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Hallermann-Streiff Syndrome: No Evidence for a Link to Laminopathies

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Key Words
Hallermann-Streiff syndrome · Hutchinson-Gilford progeria syndrome · *ICMT* · Laminopathy · *LMNA* · Mandibuloacral dysplasia · *ZMPSTE24*

Abstract
Hallermann-Streiff syndrome (HSS) is a rare inherited disorder characterized by malformations of the cranium and facial bones, congenital cataracts, microphthalmia, skin atrophy, hypotrichosis, proportionate short stature, teeth abnormalities, and a typical facial appearance with prominent forehead, small pointed nose, and micrognathia. The genetic cause of this developmental disorder is presently unknown. Here we describe 8 new patients with a phenotype of HSS. Individuals with HSS present with clinical features overlapping with some progeroid syndromes that belong to the laminopathies, such as Hutchinson-Gilford progeria syndrome (HGPS) and mandibuloacral dysplasia (MAD). HGPS is caused by de novo point mutations in the *LMNA* gene, coding for the nuclear lamina proteins lamin A and C. MAD with type A and B lipodystrophy are recessive disorders resulting from mutations in *LMNA* and *ZMPSTE24*, respectively. *ZMPSTE24* in addition to *ICMT* encode proteins involved in posttranslational processing of lamin A. We hypothesized that HSS is an allelic disorder to HGPS and MAD. As the nuclear shape is often irregular in patients with *LMNA* mutations, we first analyzed the nuclear morphology in skin fibroblasts of patients with HSS, but could not identify any abnormality. Sequencing of the genes *LMNA, ZMPSTE24* and *ICMT* in the 8 patients with HSS revealed the heterozygous missense mutation c.1930C>T (p.R644C) in *LMNA* in 1 female. Extreme phenotypic diversity and low penetrance have been associated with the p.R644C mutation. In *ZMPSTE24* and *ICMT*, no pathogenic sequence change was detected in patients with HSS. Together, we found no evidence that HSS is another laminopathy.

Hallermann-Streiff syndrome (HSS, MIM 234100) is a rare congenital disorder characterized by cranial and facial bone malformation, hypotrichosis, microphthalmia, cataracts, skin atrophy, dental anomalies, and pro-

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portionate short stature. The condition can be immediately recognized by a characteristic facial gestalt with small face, prominent forehead, thin narrow nose, and micrognathia. Although a few familial cases have been reported [Guyard et al., 1962; Koliopoulos and Palimeris, 1975; Gerinec et al., 1989], all classical cases are sporadic [Cohen, 1991]. Inheritance of this developmental disorder is still unknown, and autosomal recessive as well as autosomal dominant inheritance with de novo mutations have been discussed.

Conventional cytogenetic studies revealed normal chromosomes in the majority of cases. However, Carones [1961] reported some discrepancy in the size of 2 D-group chromosomes in an individual with HSS, Jalbert et al. [1968] noted a 4p anomaly in 1 patient with HSS, and Fryns et al. [1993] described a girl with clinical features compatible with HSS and a partial 4q monosomy and a partial 14q trisomy. However, no clue to the localization of the causative gene for HSS in the genome exists so far. Thus, by screening candidate genes implicated in congenital disorders with clinical manifestations overlapping HSS, the underlying genetic defect in HSS-affected patients could be identified. For example, dominantly inherited oculodentodigital dysplasia (ODDD, MIM 164200) that shares several clinical characteristics with HSS, such as microphthalmia, small nose, hypotrichosis, and dental anomalies, is caused by mutations in *GJA1* [Paznekas et al., 2003]. In a patient with phenotypic overlap with HSS [Damiano Salpietro et al., 2004], the homozygous missense mutation c.227G>A (p.R76H) in *GJA1* was found, while no sequence alteration in this gene was detected in another patient with full-blown HSS [Pizzuti et al., 2004].

