analysis of the FALDH gene as described elsewhere.[1,4]

Image acquisition by OCT

We performed OCT scanning with a third-generation OCT (Stratus OCT, Carl Zeiss Meditech, Dublin, CA) using the custom software, version 4.0.1, to acquire and analyze images. Before OCT examination, sufficient pupil dilation was achieved with 1 drop of tropicamide 0.5% and 1 drop of phenylephrine 5%. One OCT image of the central retina, consisting of 6 B-scans, was recorded in each eye of the patients. In detail, we used radially oriented scans with a scan length of 6 mm and 512 A-scans per B-scan (macular thickness map scan protocol of the custom OCT software), resulting in approximately 10 μm axial and 12 μm transversal resolution. Only sections through the fovea were used for further analysis. Foveal sections were identified by the foveal depression and the lack of the inner retinal cellular layers. In addition, the infrared video fundus image of the OCT was used to ensure a transfoveal scan. Changes in morphology and reflectivity of the retinas of all SLS patients on OCT were analyzed by comparison with transfoveal OCT images of healthy maculae from age-matched patients.

Cerebral imaging

In a previous study, cerebral magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) results were systematically analyzed for the same cohort of patients described here.[1] In short, cerebral MRI showed normal brain anatomy, but white matter abnormalities in all patients. Cerebral white matter was systematically studied and graded normal (0), mildly (1), or severely (2) affected. Cerebral MRS showed an abnormal accumulation of lipids compared to a reference metabolite (*N*-acetylaspartate) in white matter that was graded as 0 (no lipid resonance visible), 1 (lipid resonance < resonance of N-acetylaspartate), or 2 (lipid resonance > *N*-acetylaspartate).

Because the retina originates from the diencephalon, one may expect changes in both retina and brain in this complex metabolic disease. We therefore compared the degree of cerebral abnormalities in MRI and MRS to macular affection on OCT.
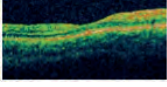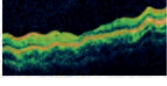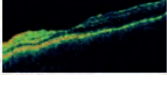
Results

Population
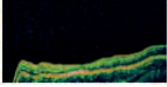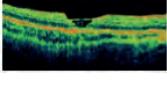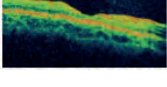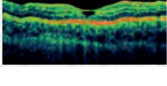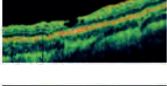
We found 28 eyes of 14 patients from 11 different families eligible to be included in our study and we obtained informed cons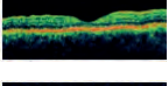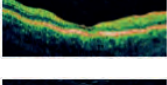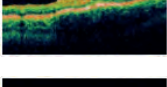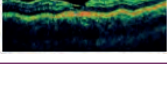ent from all patients. In one eye of one patient, the quality of the OCT scan was not suitable for further analysis, and in 14 eyes of 8 patients we were unable to gain the complete OCT dataset to create a retinal map. In all of these eyes, however, we were able to obtain at least one transmacular B-scan for a detailed morphological analysis of the fovea. Eventually, a total of 27 (14 right and 13 left) eyes of 14 patients (6 male, 8 female; mean age, 14.6 years; range 3–24 years) were included in our study.

All patients showed the classic clinical phenotype of SLS with ichthyosis, spasticity, and mental retardation. In addition, a crystalline maculopathy could be detected by ophthalmoscopy in all study participants. Figure 2 depicts our major study results, including patients' demographics, visual acuity, and results of OCT, MRI, and MRS.

Optical coherence tomography

The OCT analysis of normal maculae showed the clearly distinguishable bands presumed to correlate with the normal anatomic configuration of a healthy macula as described elsewhere.[5] When compared with healthy eyes, changes in macular OCT scans were observed in all eyes of all SLS patients studied. These changes were symmetric for both eyes of a study participant in all cases.

Focal hyperreflective spots on OCT corresponding to retinal crystals were observed in all study eyes without exception. The focal increased reflectivities on OCT could be observed within inner perifoveal layers of the retina, that is, within the

Patient	Sex; Age (years)	Visual acuity OD – OS	Cerebral MRI grading	Cerebral MRS grading	OCT Grading	OCT Image
1	F; 3	NR	2	0	1	
2	F; 5	NR	1	0	1	
3	M; 8	NR	1	1	2	
4	M; 9	NR	1	2	1	
5	M; 13	20/125 - 20/125	1	2	3	
6	F; 14	20/50 - 20/50	1	1	1	
7	M; 15	20/50 – 20/50	1	2	3	
8	M; 15	20/50 – 20/40	1	2	3	
9	F; 15	20/63 – 20/32	1	1	1	
10	F; 19	20/32 – 20/50	1	0	3	
11	F; 20	20/32 – 20/40	1	1	2	
12	M; 22	20/80 – 20/80	2	2	3	

| 13 | F; 23 | 20/63 – 20/63 | 2 | 1 | 1 | |
| 14 | F; 24 | 20/80 – 20/80 | 1 | 2 | 2 | |

Figure 2 Demographics, visual acuities, and imaging results of patients with macular dystrophy in Sjögren-Larsson syndrome. To facilitate data comparison, the mild, moderate, and severe forms of OCT results are simplified to OCT grades 1, 2, and 3, respectively (grading here does not allow any assumptions about retinal function or longitudinal disease progression).

F = female; M = male; MRI = magnetic resonance imaging; MRS = magnetic resonance spectroscopy; NR = not recordable; OCT = optical coherence tomography; OD = right eye; OS = left eye.

ganglion cell layer and the inner plexiform layer (Figure 3A). The hyperreflective spots were located at the site of the intraretinal yellow-white dots as documented by color fundus photographs, and their number increased by a clinically larger number of crystals.

In 10 eyes of 5 patients an inconsistent number of intraretinal microcystoid spaces were present on OCT within the foveal and parafoveal inner retinal layers (Figure 3B). An additional 8 eyes of 4 patients showed a single, large cystoid space right within the fovea, which was covered by a thin, intermediate to high reflective membrane. Occasionally, spots of increased reflectivity, suggesting floating material, were observed within these large foveal cystoid spaces (Figure 3C).

From comparison of the patients' OCT scans and the corresponding MRS results, one may consider a positive data relationship, because most patients with severe macular changes on OCT also revealed strong abnormalities on MRS. However, to verify this possible relationship, a larger sample is required to allow statistical analysis. Changes on conventional MRI scanning did not show a clear association with the OCT results. Because of the small datasets, no statistical analysis was performed.

We could gain retinal maps in 14 eyes (6 right eyes) of 8 SLS patients (4 males) of a mean age of 17 (range 13–24) years. The mean central macular thicknesses were $144.6\pm28.8\,\mu$m and $146.6\pm24.9\,\mu$m in the right and left eyes, respectively. The mean central foveal thickness in our study eyes was thinner than recently published normal measurements, which range from $203.8\pm19.5\,\mu$m to $212.0\pm20\,\mu$m.[6,7] We were able to achieve follow-up maps in 5 eyes (3 patients) after a period of 15 ± 2 months, and observed a slight increase in macular thickness on OCT in these eyes. The mean central macular thicknesses after 1 year in this subpopulation changed from $124.8\pm19.3\,\mu$m to $137.7\pm24.8\,\mu$m.
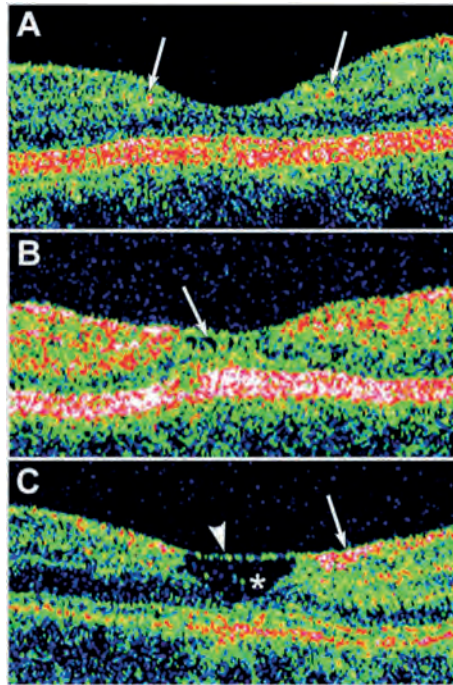
Figure 3 A. Optical coherence tomography (OCT) view of the mild form of juvenile macular dystrophy in Sjögren-Larsson Syndrome (SLS). Note the focal hyperreflectivities (arrows) sited at locations of clinically visible intraretinal crystals. The shape of the fovea as well as structure and reflectivity of the photoreceptors seem normal. **B.** An OCT view of the moderate form of juvenile macular dystrophy in SLS. The fovea is thinned with a trodden shape. Microcystoid intraretinal spaces of low reflectivity emerge within the inner parts of the fovea (arrow). **C.** An OCT view of the severe form of juvenile macular dystrophy in SLS. A large cystoid space including some hyperreflective spots has appeared within the fovea (asterisk). A thin structure, presumably being a complex of the retinal inner limiting membrane and the posterior vitreous membrane, covers the foveal cystoid space (arrowhead). A prominent hyperreflective area corresponds to the perifoveal crystals observed clinically (arrow). Note the structural change and increased reflectivity of the central parts of the photoreceptor inner segments–outer segments complex.

Discussion

In this paper, we describe a peculiar macular dystrophy in patients suffering from an autosomal-recessive inherited deficiency of FALDH that causes a neurocutaneous disease called SLS. Beside a thinned central fovea, we found typical changes of the macular anatomy on OCT scans in all study participants. We observed focal hyper-reflectivities, which corresponded to clinically visible intraretinal crystals. Most interestingly, we frequently found clinically invisible intra-foveal cystoid changes on OCT scans in the majority of our patients.

The focally increased reflectivity on OCT seems to be located in the perifoveal ganglion cell layer and inner plexiform layer. These layers are mainly composed of axons and dendrites of ganglion cells and retinal interneurons. The arrangement of crystals both on OCT and fundoscopy suggests participation of the perifoveal ganglion cells in the deposition of abnormal lipids in SLS. This finding correlates with the known involvement of ganglion cells in other lipid storage diseases showing fundus abnormalities, for instance in lysosomal storage disorders.[8] In the present study, no abnormalities were found by fundoscopy in the peripheral parts of the retina. Previously described biochemical and metabolic differences between the macula and the peripheral parts of the retina may explain this dissimilarity, and an increased lipid turnover in the central retina may play a role in the location of the crystalline deposits in the perimacula.[9,10]

The development of macular cystoid spaces does not seem to correlate to the patients' age; patients of more or less the same age may have quite different stages of cystoid changes. Even within the same family a younger patient may show a more severe stage of maculopathy than an older sibling. Furthermore, follow-up OCT in 5 eyes of 3 patients after a period of 15 months did not reveal any major changes of the cystoid macular dystrophy. Individual variation in progression of the maculopathy and differences in genetic background may play a part in the creation of this heterogenic subclinical picture.

According to our present findings, we propose a differentiation of the OCT results regarding patients with SLS maculopathy into 3 forms, namely, mild, moderate, and severe. In the mild form, the OCT image shows focal perifoveal intraretinal hyper-reflectivities (Figure 3A). The moderate form of maculopathy is characterized by microcystoid changes within the thinned fovea in addition to perifoveal intraretinal hyperreflectivities (Figure 3B). Finally, in severe maculopathy, a foveal macrocystoid space appears within a ring of strong perifoveal hyperreflectivities (Figure 3C). The thin, highly reflective membrane on top of the solitary large cystoid spaces may represent a structural complex of the posterior vitreous cortex and the retinal inner limiting membrane.

Multiple, small, intraretinal cystoid structures as well as single, large, central foveal cystoid spaces were found on OCT and seemed to be located in the outer

plexiform layer and inner nuclear layer of the retina. The inner nuclear layer mainly contains nuclei and cell bodies of Müller glia cells whereas the outer plexiform layer is composed of synapses between photoreceptors and horizontal cells or bipolar cells and the cytoplasm of Müller glia cells. The typical localization of the cystoid spaces in our patients, together with the only mild-to-moderate loss of visual acuity, suggests a major affection of the foveal Müller cells and not the photoreceptors. If neuronal retinal cells or photoreceptors would be primarily involved in this macular dystrophy, one would expect a more severe visual impairment in these patients. Increasing loss of Müller cells with disease progression, however, may lead to foveal thinning and eventual formation of a cystoid space under partial preservation of visual acuity. We were unable to detect significant changes within fifteen months of observation in 3 of 14 study participants. Therefore, we believe that foveal atrophy in SLS may be a time-consuming process and a prolonged follow-up period in a larger number of patients may be necessary to illustrate disease progression.

In pediatric neurology, SLS is considered to be a typical example of a white matter disorder, and therefore the affection of oligodendrocytes located in the CNS may play a pivotal role in disease development. Müller cells and oligodendrocytes, however, share many structural and functional similarities.[1,11] The classification of SLS as a white matter disorder and the OCT findings in the present study point to the Müller cell and the retinal ganglion cell layer as the most probable retinal tissue components involved in the pathogenesis of the juvenile macular dystrophy in SLS. Here, direct effects of the FALDH enzyme deficiency or the subsequent accumulation of fatty alcohols may be the underlying biochemical processes for crystal deposition and formation of cystoid spaces.[1]

In conclusion, to the best of our knowledge, we offer the first description of the subclinical foveal alterations on OCT in patients with crystalline macular dystrophy in SLS. After an extensive literature research, we were unable to find any other reports covering this issue. In addition to the perifoveal deposition of intraretinal crystals, a cystoid foveal atrophy was visible on OCT. Because the latter was highly variable between individual SLS patients, we propose a differentiation into 3 forms to offer a simplified description of these microscopic changes. Our study illustrates the potential of OCT for determination and ranking of the maculopathy in SLS patients, which may allow assumptions about the CNS of SLS patients. Other than MRI and MRS, examination by OCT provides a straightforward low-cost approach to the outermost part of the CNS, the retina, which may be helpful in future SLS treatment trials.

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5

Patients with Sjögren-Larsson syndrome lack macular pigment

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Abstract

Purpose: Sjögren-Larsson syndrome (SLS), an autosomal recessive hereditary disorder with congenital ichthyosis, spastic diplegia or tetraplegia, and mental retardation, reveals a characteristic macular dystrophy with intraretinal crystals and foveal pseudo-cysts. Ophthalmic symptoms in SLS are reduced visual acuity and photophobia. This article reports the deficiency of macular pigment as a novel finding in this peculiar, congenital maculopathy.

Design: Cross-sectional, observational case study.

Participants: Patients with clinically and genetically proven SLS.

Methods: Besides general ophthalmologic examination, 2 different methods were used; fundus autofluorescence (FAF) and fundus reflectometry with the macular pigment reflectometer (MPR), for measuring macular pigment (MP).

Main Outcome Measures: Distribution profiles and quantity of MP in eyes of SLS patients.

Results: Twenty-eight eyes of 14 patients were included. The technique to measure MP depended on the ability of the mentally handicapped patients to cooperate. Fundus autofluorescence images providing qualitative estimates were obtained from 9 eyes of 5 patients, and MPR measures providing quantitative estimates were obtained from 19 eyes of 10 patients. Fundus autofluorescence images of SLS patients lacked the typical attenuation of macular FAF signal expected in normal eyes. Mean foveal MP levels measured by MPR showed significantly lower values in SLS patients (0.10 ± 0.07) than in healthy individuals (0.69 ± 0.17; $P<0.001$, Student t test).

Conclusions: The group of SLS patients studied here had significantly reduced levels of foveal MP. The crystalline macular dystrophy in SLS seems to be the first known disease with a genetically caused deficiency of MP.

Introduction

Sjögren-Larsson syndrome (SLS), as first described by Sjögren and Larsson in 1957, is an autosomal recessively inherited disorder characterized by mental retardation, spastic diplegia, and congenital ichthyosis.[1] The disease is caused by an enzymatic defect in lipid metabolism, namely microsomal fatty aldehyde dehydrogenase (FALDH) deficiency. Fatty aldehyde dehydrogenase catalyzes the oxidation of many different medium-and long-chain fatty aldehydes into fatty acids. Fatty aldehyde dehydrogenase deficiency results in the accumulation of fatty aldehydes and fatty alcohols in body tissues, which is considered the principal causative mechanism leading to the clinical symptoms of SLS.