Patients with HSS have a progeroid appearance with small face, pointed nose, micrognathia, and hypotrichosis, all features that are also present in individuals with Hutchinson-Gilford progeria syndrome (HGPS, MIM 176670) and mandibuloacral dysplasia with type A or B lipodystrophy (MADA, MIM 248370; MADB, MIM 608612). Indeed, in a female patient with MAD, the clinical diagnosis of HSS has been suggested at the age of 2 years because of her characteristic facial appearance [Schrander-Stumpel et al., 1992]. HGPS is a rare progressive disorder characterized by extreme premature ageing. Patients with MAD, a progeria-like syndrome with autosomal recessive inheritance, have growth retardation, craniofacial anomalies, skeletal abnormalities with progressive acro-osteolysis, pigmentary skin changes, and lipodystrophy type A or B. HGPS and MADA are both caused by mutations in the *LMNA* gene encoding lamins A and C [Novelli et al., 2002; De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003]. Lamins are part of the nuclear envelope of all somatic cells. *LMNA* mutations have been implicated in a wide spectrum of diseases that include disorders of the striated muscle, peripheral neuropathy, syndromes affecting adipose tissue and accelerated ageing disorders [Novelli and D'Apice, 2003; Worman et al., 2009]. In individuals affected by MADB, no sequence alteration in *LMNA* has been detected, suggesting a different genetic cause than in MADA [Simha et al., 2003]. Indeed, biallelic mutations in the *ZMPSTE24* gene have been identified in patients with MADB [Agarwal et al., 2003; Miyoshi et al., 2008; Cunningham et al., 2010; Ahmad et al., 2011; Yaou et al., 2011]. *ZMPSTE24* encodes a zinc metalloproteinase which is required for posttranslational processing of the precursor molecule of lamin A, called prelamin A [Barrowman and Michaelis, 2009]: first, ZMPSTE24 cleaves off the last 3 amino acids of the farnesylated prelamin A. Second, following methylation of the farnesylated cysteine residue at the C-terminus by the isoprenylcysteine carboxyl methyltransferase (ICMT) [Sinensky et al., 1994], ZMPSTE24 cleaves the last 15 amino acids to generate mature lamin A. Involvement of ICMT in prelamin A processing suggests that *ICMT* is a candidate gene for MAD-related disorders. However, no mutation in the *ICMT* gene has yet been linked to human diseases.

Due to the clinical overlap between progeria, MAD, and HSS, we hypothesized that the 3 conditions are allelic. Here we summarize clinical data of 8 patients with HSS and describe our results on chromosome breakage, nuclear morphology, and sequence analysis of the genes *LMNA*, *ZMPSTE24*, and *ICMT* in our cohort of HSS-affected individuals.

Material and Methods

Patients

We obtained clinical data and blood or DNA samples from 8 patients with a phenotype compatible with the clinical diagnosis of HSS who were assessed by experienced clinical geneticists and/or pediatricians. Routine chromosome analysis was performed on a clinical basis for all patients. The clinical data and samples were obtained with informed consent, including consent to use the photographs in this report, under protocols approved by Institutional Review Boards at all participating institutions.

Chromosome Breakage Studies

Blood samples were obtained from patients 2 and 3 as well as a healthy individual for chromosomal instability studies, i.e. testing for hypersensitivity to the clastogenic effect of the DNA cross-

linking agent diepoxybutane (DEB; Sigma-Aldrich, Taufkirchen, Germany). For these studies, cultures of stimulated peripheral blood lymphocytes were paired with a replicate set of untreated controls used to measure spontaneous breakage. After initial incubation for 24 h at 37°C, DEB (final concentration 0.1 μg/ml) was added to the treated cultures for further 48 h. Cultures were harvested and chromosomes were prepared according to standard protocols. Breakage analysis was performed on 100 Giemsa-stained metaphase spreads from both the untreated and the clastogen-treated cultures of the patients and the control individual. Each cell was assayed for the number of chromosomes and the number and type of structural abnormalities. Chromosome damage was reported as breaks per cell. To study chromosomal instability in patients 1 and 5, fibroblast cells derived from skin biopsies of these patients and a healthy individual were cultured under standard conditions. Confluent cultures were split, and 24 h later 1 subculture from each individual was exposed to 0.01 μg/ml DEB for 48 h, while a second one served as untreated control. After harvesting the cells, 50 Giemsa-stained metaphase spreads each were examined as described above. Testing for hyper-radiosensitivity was not performed.

Immunocytochemistry

Fibroblast cells (passage 9–11) derived from patients 1 and 5 and 1 healthy individual were grown at low density on sterile coverslips in DMEM (Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal calf serum (Invitrogen) and penicillin-streptomycin (100 U/ml and 100 mg/ml) (Invitrogen) at 37°C in 5% CO_2 for 20 h. Following rinsing with PBS, cells were fixed with 4% paraformaldehyde (Sigma-Aldrich) in PBS. After washing 3 times with PBS, cells were incubated in permeabilization/blocking solution (2% BSA (Sigma-Aldrich), 3% goat serum (Sigma-Aldrich), and 0.5% Nonidet P40 (ICN, Eschwege, Germany) in PBS). Subsequently, cells were overlaid with antibody solution (3% goat serum and 0.1% Nonidet P40 in PBS) containing monoclonal mouse anti-human lamin A/C antibody (1:10 dilution; clone JOL2, Chemicon, Hofheim, Germany). After extensive washing, cells were incubated with 4 μg/ml Alexa Fluor 546 goat anti-mouse IgG (Invitrogen) in antibody solution. Finally, cells were washed with PBS and embedded in glycerol gelatine containing 1% phenol (Sigma-Aldrich) on microscopic slides. Images were acquired and processed with a Leica TCS-NT confocal microscope system (Leica Microsystems, Wetzlar, Germany) using an APO 40-by-1.25 oil immersion objective lens (Leica Microsystems).

Sequencing of the Genes LMNA, ZMPSTE24, and ICMT

Genomic DNA was isolated from blood lymphocytes or fibroblasts according to standard procedures. The coding region, including flanking intronic sequences of the genes *LMNA* (12 exons; GenBank accession nos. NM_170707 and NM_005572), *ZMPSTE24* (10 exons; GenBank accession no. NM_005857) and *ICMT* (6 exons; GenBank accession no. NM_012405), was amplified from genomic DNA. Primer sequences and PCR conditions are available on request. Amplicons were directly sequenced using the ABI BigDye Terminator Sequencing Kit (Applied Biosystems, Darmstadt, Germany) and an automated capillary sequencer (ABI 3130 or ABI 3500; Applied Biosystems). Sequence electropherograms were analyzed using Sequence Pilot software (JSI medical systems, Kippenheim, Germany).

Computational Analysis

Splice site prediction of 1 intronic variant identified in *LMNA* was calculated by using the online tools SpliceView (http://bioinfo.itb.cnr.it/oriel/splice-view.html), the Berkeley Drosophila Genome Project (BDGP) (http://www.fruitfly.org/) [Reese et al., 1997], and the NetGene2 server (http://www.cbs.dtu.dk/services/NetGene2/) [Hebsgaard et al., 1996].

LMNA Transcript Analysis

Total RNA was isolated from fibroblast cells of patient 1 and of 2 controls using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. One microgram of RNA was reverse transcribed into cDNA using the Omniscript Reverse Transcriptase Kit (Qiagen) and random hexanucleotides (Invitrogen) according to the manufacturer's recommendations. Of a total of 20 μl reaction, 1 μl was used as template to amplify transcripts by using primer pairs spanning all *LMNA* exon-exon junctions, thereby covering the complete cDNA. Amplicons were visualized on an agarose gel. The amplicon band pattern from patient 1 was compared with that of the 2 controls. Subsequently, amplicons of the 2 controls and patient 1 were directly sequenced. Primer sequences and PCR conditions are available on request.

Results

Clinical data of the 8 individuals with HSS are summarized in table 1, and selected photographs are shown in figure 1.

Conventional cytogenetic analysis revealed a normal karyotype in the 8 patients with HSS (data not shown). In 2003, a patient with HSS showing a markedly increased chromosome breakage rate has been described, suggesting that defective DNA repair may underlie HSS [Hou, 2003]. Chromosome breakage studies using fibroblast cells from patients 1 and 5 and lymphocytes from individuals 2 and 3 did not reveal an increased breakage rate compared with cells (fibroblasts and lymphocytes) from healthy individuals (online suppl. table 1; for all online material, see www.karger.com/doi/10.1159/000334317).