Besides the neurocutaneous features, patients with SLS exhibit a typical crystalline juvenile macular dystrophy with onset in childhood, which is characterized clinically by reduced visual acuity and photophobia.[2] In addition to the crystalline deposits located at the inner retinal layers, a cystoid foveal atrophy has been detected, possibly based on an underlying Müller cell disorder, by optical coherence tomography (OCT).[3]

Macular pigment (MP) is a mixture of 2 carotenoids, lutein and zeaxanthin. The concentration[4] and spatial distribution[5–7] of lutein and zeaxanthin varies greatly among individuals. In humans, carotenoids are acquired only through dietary intake. Peak concentration of MP depends on many factors, such as geographic location and lifestyle, that is, nutritional habits, smoking, and supplemental intake.[8–11] Regardless of the wide ranges of MP concentrations under physiologic circumstances, MP is not totally absent in a healthy individual, and optical density levels of MP below 0.2 are extremely rare.[12,13] Macular pigment is predominantly located in the photoreceptor axon layer and the inner plexiform layer of the macula,[14] where it probably plays a role in photoreceptor protection by neutralizing free oxygen radicals and by absorbing potentially phototoxic, high-energetic blue light (400–530 nm).[15,16]

The macula of SLS patients lacks the normal dark yellowish appearance, a feature that had not been appreciated fully previously.[2,3] This is probably because the phenomenon easily could be overlooked by the considerable number of bright, glistening crystals at the macula. To decide whether an altered distribution or concentration of MP could be responsible for the changed macular shades, previous ophthalmologic studies on SLS patients with fundus autofluorescence (FAF) and spectral fundus reflectometry were expanded to compare the levels of lutein and zeaxanthin in the eyes of SLS patients with those of healthy controls.[17,18]

Patients and Methods

A cross-sectional, observational study was performed on patients from an earlier investigated population with clinically and genetically proven SLS,[3] treated at the Departments of Ophthalmology and Pediatric Neurology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. The study was performed in accordance with the tenets of the Declaration of Helsinki (1983 revision). Local ethics committee approval was obtained, and informed consent to participate in this study was acquired from all subjects. Because of the mental retardation in SLS, informed consent for study participation also was received from the patient's parents or legal guardian.

Limited cognitive capacities, photophobia, and inability for stable fixation resulting from spasticity complicated the clinical examinations, especially the acquisition of autofluorescence images and fundus reflectometry. Therefore, data using these methods could be obtained only in a subset of patients. The diagnosis of SLS was proven in all patients by deficient FALDH activity in leukocytes or cultured skin fibroblasts and mutation analysis of the FALDH gene.[19]

Besides a general ophthalmic examination that included best-corrected visual acuity, slit-lamp biomicroscopy, binocular ophthalmoscopy, digital color fundus photographs (Imagenet; Topcon Corporation, Tokyo, Japan) also were obtained. In addition, OCT scans (transmacular line scans) and FAF images were performed by a hybrid OCT– confocal scanning laser ophthalmoscope (Spectralis; Heidelberg Engineering, Heidelberg, Germany).

The protocol to derive FAF images was applied as described elsewhere.[20] In the presence of MP, decreased levels of autofluorescence usually are visible in the macular area because of the blue light-absorbing properties of MP. To quantify MP, a 2-wavelength method (blue λ, ~460 nm; green λ, ~530 nm) should be used, which is technically impossible with Spectralis; therefore, only a qualitative estimation on the presence of MP was realizable in this study arm.

To show a possible loss of MP objectively, the optical density of macular pigment (MPOD) by fundus reflectance spectroscopy using the Macular Pigment Reflectometer (MPR) was measured.[21,22] Details of this setup and the spectral analysis used are described by van de Kraats et al[17] and by Berendschot et al[23] and Berendschot and van Norren,[24] respectively. To determine MPOD values at different eccentricities, the MPR was modified by adding 5 fixation points at 1, 2, 4, 6, and 8 degrees next to the central measurement field of 1 degree.[18,25] Because of a fixed location of the eccentric fixation points in the MPR, they are always presented on the right side of the central white light (i.e., temporally for the right eye and nasally for the left eye). A single measurement on the MPR takes 1 second. The mean of 5 measurements was used. For comparison with normals, MPOD spatial profiles were taken from van de Kraats

et al,[18] who measured 23 healthy subjects (mean age $\pm$ standard deviation, 24.3$\pm$2.7 years) at 0, 1, 2, 4, and 8 degrees using a similar device for determination of MPOD.

Results

Twenty-eight eyes of 14 patients with SLS (7 male, 7 female; mean age $\pm$ standard deviation, 20$\pm$9 years) were included. Patient demographics, best-corrected visual acuity, FAF data, and MPOD measurements are summarized in Table 1. All patients showed the characteristic crystalline macular dystrophy on ophthalmoscopy and OCT. In contrast to normal fundus appearance, a relatively homogenous orange background reflectance was observed beneath the area of the obvious glistening white crystals (Figure 1).

Confocal Fundus Autofluorescence Imaging

Fundus autofluorescence images were obtained in 9 eyes of 5 patients with a mean age $\pm$ standard deviation of 23.2$\pm$9.1 years and revealed important differences as compared with 20 eyes of 10 healthy controls with a mean age $\pm$ standard deviation of 24$\pm$3 years (Figure 2).

In all patients, the typical FAF attenuation in the fovea seen in healthy eyes was absent. Instead, the background FAF as seen in the periphery was continued within the macular region. In addition, focal FAF increase was observed at the foveal center in 7 of 9 eyes examined. Occasionally, spots of decreased FAF were observed perifoveally, which were not co-located with intraretinal crystals or pseudocysts. No FAF abnormalities were discerned outside the macular region.

Fundus Reflectance Spectroscopy

Fundus reflectometry was performed successfully with central fixation in 19 eyes of 10 patients (mean age $\pm$ standard deviation, 17.4$\pm$10.5 years; median, 16.5 years; range, 5–38 years). Although the mean age of SLS patients differed significantly from the mean age of the control subjects for MPR measurements (mean age $\pm$ standard deviation, 24.2$\pm$2.7 years; median, 23.9 years; range, 20 –32 years; $P = 0.005$), the data comparison was considered appropriate. The small but significant age difference between cases and controls is unlikely to have an impact on MP density, because the relationship between MPOD and age, if any, is marginal.[26] Furthermore, MP also has been perceived in otherwise healthy premature infants and children.[9,27]

Peripheral fixation proved to be difficult for these subjects, resulting in fewer peripheral data. The spatial profile of MPOD from SLS patients showed very low values at all eccentricities and lacked the central peak present in healthy eyes (Figure 3).

Table 1 Demographics, visual acuity, and macular pigment optical density of patients with Sjögren-Larsson syndrome.

Patient	Age (yrs)	Gender	Macular Pigment Optical					
			Best-Corrected Visual Acuity		Fundus Autofluorescence		Density at Central Fixation (Fovea)	
			Right Eye	Left Eye	Right Eye	Left Eye	Right Eye	Left Eye
1	5	M	NA	NA	NA	NA	0.012	0.000
2	6	F	NA	NA	NA	NA	0.031	0.077
3	8	F	0.16	0.2	NA	NA	0.052	0.263
4	13	M	0.3	0.4	NA	NA	0.041	0.095
5	15	M	0.3	0.3	NA	NA	0.216	0.096
6	16	M	0.25	0.25	Abnormal*	Abnormal*	NA	NA
7	18	M	0.4	0.4	Abnormal*	Abnormal*	0.096	0.065
8	18	F	0.6	0.6	Abnormal*	Abnormal*	0.067	0.074
9	26	F	0.003	0.2	NA	Abnormal*	NA	0.125
10	27	F	0.3	0.3	NA	NA	0.100	0.103
11	38	M	0.2	0.2	Abnormal*	Abnormal*	0.231	0.179

F = female; M = male; NA = not assessable because of mental retardation or photophobia.

* : for description see text and Figure 2.

Figure 1 Fundus appearance of the macula in Sjögren-Larsson syndrome. Color fundus photographs of (**A**) a patient with Sjögren-Larsson syndrome and (**B**) a normal control. The brightness of the macular background is relatively homogenous in the patient, whereas a considerable central darkening resulting from macular pigment is present in the healthy control. Note the large number of intraretinal crystals in the Sjögren-Larsson syndrome patient.
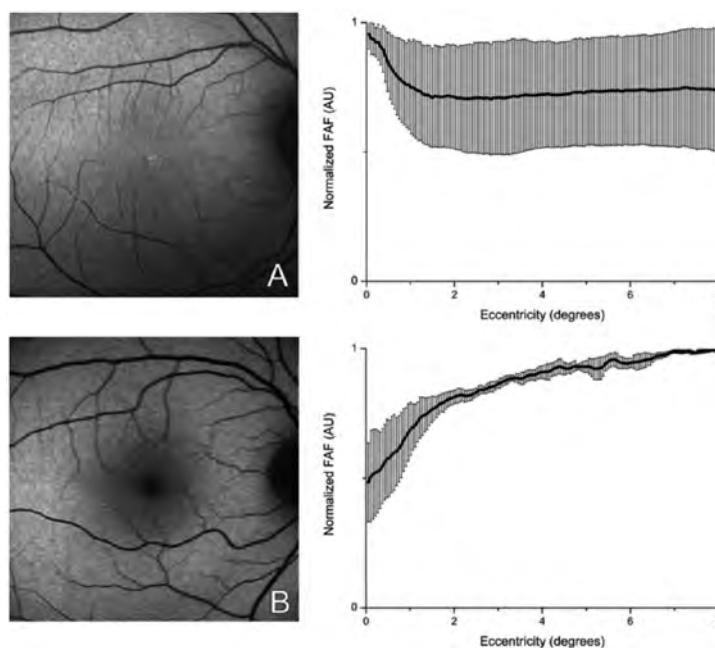

Figure 2 Fundus autofluorescence (FAF) characteristics in Sjögren-Larsson syndrome. Fundus autofluorescence sample images and profiles of mean (error bars indicate standard deviation of mean) in (**A**) patients with Sjögren-Larsson syndrome and (**B**) healthy, age-matched controls. Study subjects show an autofluorescence maximum within 1-degree eccentricity, which is roughly 25% more than the peripheral levels. Instead, healthy individuals show central attenuation of autofluorescence of approximately 50% and highest values in the periphery. AU = arbitrary units.
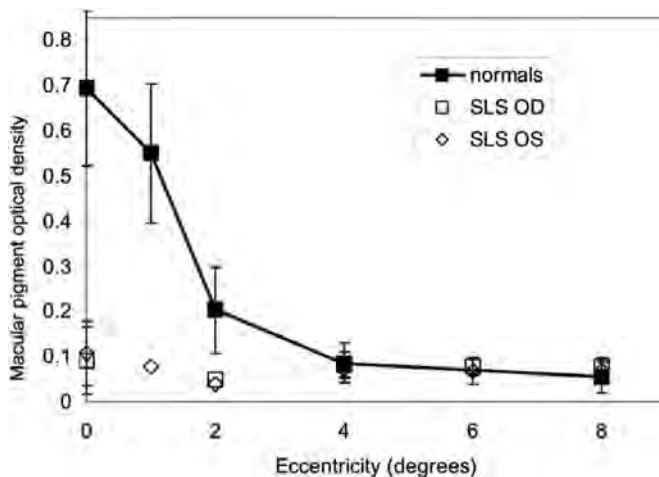
Figure 3 Graph showing macular pigment optical density measurements in Sjögren-Larsson syndrome (SLS). Macular pigment optical density (MPOD) is expressed on the vertical axis, against foveal eccentricity in degrees on the horizontal axis. Data of patients with SLS, obtained from 9 right eyes (OD) and 10 left eyes (OS; see Table 1), had significantly lower foveal MPOD values compared with healthy controls ($P<0.001$, Student t test). Error bars indicate the standard deviation. The line is drawn as a guide to the eye. At 1 degree, only one measurement was available in the SLS cohort, and at 2 degrees, only two measurements were available in the SLS cohort. Therefore, no error bars were placed here.

Mean MPOD values at 0, 4, 6, and 8 degrees of eccentricity were always less than 0.13. At the fovea, mean MPOD (mean ± standard deviation, 0.10±0.07) was decreased significantly compared with that of control subjects (mean ± standard deviation, 0.69±0.17; $P<0.001$, Student t test). Spectral subanalysis revealed that MP in SLS almost solely constituted of lutein and that zeaxanthin was virtually absent. Average foveal zeaxanthin fraction (zeaxanthin/lutein plus zeaxanthin) was 0.14, compared with 0.24 in healthy controls ($P<0.001$, Student t test).

Discussion

This article reports on the lack of macular pigment in patients with juvenile, crystalline macular dystrophy resulting from SLS. This was indicated by the absent yellowish reflex of macular pigment on fundoscopy and the missing macular FAF attenuation in SLS patients. Quantitative analysis by MPR revealed virtually complete absence of carotenoids, especially zeaxanthin, within the central retina of SLS patients. Recently,

acquired partial MP loss has been reported in patients with macular telangiectasia type 2[25]; however, the authors are not aware of any earlier description of virtually complete absence of MP resulting from a genetic disorder. Normally the MPOD peaks at, or near, the foveal center and then decreases rapidly with eccentricity.[5,7] The shape of MPOD distribution in SLS, however, seems to be flat over the central 8 degrees of eccentricity, lacking the well-known profile of MPOD observed in healthy subjects.

The findings of this study correspond with the ophthalmic symptoms observed in SLS patients. Short-wavelength light, which is filtered by MP in large part, has been shown to induce more intraocular stray light than light of longer wavelengths.[28] Reduction or absence of MP therefore may lead to increased intraretinal straylight and hence, photophobia. Furthermore, it has been demonstrated that the amount of MP, clearly reduces the deleterious effects of glare, an effect that was supposed to be based mainly on filtering.[29] In addition, the central levels of MP seem to contribute to improved visual performance, mainly in the short-wavelength spectrum (Snodderly DM, et al. Invest Ophthalmol Vis Sci:E-Abstract 2009;50:1702). Besides retinal thinning and pseudocyst formation, the absence of macular pigment together with the presence of highly refractive intraretinal crystals may induce increased amounts of stray light at the fovea, and hence, may play an important role in the ophthalmic symptoms of SLS patients, such as reduced visual acuity and photophobia. The lack of MP-related protection from high-energetic photons also may add to the degenerative processes, leading to macular thinning and pseudocyst formation.

Optical coherence tomography imaging in SLS patients displayed intraretinal crystals within the central 8 degrees of foveal eccentricity.[3] These crystalline deposits seemed to be located mainly in the ganglion cell layers and inner plexiform layer. Thus far, the biochemical nature of the glistening dots remains unknown; however, the site of their location on OCT corresponds with the histologic position of MP in the normal retina.[14] This co-localization suggests a relationship of the formation of intraretinal crystals and the absence of MP.

The human body is unable to synthesize any carotenoids, and therefore is completely dependent on dietary carotenoids, including lutein and zeaxanthin. Lutein and zeaxanthin are absorbed by intestinal mucosal cells and incorporated into chylomicrons that are secreted into the lymphatic system and subsequently enter the circulation. Hepatic cells incorporate the carotenoids from the chylomicrons into lipoproteins that facilitate further transport of the carotenoids to the various body tissues. Transportation, distribution, and uptake of plasma lipoproteins are regulated by apolipoproteins that are produced by many organs, including the liver. Apolipoprotein-E, which is produced in the eye by Müller cells and in the retinal pigment epithelium (RPE), is known to play a part in lipid transportation and binding of lipoproteins to target sites within the central nervous system and in the targeted

uptake of the lipoproteins carrying lutein and zeaxanthin within the retina. Interestingly, Müller cell degeneration seemed to be involved in the development of the juvenile maculopathy in SLS.[3] Furthermore, normal plasma levels of lutein and zeaxanthin were found in SLS patients (Willemsen MAAP, doctoral thesis, Radboud University Nijmegen, Faculty of Medicine, Nijmegen, The Netherlands, 2001; available at: http://ubn.ruhosting.nl/dissertatie/dissertaties_1J2001.html; accessed September 6, 2009). Therefore, a disturbed retinal MP metabolism, presumably located within the retinal Müller cells, seems to be the most probable reason for MP deficiency in SLS. The characteristic crystals seen in patients with SLS typically develop in the first 2 years of life. The metabolic pathway, in which the FALDH deficiency contributes to the appearance of these deposits, is yet undiscovered. The location and time of manifestation, together with absence of MP, suggests that the glistening dots are spin-offs of a disturbance in MP metabolism.

The macula has a very active lipid metabolism that could be affected by FALDH deficiency in many different ways. A literature search revealed only a single histo-pathological case report, describing the postmortem retinal changes in a 23-year old SLS patient.[30] At the level of the macular retinal pigment epithelium, an increased amount of lipofuscin granules and a significant decrease in the amount of melanin and melanolipofuscin granules was observed. The lack of MP probably leads to uncontrolled oxidative stress, which may cause photoreceptor damage and accumulation of waste products indigestible for the RPE. Furthermore, FALDH deficiency itself results in the accumulation of many fatty alcohols and fatty aldehydes that in vitro also can react with phosphatidylethanolamine, forming lipofuscin like substances.[31]

The aforementioned pathophysiological processes are very likely to cause the focally increased FAF in most of the SLS patients examined here. Recently, 2 additional cases of with similar changes of foveal FAF have been described.[32] The authors concluded that the glistening dots may be the source of increased FAF in SLS. However, in the current series of 9 eyes, spots of increased FAF were not co-located with the crystals seen on fundus photography in any case. In contrast, increased FAF occurred mostly at locations where no crystals were visible. Therefore, foveal hyperfluorescence in SLS most likely originates from increased levels of retinal and subretinal fluorophores, that is, lipofuscin and its derivates.

In SLS, the presumed Müller cell degeneration with subsequent absence of MP leads to increased photooxidative stress. The formation of highly active free oxygen radicals and oxidation of lipofuscin causes damage to the retinal pigment epithelium and eventually photoreceptor degeneration. This additionally may lead to increased accumulation of lipofuscin and retinal pigment epithelium degeneration, reflected by focally increased and decreased FAF in SLS, respectively.