Nuclear abnormalities, including irregular shape and lobulation of the nuclear envelope, have been reported in fibroblasts of patients with different types of laminopathies and *LMNA* mutations [Ostlund et al., 2001; Muchir et al., 2004]. To study, if the lamin A/C pathway is affected in HSS, we first analyzed the nuclear shape in fibroblast cells of HSS-affected individuals 1 and 5 by immunofluorescence analysis with an anti-lamin A/C antibody. We did not detect any abnormality in nuclear morphology of the patients' fibroblast cells compared with normal cells. Nuclei showed a normal ovoid morphology (fig. 2).

Table 1. Clinical data from individuals with HSS

Patient No.	1	2	3	4	5[a]	6	7	8[b]
Ethnic origin	Arabian	German (C)	Kosovan (C)	Czech (C)	Turkish	Czech (C)	German (C)	German (C)
Sex	F	F	F	M	M	M	F	M
Birth weight (SD)	−1.5	−0.5	−0.6	−1	−1.9	+1.7	−0.6	−2.3
Birth length (SD)	−2.6	+0.1	−0.6	0	−1	0	+0.8	−3.8
OFC birth (SD)	−0.9	+0.9	microcephaly	nd	−2	0	−0.9	−2.8
OFC* (SD)	−2.6	+0.9	−2.9	−1	nd	−0.2	−2.0	−10
Height* (SD)	−4.2	−1	−4.4	+0.5	nd	−1.5	−1.6	−10.1
Weight* (SD)	−2.6	−0.8	−2.5	+0.3	nd	−0.8	−2.0	−7.1
Age*	9 y 9 mo	3 y 2 mo	4 y 9 mo	2 y 4 mo	nd	7 y 7 mo	2 y 7 mo	7 mo
Development/ID	mildly retarded	mildly retarded	delayed at the beginning, now normal	slightly delayed	nd	delayed at the beginning, now normal	normal	markedly delayed
Speech	36 mo	first words: <12 mo	first words: 30 mo	first words: 12 mo	nd	first words: 12 mo, dyslalia until 6 y, rhinolalia until now because of velopharyngeal insufficiency	first words: 9 mo	nd
Sitting and walking	walking: 30 mo	sitting: 8 mo walking: 19 mo	sitting: 14 mo walking: 20 mo	sitting: 10 mo walking: 15 mo	nd	sitting: 6 mo walking: 15 mo	sitting: 10 mo walking: 13 mo	nd
Hair and skin	sparse hair, high anterior hairline	full hair, high anterior hairline	sparse hair, high anterior hairline, atrophic skin of the head	sparse hair, high anterior hairline	high anterior hairline	atrophic skin of the head, face and belly in infancy, high anterior hairline	sparse hair, high anterior hairline, atrophic skin on the nose, several telangiectasias	sparse hair, high anterior hairline, eyebrows and eyelashes, prominent vessels, leathery skin, more deeply pigmented back, lack of subcutaneous fat
Craniofacial features								
Head	broad forehead	frontal bossing	broad forehead	frontal bossing, short neck	broad forehead	large upper head, hypoplastic facial bones, short neck	frontal bossing	prominent and broad forehead, short neck
Eye	congenital cataracts, right eye smaller than left, nystagmus, blue sclerae	congenital cataracts, microphthalmia bl (left more pronounced)	congenital cataracts, downslanted palpebral fissures	microphthalmia, strabism	congenital cataracts, microphthalmia bl	microphthalmia, nystagmus, convergent strabism, amblyopia	congenital cataracts, microphthalmia bl, papilledema bl	congenital cataracts, microphthalmia, deeply set eyes
Nose	long, thin	small, pointed	small, narrow nasal tip and rigde, underdeveloped alae nasi	small, underdeveloped alae nasi	small and pointed, underdeveloped alae nasi	short in infancy, now thin	small, narrow	small, narrow
Mouth	narrow, protruding tongue	narrow, thin vermillion border, high-arched palate	narrow with thin lips	narrow	nd	narrow, high arched palate	narrow, high-arched palate	narrow
Teeth	neonatal	neonatal, maxilla: lacks 2 incisors, mandible: partly double-rowed	neonatal	abnormal, overcrowded, malocclusion, open bite	neonatal	abnormal, overcrowded incisors, malocclusion, open bite	first tooth >20 mo, 1 maxilla incisor right lacking, inclined arrangement of the teeth	–
Mandible	micro-retrognathia	microretrognathia	microretrognathia	microretrognathia, flat mandibular angle	receding	hypoplastic mandible with increased angle due to anterior displacement of the temporomandibular joint	micrognathia	micrognathia
Other anomalies	body asymmetry with a smaller left side, recurrent infections of the upper airways		low-set ears	dysplastic auricles, hip dysplasia	apnoea, recurrent pneumonias	dysplastic auricles, hip dysplasia, cervical-thoracic scoliosis, thin calvarium with ossal defects, narrow vertebral tube, scapulae alatae, myotonic reaction in EMG, umbilical hernia o	progressive snoring with aggravate breathing, recurrent infections of the upper airways, apnoea	polymicrogyria, hypoplasia of cc, cb and bg, narrowing of foramen magnum and of spinal canal at craniocervical junction, large perimembrane VSD, overriding of aorta, DCRV, hepatomegaly, hip dysplasia, clubfoot r, planovalgus l, thumb malposition and flexion contracture bl, hypospadia, inguinal hernia l, bend in penis shaft, poor weight gain