Dietary lutein supplementation, especially for the purpose of age-related macular degeneration prevention, has been investigated extensively in past decades. The

macular response to increased lutein and zeaxanthin serum concentrations is not similar in all patients, and the increase of MPOD varies strongly.[8,33,34] The role of MP increase in late age-related macular degeneration prevention remains controversial. It is also uncertain whether supplementation of carotenoids is beneficial in SLS patients. First, blood levels of carotenoids are supposed to be normal, as found by Willemsen (Willemsen MAAP, doctoral thesis, 2001. Available at: http://ubn.ruhosting.nl/dissertatie/dissertaties_ 1J2001.html; accessed September 6, 2009) and therefore whether uptake in the retina is appropriate is uncertain because the SLS patients measured herein displayed very low levels of MP despite normal carotenoid blood levels. Second, highly concentrated carotenoids may undergo oxidation into so-called carotenoid derived aldehydes, which are highly reactive and can cause significant oxidative stress that is comparable with the oxidative stress from lipid peroxidation products.[35] Therefore, a risk for additional retinal damage resulting from carotenoid supplementation in SLS patients cannot be excluded.

In conclusion, the juvenile, crystalline maculopathy in SLS shows a significant lack of central macular pigment compared with healthy eyes. This may be caused by a reduced carotenoid accumulation in the macula. Additional focal changes of fundus autofluorescence point to increased foveal lipofuscin content and parafoveal atrophy of the retinal pigment epithelium. This may be the result of increased photo-oxidation and cellular toxicity as a consequence of macular pigment deficiency. Based on the complex pathobiochemistry in SLS, a complementary diet with carotenoids may be harmful.

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Part three

Dermatological findings

6

Zileuton for pruritus in Sjögren-Larsson syndrome

A randomized double-blind placebo-controlled crossover trial

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Abstract

Patients with the Sjögren-Larsson syndrome (SLS) suffer from a generalized ichthyosiform hyperkeratosis with a striking pruritic character resulting in excoriations and scaling. We performed a randomized double-blind, placebo-controlled trial in crossover design to assess the effect of blocking leukotrienes synthesis with zileuton on general skin condition and pruritus in ten SLS patients. Erythema, desquamation, lichenification and excoriations were scored each visit complemented with a Physician Global Assessment (PGA) of the skin. Primary outcome measures were changes in scores during treatment with zileuton. Also, self-reported visual analogue scales (VAS) for pruritus and excoriations were studied. Individual data analysis showed a consistent and convincing response to treatment in one patient (success rate approximately 10%). Therefore, we suggest a therapeutic trial with zileuton during 4-6 weeks in all patients with SLS ≥5 years old with severe pruritus. If no clear beneficial response is noted during this period, treatment should be discontinued.

Introduction

Sjögren-Larsson syndrome (SLS) is an autosomal recessive neurometabolic disorder characterized by ichthyosis, spastic di- or tetraplegia and intellectual disability. Mostly, the ichthyosis is congenital or develops early in life. The neonatal skin has an erythrodermic appearance gradually evolving into a generalized ichthyosiform hyperkeratosis during infancy. Incidentally, SLS presents with a collodion membrane.[1] Skin lesions follow a generalized distribution pattern being more prominent in the flexural areas, neck and lower abdomen sparing the central face. The hyperkeratosis thickens the skin, resembling lichenification, and produces a yellowish dark-brown color (Figure 1). Ichthyosis in SLS differs from other cornification disorders by the pruritic character resulting in excoriations and scaling.

Figure 1 Ichthyosis in SLS. The hyperkeratosis thickens the skin, resembling lichenification, and produces a yellowish dark-brown color.

SLS is caused by mutations in the *ALDH3A2* gene located on chromosome 17 which results in a deficiency of microsomal fatty aldehyde dehydrogenase (FALDH) causing a disturbed bodily lipid metabolism.[2,3] The lipid metabolism plays an important part in the normal formation of the water barrier in the stratum corneum. In SLS patients, lamellar bodies, synthesized in the stratum granulosum and normally containing essential precursor membranes, are misshapen and empty. This results in defective membrane formation and a leaky water barrier. To restore function, the skin reacts by hyperkeratosis resulting in ichthyosis.[4-6]

FALDH is important for formation and excretion of lamellar bodies. Deficiency leads to several disrupted lipid pathways, but it is unclear which specific pathways are involved in the SLS dermatological phenotype. Pruritus may, however, have another pathophysiological origin. Previously, we showed an association between pruritus and elevation of pro-inflammatory leukotriene B4 (LTB4), which is also FALDH-dependent for its breakdown. This association was later confirmed in experimental mice studies.[7,8] In allergic skin inflammation (e.g. atopic dermatitis, psoriasis etc.), LTB4 plays a crucial part in the inflammatory response.[9] In SLS, elevation of dermal LTB4, by either increased production or defective breakdown or a combination of both, probably plays an important role in its dermatological phenotype. Zileuton is an active oral inhibitor of 5-lipoxygenase, an enzyme forming leukotrienes from arachidonic acid. Blocking this pathway results in reduced formation of leukotrienes, including LTB4.

Before, we studied zileuton treatment in 5 SLS patients (non-placebo controlled study).[10,11] Treatment reduced urinary concentrations of LTB4 and 20-OH-LTB4, indicating in vivo effectiveness. Clinically, beneficial effects on pruritus and general well-being were described. To further investigate the effects of zileuton on the skin, we performed the current randomized controlled trial (RCT).

Materials and methods

Study design and participants

We used a crossover design in this single-center RCT, resulting in each participant being its own control. Approval of the Regional Committee on Research involving Human Subjects Arnhem-Nijmegen was obtained. From June 1st 2012 to November 1st 2012, patients were enrolled at the Radboud university medical center (Radboudumc) in Nijmegen, the Netherlands. Because of substantial intellectual disability in all patients, informed consent was obtained from their parents.

Patients with genetically and biochemically confirmed Sjögren-Larsson syndrome, aged 5-60 years were included in the study. Exclusion criteria were (1) hepatic failure; (2) usage of theophylline, cumarin derivates, beta-blockers, terfenadine and/or pimozide; (3) alcohol consumption or smoking; and (4) pregnancy or lactation. All patients continued current dermatological treatment ('daily practice') during the study period.

Treatment

Zileuton is US FDA approved for chronic treatment of asthma in patients ≥ 12 years. Zileuton is not approved by the European Medicines Agency (EMA) or the Dutch Medicines Evaluation Board. Inhibition of LTB4 biosynthesis in whole blood is directly

related to zileuton plasma concentration.[12,13] Since we expected the same mechanism of action to be responsible for reduction of pruritus in SLS, our aim was to achieve similar exposure to zileuton as in asthmatic patients. Zileuton pharmacokinetics in asthmatic adult patients are comparable to those in healthy volunteers.[13-15] Also, pharmacokinetics of zileuton in children (9-12 years) are comparable to those in adults after the adjustment of dose for body weight or body surface area.[13]

For patients ≥ 12 years of age, dosages as currently approved for the treatment of asthma (zileuton 600mg quater in die; qid) were used. To determine dosages for younger patients, the guidance for pediatric dosing was used.[16] Zyflo® 600mg tablets were provided by the manufacturer (Cornerstone Therapeutics Inc., US). Placebo or zileuton capsules with identical appearances were prepared in the hospital pharmacy.

Randomization and allocation to treatment

All patients received treatment with either zileuton or placebo in two periods of 8 weeks, separated by a wash-out period of 4 weeks. Based upon the pharmaco-kinetics and pharmacodynamics of zileuton, these treatment periods were considered long enough to demonstrate clinical effects.[17] A 4-week wash-out period was considered sufficient because of the short half-life of both zileuton (2.5h) and leukotrienes.[18] Treatment order was randomly assigned and randomization was performed by the hospital pharmacy. Assessors and patients were blinded to assignment of treatment.

Data collection

Visits were scheduled for all patients at t=0 (start first treatment period), t=8 weeks (stop first treatment period), t=12 weeks (stop wash-out period and start second treatment period), t=20 weeks (stop second treatment period) and t=24 weeks (stop second wash-out period). At t=0, baseline measurements were performed by two assessors (M.S. & J.F.) producing one mutual assessment for all patients. All subsequent measurements were performed by one assessor (J.F.).

During all visits, structured dermatological assessment was performed using the Sjögren-Larsson Severity Index (SLaSI), specifically designed for this study using items from the Psoriasis Area and Severity Index (PASI).[19] The SLaSI outcomes *erythema*, *desquamation*, *lichenification* and *excoriations* were scored separately on a 5-points severity scale (0-4 with 4 being most severe) assessing four different areas of the body (head, trunk, upper and lower extremities) each visit. This was done to improve detection of partial effects and differs from PASI. For each SLaSI outcome, the arithmetic mean of all body areas scored was calculated for each patient at each visit leading to 'SLaSI mean scores'. To measure global changes, the Physician Global Assessment (PGA) tool (range 0-5; 5 being most severe) was performed during all visits.[20]

Urine samples were collected at each visit and stored at -80 °C within maximally 6 hours after collection.

Primary caregivers weekly scored pruritus and excoriations in all patients during the entire study period using a Visual Analog Scale (VAS) instrument consisting of 100-mm horizontal lines marked with 'no pruritus' or 'no excoriations' on the left and 'severe pruritus' and 'severe excoriations' on the right. The scoring forms were weekly sent to the primary investigator.

Primary outcome measures (POM)

POM were the differences in SLaSI mean scores and PGA scores before and after treatment with zileuton or placebo.

Secondary outcome measures (SOM)

SOM were individual changes in VAS scores. To investigate zileuton's safety profile in SLS, adverse events were reported and serum aminotransferases were determined at each hospital visit.

Statistical analysis

Primary analysis involved studying changes in SLaSI mean scores and PGA scores during treatment. Data were analyzed for the two treatment periods (t=0-8 weeks and t=12-20 weeks) combined and separately using paired samples t-tests, comparing: 1) the scores at the start of the treatment period with those at the end of the treatment period (treatment); 2) the scores at the end of the treatment period with those at the end of the wash-out period (wash-out); and 3) the scores at the start of the treatment period with those at the end of the wash-out period (treatment + wash-out). Comparison 2 (wash-out) was done to detect reversal of potential beneficial effects from zileuton after discontinuation of treatment. Comparison 3 (treatment + wash-out) was added post-hoc to check for potentially longer-lasting therapeutic effects of zileuton after treatment discontinuation. To detect potential bias by seasonal influences on skin condition, the mean changes in scores from the patients receiving treatment with zileuton were compared with those from the placebo group in the same treatment period using independent samples t-tests. To assess intra-observer variability, individual SLaSI mean scores and PGA scores from patients receiving zileuton in the second treatment period were compared between t=0, t=8 and t=12 weeks, before they received any treatment.

SOM were studied by calculating individual mean VAS scores during treatment periods with zileuton and periods with placebo treatment for the outcomes pruritus and excoriations separately. Previous experience led to the assumption that only a minority of patients would respond to zileuton. Therefore, POM were also analyzed in each individual patient to detect responders. Because we analyzed SLaSI outcomes

separately, general PASI response definitions could not be used.[21] Regarding SLaSI and PGA scores, we defined a positive response to zileuton as a decrease of ≥0.5 after treatment. Based upon use in psoriasis, a positive VAS response was defined as a decrease of ≥20 during treatment.[22] All separate outcomes scoring positive were given one point (maximum score 7 points). Patients scoring ≥5 points were considered responders.

Leukotrienes analysis

For LTB4 and 20-OH-LTB4 analysis samples were purified using anion exchange solid phase extraction with subsequent HPLC fractionation on a Acquity HSS T3 column (100 x 1 mm/ dp = 1.8 μm; Waters Chromatography, Etten-Leur The Netherlands). Relevant fractions were measured using an enzyme immunoassay (Leukotriene B4 Express EIA Kit, Cayman Chemical Company nr 10009292, USA and standard for 20-OH-LTB4 from the same company nr 20190). Reference values for age matched healthy controls were determined in house. Total procedure was validated by performing recovery experiments for LTB4 and 20-OH-LTB4 which gave satisfactory results.

Results

Eighteen patients were assessed for eligibility. Eight patients did not participate because of logistic difficulties (too many study visits, travel distance etc.). Included patients were randomized in one of two treatment arms. Patient characteristics are given in Table 1. Three patients participated in earlier zileuton studies using the same dosages as given in this study, but discontinued treatment afterwards because of supposed treatment failure. All patients completed the current study.

Primary outcomes measures (POM)

Results of analyses on the POM are presented in Table 2 by mean changes in scores (plus 95% confidence intervals (95%CI)) for the two treatment periods combined and separate. In the combined analysis, a treatment effect was only seen for the PGA scores, which improved on average by 0.7 (95%CI: 0.1-1.3) during treatment with zileuton. When analyzing the two treatment periods separately, treatment effects of 1.0 (0.1-1.8) and 1.2 (0.2-2.2) were seen for the SLaSI outcome desquamation and the PGA, respectively, both in the second period and both largely maintained during wash-out period. Thus, a reversal of beneficial effects from zileuton after discontinuation of treatment could not be detected. Simultaneously, the placebo group showed no effects for desquamation and PGA, thereby excluding seasonal influences upon these scores.

Table 1 Patient characteristics.

Patient	Age (years)	Gender (M/F)	Weight and body surface area (BSA)[a]	Daily dermatological treatment	Remarks	Zileuton treatment previously	Zileuton treatment period (weeks)	Zileuton dosage (qid)
1	26	F		Calmurid bid		No	t=0-8	600mg
2	27	F		Calmurid bid		No	t=0-8	600mg
3	9	M	22.8 kg 0.85 m²	Daily bath and scrubbing Udder cream qid		No	t=12-20	400mg
4	8	F	19.7 kg 0.79 m²	Cetomacrogol bid		No	t=12-20	400mg
5	12	F		Calmurid and Vaseline bid		No	t=12-20	600mg
6	40	M		Oral Acitretine and Calmurid bid	UTI at t=8	No	t=12-20	600mg
7	29	M		Vaseline		Yes	t=0-8	600mg
8	22	M		Vaseline		Yes	t=0-8	600mg
9	7	F	21.7 kg 0.84 m²	Vaseline	Chickenpox at t=24	No	t=12-20	400mg
10	22	M		Calmurid bid		Yes	t=0-8	600mg

M = male; F = female. Bid = bis in die (twice a day). Qid = quater in die (four times a day). UTI = urinary tract infection

[a] Weight and BSA used for dosage calculations (only in patients <12 years).

Dosage calculation: 15-<20 kg or < 0.60 m² : 200 mg qid
20-<32 kg or 0.60-1.0 m² : 400 mg qid
≥32 kg or >1,0 m² : 600 mg qid

In contrast, the placebo group scored better at the second period for erythema and excoriation, while they did much worse during the ensuing wash-out period. In the first period, the treatment group scored slightly better for excoriations, but worse again at the end of the wash-out period. The severity of desquamation seemed to increase during the first treatment and wash-out periods for both the zileuton-treated and placebo group. All other scores did not change substantially from t=0 to t=8 and t=12 weeks in the placebo group in the first treatment period, indicating limited intra-observer and seasonal variability.

Secondary outcomes measures (SOM)

due to a logistical error, no scoring forms were collected during t=9-11 weeks. During the other periods, 181/210 (86%) scoring forms were returned. When treated with zileuton, none of the patients missed more than 2 forms. Large intra-individual variability in scores was seen in subsequent study weeks in some patients. Substantial decreases in mean VAS scores were only detected in one patient (Table 3).

Individual responders

one patient met our criteria for 'responder' with a score of 6 out of 7 (Table 4). Subsequent retrospective analysis of the medical records from all patients showed that for this patient only, the parents reported a tremendous improvement of the clinical condition (especially regarding pruritus) in the period zileuton was administered. In the placebo phase, they reported to have seen no relevant effects, while they reported a complete setback within days after discontinuation of zileuton at the wash-out visit.

Leukotrienes analysis

Urinary samples were collected for all patients during nearly all visits (except for patient 9 at visit T=12). Measurement of baseline concentrations of LTB4 and 20-OH-LTB4 however failed to detect the expected differences in LTB4 and 20-OH-LTB4 excretion between SLS patients and healthy controls due to as yet unknown reasons. We therefore could not reliably use urine leukotriene measurements in this study.

Adverse events

No adverse events related to the study drug were observed. Patient 6 suffered from a urinary tract infection treated with co-trimoxazole (during placebo treatment). Patient 9 had chickenpox during her visit at t=24 weeks. The serum aminotransferases remained within normal limits in all patients.

Table 2 Primary Outcome Measures: SLaSI & PGA scores.

SLaSI & PGA		Any treatment period	Treatment period T0 – T8			Treatment period T12 - T20		
		Zileuton group (n=10) mean change + 95%CI	Zileuton Group (n=5) mean change + 95%CI	Placebo Group (n=5) mean change + 95%CI	P value	Zileuton Group (n=5) mean change + 95%CI	Placebo Group (n=5) mean change + 95%CI	P value
Erythema	treatment	0.05 (-0.31 – 0.41)	0.05 (-0.41 – 0.51)	0.00 #	0.78	0.05 (-0.75 – 0.85)	**0.60 (0.04 – 1.16)***	0.16
	wash-out	-0.05 (-0.42 – 0.32)	-0.30 (-0.86 – 0.26)	0.30 (-0.26 – 0.86)	0.07	0.20 (-0.40 – 0.80)	-0.35 (-0.82 – 0.12)	0.08
	treatment + wash-out	0.00 (-0.36 – 0.36)	-0.25 (-0.91 – 0.41)	0.30 (-0.26 – 0.86)	0.11	0.25 (-0.19 – 0.69)	0.25 (-0.33 – 0.83)	1.00
Desquamation	treatment	0.40 (-0.19 – 0.99)	-0.15 (-0.76 – 0.46)	-0.35 (-0.87 – 0.17)	0.51	**0.95 (0.06 – 1.84)***	0.25 (-0.24 – 0.74)	0.09
	wash-out	-0.40 (-1.03 – 0.23)	-0.60 (-2.13 – 0.93)	**-0.60 (-1.08 – -0.13)***	1.00	-0.20 (-0.66 – 0.26)	-0.15 (-0.83 – 0.53)	0.87
	treatment + wash-out	0.00 (-0.83 – 0.83)	-0.75 (-2.22 – 0.72)	**-0.95 (-1.62 – -0.28)***	0.74	**0.75 (0.17 – 1.33)***	0.10 (-0.82 – 1.02)	0.14
Lichenification	treatment	0.23 (-0.22 – 0.67)	0.05 (-0.78 – 0.88)	-0.10 (-0.38 – 1.78)	0.65	0.40 (-0.31 – 1.11)	0.30 (-0.21 – 0.81)	0.76
	wash-out	-0.05 (-0.56 – 0.45)	-0.20 (-1.21 – 0.82)	-0.30 (-1.07 – 0.47)	0.83	0.10 (-0.68 – 0.88)	-0.50 (-0.76 – 0.66)	0.70
	treatment + wash-out	0.18 (-0.29 – 0.65)	-0.15 (-0.67 – 0.37)	-0.40 (-1.27 – 0.47)	0.51	0.50 (-0.41 – 1.41)	0.25 (-0.80 – 1.30)	0.63
Excoriation	treatment	0.43 (-0.89 – 0.94)	0.60 (-0.15 – 1.35)	0.15 (-0.66 – 0.96)	0.29	0.25 (-0.80 – 1.30)	**0.75 (0.17 – 1.33)***	0.28
	wash-out	-0.08 (-0.72 – 0.57)	**-0.75 (-1.33 – -1.7)***	-0.25 (-0.83 – 0.33)	0.13	0.60 (-0.24 – 1.44)	**-0.65 (-1.07 – -0.23)***	0.06
	treatment + wash-out	0.35 (-0.45 – 1.15)	-0.15 (-0.90 – 0.60)	-0.10 (-0.74 – 0.54)	0.89	0.85 (-0.83 – 2.53)	0.10 (-0.71 – 0.91)	0.30

PGA							
treatment	**0.70** **(0.11 – 1.29)***	0.20 (-0.36 – 0.76)	1.00	**1.20** **(0.16 – 2.24)***	0.20 (-0.36 – 0.76)	**0.05**
wash-out	-0.20 (-0.50 – 0.10)	-0.20 (-0.76 – 0.36)	1.00	-0.20 (-0.76 – 0.36)	0.00 (-0.88 – 0.88)	0.61
treatment + wash-out	0.50 (-0.50 – 1.00)	0.00 # (-0.88 – 0.88)	1.00	**1.00** **(0.12 – 1.88)***	0.20 (-0.36 – 0.76)	0.07

95%CI = 95% confidence interval around the mean change in SLaSI and PGA scores.

treatment = score at the start of the treatment period minus score at the end of the treatment period
wash-out = score at the end of the treatment period minus score at the end of the wash-out period
treatment + wash-out = score at the start of the treatment period minus score at the end of the wash-out period
95%CI could not be computed as the standard error of the difference was zero
* relevant effects

Table 3 Secondary outcome measures.

Patient	Mean VAS scores Pruritus			Mean VAS scores Excoriations		
	During placebo period	*During treatment period*	*Difference*	*During placebo period*	*During treatment period*	*Difference*
1	40	69	+29	49	60	+11
2	63	49	-14	54	49	-5
3	77	87	+10	86	87	+1
4	90	60	**-30***	89	60	**-29***
5	50	50	0	51	50	-1
6	49	63	+14	45	61	+16
7	80	77	-3	77	78	+1
8	92	87	-5	48	52	+4
9	93	75	-18	32	37	+5
10	5	19	+14	7	21	+14

Individual VAS scores. During placebo period is defined as t=0-8 weeks or t=12-20 weeks. During treatment period is defined as t=1-8 weeks or t=13-20 weeks, starting one week after the beginning of treatment (t=0 or t=12). The scores were derived from a scale of 0-100 mm.
* = relevant effects

Table 4 Individual responder's analysis.

Patient	SLaSI				PGA	VAS		Total score	Responder
	Erythema	Desquamation	Lichenification	Excoriations		Pruritus	Excoriations		
1	-	-	0.75	1.25	-	-	-	2	-
2	0.5	-	0.5	1.0	1.0	-	-	4	-
3	-	1.0	-	1.25	2.0	-	-	3	-
4	-	1.0	0.75	0.75	2.0	+	+	6	+
5	-	0.75	0.5	-	1.0	-	-	3	-
6	-	2.0	1.0	-	1.0	-	-	3	-
7	-	-	-	-	-	-	-	0	-
8	-	-	-	-	-	-	-	0	-
9	0.75	-	-	-	-	-	-	1	-
10	-	-	-	0.75	-	-	-	1	-

SLaSI and PGA outcomes in individuals: sum of SLaSI mean score before zileuton treatment minus SLaSI mean score after zileuton treatment.
Results are only given if sum was ≥0.5; otherwise the patient is considered a non-responder.
VAS outcomes: positive score if difference in mean VAS scores during the treatment and placebo periods was ≥20 (Table 3).
Patient is considered to be a responder to zileuton treatment if the total score was ≥5

Discussion

Key findings

Except for small effects on SLaSI scores for desquamation and PGA scores in the second treatment period, changes in mean scores were not different between the zileuton and placebo groups. However, one patient responded on zileuton treatment with substantial changes in almost every scoring item. Upon parental request, the patient continued zileuton and was monitored closely. Follow up (~1 year) after the study showed a consistent beneficial therapeutic effect. Discontinuation of treatment (due to a lack of zileuton supplies) led to a setback shortly after stopping zileuton, which was reversed upon resuming treatment. This observation of prompt and clear response to starting and stopping zileuton treatment corresponds to previous findings.[11] From the patients treated previously, one other patient (female, 24 years) still uses zileuton (600mg qid) and reports lasting beneficial effects. It remains unclear, however, why only some patients respond to treatment.

Although genotypes in SLS differ, the corresponding clinical phenotype as well as degree of enzyme deficiency are usually quite homogeneous making it impossible to predict responders using clinical (or biochemical or genetic) decision models. Both responders from these two studies have different genotypes and lack residual FALDH activity. Nonetheless, minimal but perhaps relevant variations in concentrations of abnormal metabolites may still exist in patients, but cannot be detected because of insufficient sensitivity of biochemical assays. To improve general condition of the skin and pruritus, some adult patients are treated with acitretin, but adverse effects are frequently reported.[23] In this study, no adverse events were detected and zileuton is considered safe for usage in SLS. This is a major advantage over the use of acitretin when treating pruritus in SLS patients.

Limitations

Despite state of the art study design and realization, some limitations have to be mentioned. Due to the small sample size, it is difficult to draw firm conclusions. However, it is near impossible to perform studies with larger patient groups, given that SLS is a rare disease.

LTB4 is produced by epidermal keratinocytes upon stimulation of specific receptors.[8] Research in mice proved that orally administered zileuton has the ability to inhibit epidermal LTB4 production and decrease pruritus.[24] However, correlations between zileuton dosages used in animal research and the dosages used in this study are unclear.

Use of zileuton in SLS is off-label and no formal dose finding studies have been performed. Dosages used were based on the treatment of asthma, in which leukotrienes are formed by mast cells that may have different biochemical responses to zileuton

than keratinocytes.[25] In addition, it is possible that dosages of zileuton in SLS should be higher than used here to sufficiently penetrate epidermal layers or have stronger inhibitory effects on leukotriene formation. As the one responder had the lowest body weight of all patients, she received a relatively high dosage for weight (81mg/kg total daily dose) compared to other participants <12 years (respectively 70 and 74 mg/kg total daily dose). Higher dosages of zileuton may be needed to increase the number of responding patients.

Due to unsuccessful urinary leukotriene analysis, we could not confirm bio-chemically that exposure to zileuton in this study was sufficient to decrease leukotriene production.

In our previous study, however, we reported effects on urinary concentrations using the same dosages of zileuton for patients ≥12 years.[11] Nonetheless, the correlation between decreasing urinary LTB4 concentrations (representing total body leukotriene production) and a decrease of locally formed LTB4 in keratinocytes has not yet been studied.

The intellectual disability in SLS patients may result in scratching becoming habitual behavior. Therefore, scratching may continue although the pruritus is diminished by zileuton treatment, disguising potential beneficial effects.

Data from the treatment plus wash-out period suggest prolonged beneficial effects of zileuton on the skin. However, the responding patient repeatedly reported setbacks within days after discontinuation of zileuton treatment. Consequently, we presume that beneficial effects of zileuton treatment, if present, will appear within days to weeks after treatment initiation and will disappear shortly after discontinuation.

Conclusion

Zileuton in the currently used dosages is safe to treat pruritus in SLS. Approximately 10% of the patients seem to respond and responders can easily be detected and will experience a significant improvement in quality of life. We suggest a therapeutic trial with zileuton during 4-6 weeks in SLS patients ≥5 years, especially when medical treatment for severe pruritus is warranted. If no clear beneficial response to zileuton is noted, treatment should be discontinued. Dose-finding with higher doses of zileuton in non-responders should not take place in individual patients, but would necessitate further research focusing on pharmacokinetic – pharmacodynamic relationships of zileuton in SLS patients.

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Part four

Current pathophysiological concepts

7

Sjögren-Larsson syndrome in clinical practice

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Abstract

This review article gives a state-of-the-art synopsis of current pathophysiological concepts in Sjögren-Larsson syndrome (SLS) mainly based upon original research data of the authors in one of the world's largest clinical SLS study cohorts. Clinical features are discussed in order of appearance, and diagnostic tests are set out to guide the clinician toward the diagnosis SLS. Furthermore, current and future treatment strategies are discussed to render a comprehensive review of the topic.

Introduction and historical perspective of the research group

In 1957, the Swedish physicians Sjögren and Larsson reported a cohort of 28 familial interrelated patients diagnosed with the clinical triad of mental retardation, spastic diplegia, and congenital ichthyosis following a seemingly autosomal recessive pattern.[1] Although, in retrospect, other patients with apparently the same disorder had been described previously, the name Sjögren-Larsson syndrome (SLS) has been used since then. In succeeding decades, more patients were reported, adding up to a clinical picture with variable predominance, especially toward the degree of spasticity and ichthyosis. Additional clinical features were described, such as a distinctive crystalline maculopathy and impaired speech-language performance. In 1988, Rizzo et al. discovered that a deficient microsomal fatty aldehyde dehydrogenase (FALDH) underlies SLS.[2] FALDH is part of the fatty alcohol nicotinamide adenine dinucleotide (NAD) oxidoreductase complex (FAO) and catalyzes oxidation of many different medium-and long-chain fatty aldehydes into fatty acids. Deficiency results in the accumulation of fatty aldehydes and fatty alcohols in body tissues, which is considered the principal causative mechanism leading to the overall clinical phenotype of SLS.[3] Besides its important role in fatty alcohol metabolism, FALDH is also involved in the breakdown of pro-inflammatory leukotrienes (Figure 1). Accumulation of precursor metabolites is thought to be particularly involved in the dermatological phenotype of SLS.

Later on, in 1996, the genetic basis of SLS was unravelled by De Laurenzi et al.[4] The FALDH gene, recently renamed ALDH3A2, is located on chromosome 17p11.2, consists of 11 exons, and spans about 31 kb. By now, more than 70 different mutations in the ALDH3A2 gene have been identified in SLS patients originating from about 120 different families. As recently reviewed by Rizzo and Carney, mutations in SLS are scattered throughout the ALDH3A2 gene and are generally private mutations, occurring in single cases.[5] Most mutations result in a complete loss of catalytic activity of FALDH, although there are several (missense) mutations associated with residual FALDH activity. For more than two decades, our Dutch research group has studied clinical, biochemical, and genetic aspects of SLS. During the years, we have extended our study cohort to > 30 genetically proven SLS patients. This clinical review summarizes the main study results and provides a state-of-the-art synopsis of current pathophysiological concepts.
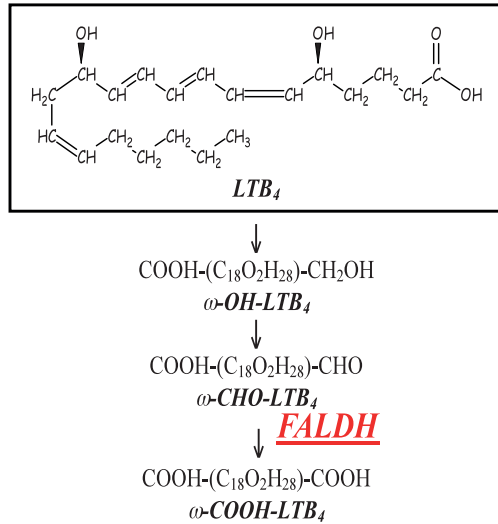
Figure 1 LTB4 degradation as an example of one of the pathways disturbed in fatty aldehyde dehydrogenase (FALDH) deficiency.

Clinical features

We discuss details of the main clinical features in SLS in order to guide the clinician toward a clinical diagnosis of SLS and to offer a framework for appropriate follow-up and care.

Skin disorder

In the majority of the patients, ichthyosis is congenital or develops very early in life. The neonatal skin may first have an erythrodermic appearance, gradually evolving into a generalized ichthyosiform hyperkeratosis during infancy. Ichthyosis follows a generalized distribution pattern and is more prominent in the flexural areas, neck, and lower abdomen. The central face is often spared. Hyperkeratosis thickens the skin, resembling lichenification, and produces a yellowish, dark-brown color (Figure 2). Ichthyosis in SLS is different from other cornification disorders by the striking pruritic character, resulting in excoriations and more scaling. It is the result of a defective lipid barrier in the stratum corneum of the skin. Seemingly, FALDH plays an important role in epidermal functioning, and its deficiency results in a leaky water barrier causing ichthyosis.[6] Besides the skin, other ectodermic structures are not affected, though most patients from our cohort have relative heat intolerance probably due to hypohydrosis.

Figure 2 Skin. Hyperkeratotic thickening of the skin, with a yellowish, dark-brown color.

Neurological disorder

The motor problem in SLS is summarized as bilateral spastic paresis involving the legs more than the arms. In 2008, we performed a cross-sectional study of motor performance and everyday functioning in 17 of our patients.[7] In doing so, we found that none of the adolescent participants was able to walk without restrictions, and most patients with SLS use a wheelchair to move around outdoors. In five patients, parents reported that their child had been able to walk with or without support but gait had deteriorated gradually over time. There seems to be a limit in motor performance and everyday functioning, with most participants reaching a developmental age of maximally 12 years. Later, in adolescence, neurological features stabilize, and the clinical picture behaves rather like stationary cerebral palsy than the progressive deterioration often seen in other neurometabolic disorders.

Most of the patients suffer from moderate cognitive impairment and typically reach a developmental age of 5–6 years. In our study cohort, some patients reached a certain level of independence, self-care, and mobility in an adapted environment, though most of them had limited social interaction with peers and their participation was restricted. In assessments of cognitive capacity of adult patients (n=6) from our cohort, history—including well-documented school performances of many other patients, as well as the available data in the literature—all point to stable intellectual capacities in SLS without any loss of acquired cognitive abilities during at least the

first three to four decades of life.[8] Epileptic seizures are encountered in the minority of patients with SLS; they do not dominate the clinical picture and are generally easily treatable with antiepileptic monotherapy.

Almost all patients suffer from a mild-to-moderate (pseudobulbar) dysarthria, which influences speech audibility and, together with a delay in language development, aggravates their already poor communicative functioning in daily life. Although most patients learn to talk, some are left with complete anarthria. Oral facial motor functioning is impaired, and most SLS patients have poor upper facial musculature functioning. Remarkably, severe dysphagia was not encountered in these patients, and accordingly, none depended on gastric tube feeding in daily life. The degree of cognitive impairment relates to the degree of language impairment, as was demonstrated in our previous study.[8] No clear relationship between motor functioning (spasticity) and dysarthria or cognitive development was observed in our study series. Thus, given the variability in cognitive and speech–language development within different levels of motor impairment, spasticity is not likely the limiting factor in cognitive and speech–language development in these patients.

Magnetic resonance imaging (MRI) in SLS shows normal gross anatomy of the brain, with periventricular white matter abnormalities ranging from (very) mild to severely increased signal intensities on T2–weigthed images (Figure 3). These abnormalities occur in the first 2 or 3 years of life and stay stable thereafter. Myelination is generally slightly delayed, and most patients show a discrete permanent deficit in myelination. Only at ages >10 years may slight cerebral atrophy be seen.

Proton magnetic resonance spectroscopy (H-MRS) shows moderate changes of the known metabolites (normal N-acetylaspartate and glutamine/glutamate; increased choline, creatine and myoinositol), reflecting white matter gliosis without significant axonal damage or loss. In patients from the age of 1 or 2 years onward, H-MRS shows highly characteristic, almost pathognomonic, abnormal resonances at 1.3 and 0.8–0.9 ppm in cerebral white matter but not in grey matter spectra, reflecting lipid accumulation (Figure 4).[9] To date, the exact nature of the accumulating lipids is unknown. Over the years, we have seen some very rare exceptions to the rules, i.e., patients with very severe MRI signal changes or with hardly any lipid peak on H-MRS. Nevertheless, we think that combined MRI and H-MRS data in patients >2 years almost always are highly suggestive, if not pathognomonic, for SLS, especially in the context of a patient with a neurocutaneous disorder.