bg, Basal ganglia; bl, bilateral; C, Caucasian; cb, cerebellum; cc, corpus callosum; DCRV, double-chambered right ventricle; EMG, electromyography; F, female; ID, intellectual disability; l, left; M, male; mo, months; nd, not documented; o, operated at the age of 3 months; r, right; SD, standard deviation; VSD, ventricular septal defect; y, year(s); +, present; –, absent; *, at last follow-up.

[a] Died at the age of 13 months from pneumonia; [b] died at the age of 7 months.

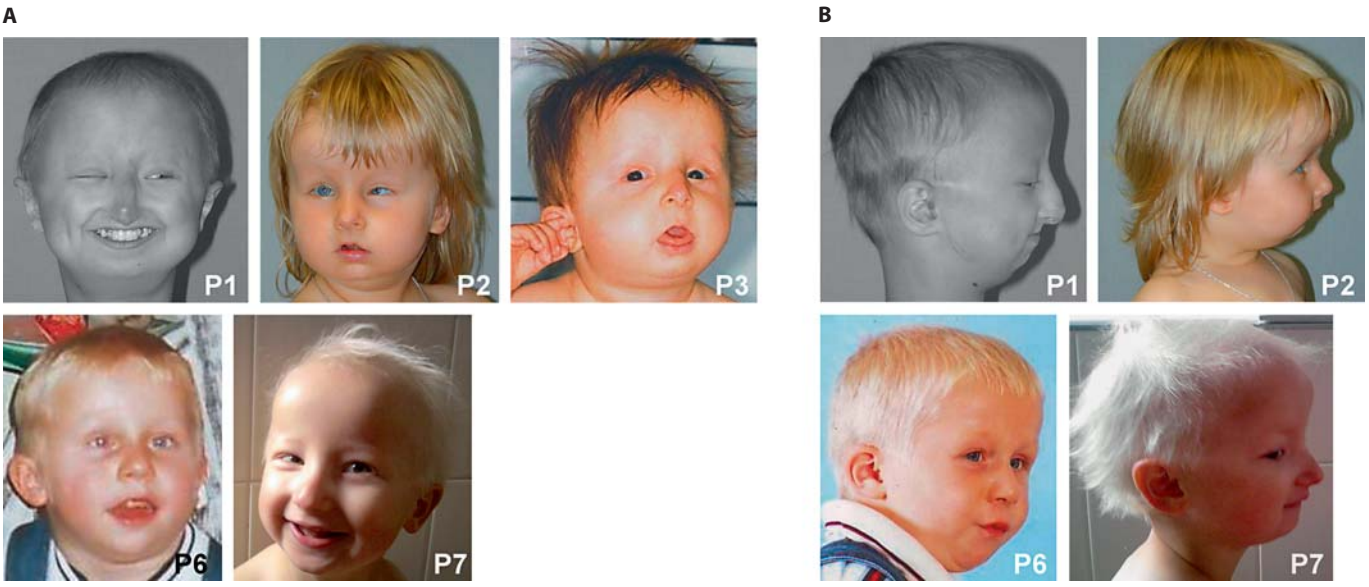

Fig. 1. Facial appearance of patients 1, 2, 3, 6 and 7 with HSS. Numbers refer to patient numbers. **A** Frontal view and **B** lateral view of the patients. All patients show the typical facial appearance with malformations of the cranium and facial bones, frontal bossing, pointed nose, narrow mouth, and micrognathia. Microphthalmia is present in patients 1, 2, 6 and 7. Except for patient 2, all individuals have sparse hair. (Photographs submitted with written consent from the patient's legal guardian for publication in print and online.)

Next, we sequenced the coding region and adjacent intronic sequences of the 3 genes *LMNA*, *ZMPSTE24*, and *ICMT* in the 8 patients with HSS. Patient 1 was homozygous for the intronic sequence variation c.1158–44C>T in the *LMNA* gene that was found in her mother in the heterozygous state, while the father was not available (data not shown and online