Ophthalmological abnormalities

In early infancy, a peculiar crystalline macular dystrophy develops in patients with SLS. Clinically, this maculopathy is accompanied by decreased visual acuity of a certain degree and by photophobia. Ophthalmoscopy reveals perifoveal crystalline deposits located in the inner retinal layers, which may reflect accumulation of
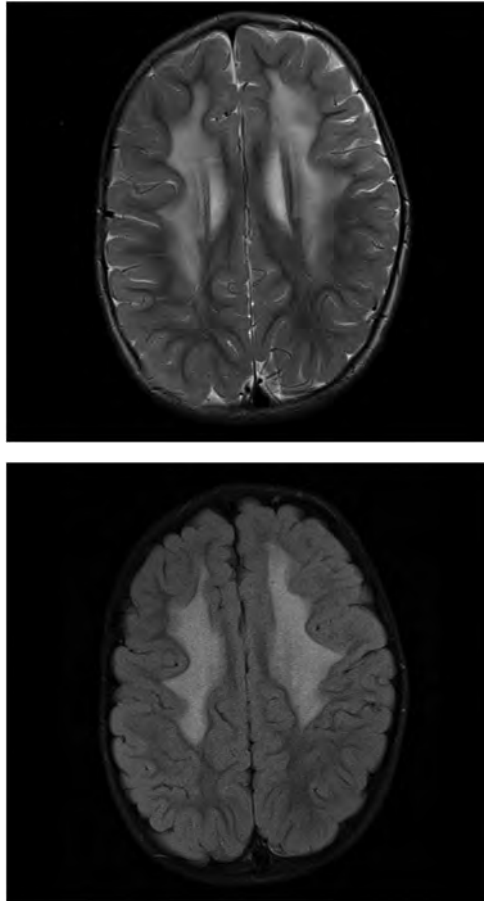
Figure 3 Magnetic Resonance Imaging (MRI) in a 5 year old girl with SLS. Severe periventricular signal changes on T2 (**top**) and T2-FLAIR (**bottom**) images.

pathological lipids (Figure 5). In recent years, we extended our studies on this macular dystrophy by using optical coherence tomography (OCT). This technique, which allows depiction of single retinal layers, showed a clear change in macular morphology and reflectivity compared with healthy eyes (Figure 6).[10] By OCT, we were able to localize crystals within the perimacular ganglion cell layer and the inner plexiform layer. Interestingly, we detected a cystic foveal degeneration by OCT in the majority of patients with SLS, varying from multiple microcysts to cystic foveal atrophy. In general, patients who were severely affected on OCT also showed intense changes on previously performed cerebral MRS.
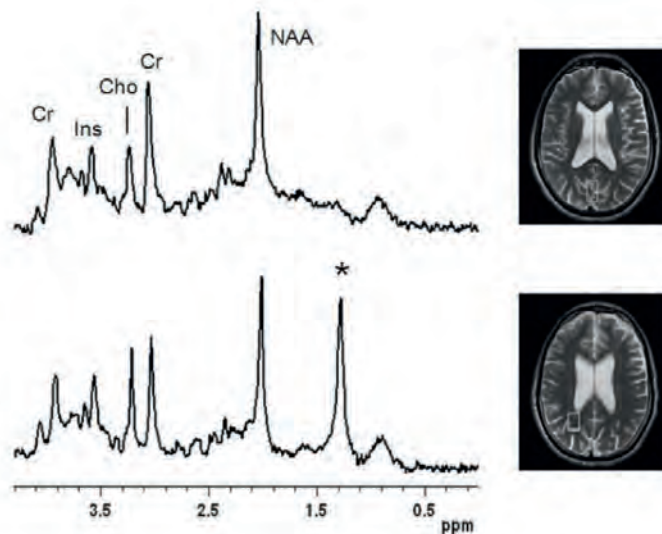
Figure 4 Magnetic resonance spectroscopy (MRS) (3 Tesla, short echo time 30 ms) in a 39-year-old man with Sjögren-Larsson syndrome (SLS).

Normal spectrum of grey matter (**top**). High lipid peak (*) in normal-appearing white matter occipitally (**bottom**). NAA N-acetyl aspartate, Cr creatine, Cho choline, Ins myoinositol

Furthermore, we studied the macula in these patients by using fundus autofluorescence and the macula pigment reflectometer. These examinations revealed significantly reduced levels of central retinal macular pigment, indicating a lack of retinal carotenoids.[11] The cystic foveal degeneration together with the lack of macular pigment are presumably a sign of retinal Müller cell degeneration in patients with SLS. The changes observed with the macular pathology in SLS correlates well with the generally observed clinical features of reduced visual acuity and photophobia.

Preterm birth

We were the first to recognize that preterm birth is a clinical hallmark, in fact the very first sign, of SLS. In out cohort, we have encountered preterm birth in 18 of 25 (72 %) of all patients with exactly known gestational ages at birth. In this series, we calculated a mean gestational age at birth of 35.6 [standard deviation (SD) 2.1] weeks. Only three cases were born < 33 weeks or > 37.5 weeks, namely 28 weeks (n=1) and 38 weeks (n=2). In many case descriptions in the literature, preterm birth is also reported. Apparently, a fetus with SLS induces its own (preterm) birth, e.g., by excessive urinary

Figure 5 Fundus. Representative color fundus photograph of juvenile maculopathy in Sjögren-Larsson syndrome. Note the huge amount of yellowish-white glistening dots around the central macula.
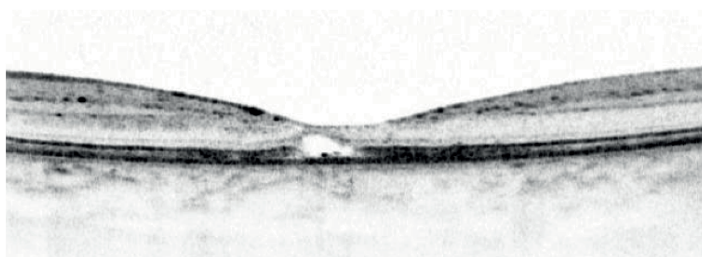

Figure 6 Optical Coherence Tomography (OCT). Trans-foveal OCT scan of a patient with Sjögren-Larsson syndrome. Note the large central pseudocyst with interruption of the photoreceptor layer. Glistening white crystals appear as dark, high-reflective dots within the nerve fibers and the inner and outer plexiform layers of the retina

excretion of highly active lipids (such as leukotrienes) in amniotic fluids, thus triggering labor through inflammatory responses.

Other signs & symptoms

In contrast to what has been reported in some cases in the literature, we could not confirm that the following features are part of the phenotype in SLS: microcephaly, short stature, peripheral neuropathy, dental enamel hypoplasia and widely spaced teeth, and hypertelorism.

Diagnostics

Clinical diagnosis

Though the classical description of its most prominent features seems to make SLS easy to diagnose, its phenotype can be variable and develops following a typical age dependent pattern. The latter can make diagnosing SLS difficult, especially in young children. We therefore categorize the gradual development of the complete SLS phenotype into different age-dependent phases.

Preterm birth and the typical congenital erythrodermic skin or ichthyosis are the first symptoms of SLS. Infants presenting in this manner do not yet exhibit cognitive or motor abnormalities, and the typical crystalline macular dystrophy is lacking. Therefore, SLS is generally not suspected clinically in this very early phase. During early childhood (1–2 years of age) mental and motor retardation gradually become clear and should be followed up by cerebral imaging studies. Typical MRI and H-MRS abnormalities, as well as crystalline maculopathy, may be absent, and normal radiologic and ocular findings thus absolutely do not exclude SLS at this stage. Later on in childhood (from 3 years of age), the full-blown phenotype of SLS with the classical triad of ichthyosis, spasticity, and mental retardation is present and is completed with the typical findings during ophthalmological and MRI/H-MRS studies. Only at this stage of the disease is a clinical diagnosis of SLS generally made.

Genetic & biochemical diagnostics

Once the clinical suspicion of SLS has arisen, diagnosis can be confirmed by demonstrating the enzymatic defect and mutation analysis of the ALDH3A2 gene. Traditionally, and in contrast to many other inborn errors of metabolism, it is very difficult to prove SLS at the metabolite level in body fluids. Only concentrations of long-chain fatty alcohols and leukotriene B4 (LTB4) metabolites have been found elevated in blood and urine, respectively, but these metabolites are extremely difficult to measure, and routine analysis is unavailable in most dedicated laboratories for metabolic diseases.

Enzyme analysis is preferably performed in cultured skin fibroblasts and can be done with the use of several substrates (Table 1).

FALDH deficiency can also be demonstrated in polymorphonuclear leukocytes using LTB4 as the substrate, but this assay demands fresh blood cells and is very complex and laborious. Only very recently did we develop a much easier and also highly reliable assay for FALDH enzyme activity measurements in blood cells. This is possible using the artificial substrate pyrenedecanal, first introduced by Keller et al.[12] Sequence analysis of the coding region of the ALDH3A2 gene in patients displaying FALDH deficiency results in the identification of two pathogenic ALDH3A2 gene mutations in > 95 % of patients. Although many patients harbor unique mutations,

Table 1 Substrates for fatty aldehyde dehydrogenase (FALDH): metabolites used to determine FALDH enzyme activity in vitro and can be measured as biomarkers in different bodily fluids.

Substrate	In vitro	In vivo	Remark	Reference
Hexa- and octadecanol	+ (F)	+ (B)		Rizzo et al. 2000 [24]
Phytol	+ (F)	-	In blood normal values	Van den Brink et al. 2004 [19]
Omega-hydroxy-C22:0	+ (F)	NM		Sanders et al. 2009 [25]
Dihydrophytal	+ (F)	NM		Kelsson et al. 1997 [3]
Pristanal	+ (F)	-	In blood normal values	Verhoeven et al. 1998 [26]
20-CHO-LTB4	+ (L)	+ (U)	In CSF normal values	Willemsen et al. 2001 [17]
Pyrenedecanal	+ (F;L[a])	NM	Artificial substrate	Keller et al. 2010 [12]

F= cultured skin fibroblasts; L= leukocytes of lymphocytes; B= blood (plasma or serum); U= urine; CSF= cerebrospinal fluid; LTB4= leukotriene B4; NM= not measured
[a] = unpublished data

two mutations, namely, c.1297–1298delGA (leading to protein truncation at position 435 along with amino acid substitutions at 433 and 434) and c.943 C>T (p.Pro315Ser), are quite commonly found and considered founder mutations in Europe. Missense mutations are the most frequent mutation type, followed by small exonic alterations (deletions or insertions) that lead to protein truncation.

Treatment options

Classical treatment: symptomatic

Treatment should focus on the spasticity and prevention of contracture development, as both can bear important effects upon patients' daily life functioning. Physical therapy, as well as bracing and ergonomic support, is important, as are aids to stimulate spontaneous movements of the child as varied as possible. Furthermore, speech-language therapy when started early may play an important role in optimizing speech-language performance and (augmented) communication and thereby improve daily functioning. Daily skin care focuses on diminishing scaling and pruritus by life-long application of different keratolytic or urea-containing emollients.

Alternative options originating from psoriasis treatment, such as the topical use of calcipotriol, have also been used in our patients with varying degrees of success.[13] Moreover, a review from the original Swedish cohort of SLS patients described favorable effects of retinoids (acitretin) taken orally, mainly upon ichthyosis.[14] However, this was based upon single-case observations, and a placebo controlled trial has not yet been performed to obtain objective proof.

Alternative treatment strategies

In past decades, and inspired by growing insights into pathophysiological mechanisms, multiple treatment regimes have been postulated in the literature, often with disappointing results. Despite some hopeful case reports of dietary treatment in which long-chain fatty acids were replaced by medium-chain fatty acids in an attempt to decrease fatty alcohol accumulation, no biochemical or clinical improvement were seen in the five SLS patients treated in this way. Dietary supplementation of certain polyunsaturated fatty acids, the deficiency of which has been described in SLS patients, by using primrose oil was also unsuccessful in the same study.[15] Although the presence of biochemical variability in terms of residual FALDH activity in different SLS patients urges caution, so far, dietary treatment of SLS has not been successful.

The accumulation of leukotrienes subsequent to FALDH-dependent deficient degradation is presumed to play an important part in the inflammatory reactions seen in the skin of SLS patients. The striking pruritus from which these patients suffer and which is generally not seen in other ichthyotic diseases may originate from LTB4 accumulation. Zileuton is a drug that inhibits leukotriene formation by blocking its biosynthesis pathway and has proven its use in managing chronic (severe) asthma. We investigated the effect of zileuton treatment both clinically and biochemically in SLS patients. Treatment of one and five patients with zileuton during a period of 5 weeks and 3 months, respectively, led to a significant reduction of urinary concentrations of LTB4 and omegaOH-LTB4.[16,17] Clinically, advantageous effects upon pruritus, general well-being, and the electroencephalographic background pattern were seen. No important side effects or complications were reported. Though these studies have proven a potent biochemical effect of zileuton in SLS patients, the potential clinical effects, especially upon pruritus, require further research. To prove the postulated effect of zileuton upon (some) dermatological characteristics of SLS, a randomized double-blinded placebo controlled trial is warranted.

Future perspective

Bezafibrates

Recent research indicates the involvement of FALDH in the oxidative breakdown of phytol.[18,19] Phytol is a branched-chain fatty alcohol and compound of the chlorophyll molecule and therefore part of our regular diet. Its breakdown is augmented by FALDH in two stages of the cascade: oxidation of phytenal into phytenic acid, and pristanal into pristanic acid subsequently. Phytanic and pristanic acid, both fatty acids, act in vitro as natural ligands for the nuclear hormone receptor peroxisome proliferator activated receptor-α (PPAR-α). PPAR-α is a ligandactivated transcription factor that modulates the expression of target genes. The target genes of PPAR-α are involved in various aspects of lipid metabolism. PPAR-α is mostly expressed in tissues with high rates of fatty acid catabolism, such as liver, heart, and kidney. They are thought to play an important part in the pathogenesis of disease modalities such as hypertension and dyslipidemia. Besides the natural ligands mentioned above, there are several artificial ligands known to activate PPAR-α, such as bezafibrate.

Interestingly, recent animal studies have shown that a phytol-enriched diet induces FALDH activity in a PPAR-αdependent manner.[20] Elevated levels of phytanic and pristanic acid act as natural ligands and activate PPAR-α, which modifies the activity of the target gene by binding to a response element. Strikingly, the ALDH3A2 gene (responsible for modulating FALDH activity) has not yet shown to have such a PPAR-response element and thus the molecular–genetic explanation of the PPAR-α-induced FALDH up regulation remains to be elucidated.

Nevertheless, in 2006, Gloerich et al. investigated the in vitro capability of bezafibrate, acting as a artificial ligand to PPAR-α, to induce FALDH expression and activity in human fibroblasts of healthy controls as well as SLS patients with residual FALDH activity.[21] They found a 1.4-fold increase in FALDH activity (measured using phytol as substrate) in healthy control fibroblasts after incubation with 800 μM bezafibrate for 3 days. In fibroblasts of four different SLS patients with different mutations in the ALDH3A2 gene but whom all had some residual FALDH activity, in vitro treatment with 800 μM bezafibrate for 3 days resulted in a 2.5-and 2.8-fold increase of FALDH activity in two SLS patients, respectively. This represents an improvement in FALDH activity to 34 % and 39 % of the normal FALDH activity found in healthy controls.

This research project proves the in vitro capability of bezafibrate to induce FALDH activity in controls and selected SLS patients. Based upon these results, comparable ALDH3A2 mutations (resulting in residual FALDH activity) in SLS patients are suggested as candidates for bezafibrate FALDH induction. However, residual FALDH activity is a strict requirement for bezafibrate to be effective and is unfortunately not seen in many SLS patients, which limits its potential use. Besides this limitation, clinical implications of bezafibrate induction in SLS still need to be elucidated.

Carotenoids

We found a virtually complete absence of retinal carotenoids in those SLS patients available for macular pigment examination.[11] Macular pigment, consisting of the dietary-derived carotenoids lutein and zeaxanthin, plays an important role in protecting the retinal photoreceptors by neutralizing free oxygen radicals and by absorbing potentially phototoxic, high-energy blue light. Research in a limited number of our SLS patients showed normal plasma levels of lutein and zeaxanthin, therefore excluding gastrointestinal or dietary causes of carotenoid deficiency (unpublished data). A disturbed retinal macular pigment metabolism, presumably located within retinal Müller cells, appears the most probable reason for macular pigment deficiency in SLS. It is uncertain whether supplementation of carotenoids would be beneficial in SLS patients. Surely, normal plasma concentrations of lutein and zeaxanthin indicate that there is no strict bodily depletion of carotenoids. Furthermore, carotenoids undergo oxidation at the ocular level and form highly active aldehydes (carotenoid-derived aldehyde).[22] In general, oxidation of aldehydes is disturbed in SLS patients. Supplementation of carotenoids in SLS might therefore lead to an accumulation of these toxic aldehydes due to insufficient breakdown and cause additional retinal damage. The absence of macular pigment increases the risk for light toxicity and may augment the development of macular degeneration.