suppl. table 2). In silico analysis using 3 splice site prediction programs revealed that c.1158–44C>T potentially creates a new splice donor site (online suppl. table 3) and could result in altered splicing. However, our RNA analysis did not uncover any aberrant *LMNA* transcript in patient 1 compared with 2 healthy individuals (data not shown). In patient 2, we identified the heterozygous missense mutation c.1930C>T (p.R644C) in exon 11 of *LMNA* (online suppl. table 2) that has been described in patients with a wide spectrum of phenotypes [Rankin et al., 2008]. This mutation was also found in the asymptomatic mother of patient 2 (data not shown). In addition, 5 known single-nucleotide polymorphisms (SNPs) were detected in the *LMNA* gene (online suppl. table 2). Sequencing of the *ZMPSTE24* gene revealed 2 SNPs (online suppl. table 2). As the only sequence alteration in the *ICMT* gene, we found a heterozygous duplication of 24 bp (c.–41_–64dup24) in the 5′ untranslated region in patient 1 (online suppl. table 2).

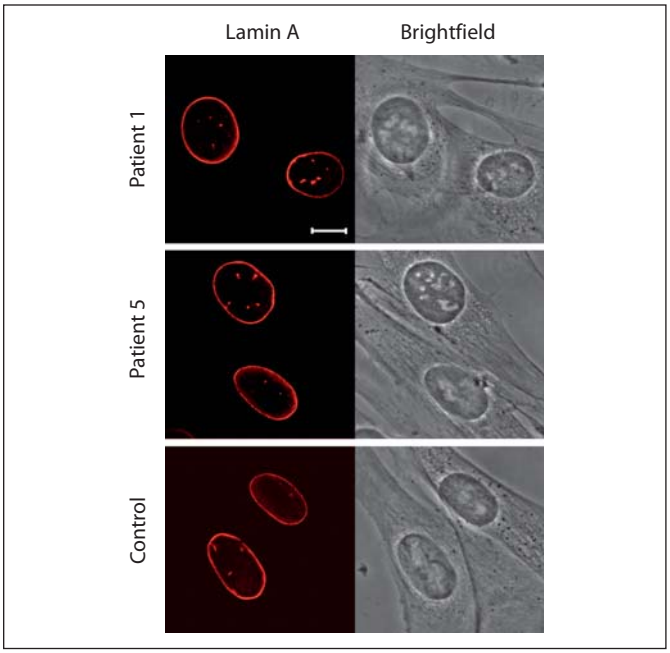

Fig. 2. Representative nuclear morphology of fibroblast cells derived from patients 1 and 5 with HSS and a control. Cells were labeled with a monoclonal mouse anti-human lamin A/C antibody and a secondary Alexa Fluor 546 anti-mouse IgG (left panel). The same cells are shown by phase-contrast microscopy in the right panel. Scale bar: 10 μm.