Gene therapy

In 2006, Haug et al. demonstrated the in vitro capability of FALDH activity induction through gene transfer using an adeno-associated viral vector in keratinocytes of two SLS patients.[23] FALDH activity was augmented up to 60–70 % of normal activity (measured by standardized procedure using octadecanol as substrate). Histologically, reduced thickness of the stratum corneum was observed, which may clinically relate to improvement of ichthyotic skin in these patients. Strict limitations and cautions are mentioned by the authors regarding the scientific interpretation of this observation. Also, it is questionable whether these in vitro results are reproducible in vivo when tested, for example, in an appropriate animal model. Furthermore, the achievability of gene transfer into neuronal cells in SLS has not yet been studied. Although in vitro results are promising, this illustrates that FALDH activity induction through gene transfer needs more research into feasibility and clinical implications in vivo using animal models. The difficulty here is that, to the best of our knowledge, such animal models do not exist at this time.

Conclusions

Primarily based upon findings from our own study cohort, we here provided a state-of-the-art synopsis of current pathophysiological concepts in SLS to guide the clinician when making the diagnosis SLS. We show the distinctive clinical features in SLS and provide a cross-sectional overview of psychomotor functioning, which helps the clinician when counseling parents of new SLS patients. By focusing upon treatment strategies and research topics in SLS, we provide greater insight into pathophysiological concepts that play part in SLS and help clinicians understand its current challenges.

Acknowledgements

Sjögren-Larsson syndrome in clinical practice

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8

Current insights
& future perspectives

Clinical features

Daily functioning

In Chapter 2 and Chapter 3 of this thesis we studied daily functioning in our cohort of SLS patients. Regarding motor functioning, we found that most of our patients had limitations, and these limitations were present in all gross motor dimensions, except for lying and rolling. Most of the patients suffer from moderate intellectual disability which typically seems to be limited around 5–6 years of developmental age. Though SLS is a neurometabolic disorder and the ongoing accumulation of both fatty aldehydes and alcohols in tissues is considered one of the principal causative mechanisms, the level of daily functioning remains rather stable throughout patients lives.[1] We previously reported that SLS behaves clinically in a non-progressive way and its course has more of a resemblance to the course seen in patients with cerebral palsy.

Currently our study cohort contains 31 patients aging from 7-60 years some of which we have been following for almost 2 decades yet daily functioning still remains rather stable. Surely some patients encounter complications such as scoliosis and spontaneous fractures that impair daily functioning but these usually result from their longstanding spasticity rather than indicate progressive disease.

Ophthalmological features

Though SLS seems to behave in a non-progressive way when cognitive and motor performances are brought into consideration, this may not apply to the maculopathy seen in these patients.

In Chapter 4 we studied the maculopathy in SLS and proposed a differentiation of the optical coherence tomography (OCT) results into 3 forms, namely, mild, moderate, and severe. In general, patients who were severely affected on OCT showed intense changes on previously performed cerebral magnetic resonance spectroscopy, indicating a positive relationship. The follow-up on OCT in 3 of 14 study participants was unable to detect significant changes within fifteen months of observation. We postulated that foveal atrophy in SLS may be a time-consuming process and a prolonged follow-up period in a larger number of patients may be necessary to detect disease progression.

In addition, the crystalline maculopathy in SLS showed a significant lack of central macular pigment (MP). The lacking macular pigment resulting in poor protection from high-energetic photons may explain some of the clinical features in SLS (e.g. photophobia) but may also play an important part in progressive degenerative processes, leading to macular thinning and pseudocyst formation.

Recently, one of our patients (female, 31 years) suffered from a severe, consecutive loss of visual acuity in both eyes. Evaluation revealed an exudative macular degeneration not previously encountered in other SLS patients. It is yet

unclear if this results from the ongoing accumulation of FALDH-related metabolites and therefore an indication of a progressive disease. In addition, the lack of macular pigment in SLS may add to increased retina vulnerability due to light toxicity. If so, therapeutic interventions may aim to protect the retina from light in order to slow down or even stop the degenerative processes within the retina.

Oxidative stress from high energetic photons is thought to play an important part in the development of age-related macular degeneration.[2] When lutein is combined with zeaxanthin macular pigment is formed; eliminating lutein from the diet resulted in signs of early retinal degeneration in primates.[3] One could postulate that longstanding MP deficiency and subsequent light toxicity is responsible for the development of macular degeneration in the aforementioned patient. Also, the motor and cognitive performances in this patient have not deteriorated overtime; therefore no other signs of progressive disease due to the ongoing accumulation of fatty alcohols and aldehydes are currently seen in this patient. Further research is needed to find out what kind of a role MP deficiency plays in SLS maculopathy and what complications can evolve from this deficiency.

Histological analysis of the retina in two patients with macular telangiectasia - a disorder in which patients gradually lose macular pigment - showed the absence of Müller cells. In addition to our previous findings this analysis points to a pivotal role of the Müller cell in macular pigment metabolism. [3-5] Results from future SLS studies should be compared with studies from patients with macular telangiectasia to increase further development in pathophysiological concepts.

Light protection by filtering glasses has been studied in adults in order to either prevent further macular degeneration or to improve visual functioning but results remained inconclusive.[6]

In SLS, MP deficiency and subsequent light toxicity probably already develops in childhood which is much earlier than patients with adult macular degeneration or macular telangiectasia. It may be useful to early apply short-wavelength filtering glasses for SLS patients to reduce toxic light effects and to prevent the worsening of maculopathy which can lead to macular degeneration. Future studies are needed to back up these postulations.

Dermatological features

In Chapter 6 we studied the effects of inhibition of leukotrienes upon the skin. Although we could only find a convincing clinical response to the treatment with zileuton in one patient, this response was in such a way that a positive relationship between leukotrienes formation by keratinocytes and pruritus in our patients must exist. Difficulties in demonstrating effects of zileuton presumably are dosage related. The following factors need to be considered: (1) the off-label usage of zileuton (namely dose-finding data are from asthmatic patients); (2) different cell types involved

(namely mast cells versus keratinocytes in asthmatic and SLS patients respectively); and (3) different end organs that react (namely the lung versus the skin in asthmatic and SLS patients respectively). Further studies of pharmacokinetic–pharmacodynamic relationships of zileuton are needed in SLS to find out what dosage is sufficient to decrease dermal leukotrienes formation.

What causes keratinocytes to produce more leukotrienes in SLS? Aside from a defective water barrier in the stratum corneum which resulted from abnormal lamellar body formation, the skin in SLS also displays a reduced level of ceramides.[7] Ceramides also play a crucial part in water barrier formation and thus not only is the FALDH-related accumulation of alcohols and aldehydes responsible for the ichthyosiform phenotype in SLS but also the deficiency of ceramides. Interestingly enough, patients with atopic dermatitis also demonstrated to have a markedly reduced amount of ceramides. Ceramide is synthesized from sphingomyelin in the stratum corneum by sphingomyelinase. In patients with atopic dermatitis however, sphingomyelin metabolism is altered. Instead of being hydrolyzed into ceramide, sphingomyelin is preferentially hydrolyzed into sphingosyl phosphorylcholine (SPC) by a different enzyme. The result is not only a deficiency of ceramide but also an increased production of SPC in the stratum corneum of these patients.[8] Moreover, intradermal SPC injection was demonstrated to produce itch-associated responses in a mouse model and cultured mouse keratinocytes significantly increased the production of leukotriene B4 upon exposure to SPC.[9] Presumably, SPC exposure triggers keratinocytes to produce LTB4 thereby causing pruritus. In addition, treatment with zileuton after SPC exposure, led to a dose-dependent reduction of scratching, again confirming the causative relationship between LTB4 and pruritus.

Perhaps in SLS the sphingomyelin metabolism is also altered in an atopic dermatitis type manner, thereby explaining the lack of extracellular ceramide (also contributing to the defective water barrier) and an increased leukotriene formation in the stratum corneum causing pruritus. This may yield other future therapeutic approaches for new drugs targeting the SPC receptor of keratinocytes or inhibiting the enzyme responsible for SPC formation from sphingomyelin.

However, data from SPC measurements in SLS skin are lacking, and thus further research is needed to back up these findings.

In addition to these findings, intracellular ceramide catabolism generates sphingosine-1-phosphate (S1P) which is degraded into hexadecenal to be oxidized by FALDH into fatty acids.[10] In SLS this ceramide catabolism may also be disturbed due to a defective breakdown of hexadecenal and subsequent accumulation of S1P. However, due to apparent extracellular ceramide deficiency in SLS, it seems unlikely that large amounts of S1P are generated at the intracellular level.

Biochemical features

As discussed in the general introduction section of this thesis, FALDH is involved in the breakdown of many substrates from several diverse lipid pathways.

To what extent the defective breakdown of these substrates contributes to the clinical signs and symptoms in SLS remains largely to be elucidated. As stated previously, it is generally believed that clinical symptoms evolve from one of the following two factors: (1) an accumulation of (toxic) lipid metabolites which cannot be metabolized by FALDH (or FAO), their diversion into other metabolic products or a combination of both, or (2) the deficiency of critical fatty acid products of FALDH. However, it is not likely that all substrates that depend on FALDH for their breakdown contribute equally to the clinical picture seen in SLS. Also, there may be other unknown substrates that are relevant and cause symptoms as shown in Figure 1.
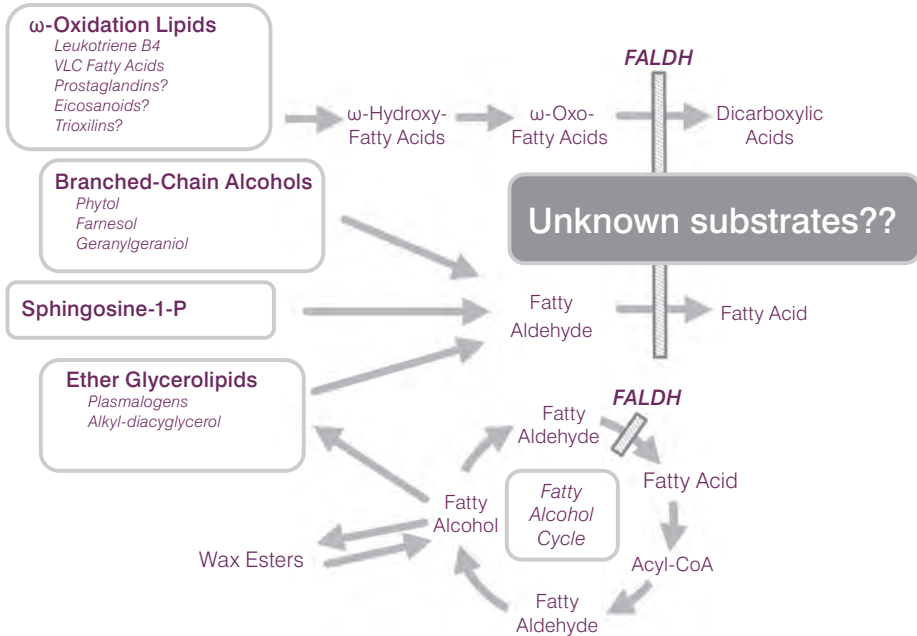

Figure 1 Lipid pathways involved in SLS. Adapted from Rizzo et al.[10]

Future biochemical studies will contribute to a growing insight into FALDH and its role in SLS. As was demonstrated by the histopathological and biochemical parallels seen in both macular telangiectasia and atopic dermatitis, it is important to use different disease models to gain a greater insight into SLS.

Genetic features

As was stated previously, more than 70 different mutations in the ALDH3A2 gene have been identified in patients with SLS to date. Despite this rather variable genotype, the phenotype in SLS is usually quite homogeneous. Recently, Rizzo et al. reported a patient with a very large deletion to the ALDH3A2 locus, leading to a complete loss of ALDH3A2. Yet, the phenotype remained within the range of what might be expected in SLS.[11]

However, genetic testing is usually performed in those patients with an already high clinical suspicion of having SLS. This may create a bias in our view of the clinical phenotype in SLS. It is imaginable that with new techniques such as whole exome sequencing being performed in patients with different signs and symptoms, the clinical spectrum of SLS symptoms changes. By using exome sequencing it is also possible to detect other genetic defects in new disease entities that resemble SLS clinically but lack FALDH deficiency. This was recently demonstrated in two cases with SLS-like symptoms but normal FALDH activity. Exome sequencing revealed mutations in the ELOVL4 gene, coding for fatty acid elongases which are enzymes involved in the synthesis of very-long-chain fatty acids (which in turn are components of sphingolipids and phospholipids).[12]

It is possible that in the future other mutations in different genes are found that produce a SLS-like phenotype. This will again generate a greater understanding of the complex biochemical pathways involved in SLS.

Treatment strategies

In general, the current treatment is mainly symptomatic. Nevertheless, aside from zileuton, several new treatment concepts in the past years have evolved from a greater understanding on the pathophysiological mechanisms responsible in SLS.

Bezafibrates

The expression of the ALDH3A2 gene is regulated by a transcription factor called *peroxisome proliferator activated receptor* α (PPARα).[13] PPARα is part of a family of PPARs which are ligand-inducible transcription factors belonging to the family of nuclear hormone receptors. It binds to a specific PPARα response element in the promoter of the ALDH3A2 gene leading to the up regulation of its transcription. Several ligands are known to be capable of activating PPARα among which are natural ligands (such as phytanic and pristanic acid) and also pharmacological agents (such as bezafibrate).[14,15]

Interestingly, mouse model studies have shown that a phytol-enriched diet is capable of the activation of PPARα through increased levels of the phytol metabolites

phytanic and pristanic acid in plasma.[16] A subsequent induction of ALDH3A2 gene expression however has not been studied.

Bezafibrate, a drug used for treatment of hyperlipaedemia, has also been demonstrated to act as a PPARα ligand and has the in vitro capability of inducing ALDH3A2 expression and subsequent improvement of residual FALDH activity - measured using phytol as substrate - in fibroblasts from SLS patients.[15] This can only be accomplished in patients with some residual FALDH activity which occurs only in a minority of the genotypes in SLS and particularly in some specific missense mutations. Potential therapeutic effects of bezafibrate treatment in selected SLS patients should be part of future research.

Gene therapy

As was discussed in Chapter 7, the in-vitro induction of FALDH activity in 2006 was accomplished in keratinocytes by gene transfer using an adeno-associated viral (AAV) vector.[17] Recently, advances have been made towards a safer and more effective intraocular administration of AAV vectors in humans underlining possibilities in retinal gene therapy for inherited retinopathies.[18] Previously, research in rats detected a new AAV vector serotype which is capable of highly selective Müller cell transduction upon intravitreal injection.[19] Combining these findings, one could postulate that in SLS there are future possibilities to selectively induce FALDH activity in Müller cells using intravitreal-administered AAV vectors by gene transduction. Of course, many limitations are to be made, such as the use of different vectors and the still unclear pathophysiological concepts of the SLS maculopathy and Müller cell involvement specifically. Future studies are needed to investigate the possibilities of retinal gene therapy in SLS.

What's next?

This thesis on Sjögren-Larsson syndrome is published almost 60 years after the original description was first drawn up in 1956. Although insight into underlying pathophysiological mechanisms has increased vastly since 1956, there are still many questions that need to be addressed. The work presented in this thesis could not have been accomplished without very active patient participation in our clinical trials. This can be the key to success for future clinical trials. Real progress in quality of life in SLS can be made if researchers and patients define and prioritize study goals together. Shared decision making in SLS research is the next chapter!

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Current insights & future perspectives

9

Summary

Samenvatting in het Nederlands
(Dutch summary)

Dankwoord
(Acknowledgments)

Curriculum Vitae

List of publications

Summary

The Sjögren-Larsson syndrome (SLS) is hallmarked by an original triad of clinical symptoms, namely ichthyosis, spasticity and mild-to-moderate intellectual disability. During past decades studies with partially genetically unrelated patients revealed additional clinical features such as premature birth, a peculiar crystalline maculopathy and impaired speech-language performance.

A deficient microsomal fatty aldehyde dehydrogenase (FALDH) underlies SLS. FALDH is part of the fatty alcohol nicotinamide adenine dinucleotide oxidoreductase complex (FAO) and catalyzes oxidation of many different medium- and long-chain fatty aldehydes into fatty acids. Deficiency of FALDH results in the accumulation of fatty aldehydes and fatty alcohols in body tissues. Besides its important role in fatty alcohol metabolism, FALDH is also involved in the breakdown of many other substrates among which are the pro-inflammatory leukotrienes.

It is generally believed that clinical symptoms evolve from one of the following two factors: (1) an accumulation of (toxic) lipid metabolites which cannot be metabolized by FALDH (or FAO), their diversion into other metabolic products or a combination of both, or (2) the deficiency of critical fatty acid products of FALDH.

The genetic basis of SLS is found in the FALDH gene, recently renamed ALDH3A2 and located on chromosome 17p11.2. By now, more than 70 different mutations in the ALDH3A2 gene have been identified in SLS patients originating from about 120 different families. Most mutations result in a complete loss of catalytic activity of FALDH, although there are several (missense) mutations associated with residual FALDH activity.

Part ONE of this thesis focuses on the daily functioning of patients with SLS. The purpose of this part is to provide patients, parents and clinicians a further insight into the clinical course of SLS and to provide prognostic information on motor performance and intellectual disability which can be helpful in counseling patients and parents.