Discussion

In this study, we aimed to gain insight into the pathophysiological and/or genetic basis of HSS, a rare developmental disorder for which the causative gene has not been identified to date.

We present 8 patients with clinical features of HSS, including individuals 1, 3, 5, and 7 with the characteristic manifestations of HSS (fig. 1, table 1) and male patient 8 with the typical facial appearance (facial photographs of this patient could not be shown because of lack of consent) and very severe abnormalities affecting various organ systems (table 1). In detail, he showed markedly delayed development, several brain malformations, congenital heart defects, skeletal abnormalities, hypospadia, and left inguinal hernia and died at the age of 7 months. Such a combination of severe organ manifestations is unusual in HSS; however, several patients with the classical facial appearance and additional atypical clinical features have been reported in the literature. For example, bone fractures have been described in severe (lethal and nonlethal) forms of HSS [Dennis et al., 1995; Ertekin et al., 2004]; cryptorchidism, clitorial enlargement, and hypospadia have been documented in some patients [Cohen, 1991]. Hemihypertrophy has also been associated with HSS [Dogan et al., 2010], and various congenital heart defects, comprising valvular pulmonary stenosis, patent ductus arteriosus, ventricular and atrial septal defects are not uncommon in HSS [Imaizumi et al., 1994 and references therein; David et al., 1999]. Agenesis of the corpus callosum has been seen in a 4-year-old boy with HSS and other complications, such as bilateral inguinal hernia with cryptorchidism and a small penis [Sigirci et al., 2005]. The 2-month-old girl described by Hou [2003] also had rare anomalies in addition to the characteristic features of HSS. She presented with choanal atresia, small cerebellum, hypothyroidism, and generalized organic aciduria [Hou, 2003]. Although most of the patients with HSS have normal intelligence, intellectual disability ranging from mild to severe has been documented [Cohen, 1991; David et al., 1999]. Taken together, most of the patients with HSS have the classical manifestations; however, the natural variations of the clinical spectrum might include mild and severe HSS, or 'variant forms' as speculated earlier [Verloes, 1997]. As long as the genetic etiology of this syndrome remains unknown, it is still under discussion whether the different manifestations in patients with HSS represent distinct genetic entities as they could overlap with e.g. ODDD [Spaepen et al., 1991], osteocraniostenosis [Verloes, 1997], Wiedemann-Rautenstrauch syndrome, and MAD [Cohen, 1991]. In addition, genetic heterogeneity can not be excluded in patients with HSS and different severity.

The report on a 36-fold increased chromosome breakage rate in a patient with the principal manifestations of HSS [Hou, 2003] prompted us to investigate the chromosome breakage rate in our patients. Normal chromosome breakage rate in 4 patients with HSS indicates that defective DNA recombination and/or recombination repair is not likely to be associated with this disorder.

Progeroid appearance in patients with HSS as well as dental anomalies, mandibular hypoplasia, skin atrophy, hypotrichosis, and short stature are also common features in patients with HGPS and MAD. Most of the typical patients with HGPS have a recurrent de novo *LMNA* mutation, resulting in a lamin A protein which lacks 50 amino acids near the C-terminus, including the second cleavage site for ZMPSTE24. The mutant form of lamin A can be processed at the CAAX motif, including methylation by ICMT, but fails to be cleaved by ZMPSTE24 and is permanently farnesylated and carboxylmethylated [Young et al., 2006]. As mutations in *ZMPSTE24* itself also result in a progeroid disorder [Worman and Bonne, 2007], the hypothesis has been put forward that the amount of accumulated farnesyl-prelamin A determines the severity in disease [Young et al., 2006; Barrowman and Michaelis, 2009]. Farnesyl-prelamin A and other lamin A mutant proteins are targeted to the nuclear envelope, where they disrupt the integrity of the nuclear lamina and interfere with the nuclear architecture [Worman and Bonne, 2007]. We studied the nuclear morphology in skin fibroblasts of 2 patients with HSS and could not find any alteration. However, nuclear abnormalities have been identified only in a subset of fibroblasts from patients with *LMNA* mutations [Muchir et al., 2004]. We therefore analyzed the genes *LMNA, ZMPSTE24* and *ICMT* for mutations in the 8 patients with HSS. In patient 2, we identified the heterozygous *LMNA* mutation c.1930C>T (p.R644C) that has been considered as a pathogenic missense mutation [Rankin et al., 2008]. Arginine at position 644 is part of the cleavage recognition sequence for ZMPSTE24 which removes the 15 C-terminal amino acids of prelamin A [Kilic et al., 1997]. If substitution of arginine by cysteine results in abnormal processing of prelamin A, accumulation of unprocessed prelamin A would be expected in cells of individuals with this *LMNA* alteration, resulting in a progeria-like phenotype. Indeed, a patient with p.R644C and atypical progeria has been described [Csoka et al., 2004], but although the nuclear shape of the patient's cells showed some ab-