Chapter 2 describes data from a cross-sectional study of motor performance and everyday functioning in 17 patients with this rare disorder. Nine female and eight male patients with SLS (age range 1–35 years) were investigated. Data were obtained by structured interview (with parents and SLS patients) and physical examination. Motor performance was measured by the Gross Motor Function Measure; everyday functioning was assessed using the Pediatric Evaluation of Disability Inventory and the Vineland Adaptive Behavior Scale. In most patients, spasticity was bilaterally present in hamstrings, hip adductors, and gastrocnemic muscles. All participants above 7 years of age had contractures in the lower extremities. Limitations were present in almost all gross motor dimensions. Nevertheless, on the activity level these children and young adults were able to move around in the community mostly using wheelchairs. Patients had developmental ages below their chronological age.

Limited communication, social interaction with peers, and restricted participation had additional impact upon daily functioning. During this cross-sectional study motor performance and everyday functioning remained rather stable in patients, referring to the absence of signs of progressive disease.

Chapter 3 provides a detailed insight into cognitive functioning and speech-language pathology in SLS. Cognitive functioning was assessed in 16 patients with SLS using different neuropsychological tests. Speech-language pathology was studied focusing on dysarthria, oral motor functioning, speech intelligibility and language development. Potential correlations between speech-language pathology and other neurological symptoms (e.g. spasticity) were studied. Doing so, we found that the median cognitive developmental age in this study group was 5 (years);8 (months) (n =13; range 3;5 – 8;0) years of age. Besides this intellectual impairment, a variable degree of mainly pseudobulbar dysarthria was found. Speech intelligibility was influenced by dysarthria, but was also related to language pathology. Subsequently we studied potential correlations between the speech-language pathology found in this study and co-existing impairments on motor- and everyday functions (as found in the study described in Chapter 2). No intra-individual correlation between the level of motor functioning and the level of dysarthria or cognitive development was observed in patients. We concluded that dysarthria and language problems are important factors in daily life functioning of patients with SLS. Based upon the clinical profile found in this study, we recommended early speech-language therapy in order to optimize speech-language development in patients with SLS.

Part TWO focuses on the pathognomonic crystalline maculopathy seen in the eyes of SLS patients. This maculopathy, which develops gradually within early childhood, can easily be observed by ophthalmoscopy. The crystalline retinal dots are clinically scattered throughout the peri-fovea. Although the central fovea seems to be uninvolved by crystal deposition, virtually all SLS patients have subnormal visual acuity. Little is known about what specific retinal cell types are pathophysiologically involved in SLS. By studying this peculiar maculopathy we gained further insight into the retinal lipid metabolism and cells involvement in the development of the SLS-maculopathy.

Chapter 4 studies morphologic changes in the retina by optical coherence tomography (OCT). OCT provides high-resolution images of the retina enabling researchers to zoom-in on specific retinal cell layers. Doing so, we expected to be able to specify the layers involved in the SLS-maculopathy thereby gaining further insights into the possible cell types involved.

Full ophthalmologic examination and OCT was performed in all study patients (N=14; 27 eyes studied). We found that, besides the expected peri-macular crystals in the eyes of all study participants detected with ophthalmoscopy, macular morphology

and reflectivity were significantly changed on OCT compared with healthy eyes. Focal hyperreflectivities were found within the peri-foveal ganglion cell layer and the inner plexiform layer that corresponded to the clinical localization of retinal crystals. These layers are mainly composed of axons and dendrites of ganglion cells and retinal interneurons.

The arrangement of crystals both on OCT and fundoscopy suggests involvement of the peri-foveal ganglion cells in the deposition of abnormal lipids in SLS.

More interestingly, a cystoid foveal degeneration on OCT was present in the majority of patients with SLS (18/27 eyes, or 67% of all eyes studied), varying from multiple microcystoid spaces to cystoid foveal atrophy. The cystoid changes seemed to be located in the outer plexiform layer and inner nuclear layer of the retina. The inner nuclear layer mainly contains nuclei and cell bodies of Müller glia cells whereas the outer plexiform layer is composed of synapses between photoreceptors and horizontal cells or bipolar cells and the cytoplasm of Müller glia cells. The typical localization of the cystoid spaces in our patients, together with the only mild-to-moderate loss of visual acuity, suggests a major affection of the foveal Müller cells and not the photoreceptors.

We were unable to detect significant changes within fifteen months of observation in 3 of 14 study participants. Therefore, we believed that foveal atrophy in SLS may be a time-consuming process and a prolonged follow-up period in a larger number of patients may be necessary to illustrate disease progression.

Chapter 5 continues the studying of the peculiar maculopathy in SLS. It was noted that the macula of SLS patients lacks the normal dark-yellowish appearance, a feature that had not been appreciated fully previously. To decide whether an altered distribution or concentration of macular pigment (MP) could be responsible for this changed appearance, two different techniques were used in the study presented in this Chapter: (1) fundus autofluorescence (FAF) providing qualitative estimates of MP and (2) the novel macular pigment reflectometer (MPR) providing quantitative estimates of MP. Data were obtained from twenty-eight eyes of 14 SLS patients. Fundus autofluorescence images of SLS patients lacked the typical attenuation of macular FAF signal as expected in normal eyes. Mean foveal MP levels measured by MPR showed significantly lower values in SLS patients (0.10 ± 0.07) than in healthy individuals (0.69 ± 0.17; $P<0.001$, Student t test). This led to the conclusion that SLS patients must lack macular pigment.

As stated in Chapter 4, the crystalline deposits seen with ophthalmoscopy in SLS patients seemed to be located mainly in the ganglion cell layers and inner plexiform layer. Thus far, the biochemical nature of the glistening dots remains unknown; however, the site of their location on OCT corresponds with the histological position of MP in the normal retina. This co-localization suggests a relationship between the formation of intraretinal crystals and the absence of MP.

MP consists of a mixture of 2 dietary carotenoids, namely lutein and zeaxanthin. The human body is unable to synthesize any carotenoids, and therefore is completely dependent on dietary carotenoids, including lutein and zeaxanthin. Lutein and zeaxanthin are absorbed by intestinal mucosal cells and incorporated into chylomicrons that are secreted into the lymphatic system and subsequently enter the circulation. Hepatic cells incorporate the carotenoids from the chylomicrons into lipoproteins that facilitate further transport of the carotenoids to the various body tissues. Transportation, distribution and uptake of plasma lipoproteins is regulated by apolipoproteins that are produced by many organs, including the liver. Interestingly, Müller cells may as well play a part in macular pigment metabolism. Müller cells produce Apolipoprotein-E which is known to play a part in the targeted uptake of lutein and zeaxanthin from plasma and incorporation within the retina. Therefore, defective functioning of Müller cells may lead to a disturbed retinal MP metabolism which could be the underlying reason for MP deficiency in SLS.

Part THREE focuses on the skin. Epidermal dysfunction in SLS is rather complex since it contains various FALDH-dependent lipid pathways and hence it offers multiple potential approaches to reduce cutaneous symptoms. Previously, Willemsen et al. showed an association between pruritus and elevation of pro-inflammatory leukotriene B4 (LTB4), which is also FALDH-dependent for its breakdown. In SLS, elevation of dermal LTB4, by either increased production or defective breakdown or a combination of both, probably plays an important role in its dermatological phenotype.

Chapter 6 describes results from a prospective, randomized, double-blinded and placebo-controlled trial studying the effect of blocking leukotrienes synthesis upon the general skin condition and especially pruritus in 10 patients with SLS. Erythema, desquamation, lichenification and excoriations were studied complemented with a Physician Global Assessment (PGA) of the skin. Primary outcome measures were changes during treatment with zileuton. Also, self-reported visual analogue scales (VAS) for pruritus and excoriations were studied. Individual data analysis showed a consistent and convincing response to treatment in one patient. This corresponded to findings from previous studies relating to the use of zileuton in SLS. Also, though only a minority of patients seems to respond to treatment, they can easily be detected and will experience a significant improvement in quality of life. We thus suggested a therapeutic trial with zileuton during 4-6 weeks in SLS patients ≥5 years, especially when medical treatment for severe pruritus is warranted. If no clear beneficial response to zileuton is noted, treatment should be discontinued.

Part FOUR gives a state-of-the-art synopsis of current pathophysiological concepts in SLS.

Chapter 7 focuses on clinical practice. Distinctive clinical features in SLS are reviewed in order of appearance together with diagnostics and treatment options. Furthermore, current and future treatment strategies are discussed to render a comprehensive review of the topic.

Chapter 8 presents a discussion on current insights and future perspectives focusing on research questions. Consecutively, challenges in regard to clinical-, biochemical- and genetic SLS features are discussed followed by possible future treatment modalities.

Samenvatting in het Nederlands (Dutch summary)

Het Sjögren-Larsson syndroom (SLS) wordt gekenmerkt door een drietal oorspronkelijk beschreven hoofdsymptomen: ichthyosis (droge en schilferende huid met verdikking van de opperhuid), spasticiteit en een verstandelijke beperking. Hiernaast zijn er de afgelopen jaren nieuwe symptomen ontdekt zoals prematuriteit (vroeggeboorte), zeer specifieke afwijkingen aan de retina (netvlies) en een belemmering in taal-/spraak-ontwikkeling.

SLS wordt gerekend tot de zogenaamde neuro-metabole aandoeningen. Symptomen komen voort uit een afwijking in de stofwisseling van cellen. Deze afwijking betreft een tekort aan het enzym *fatty aldehyde dehydrogenase* (FALDH). Dit enzym is in cellen onder andere verantwoordelijk voor het omzetten van specifieke vetten (fatty aldehydes) in vetzuren (fatty acids). Door het tekort aan FALDH ontstaat er stapeling van deze fatty aldehydes in het lichaam. Naast de rol in het vet metabolisme speelt FALDH ook een rol bij de afbraak van andere stoffen zoals leukotriënen (stoffen die betrokken zijn bij ontstekingsreacties). Het wordt algemeen aangenomen dat de symptomen in SLS voortkomen uit een van de volgende twee factoren: (1) opeen-stapeling van (toxische) stoffen die niet door FALDH kunnen worden afgebroken en al dan niet worden omgezet in andere (toxische) stoffen, of (2) het tekort aan essentiële stoffen die normaliter geproduceerd worden door FALDH.

Het tekort aan FALDH wordt bij het SLS veroorzaakt door een erfelijke afwijking. Door een afwijking op chromosoom 17 is een verandering (mutatie) ontstaan in het zogenaamde ALDH3A2 gen. Dit gen is verantwoordelijk voor de productie van FALDH en kan dat door deze mutatie niet of in veel mindere mate uitvoeren.

Dit proefschrift bestaat uit vier delen.

In het **eerste deel** wordt het dagelijks functioneren van patiënten met SLS beschreven. De doelstelling is hierbij om meer inzicht te verkrijgen ten aanzien van de spasticiteit, de verstandelijke ontwikkeling en de taal-/spraak problematiek. De informatie die hieruit voortkomt, kan worden gebruikt om patiënten, ouders en behandelaars meer inzicht te geven in het te verwachten ziektebeloop bij jonge patiënten met SLS. **Hoofdstuk 2** beschrijft een studie naar het functioneren van 17 patiënten met SLS. In deze studie werd gekeken naar het motorisch functioneren in algemene zin en de invloed die dit had op het dagelijks leven. De meeste patiënten hadden als gevolg van spasticiteit aanzienlijke contracturen aan (met name) de benen. Dit leidde tot aanzienlijke beperkingen in mobiliteit. Toch bleken vrijwel alle patiënten in staat om zichzelf voort te bewegen met gebruik van een rolstoel. Het dagelijks functioneren werd verder beperkt door een matige verstandelijke beperking en beperking in de communicatie door belemmering van spraak. In de studie weergegeven in **hoofdstuk 3** werd deze belemmering in taal-/spraak ontwikkeling bij

16 patiënten met SLS verder bestudeerd. Belemmerende factoren werden in kaart gebracht. Naast de verstandelijke beperking (en de daarmee samenhangende, belemmerde taalontwikkeling) werd er een spraakstoornis (dysarthrie) gevonden die voortkomt uit spasticiteit van spieren in het mondgebied (pseudobulbaire dysarthrie). De kwaliteit en de verstaanbaarheid van de spraak werd hiermee in negatieve zin beïnvloed. De verstandelijke beperking werd in kaart gebracht, waarbij een ontwikkelingsleeftijd van gemiddeld 5 jaar en 8 maanden werd vastgesteld, hoewel de werkelijke leeftijd van deze groep 17 jaar en 10 maanden bedroeg.

In **deel 2** worden de voor SLS kenmerkende afwijkingen aan het netvlies (retina) beschreven. Het netvlies kan worden gezien als een onderdeel van het centraal zenuwstelsel en meer kennis over afwijkingen aan het netvlies kan daarom wellicht meer inzicht geven ten aanzien van afwijkingen in de hersenen en onderliggende problemen in de stofwisseling.

Patiënten met SLS hebben te maken met een verminderd zicht (visus) en verdragen geen fel licht (fotofobie). Het netvlies bij SLS-patiënten vertoont al in de eerste levensjaren afwijkingen in de vorm van kleine kristallen die gerangschikt zijn rondom de gele vlek (macula). Deze kristallen kunnen met oogheelkundig onderzoek (spleetlamp) goed worden waargenomen. Het is onduidelijk hoe deze kristallen precies ontstaan en welke rol zij spelen bij het zicht van patiënten met SLS. De studie weergegeven in **hoofdstuk 4** bestudeerde het netvlies van SLS-patiënten met behulp van een relatief nieuwe techniek: optical coherence tomography (OCT). Deze techniek maakt gebruik van lichtgolven met een zeer hoge resolutie om (net als bij de geluids-golven van echografie) een dwarsdoorsnede van weefsels te verkrijgen.

Het netvlies is opgebouwd uit 10 afzonderlijke lagen en bevat ondermeer zogenaamde fotoreceptoren (staafjes en kegeltjes), zenuwcellen en steunweefselcellen. Met behulp van OCT zijn deze verschillende lagen (en eventuele afwijkingen in die lagen) goed te visualiseren. Door in kaart te brengen in welke specifieke lagen van het netvlies er bij SLS-patiënten afwijkingen worden gezien, groeit het inzicht in onderliggende pathologie.

De ogen van 14 SLS-patiënten werden op deze wijze onderzocht. Ter plaatse van de retinale kristallen werden met OCT kenmerkende reflecties gezien vanuit de zogenaamde ganglioncellaag. Dit type cel vormt de schakel tussen de fotoreceptoren en de oogzenuwcellen. Het is aannemelijk dat ganglioncellen betrokken zijn bij het ontstaan van de kristallen in SLS.

Naast deze reflecties werd er bij de meerderheid van de patiënten ook een degeneratie (verlies van weefsel) van het centrale deel van de macula (fovea) gezien. De cellagen die verloren waren gegaan, bevatten voornamelijk Müller cellen, hetgeen steuncellen zijn die in contact staan met de fotoreceptoren. Het is daarom goed mogelijk dat (problemen in) de Müller cellen een belangrijke rol spelen bij het veroorzaken van de oogheelkundige afwijkingen in SLS.

Hoofdstuk 5 betreft een vervolgstudie. Tijdens de eerdere studie was opgevallen dat de macula (gele vlek) van SLS-patiënten een duidelijk blekere kleur heeft in vergelijking met die van gezonde personen. Het netvlies krijgt zijn oranje kleur door een aparte laag van het netvlies (retinapigment epitheel laag). De macula dankt zijn specifieke gele kleur aan maculapigmenten (opgebouwd uit zogenaamde carotenoïden) die vrijwel alleen daar voorkomen. Er werd aangenomen dat de blekere kleur van de macula bij SLS-patiënten voortkomt uit afwijkingen in het maculapigment. Het maculapigment werd bestudeerd bij 14 SLS-patiënten middels het verrichten van kwalitatieve autofluorescentie metingen ('fundus autofluorescence imaging'; FAF) en kwantitatieve pigment reflectie metingen ('macula pigment reflectometer'; MPR). Hierbij werd aangetoond dat bij de onderzochte patiënten de hoeveelheid maculapigment in sterk verminderde mate aanwezig of geheel afwezig was. Deze zeer bijzondere bevinding zou de fotofobie, zoals aanwezig bij SLS-patiënten, goed kunnen verklaren.

Verder is bekend dat Müller cellen een belangrijke rol spelen bij het metabolisme van maculapigment. De uitkomsten van dit onderzoek geven daarmee een nieuwe aanwijzing dat deze cellen inderdaad aangedaan zijn door de stofwisselingsproblematiek in SLS.

Deel 3 van dit proefschrift beschrijft de huidafwijkingen bij SLS-patiënten. Zoals vermeld, hebben patiënten met SLS last van ichthyosis, die leidt tot een dikke opperhuid met veel schilfering. Dit is het duidelijkst waarneembaar aan de ellebogen, in de nek en op de romp. Patiënten hebben opvallend veel last van jeuk. Eerder onderzoek toonde aan dat het FALDH-gerelateerde vetmetabolisme ook in de opperhuid een rol speelt. Onderzoek bij andere huidaandoeningen toont verder een duidelijke relatie aan tussen leukotriënen en jeuk. FALDH is ook betrokken bij de afbraak van specifieke ontstekingstoffen (leukotriënen; LTB4). Het is daarom aannemelijk dat bij SLS stapeling van deze stoffen, hetzij door verhoogde productie dan wel door gestoorde afbraak (of een combinatie hiervan), in belangrijke mate bijdraagt aan het huidbeeld.

Hoofdstuk 6 beschrijft de resultaten van een geneesmiddelstudie waarbij onderzocht werd of met het middel zileuton het huidbeeld bij SLS-patiënten verbeterd kan worden. Zileuton is een middel dat ondermeer gebruikt wordt bij astmapatiënten. Het remt de productie van leukotriënen. Gedachte is, dat er in de huid minder stapeling van deze leukotriënen optreedt (door remming van de productie) en dat hierdoor vooral de jeuk sterk kan afnemen.

De studieopzet was geblindeerd voor artsen en patiënten en er werd gebruik gemaakt van een placebo. Bij 10 patiënten werd het effect van zileuton op de huid bestudeerd met gestructureerde beoordelingen van de huid (door de onderzoeker) en zelfevaluaties (door ouders/verzorgers van patiënten). Een sterke vermindering van de jeuk werd bij 1 patiënt geconstateerd. De andere patiënten vertoonden geen

(overtuigende) reactie. Het is niet duidelijk waarom er slechts bij 1 patiënt een dergelijke reactie werd gezien. Dit is echter wel in lijn met bevindingen uit een eerdere studie. Bij huidige doseringen reageert slechts een minderheid van de SLS-patiënten op zileuton behandeling. Verdere bestudering van de relatie tussen de verstrekte dosis zileuton en het effect bij patiënten dient in de toekomst plaats te vinden.

Zileuton kan als een veilig middel aangemerkt worden. Omdat een eventuele positieve reactie bij patiënten duidelijk herkenbaar is en daarmee leidt tot verbetering van kwaliteit van leven, wordt een korte (proef)behandeling met zileuton aanbevolen bij SLS-patiënten die veel last hebben van jeuk.

In **deel 4** wordt een uitgebreide samenvatting gegeven ten aanzien van de huidige inzichten rondom SLS. **Hoofdstuk 7** beschrijft aan de hand van de symptomen bij SLS de verschillende diagnostische en therapeutische mogelijkheden. Dit is bedoeld om behandelaars van SLS-patiënten inzicht te geven in de huidige stand van zaken en hen zo te helpen deze patiënten wereldwijd goed te diagnosticeren en behandelen. In **hoofdstuk 8** wordt de discussie met betrekking tot de huidige inzichten weergegeven en worden onderzoeksvragen voor de toekomst belicht.

Dankwoord (Acknowledgments)

Ik schrijf deze woorden enkele dagen voor kerstmis, de mooiste tijd voor reflectie en dank.

Allereerst: dit proefschrift had nooit tot stand kunnen komen zonder de patiënten en hun ouders die aan de beschreven onderzoeken hebben meegewerkt. Zij verdienen dan ook de eerste woorden van dank. Mijn allereerste kennismaking met jullie was in 2006 op de SLS-dag in de Hoogstraat te Utrecht. Ik zag een groep betrokken en zelfbewuste mensen. Een groep die gemotiveerd was om de kennis rondom het Sjögren-Larsson syndroom te vergroten en die verantwoordelijkheid voelde om hier zelf ook een rol in te nemen. Voor mij als jonge onderzoeker was dit zowel inspirerend als ook wat beangstigend. Immers, het in ons gestelde vertrouwen bracht ook verantwoordelijkheid met zich mee. De afgelopen jaren hebben we elkaar meermaals op dergelijke bijeenkomsten ontmoet en de stand van zaken met elkaar besproken. Samen hebben we gekeken naar nieuwe onderzoeksrichtingen en plannen gemaakt. Soms ging het snel en met relatief weinig tegenslagen maar regelmatig liep het anders en moesten plannen worden bijgesteld. Jullie flexibiliteit was daarbij cruciaal. Ik heb een groot respect ontwikkeld voor de wijze waarop jullie omgaan met de zorgen van het dagelijks leven en de missie om de kennis ten aanzien van SLS te vergroten, ook als dit niet direct iets oplevert voor de persoonlijke situatie. Dank voor jullie bijdrage en het in mij gestelde vertrouwen al die jaren. Ik besef me daarbij terdege dat het werk nog niet af is…

Een groot woord van dank moet ook uitgaan naar mijn promotor, prof. dr. Michèl Willemsen. Het is jouw werk geweest dat ik mocht voortzetten. Ik weet nog goed hoe we hier de eerste keer over spraken onder het genot van een goed glas wijn bij jou thuis. En hoewel me nog veel onduidelijk was, kreeg ik wel direct een goed gevoel bij het onderwerp, de opzet, maar vooral jouw begeleiding. Het is een goede keuze gebleken. Ik heb veel mogen leren van je als onderzoeker, specialist en mens. Je prikkelende en kritische reflecties op mijn werk motiveerden telkens weer om verder te komen. De wijze waarop je patiënten betrok bij de richting en invulling van het onderzoek en je omgang met patiënten en collegae inspireerden mij keer op keer. Ik heb er veel van geleerd en het heeft bijgedragen aan de wijze waarop ik vandaag mijn vak uitoefen. Ik ben je hiervoor zeer dankbaar. Uiteindelijk heeft het promotietraject wel wat langer geduurd dan oorspronkelijk de bedoeling was, maar dat heeft er uiteindelijk ook toe geleid dat je vandaag als mijn promotor mag optreden!

Mijn copromotor, dr. Thomas Theelen, ben ik ook veel dank verschuldigd. Thomas, je bijdrage aan dit proefschrift is groter dan enkel de oogheelkundige studies. Naast je directe betrokkenheid bij de uitvoering van deze studies heb je ook op tal van andere momenten bijgedragen aan het werk dat nu voorligt.

De leden van de manuscriptcommissie. Prof. dr. Hans Cruysberg. Beste Hans, je geldt als oogheelkundig expert op het gebied van zeldzame aandoeningen en hebt vanuit die rol betrokkenheid gehad bij de eerste stappen van mijn promotietraject. Ik ben dan ook vereerd dat je ook bij de afronding ervan betrokken bent als voorzitter van de manuscriptcommissie. Veel dank hiervoor.

Prof. dr. Oebo Brouwer. Beste Oebo, veel dank voor de grondige beoordeling van mijn manuscript.

Dr. Jos Draaisma. Beste Jos, ik vind het een grote eer dat je onderdeel uitmaakt van deze manuscriptcommissie en op die manier betrokken bent bij dit proefschrift. Je hebt een meer dan belangrijke rol gespeeld bij het halen van deze mijlpaal waarvoor ik je zeer erkentelijk ben. Je bevlogenheid als kinderarts is voor mij een grote inspiratiebron. Ik hoop dat we nog vele jaren samen vorm kunnen geven aan de algemene pediatrie in ons ziekenhuis.

Collega kinderartsen drs. Petra Bot en drs. Ester Coolen. Samen met Jos geven we vorm aan de vakgroep algemene pediatrie in ons kinderziekenhuis. Ons adagium 'dingen voor elkaar mogelijk maken' heeft ook geholpen bij de totstandkoming van dit proefschrift. Ik ben jullie veel dank verschuldigd voor het overnemen van allerhande (supervisie) taken en het bieden van ruimte om dit werk tot een goed eind te brengen. Ik hoop nog vele jaren samen met jullie vorm en inhoud te mogen geven aan academische algemene pediatrie!

Dank gaat ook uit naar collega en afdelingshoofd prof. dr. Kees Noordam. Beste Kees, de afgelopen jaren heb je mij op tal van momenten kansen en mogelijkheden geboden om mijzelf als kinderarts verder te ontwikkelen. Daarbij hoort zeker niet in de laatste plaats de mogelijkheid tot het volgen van de NFU-master opleiding in kwaliteit en patiëntveiligheid. Ik wil je danken voor het vertrouwen dat je daarbij steeds in mij hebt gesteld.

Ik wil iedereen danken die op enigerlei wijze heeft bijgedragen aan het tot stand komen van de verschillende artikelen. In het bijzonder wil ik daarbij de volgende personen noemen.

Dr. Jan Willem Gorter en drs. Judith Verhoog voor het gedegen werk bij het in kaart brengen van het motoor functioneren van SLS patiënten.

Marjo van Gerven en prof. dr. Ben Maassen voor de samenwerking bij ons onderzoek naar taal-/spraakontwikkeling.

Ik dank dr. Tos Berendschot en dr. Rob van der Veen voor de mogelijkheid die we hebben gekregen om macula pigment metingen bij SLS patiënten te gaan verrichten. Prof. dr. Ron Wevers dank ik voor de zinvolle inhoudelijke discussies die wij in dit traject meermaals mochten hebben en de hulp van zijn laboratorium bij de uitvoering van ons onderzoek. Ing. Alexandra Versleijen en ing. Ed van der Heeft wil ik hierbij eveens noemen. Dank voor al het werk dat er is gaan zitten in de metingen van leukotriënen.

Dr. Marieke Seyger en drs. Diane Bastiaans. Het heeft even geduurd maar uiteindelijk hebben we onze zileuton studie toch kunnen uitvoeren. Dank voor jullie meer dan noodzakelijke bijdrage hierbij. Dr. Nel Roeleveld dank ik voor haar waardevolle bijdrage aan het artikel.

Mijn collega in het werkplekmanagement, Margriet van Buuren. Al enkele jaren geven we samen vorm en inhoud aan het 'duaal leiderschap' van de medium care. Ik realiseer me dat alle drukte rondom de afronding van mijn proefschrift heeft gemaakt dat er minder tijd was dan voorheen. Ik wil je danken voor het begrip en geduld dat je hierbij hebt getoond.

Ook alle collega specialisten, AIOS, teamleiders en verpleegkundigen van het Amalia kinderziekenhuis wil ik danken voor de samenwerking de afgelopen jaren. Hedwig Dillerop wil ik hierbij in het bijzonder noemen. Jouw gedegen werk in de coördinatie van de bedcapaciteit neemt mij dagelijks veel werk uit handen.

Medewerkers van het secretariaat wil ik danken voor de dagelijkse ondersteuning en flexibiliteit. In het bijzonder wil ik hierbij Sandra van Hugten en Wilma Kosman noemen. Sandra, je bent al jaren dé spil in onze vakgroep. Het lijkt een onmogelijke opgave om al onze taken en agenda's te stroomlijnen en toch lukt je dit iedere keer weer. Wilma, je komst naar de Medium Care is van grote meerwaarde gebleken. Ik weet niet hoe je het doet maar iedere keer lukt het je weer om de meest onmogelijke opgaven binnen korte tijd geregeld te hebben.

Lieve familie en vrienden, ik wil jullie danken voor alles wat jullie voor mij betekenen en de rol die jullie direct of indirect hebben gehad bij het tot stand komen van dit proefschrift.

Ik wil graag beginnen bij mijn goede vrienden Martijn Bevers, Frank Eleveld, Johan Spierings, Olivier ten Hallers, Luc Roelofs en Stijn Hazenberg. 'Vrienden zijn familie-leden zonder bloedverwantschap' schreef Ronald Giphart en dat is zeker op onze vriendschap van toepassing. Wat is begonnen als vriendschap tijdens onze studie is

inmiddels uitgegroeid tot een hechte band. We concludeerden laatst dat onze vriendschap in al die jaren ook onszelf heeft gevormd tot wie we nu zijn. Ik ben enorm trots op die band en de onvoorwaardelijke steun die ik van jullie ervaar. Ik realiseer me dat ik de afgelopen periode wat minder vaak op 'de donderdagavond' aanwezig ben geweest, ik zal snel een pot verf meenemen voor ons bankje!

Mijn schoonouders, Fred en Willeke Kok. Ik heb het enorm getroffen met jullie. Vanaf de eerste ontmoeting heb ik een warm, liefdevol welkom ervaren en dat is al die jaren zo gebleven. Ik ben afgelopen zomer aan de laatste onderdelen van dit proefschrift begonnen in jullie fantastische maison en France. 'La Coquille' staat misschien wel het meest symbool voor jullie gastvrijheid en warmte. Met mijn hele schoonfamilie heb ik er een geweldige tweede familie bijgekregen. Ik hoop dat we nog vaak met elkaar van het leven mogen genieten! Nienke, ik zie uit naar jouw boekje, het gaat je ongetwijfeld lukken.

Mijn zus en zwager, Eveline en Bas Oonk.
Bas, daar waar ik eerder schreef over vrienden die familie worden, geldt voor jou het omgekeerde. Je bent veel meer dan een zwager, je bent een echte vriend die onvoorwaardelijk voor ons klaarstaat. Ik dank je voor alle mooie momenten die we samen hebben mogen delen en zie uit naar wat nog gaat komen!
Lieve Eveline, ik bewonder je om wie je bent en hoe je in het leven staat. Onze band heb ik eigenlijk altijd als sterk ervaren en je hebt meer invloed gehad op wie ik ben geworden dan je zelf vermoedelijk denkt. Ik ben er supertrots op dat je vandaag als paranimf aan mijn zijde staat!

Mijn ouders, Arjen en Marjon Fuijkschot.
Lieve pap en mam. Dit proefschrift is aan jullie opgedragen. Het feit dat ik zover gekomen ben, komt voort uit de opvoeding die jullie me hebben gegeven. Ik ben jullie enorm dankbaar voor de 'kansen en mogelijkheden' die jullie me hebben geboden en de warmte en liefde die daar altijd aan ten grondslag lag.
Pap, misschien is het samen reviseren van een compleet motorblok uit onze oude Volvo wel een mooie metafoor voor hetgeen ik van je heb geleerd: technisch inzicht, doortastendheid, volharding en vooral: uitdagingen niet uit de weg gaan.
Mam, 'mannen zijn wat moeders van hen gemaakt hebben', wordt wel gezegd. Daar valt niets op af te dingen.

Tot slot de 4 allerbelangrijkste mensen in mijn leven.
Lieve Hannah, mijn prinses. Wat heb je een boel liefde en levendigheid in ons huis gebracht. Je ijver, geduld en nieuwsgierigheid zijn geweldig om te zien. Ik ben heel benieuwd wat komen gaat en wees gerust, ik zal altijd je 'pappa van de prinses' blijven!

Lieve Jochem, jouw ondernemingszin en doorzettingsvermogen zijn nu al ongekend. Wat ben je toch een geweldig ventje. Ik zie ernaar uit om samen met jou ons motorblok te reviseren!

Lieve ……, jouw aankondiging heeft gemaakt dat dit werk een prachtige deadline kreeg. Ik zie uit naar je komst in ons gezin. Welkom kanjer!

So now I'll sing into the world a simple message to my girl. Lieve Ellemieke, wat ben ik blij dat ik jou ben tegengekomen. Ik bewonder je iedere dag weer om wie je bent en hoe dat mij maakt. Je bent the change in mijn world. Het zonlicht in ons gezin komt écht van jou!

Curriculum Vitae

Joris Fuijkschot was born on November 10th, 1977 in Eindhoven, the Netherlands. After finishing secondary school at the Lorentz Lyceum in Eindhoven (1996) he started Medical School at the Radboud University Nijmegen. In his final year he performed a research traineeship studying pulmonary development at the Hospital for Sick Children in Toronto, Canada. After obtaining his medical degree he started working as a resident in pediatrics at the Canisius-Wilhelmina hospital in Nijmegen (supervisor dr. Paul van Wieringen). His residency in pediatrics continued at the Radboud University Nijmegen Medical Center in 2005 (successive supervisors prof. dr. Louis Kollée and dr. Jos Draaisma). During this training he became especially interested in the Sjögren-Larsson syndrome and initiated his PhD project under the supervision of prof. dr. Michèl Willemsen and dr. Thomas Theelen.

He finished his residency pediatrics in 2008 after which he started to work as a general pediatrician at the Department of Pediatrics of the Radboudumc Amalia Children's hospital in Nijmegen. He became a full instructor of the European Resuscitation Council in 2009 and is at present still a member of staff at the Dutch Foundation for Emergency Medical Care of Children ('Stichting Spoedeisende Hulp bij Kinderen'). In 2010 he became clinical director of the medium care pediatric wards of the Radboudumc Amalia Children's hospital. During the past years he became especially interested in the subject 'Patient Safety and Healthcare improvement'. In 2014 he started the Master's Degree program 'Quality and Safety in Healthcare' from the Netherlands Federation of University Medical Centers (Nederlandse Federatie van Universitair Medische Centra; NFU) under the supervision of dr. Hub Wollersheim and prof. dr. Paul Robben.

Joris is happily married to Ellemieke Kok. They are living their dreams in Nijmegen together with Hannah (18-09-2011), Jochem (20-09-2013) and ……

List of publications

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5. **Fuijkschot J**, Maassen B, Gorter JW, Gerven Mv, Willemsen M. Speech-language performance in Sjögren-Larsson syndrome. *Dev Neurorehabil* 2009; 12:106-12.
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7. Verhoog J, **Fuijkschot J**, Willemsen M, Ketelaar M, Rotteveel J, Gorter JW. Sjögren-Larsson syndrome: motor performance and everyday functioning in 17 patients. *Dev Med Child Neurol* 2008; 50:38-43.

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1. **Fuijkschot J**, Vernhout B, Lemson J, Draaisma JM, Loeffen JL. Validation of a Paediatric Early Warning Score: first results and implications of usage. *Eur J Pediatr* 2015; 174:15-21.
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3. **Fuijkschot J**, Wijnen RM, Gerrits GP, Dubois SV, Rieu PN. A neonate with an intact congenital umbilical appendix: an alternative theory on the etiology of the appendico-umbilical fistula. *Pediatr Surg Int* 2006; 22:689-93.