normalities, accumulation of prelamin A has not been detected [Toth et al., 2005]. The p.R644C mutation has been previously linked to extreme phenotypic diversity including lipodystrophy, insulin resistance, motor neuropathy, dilated cardiomyopathy, left ventricular hypertrophy, limb girdle muscle weakness, hepatic steatosis, and atypical progeria [Genschel et al., 2001; Csoka et al., 2004; Mercuri et al., 2005; Muntoni et al., 2006; Rankin et al., 2008; Møller et al., 2009; Perrot et al., 2009]. All patients with the p.R644C mutation had features of laminopathies, but some showed additional complications such as renal disease with focal segmental glomerulosclerosis, growth retardation, micrognathia, sensorineural hearing loss, and congenital contractures with club foot [Rankin et al., 2008]. The phenotype of patient 2 is not typical of laminopathies as she presented with ocular anomalies, such as bilateral congenital cataracts, microphthalmia of the left eye in addition to short stature, a small and thin nose, a thin vermillion border, and microretrognathia that are all manifestations seen in patients with HSS. Although compelling evidence exists that the p.R644C mutation in *LMNA* is pathogenic, nonpenetrance of this sequence change in many first-degree relatives [Rankin et al., 2008; this report] and the presence of this alteration in 0.8% of the Danish population [Møller et al., 2009] suggested that a second mutation could be considered in affected individuals with p.R644C [Muntoni et al., 2006]. Accordingly, the p.R644C mutation might have contributed to the phenotype of patient 2; however, the failure to identify any pathogenic *LMNA* mutation in the other 7 patients with HSS suggests that *LMNA* is not the major gene for this syndrome. In line with this conclusion, the homozygous c.1158–44C>T mutation in *LMNA* in patient 1 did not turn out to be pathogenic. Although in silico analysis predicted the creation of a new splice donor site in intron 6, *LMNA* transcript analysis in this patient did not reveal any evidence for aberrant splicing.

We identified 2 SNPs and 1 small rearrangement in the 5′-untranslated region of the genes *ZMPSTE24* and *ICMT*, respectively, which we believe are rather benign polymorphisms than pathogenic mutations (online suppl. table 2). Nonetheless, we can not rule out molecular lesions in the 3 genes that escaped detection by our mutation screening, such as large exon-spanning deletions, inversions and duplications, changes in the 5′ and 3′ untranslated regions, and promoter defects. Taken together, our data provide evidence that HSS does not belong to the laminopathies, although we cannot yet exclude that the underlying genetic basis of HSS may be linked to the nuclear envelope and associated proteins.

Exome sequencing is the method of choice for identifying the pathogenic mutation in patients with HSS in the future, as this approach has been successfully used for other rare monogenic disorders [Ku et al., 2011]. HSS could be caused by a germline or somatic mutation. Therefore, DNA isolated from affected and unaffected tissues might be useful to detect the causative variant in the small series of patients. Indeed, exome sequencing successfully identified a mosaic mutation in the *AKT1* gene in patients with Proteus syndrome [Lindhurst et al., 2011].

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